Weight Loss and the Renin-Angiotensin-Aldosterone System

Stefan Engeli, Jana Böhnke, Kerstin Gorzelniak, Jürgen Janke, Petra Schling, Michael Bader, Friedrich C. Luft, Arya M. Sharma

Abstract—The renin-angiotensin-aldosterone system has been causally implicated in obesity-associated hypertension. We studied the influence of obesity and weight reduction on the circulating and adipose tissue renin-angiotensin-aldosterone system in menopausal women. Blood samples were analyzed for angiotensinogen, renin, aldosterone, angiotensin-converting enzyme activity, and angiotensin II. In adipose tissue biopsy samples, we analyzed angiotensinogen, renin, renin-receptor, angiotensin-converting enzyme, and angiotensin II type-1 receptor gene expression. Obese women (n=19) had higher circulating angiotensinogen, renin, aldosterone, and angiotensin-converting enzyme than lean women (n=19), and lower angiotensinogen gene expression in adipose tissue. Seventeen women successfully participated in a weight reduction protocol over 13 weeks to reduce daily caloric intake by 600 kcal. Body weight was reduced by -5%, as were angiotensinogen levels by -27%, renin by -43%, aldosterone by -31%, angiotensin-converting enzyme activity by -12%, and angiotensinogen expression by -20% in adipose tissue (all P<0.05). The plasma angiotensinogen decrease was highly correlated with the waist circumference decline (r=0.74; P<0.001). Weight and renin-angiotensin-aldosterone system reductions were accompanied by a -7-mm Hg reduced systolic ambulatory blood pressure. These data suggest that a 5% reduction in body weight can lead to a meaningfully reduced renin-angiotensin-aldosterone system in plasma and adipose tissue, which may contribute to the reduced blood pressure. (Hypertension. 2005;45:1-7.)

Key Words: adipose tissue ▪ aldosterone ▪ angiotensinogen ▪ hypertension ▪ obesity ▪ renin

Obesity leads to hypertension and increased cardiovascular risk.1,2 The renin-angiotensin-aldosterone system (RAAS) has been implicated by several authors.3 In humans, increased circulating angiotensinogen (AGT), renin, aldosterone, and angiotensin-converting enzyme (ACE) activity were reported in obese subjects.4–10 Furthermore, increased RAAS gene expression was described in adipose tissue, especially in rodent models of obesity.3,11–15 The link between adipose tissue AGT gene expression and blood pressure was recently documented in 2 mouse models. Targeted AGT expression in adipocytes of wild-type and AGT knockout mice increased circulating AGT levels and blood pressure.16 Targeted expression of 11β-hydroxysteroid dehydrogenase-1 in adipocytes increased blood pressure, plasma AGT, and adipose tissue AGT gene expression in mice with a wild-type genetic background.17,18 The relationship between blood pressure and the RAAS in obese humans comes mostly from observational and not from intervention studies. The influence of weight loss on RAAS activity, especially on AGT plasma levels and the adipose tissue RAAS, has not been explored.

Methods

The institutional review board approved both studies; all volunteers gave informed written consent. Thirty-eight white menopausal women participated in the cross-sectional study, 30 menopausal women started the weight reduction protocol, and 17 achieved the 5% body weight reduction goal. None had diabetes mellitus, liver disease, congestive heart failure, coronary heart disease, or microalbuminuria. Hormonal replacement therapy was stopped 4 weeks and all other medication 7 days before the studies. No concomitant medication was allowed during weight loss. We took the precaution that no subject lost >4 kg in weight during the 3 months before both protocols. Anthropometric measurements and fasting blood samples were obtained at 9:00 A.M. Adipose tissue biopsies were taken by needle biopsy from the periumbilical region.11 Appropriate cuff size was used for 24-hour ambulatory blood pressure measurement (SPACELABS 90207). Homeostasis model assessment (HOMA) index of insulin resistance was calculated.13 In the weight loss study, dietary consultation to reduce energy intake by 600 kcal/d and water gymnastics exercises were begun the day after clinical assessments. Adipose tissue biopsies and clinical measurements were repeated after a 5% body weight loss was achieved. Four-day nutrition diaries were kept. Urine was collected for 24 hours at the beginning and at the end of the weight loss study in parallel to ambulatory blood pressure measurement.

We isolated and processed mRNA for real-time polymerase chain reaction (TaqMan technology by PE Biosystems, Weiterstadt, Germany) as described in detail previously.13 The standard curve
TABLE 1. Clinical Variables From the Cross-Sectional Study (mean±SD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lean</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Age, y</td>
<td>56±3</td>
<td>58±4</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.5±1.9</td>
<td>37.6±3.7*</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>76±6</td>
<td>106±7*</td>
</tr>
<tr>
<td>ABPM_systolic, daytime, mm Hg</td>
<td>132±20</td>
<td>139±11</td>
</tr>
<tr>
<td>ABPM_diastolic, daytime, mm Hg</td>
<td>81±12</td>
<td>82±8</td>
</tr>
<tr>
<td>Mean daily heart rate, min⁻¹</td>
<td>82±10</td>
<td>83±10</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.4±0.9</td>
<td>5.6±0.7</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.4±0.4</td>
<td>1.3±0.3</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.5±0.8</td>
<td>3.8±0.9</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.0±0.4</td>
<td>1.3±0.6</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.0±0.3</td>
<td>5.5±0.6*</td>
</tr>
<tr>
<td>Insulin, μU/L</td>
<td>2.6±1.6</td>
<td>7.7±4.1*</td>
</tr>
<tr>
<td>HOMA index</td>
<td>0.7±0.4</td>
<td>2.0±1.0*</td>
</tr>
</tbody>
</table>

*P<0.05 vs lean.

Group comparison by Student t test for independent samples. ABPM indicates ambulatory blood pressure measurement; BMI, body mass index; HDL, high-density lipoprotein; HOMA, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein.

method was used for the target genes (AGT, renin, renin-receptor, ACE, angiotensin II type-1 [AT1] receptor) and the internal control gene (human glycerolaldehyde-3-phosphate dehydrogenase, GAPDH) in identical RNA samples. Expression of the target genes was normalized by GAPDH expression in each sample and is given in arbitrary units. Expression of the renin receptor gene in isolated human adipocytes was detected by our group (data not shown) and has not been reported before. The sequences used for real-time polymerase chain reaction were: forward primer, 5′ CACTGGCAATGTTGGGAA3′; reverse primer, 5′ CACTCCCCCC-TCACCTACCACAT3′; fluorescently labeled probe, 6-FAM-5′ TGGTTTCATCGTCCTCGGCTACCG3′-TAMRA. Interassay coefficients of variation were 1.8% for GAPDH, 6.7% for AGT, 6.4% for renin, 3.1% for the renin receptor, 6.6% for ACE, and 6.8% for the AT1 receptor.

Fasting plasma and serum samples were collected after 30 minutes of rest in the supine position. Plasma AGT was determined by radioimmunoassay after the cleavage to Ang I by exogenously added human renin as described.19 Serum Ang II was measured by enzyme radioimmunoassay after the cleavage to Ang I by exogenously added renin, aldosterone, and ACE activity in obese subjects (Figure 1).

In adipose tissue, decreased expression was found for the AGT gene in obese subjects, whereas expression of the other genes was not different between lean and obese women (Figure 2).

Weight loss of 5% within 16 weeks was achieved by 17 of 30 women. These women were aged 59±7 years and lost 5.6±1.0% body weight during 13±2 weeks. Table 2 summarizes the changes in clinical variables, diet composition, and electrolyte excretion with weight reduction. These data demonstrate that the obese women in the cross-sectional and the weight loss studies were similar, allowing a systematic study of the RAAS in obesity and weight loss. Besides anthropometric variables, changes in systolic daily mean ambulatory blood pressure measurement, fasting insulin, and in the HOMA index were observed. Weight loss was achieved by a reduction in total food consumption; no major changes in food composition were seen. Sodium and potassium intake and excretion were not significantly decreased at the end of the study.

Reduced levels were found for circulating AGT, renin, aldosterone, and ACE after weight loss (Figure 3). In adipose tissue, decreased expression was found for AGT, renin, aldosterone, and ACE (Pearson coefficient of correlation, data not shown). However, weight loss is nonspecific, whereas a decrease in waist circumference is a valuable surrogate for the loss of visceral adipose tissue. We found a highly significant correlation between the decline in AGT plasma levels, and waist circumference that was independent of the reduction in body weight or body mass index (BMI) (r=0.71; P=0.004; after correction for weight loss and reduction of BMI; Figure 5). Furthermore, the decrease of circulating AGT was strongly correlated with the decrease of AGT gene expression in adipose tissue (Figure 5). The reduction in systolic blood pressure was correlated with both plasma AGT (r=0.61; P=0.006) and AGT gene expression in adipose tissue (r=0.51; P<0.05).

Discussion

The higher AGT, renin, aldosterone, and ACE activity levels in obese compared with lean menopausal women suggest that the RAAS was activated in our obese subjects. This activation was reduced by 5% body weight reduction which was accompanied by a 7-mm Hg decrease in systolic 24-hour ambulatory blood pressure. In adipose tissue, AGT gene expression was decreased in obese women and decreased even further with weight loss. Besides being obese, all the women were healthy, with slightly increased cholesterol levels. None had signs and symptoms of obesity-associated end-organ damage.
Increased circulating AGT plasma levels in obesity have been described before. We confirmed this finding and demonstrate for the first time to our knowledge that increased AGT plasma levels in obese subjects can be reduced by 5% weight loss, close to levels in lean subjects. Furthermore, the decrease in waist circumference, a surrogate for reduced body fat mass, was a better predictor of decreased AGT plasma levels than weight loss per se. This finding directly leads to the question of whether adipose AGT secretion is involved in the determination of AGT plasma levels, as has been sug-
gested by animal studies.16,22 This question is difficult to study in humans. Microdialysis cannot be used because of the molecular size of AGT and arteriovenous differences of AGT over adipose tissue depots have never been measured. Studying AGT gene expression instead yielded conflicting results.

We found decreased AGT expression in subcutaneous adipose tissue of obese subjects, confirming our earlier results.13 Decreased or unchanged AGT expression levels in adipose tissue of obese or hypertensive subjects have also been published by others.14,15,23 Furthermore, AGT secretion from isolated subcutaneous adipocytes was not different between lean and obese donors.24 Only 1 group reported increased expression of the AGT gene in subcutaneous and visceral adipose tissue with increased BMI or increased waist circumference.11,12 In clear contrast to animal data,16–18,22,25–27 most human studies did not support increased adipose tissue AGT expression in obesity. Decreased adipose tissue AGT expression after weight loss has not been reported previously. Although AGT secretion from adipocytes is well documented, we cannot exclude the possibility that other cell types than adipocytes (eg, endothelial cells, lymphocytes, monocytes/macrophages) contribute to decreased AGT formation in adipose tissue. Furthermore, we cannot exclude the possibility that the secretion of AGT from the liver decreases with weight loss in our study. Animal data, however, strongly suggest that AGT secretion from the liver is not influenced by obesity or weight loss.22,27

If adipocytes contribute to circulating AGT levels in humans, then increased adipose tissue mass itself would be sufficient to increase AGT plasma levels in the obese. Increased AGT expression on the adipocyte level is not a necessary requirement. Decreased adipose tissue mass, together with decreased adipose tissue mass, could contribute to the decline of plasma AGT with weight loss. A strong relationship between the

<table>
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<th>Variable</th>
<th>Baseline</th>
<th>Weight Loss</th>
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<tr>
<td>BMI, kg/m²</td>
<td>33.1±4.6</td>
<td>31.2±4.3*</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>101±11</td>
<td>97±11*</td>
</tr>
<tr>
<td>ABPM&lt;sub&gt;systolic&lt;/sub&gt;</td>
<td>138±12</td>
<td>131±10*</td>
</tr>
<tr>
<td>ABPM&lt;sub&gt;diasstolic&lt;/sub&gt;</td>
<td>82±6</td>
<td>80±5</td>
</tr>
<tr>
<td>Mean daily heart rate, min⁻¹</td>
<td>82±10</td>
<td>80±10</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.7±1.0</td>
<td>5.5±1.1</td>
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<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.7±0.4</td>
<td>1.6±0.4</td>
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<td>LDL cholesterol, mmol/L</td>
<td>3.5±0.9</td>
<td>3.3±1.0</td>
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<tr>
<td>Triglycerides, mmol/L</td>
<td>1.2±0.5</td>
<td>1.3±0.6</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.7±0.8</td>
<td>5.7±0.8</td>
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<tr>
<td>Insulin, μU/L</td>
<td>4.8±3.3</td>
<td>3.9±2.5*</td>
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<tr>
<td>HOMA index</td>
<td>1.2±0.9</td>
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</tr>
<tr>
<td>Calorie intake, kcal/d</td>
<td>2164±699</td>
<td>1423±421*</td>
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<tr>
<td>Fat content, %</td>
<td>37±9</td>
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<td>Carbohydrate content, %</td>
<td>47±9</td>
<td>47±8</td>
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<tr>
<td>Protein content, %</td>
<td>16±3</td>
<td>20±5*</td>
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<td>Sodium intake, mmol/24 h</td>
<td>109±39</td>
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<tr>
<td>Potassium intake, mmol/24 h</td>
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<td>73±23</td>
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<td>Sodium excretion, mmol/24 h</td>
<td>105±59</td>
<td>96±51</td>
</tr>
<tr>
<td>Potassium excretion, mmol/24 h</td>
<td>49±25</td>
<td>47±22</td>
</tr>
</tbody>
</table>

Group comparison by t test for paired samples.

Seventeen postmenopausal women (aged 59±7 years) lost 5.6±1.0% body weight during 13±2 weeks.

*P<0.05 vs baseline.

Figure 3. The circulating renin-angiotensin-aldosterone system before and after 5% weight loss in 17 obese postmenopausal women. Data are given as mean±SD. Group comparison by t test for paired samples. *P<0.05.
decrease in adipose tissue AGT expression and circulating AGT levels was found in our study. We thus propose a negative feedback loop that controls adipocyte AGT expression in the situation of increasing AGT plasma levels in the obese. Weight loss may add a regulatory mechanism that further reduces AGT expression in adipose tissue. Decreased AGT plasma levels may then foster the decreased blood pressure. This model is based on the assumption that adipose tissue AGT enters the systemic circulation. In mice, this state of affairs is the case.16

The mechanisms that may control AGT expression in the obese and reduce AGT expression during weight loss are not known. No convincing hormonal regulators of the AGT gene have been identified in human or animal adipocytes.3 Several studies suggested the importance of AGT genotypes for the body weight–blood pressure relationship.28–31 How these variants (AGT-6, AGT-20, AGT174, AGT235) might control AGT expression and plasma AGT levels is not known. Furthermore, negative results have also been obtained for the AGT235 genotype and obese phenotypes.5,32 AGT secretion from isolated human adipocytes was not influenced by the AGT235 genotype.34 With respect to weight loss, AGT-6 genotypes were associated with the reduction of blood pressure, but not with weight loss itself.33

Our data confirm higher renin and aldosterone levels in obese subjects.8–10,34 Increased renin and aldosterone levels are not necessarily expected, because obese subjects typically present with sodium retention and volume expansion.35 Overactivity of the renal sympathetic nervous system may stimulate renin release in the obese.36 The renal sympathetic nerve activity may be stimulated by leptin that could represent the link between increased renin levels and increased fat mass.37 An oxidized derivative of linoleic acid was a potent stimulator of aldosterone secretion in an earlier in vitro study.38 Furthermore, conditioned media of human adipocytes contained biochemical substances that increased aldosterone secretion in vitro independent of potassium or AT1 receptor activation.39 Weight loss decreased circulating renin and
aldosterone levels in our study, confirming earlier findings. High renin levels have been shown to predict the decline in blood pressure induced by weight loss, but we did not see a close relationship between renin or aldosterone reduction and weight or blood pressure reduction in our study (data not shown). The mechanisms that may increase renin in the obese are reduced by weight loss. The mechanisms that decrease circulating aldosterone in weight reduced subjects are less clear, but decreased renin activity per se may contribute, as well as the possible reduction of adipocyte products and oxidized fatty acid derivatives. Sodium and potassium intake did not change during the weight loss period and are thus unlikely to be involved. Weight loss may reduce renin and aldosterone by different mechanisms, because the baseline renin and aldosterone levels were highly correlated ($r=0.75$; $P<0.01$), but not after the weight loss levels.

Higher ACE activity in obesity and the decrease in ACE activity with weight loss have been described previously. The DD genotype of the ACE gene may predict abdominal obesity and larger increases in body weight and blood pressure with aging in men. Furthermore, the DD genotype influenced the sensitivity of blood pressure to weight loss, but not the amount of weight loss per se. The decrease of ACE activity with weight loss, however, was not closely linked to the reduction in blood pressure in our study (data not shown).

In obese mice, renal ACE activity was significantly increased in an endothelin receptor type A-dependent manner. Other tissues have not been examined in this study.

Whereas circulating levels of the RAAS were increased in obese subjects and reduced by weight loss, the adipose tissue RAAS gene expression, with the exception of the AGT gene, was not influenced by obesity or weight loss. This finding is consistent with earlier results. If the lack of RAAS gene regulation in obesity is transformed into local Ang II production in adipose tissue, we can speculate that a dysregulated Ang II formation and action is of great importance for the disturbed adipose tissue metabolism in obesity. Findings using the microdialysis technique in adipose tissue corroborate this speculation. The microdialysis data, as well as the data presented here, have been obtained in subcutaneous adipose tissue. It is known that at least the expression of AGT is 2-fold higher in visceral adipose tissue compared with subcutaneous adipose tissue. Furthermore, several metabolic complications of obesity are more closely linked to the presence of increased visceral adipose tissue than to BMI itself. Thus, our findings are restricted to a specific adipose tissue depot. However, subcutaneous adipose tissue represents $\approx 75\%$ of the total body fat mass. Changes in regulation of genes encoding secreted proteins in subcutaneous adipose tissue are therefore likely to have an important impact. In clinical practice, visceral adipose tissue accumulation is determined by measuring waist circumference. The close relationship between decreased AGT plasma levels and the reduction of waist circumference in our study supports the assumption that visceral adipose tissue reacts similar to subcutaneous adipose tissue under the condition of weight loss.

### Perspectives

Obesity is associated with increased levels of the circulating RAAS (AGT, renin, aldosterone, ACE). These increased levels were significantly decreased by 5% body weight loss. The downregulated AGT expression in adipose tissue in response to weight loss supports the assumption that AGT plasma levels are linked to AGT gene expression in adipose tissue. Furthermore, the reductions in AGT expression in adipose tissue and circulating AGT were correlated to the reduction in systolic blood pressure. These data suggest that reduced body fat mass may lower RAAS activity in plasma and adipose tissue, a finding with therapeutic implications.

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