Weight Loss and the Renin-Angiotensin-Aldosterone System

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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;45:356-362.)

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

Obesity leads to hypertension and increased cardiovascular risk. The renin-angiotensin-aldosterone system (RAAS) has been implicated by several authors. In humans, increased circulating angiotensinogen (AGT), renin, aldosterone, and angiotensin-converting enzyme (ACE) activity were reported in obese subjects. Furthermore, increased RAAS gene expression was described in adipose tissue, especially in rodent models of obesity. 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. 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. 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 comitant medication was allowed during weight loss. We took the precaution that no subject lost >1 kg in weight during the 3 months before both protocols. Anthropometric measurements and fasting blood samples were obtained at 9:00 a.m. Abdominal subcutaneous adipose tissue samples were taken by needle biopsy from the periumbilical region. Appropriate cuff size was used for 24-hour ambulatory blood pressure measurement (SPACELABS 90207). Homeostasis model assessment (HOMA) index of insulin resistance was calculated. 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. The standard curve

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method was used for the target genes (AGT, renin, renin-receptor, ACE, angiotensin II type-1 [AT1] receptor) and the internal control gene (human glyceraldehyde-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′CACATCTTC-TCACCATCACCATT3′; reverse primer, 5′TGTTTCATCGTCCTCGGGCTACCC3′; fluorescently labeled probe, 6-FAM-AATCCATCGTAAACATTTCGATTGGGCCGTATGAC-3′-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.18 Serum Ang II was measured by enzyme immunoassay after extraction with ice-cold ethanol using the Ang II ELA kit (Bachem, Germany).20 ACE activity in the serum was determined by a calorimetric assay (Sigma Diagnostics, Deisenhofen, Germany). Plasma renin and activated prorenin concentration was determined by an immunochemiluminometric assay (Nichols Institute Diagnostics, Advantage Direct Renin Assay, San Clemente, Calif). Serum aldosterone was determined by a solid-phase radioimmunoassay (DPC Biermann, Bad Nauheim, Germany). Interassay coefficients of variation were 3.4% for AGT, 17% for Ang II, 7.2% for ACE activity, 6.1% for renin, and 5.6% for aldosterone.

Data were analyzed by SPSS 11.5.1 (SPSS Inc, Chicago, Ill). All variables (mean±SD) were normally distributed. Student t test was used for group comparisons. A paired sample t test was used for baseline and weight loss data. Pearson coefficient of correlation described relationships between variables. Results were considered statistical significant at P<0.05.

Results

Table 1 shows the clinical variables from the 38 women participating in the cross-sectional study. Fasting levels of glucose, insulin, and the HOMA index of insulin resistance were increased in the obese subjects, but were not in the diabetic range. Ambulatory blood pressure and blood lipids were similar; slightly increased levels of total and low-density lipoprotein cholesterol were found in both groups. For the systemic RAAS, increased levels were found for AGT, 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 (Figure 4). The differences between baseline and weight loss mean values was not reflected by relationships between the degree of weight loss and the degree of reduction in AGT expression, circulating AGT, renin, aldosterone, or ACE (Pearson coefficient of correlation, data not shown). However, weight loss was 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 weight loss 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.\(^{4-7*31}\) 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-

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**Figure 1.** Comparison of the circulating renin-angiotensin-aldosterone system between 19 lean and 19 obese postmenopausal women. Data are given as mean±SD. Group comparison by Student t test for independent samples. *P<0.05.

**Figure 2.** Comparison of adipose tissue expression of renin-angiotensin system genes between 19 lean and 19 obese postmenopausal women. Data are given as mean±SD. Group comparison by Student t test for independent samples. *P<0.05. AT1R indicates angiotensin II type-1 receptor; REN, renin; RENR, renin receptor.
gested by animal studies. 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. Decreased or unchanged AGT expression levels in adipose tissue of obese or hypertensive subjects have also been published by others. Furthermore, AGT secretion from isolated subcutaneous adipocytes was not different between lean and obese donors. Only 1 group reported increased expression of the AGT gene in subcutaneous adipose tissue with increased BMI or increased waist circumference. In clear contrast to animal data, 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.

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 AGT expression in adipose cells during the weight loss period, together with decreased adipose tissue mass, could contribute to the decline of plasma AGT with weight loss. A strong relationship between the

**TABLE 2. Changes With Weight Reduction (mean±SD)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Weight Loss</th>
</tr>
</thead>
<tbody>
<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; daytime, mm Hg</td>
<td>138±12</td>
<td>131±10*</td>
</tr>
<tr>
<td>ABPM&lt;sub&gt;systolic&lt;/sub&gt; daytime, mm Hg</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>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.7±0.4</td>
<td>1.6±0.4</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.5±0.9</td>
<td>3.3±1.0</td>
</tr>
<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>
</tr>
<tr>
<td>Insulin, µU/L</td>
<td>4.8±3.3</td>
<td>3.9±2.5*</td>
</tr>
<tr>
<td>HOMA index</td>
<td>1.2±0.9</td>
<td>1.0±0.7*</td>
</tr>
<tr>
<td>Calorie intake, kcal/d</td>
<td>2164±699</td>
<td>1423±421*</td>
</tr>
<tr>
<td>Fat content, %</td>
<td>37±9</td>
<td>33±6</td>
</tr>
<tr>
<td>Carbohydrate content, %</td>
<td>47±9</td>
<td>47±8</td>
</tr>
<tr>
<td>Protein content, %</td>
<td>16±3</td>
<td>20±5*</td>
</tr>
<tr>
<td>Sodium intake, mmol/24 h</td>
<td>109±39</td>
<td>96±30</td>
</tr>
<tr>
<td>Potassium intake, mmol/24 h</td>
<td>83±29</td>
<td>73±23</td>
</tr>
<tr>
<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.24 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.

Figure 4. Adipose tissue expression of renin-angiotensin system genes 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.

Figure 5. Relationship between the reduction in waist circumference or adipose tissue AGT expression and the decline in AGT plasma levels in 17 postmenopausal women in the weight loss study; 95% confidential intervals are given for the regression analysis.
Ang II formation and action is not of great importance for the regulation in obesity is transformed into local Ang II production in the presence of increased visceral adipose tissue than 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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**References**


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