Serum Angiotensinogen Concentration in Relation to Gonadal Hormones, Body Size, and Genotype in Growing Young People

J. Howard Pratt, Walter T. Ambrosius, Duane A. Tewksbury, Mary Ann Wagner, Lifen Zhou, Mark P. Hanna

Abstract—Multiple factors are thought to influence the level of circulating angiotensinogen (AGT). We showed previously that the serum AGT concentration was significantly related to body mass index (BMI) in a cohort of young people. In the present study, we studied whether levels of the gonadal hormones estradiol and testosterone might also predict the AGT level and might contribute to the BMI effect, since both the production of these hormones and BMI increase with age. In boys (n=127; mean±SD age, 14.7±1.9 years) and girls (n=104; age, 14.8±1.9 years) studied as a single group, we found a significant association of AGT level with level of estradiol (P=0.015) after adjustment for haplotype, age, race, testosterone concentration, and BMI. In girls studied alone, the level of AGT showed a significantly positive relation to level of testosterone (P=0.043), possibly a result of peripheral conversion of testosterone to estradiol, after adjustment for haplotype, age, race, estradiol concentration, and BMI. In boys, on the other hand, the level of testosterone was inversely related to AGT concentration (P=0.019), again after making adjustments for the other variables. Finally, in pairs of subjects matched for BMI, age, race, and gender where 1 member of each pair had either 1 or 2 copies of an AGT gene haplotype (T235 and -1074t) and the other member had no copy, the level of AGT was higher in the carrier of a haplotype in 24 of the 34 pairs (P<0.001). In conclusion, gonadal hormones are an additional influence on the circulating level of AGT in growing young people. In addition, with matching for BMI and other covariates, there is a strong association of AGT genotype with the serum level of AGT, emphasizing the importance of AGT gene expression as a determinant of the circulating level of AGT. (Hypertension. 1998;32:875-879.)

Key Words: angiotensinogen ■ genes ■ estradiol ■ testosterone ■ race ■ body mass index

Angiotensinogen (AGT) is cleaved by renin to form angiotensin I (Ang I), the precursor to Ang II. The concentration of AGT is rate-limiting for the generation of Ang II.1,2 There is much interest in the relationship of AGT to the genesis of hypertension because blood pressure and serum AGT level are related1 and essential hypertension has been linked to the AGT gene.3,4-7

In an earlier study of young people, we found the serum level of AGT to be highly associated with body mass index (BMI).5 The basis for the relationship of BMI to serum AGT concentration was unclear; was it an effect related to body size alone or also an effect of factors that are related to age and maturation? AGT synthesis is known to be affected by certain steroid hormones,8-12 and since our cohort was at various stages of pubertal development, an effect of sex steroids, which increase as young people become older, could account for part of the relation of AGT concentration to BMI. We therefore explored in children and adolescents the association of the serum AGT concentration to estradiol (E2) and testosterone (T) concentrations during this period of development. Dehydroepiandrosterone-sulfate (DHEA-S), a weak androgen that increases during adrenarche,13 was also studied.

In an additional study, we examined further the relationship of the AGT variant M235T4 to the circulating level of AGT. We found previously that BMI was a strong confounding variable when such analyses were performed.8,14 We report here on an analysis in which we examined the relationship of serum AGT to AGT genotype in subjects who were matched for BMI as well as for gender, age, and race.

Methods

Subjects

Subjects were recruited from an ongoing longitudinal study of blood pressure in young people.15 All subjects were normotensive and in good health. None were taking medication, including birth control pills.

Procedures

Most subjects were seen at their schools; approximately a quarter of the subjects were seen in the General Clinical Research Center. Subjects’ weight and height were measured. Blood pressure was...
TABLE 1. Characteristics of Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Boys (n=127)</th>
<th>Girls (n=104)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>14.7±1.9</td>
<td>14.8±1.9</td>
<td>0.65</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>110.0±12.7</td>
<td>106.6±10.6</td>
<td>0.031</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>66.6±10.1</td>
<td>65.8±9.6</td>
<td>0.55</td>
</tr>
<tr>
<td>AGT, nmol Ang I per liter</td>
<td>1154±269</td>
<td>1255±321</td>
<td>0.0096</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.3±4.7</td>
<td>23.0±5.3</td>
<td>0.28</td>
</tr>
<tr>
<td>Estradiol, pmol/L</td>
<td>57.6±44.0</td>
<td>227.5±196.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Testosterone, nmol/L</td>
<td>11.2±8.0</td>
<td>0.8±0.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DHEA-S, nmol/L</td>
<td>3.1±1.8</td>
<td>2.7±1.9</td>
<td>0.075</td>
</tr>
<tr>
<td>Haplotype, 0/1/2 copies</td>
<td>105/16/6</td>
<td>84/18/2</td>
<td>...</td>
</tr>
<tr>
<td>Race, black/white</td>
<td>37/90</td>
<td>39/65</td>
<td>...</td>
</tr>
</tbody>
</table>

Values are mean±SD.

Measured in the right arm using a random zero sphygmomanometer (Hawksley and Sons) with the subject seated. The first and fifth Korotkoff sounds were used to designate systolic and diastolic blood pressures, respectively. Three blood pressure readings were obtained, and the average of the last 2 was used as the final blood pressure. A blood sample was taken for measurement of AGT and sex hormones and for isolation of DNA. Each sample was centrifuged (IEC-Centra-8R centrifuge, International Equipment Co) at room temperature at 2700 rpm for 10 minutes. Serum was separated from the red blood cells and then stored at −70°C until assayed. White blood cells were removed from samples collected in EDTA for subsequent extraction of DNA.

**Assay Procedures**

AGT was measured as described previously. In brief, this was a 2-step procedure consisting of conversion of AGT to Ang I by human renin followed by the measurement of Ang I by radioimmunoassay. E2, T, and DHEA-S were measured in serum samples by radioimmunoassay (Coat-A-Count, Diagnostic Products Corp).

**Genotyping**

We chose an AGT genotype that previously showed a relationship to the serum AGT concentration in both blacks and whites, a diallelic haplotype consisting of T235 and −1074T variants of AGT. T235 alone did not show a relationship to the serum AGT level in blacks in our previous study; we believe this was because of the high allele frequency of T235 in blacks. (It should be noted, however, that other investigators have shown a significant relationship to the AGT level in African blacks in whom the frequency of T235 was even higher than in the present study.) The AGT haplotype that divides the T235 carrier state into separate groups provides a potentially more informative marker of AGT expression than T235 alone. The procedure for genotyping has been described previously.

**Statistical Methods**

The means and standard deviations of the variables of interest were calculated and are presented in Table 1. Sex steroid concentration (T, E2, and DHEA-S), age, BMI, systolic and diastolic blood pressures, and serum AGT concentration unadjusted for other covariates were compared with 2-sample t tests. ANCOVA was used to model the joint effects of multiple independent predictors on the dependent variables (serum AGT concentration and blood pressure). The independent variables included haplotype (the number of copies of T235 and T235 [2 df]) as well as age, gender, race, sex steroid concentration, and BMI. The significance level of each independent variable in the multivariate analyses was adjusted for the possible effects of all of the other variables. The data were analyzed using boys and girls combined and also by gender groups separated. An added-variable plot was used to graphically display the added effect of T and E2 on the serum AGT level after adjustment for the other variables in the model. The slope of the added-variable plot is the same as the slope estimated by the ANCOVA model after adjustment for the other factors in the model. For the E2 added-variable plots, the other variables included gender, race, BMI, age, haplotype, and the T level. For T, we studied the boys and girls separately, and the other variables adjusted for included race, BMI, age, haplotype, and the E2 level. To describe the strength of the relationships seen in these plots, we calculated the correlation coefficients. We used the partial coefficient of determination to measure the marginal effects of T and E2. It measures the amount of residual variability explained by those 2 variables after adjustment for the other factors in the model.

To study the relation of the AGT gene on AGT concentration, we matched individuals who had no copies of the genotype to those who had 1 or 2 copies of the genotype. Within each matched pair, both subjects were the same race and gender, and they differed by no more than 1 year in age and by 2 kg/m² in BMI. The matching was done rather than a statistical adjustment of the data using covariates because it is more robust to misspecifications of the model. Once the pairs were constructed, the difference in AGT concentration within each pair was calculated. The Wilcoxon signed-rank test was used to test the null hypothesis of no genotype effect. In addition to examining the effect of the AGT genotype using the matching procedure, we used ANCOVA to adjust for possible race, gender, and BMI effects on the AGT level. A Bonferroni correction for multiple comparisons was used to compare the AGT levels among the 3 haplotype groups.

**Results**

Relationships to Sex Hormones After Adjustment for BMI, Age, Gender, Genotype, and Race

Table 1 displays the demographic characteristics of the subjects used for the analyses. Boys and girls were similar in age and BMI. Boys had a significantly higher T concentration (𝑃<0.0001), and the girls had significantly higher concentrations of E2 (𝑃<0.0001) and AGT (𝑃=0.0096). The DHEA-S concentration was marginally higher in the boys than in the girls (𝑃=0.075).

In Table 2, for boys and girls studied as a single group, BMI (𝑃<0.001), haplotype (𝑃<0.001), and serum E2 concentration (𝑃=0.015) were significant predictors, and race (𝑃=0.054) was a marginally significant predictor of the serum AGT concentration. There was no evidence that the relationships between serum AGT level and BMI (𝑃=0.29) and between serum AGT level and E2 level (𝑃=0.52) were different between boys and girls. The mean AGT concentration increased on average by 16 nmol Ang I per liter (AI/L) when BMI increased by 1 kg/m² and increased by 0.31 nmol AI/L when E2 increased by 1 pmol/L after adjustment for the other variables. The mean level of AGT was 129 nmol AI/L higher in those carrying a single copy of the −1074T/T235 haplotype than in those carrying 2 copies of the haplotype (there were, however, only 6 boys and 2 girls carrying 2 copies), and it was 93 nmol AI/L lower in those with no copy when compared with those with 2 copies of the haplotype. The serum AGT concentration was on average 80 nmol AI/L higher in blacks than in whites, again after adjustment for the other variables.

For boys studied alone, age (𝑃<0.001) and race (𝑃=0.005) were significant predictors of the serum AGT concentration, whereas in girls BMI was a significant predictor (𝑃=0.001) and age and race were not. Another gender-specific relationship was that of genotype, which showed a significant
association with serum AGT in girls ($P=0.002$) but not in boys. In boys, the serum AGT concentration increased by an average of 58 nmol AI/L for each year in age, and the mean level was 166 nmol AI/L higher in blacks than in whites. In the girls, the serum AGT concentration increased on average by 21 nmol AI/L when BMI increased by 1 kg/m²; with regard to genotype, the mean AGT level was 7 nmol AI/L lower in girls carrying a single copy of the haplotype than in those carrying 2 copies, and it was 259 lower in girls carrying a single copy of the haplotype than in those carrying 2 copies, and it was 259 nmol AI/L lower in girls carrying a single copy of the haplotype than in those carrying 2 copies.

In boys, the concentration of T showed a significantly negative relationship with AGT level ($P=0.019$), decreasing by 10 AI/L per 1 nmol/L of T. For girls the level of T was also a significant predictor of serum AGT concentration ($P=0.043$), but in this case the relation to the AGT concentration was in the opposite direction: it increased by 132 nmol AI/L with an increase of T of 1 nmol/L. As before, the results depict relationships after adjustment for the other variables. The relation of the E2 level to level of AGT in the girls did not show significance ($P=0.11$). DHEA-S was not significant for girls only ($P=0.31$). For this reason, DHEA-S was dropped from the final model. The models used explained 30%, 30%, and 39% of the variability in the AGT level for all subjects, for boys, and for girls, respectively. The coefficient of partial determination showed that addition of E2 and T to the model explained 2.6%, 4.6%, and 10.6% of the residual variability for all subjects, for boys, and for girls, respectively.

For the E2 added-variable plot (Figure 1), the other variables included BMI, age, gender, race, genotype, and level of T. For the T added-variable plot (Figure 2), boys and girls were studied separately and the other variables adjusted for included BMI, age, gender, race, genotype, and level of E2.

When systolic and diastolic blood pressures were examined as dependent variables, we found that diastolic blood pressure in boys was significantly related to the level of E2 ($P=0.0072$), with an average increase of 0.76 mm Hg for each 10 pmol/L increase in serum E2. There was no corresponding effect in girls ($P=0.860$).

**Relationship to Genotype in Matched Pairs**

There were a total of 8 white male, 12 black male, 6 white female, and 8 black female pairs. The mean age for all gender and racial categories was 14.5±1.8 years (range, 8.9 to 19.0 years). The mean BMI was 22.9±4.0 kg/m² (range, 16.4 to 33.4 kg/m²). Figure 3 shows the differences in the AGT concentrations within pairs where the AGT concentration of the individual with no copy of the genotype was subtracted from the level of the individual who had 1 or 2 copies of the haplotype. When the AGT level of the carrier is higher than in the noncarrier, the AGT level appears above the zero line in the figure. The mean difference was 235.4 (range, 64.2 to 589.1) nmol AI/L.

When using the ANCOVA model (in place of the matching procedure) to adjust for race, gender, age, and BMI, there was also a highly significant haplotype effect ($P=0.0015$). Those with no copy of the haplotype had an adjusted AGT level of 1529 nmol AI/L, those with 1 copy had an adjusted level of 1225 nmol AI/L, those with 2 copies of the haplotype had an adjusted AGT level of 1125 nmol AI/L per liter.

TABLE 2. **Relationships to Serum AGT Concentration**

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variable</th>
<th>All Subjects (n=231)</th>
<th>Boys (n=127)</th>
<th>Girls (n=104)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGT, nmol Ang I per liter</td>
<td>BMI, kg/m²</td>
<td>16</td>
<td>&lt;0.001</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Age, y</td>
<td>15</td>
<td>0.165</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Gender=female</td>
<td>21</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Race=black</td>
<td>80</td>
<td>0.054</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>Haplotype, 0</td>
<td>-93</td>
<td></td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>Haplotype, 1</td>
<td>129</td>
<td>&lt;0.001</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>Testosterone, nmol/L</td>
<td>0.10</td>
<td>0.97</td>
<td>-10</td>
</tr>
<tr>
<td></td>
<td>Estradiol, pmol/L</td>
<td>0.31</td>
<td>0.015</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>589</td>
<td>&lt;0.001</td>
<td>106</td>
</tr>
<tr>
<td>R²</td>
<td>0.30</td>
<td></td>
<td>0.30</td>
<td></td>
</tr>
</tbody>
</table>

Parameters are either regression slopes for continuous predictors or difference between groups for categorical predictors. For example, a 15-year-old white girl with a BMI of 22 kg/m², 2 copies of the haplotype, a testosterone level of 0.5 nmol/L, and an estradiol level of 220 pmol/L would have a predicted AGT level of 15×15 (age)+0 (race)+21 (gender)+22×16 (BMI)+0 (genotype)+0.5×0.10 (T)+220×0.31 (E2)+589 (y intercept)=1225 nmol Ang I per liter.
1801 nmol AI/L, and those with 2 copies had an adjusted level of 1616 nmol AI/L. Again, there was a significant difference between those with no copies of the haplotype and those with 1 copy ($P=0.0009$). There was no evidence that those individuals who had 2 copies of the haplotype were different from either of the other 2 groups, but because there were only 8 individuals who had 2 copies, the power for a comparison involving that group was extremely small.

**Discussion**

The circulating level of AGT is determined by multiple factors, and in the present study, we sought to extend what is known of these influences. We addressed the issue of whether some of the relationship of BMI to serum AGT concentration that we observed previously in children and adolescents $^8$ could be explained by the increase in the sex hormones that change as young people grow. We found in boys and girls studied as a single group, and after adjustment for the other covariates including BMI and age, that levels of E2 and AGT were significantly and positively related ($P=0.015$). This observation is consistent with results of studies in experimental animals $^9$–$^{11}$ and studies of women who were treated with estrogen $^{19,20}$. In girls and boys studied separately, the relationships were in the same direction, although neither was significant, possibly because of the smaller sample sizes and the variability of the E2 levels. In boys, the level of T showed a slight but significantly inverse relationship with level of AGT ($P=0.019$). On the other hand, T levels in girls, which were of course much lower than in boys, showed a significantly positive relationship to AGT levels ($P=0.043$), probably because of conversion of T to E2. Such a T to E2 conversion could take place in adipose tissue, which has the necessary aromatase enzyme activity for E2 synthesis $^{21,22}$. Adipose tissue, which can synthesize AGT $^{23–25}$, contains E2 receptors $^{22,26}$ and thus E2 generated here might directly stimulate synthesis of AGT.

BMI adjusted for all other variables showed a highly significant relationship to the AGT level when boys and girls were studied as a single group ($P<0.001$), similar to findings reported previously for adults $^{17,27–29}$. Because adipose tissue is a source of AGT $^{23–25}$, the positive relationship to BMI can be explained simply from the increase in quantity of adipose tissue. Also, higher insulin levels that are associated with greater adiposity further stimulate synthesis of AGT by both adipose tissue $^{30,31}$ and the liver $^{11}$.

In the present study, genotype was related to the serum AGT level in girls but not in boys. Jeunemaitre et al $^4$ also showed that the T235 variant associated with serum AGT level in females only. In addition, the association of serum AGT level with race in the present study was significant only in boys ($P=0.005$). Such sexual dimorphisms could result from the effects of gonadal hormones, which affect AGT expression in a variety of tissues $^{32}$.

The present study of young people at various stages of pubertal development demonstrated that E2 and T explained approximately 4.6% and 10.6% of the residual variability of the serum AGT level in boys and in girls, respectively. We feel that in studies of subjects within this young age group, in which gonadal hormone levels may fluctuate widely, it would be important to consider the levels of these hormones in any study of the determinants of AGT levels. On the other hand, there would seem to be less need for concern in studies of adults, in whom levels of gonadal hormones remain relatively constant, with the exception of E2 in women during the menstrual cycle and of the decline in E2 production with menopause. In these latter instances, an assessment of E2 levels could prove helpful.
Although the present study was directed at identifying regulators of AGT production, the importance of this work ultimately lies in its relationship to blood pressure. It seemed logical therefore to explore the relation of gonadal hormone levels to blood pressure directly. Although both E2 and T were examined in boys and girls, we found only a single significant relationship, that of E2 with diastolic blood pressure in boys ($P=0.0079$), which could have occurred by chance alone; future studies may determine whether this is in fact a true relationship, possibly mediated by the level of AGT.

Finally, we have shown in this study a clear relationship between AGT genotype and the serum AGT concentration in subjects carefully matched for the other factors known to affect the AGT level, particularly BMI. We selected for use in these studies a genotype that represents a subpopulation of the T235 group, providing us with additional statistical power to detect a relationship. The observed strong association of the serum AGT level with the T235 haplotype (in 24 of 34 matched pairs, the AGT level was higher in the individual with a T235 haplotype) provides very convincing additional evidence that AGT gene variation contributes importantly to the level of AGT.

From the present observations in growing young people, we conclude that endogenous levels of gonadal hormones affect the serum concentration of AGT. In addition, after matching for BMI and other covariates, we further substantiate the strong association of the AGT genotype with the serum level of AGT.

Acknowledgments

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