Body Fat, Resting and Exercise Blood Pressure and the Angiotensinogen M235T Polymorphism: The Heritage Family Study

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Abstract

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Objective: The association of resting and exercise blood pressure (BP) and fat mass with the angiotensinogen (AGT) M235T polymorphism was investigated in 522 sedentary Caucasian subjects from 99 families.

Research Methods and Procedures: Resting BP was measured on two separate days, three times each day, and the mean of six valid measurements was used. Exercise BP was measured during a cycle ergometer test at a constant power output (50 W). Body composition was derived from underwater weighing and the AGT M235T polymorphism was typed with a polymerase chain reaction-based method.

Results: Neither resting nor exercise BP was associated with the AGT genotypes. In mothers, the homozygotes for the T allele showed 8.8 kg and 7.1 kg greater ($p=0.017$) age-adjusted body fat mass (FM) than the MM homozygotes and heterozygotes, respectively. Sixty-nine percent of all TT homozygotes were found in the highest FM tertile, whereas only 16% of the MM homozygotes fell in the same tertile ($p=0.008$). Moreover, a significant interaction was seen between FM and T-allele carrier status in women with regard to resting diastolic BP ($p=0.002$). Among women with a FM≥24 kg, carriers of the T allele showed a 6.3 mmHg higher diastolic blood pressure (DBP) than noncarriers whereas no difference was found in women with a FM less than 24 kg. A similar trend toward an interaction term was evident with resting systolic blood pressure ($p=0.011$) and exercise DBP ($p=0.012$). Body fat was not associated with the AGT polymorphism in fathers or in offspring.

Discussion: These data suggest that the AGT M235T polymorphism is associated with body fatness in women, and that the relationship between DBP and AGT M235T polymorphism is dependent on FM in middle-aged sedentary normotensive women.

Key words: fat mass, blood pressure, genetic polymorphism, interaction

Introduction

Obesity and hypertension are two major public health problems in industrialized countries. Obesity is associated with several metabolic comorbidities, and excess body fat also increases the risk of hypertension (1,2). Hyperinsulinemia and insulin resistance, increased activity of the sympathetic nervous system, abnormal intrarenal physical forces due to cell proliferation, and increase in extracellular matrix and activation of the renin-angiotensin system have been suggested as underlying causes for obesity-induced hypertension (3). Data from the Québec Family Study also suggest that body fat and blood pressure are influenced by a gene or genes with pleiotropic effects (4).

Angiotensin II (Ang II) plays an important role in the regulation of blood pressure by inducing aldosterone excretion and thereby increasing sodium reabsorption and causing plasma volume expansion. In the peripheral arteries, Ang II induces vasoconstriction, increasing peripheral re-

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sistance. Angiotensinogen (Agt), the only known precursor of Ang II, is expressed in and secreted by adipocytes (5–8). Adipose tissue can convert Agt to Ang II (7,9), and both type 1 and type 2 receptors of Ang II are found in adipocytes (10,11). Ang II appears to have a physiological role in adipogenesis. Angiotensinogen gene expression increases drastically during maturation of adipocytes (12), and Ang II has been shown to enhance production of prostacyclin (PGI_2), which is a powerful stimulator of the differentation of preadipocytes to adipocytes (11). Glucocorticoids enhance adipocyte Agt production in vitro, whereas several other hormones known to promote hepatic Agt synthesis have no effect on adipocytes (13). In addition, the effects of feeding on Agt production seem to be adipocyte specific. Fasting decreases adipocyte Agt expression and secretion in normal weight Sprague–Dawley CD rats, whereas refeeding enhances Agt production above the ad libitum feeding level (14). It is still unclear whether adipocyte-derived Agt affects blood pressure, but it has been suggested that Agt produced locally by adipocytes in or near arterial walls could induce vasoconstriction without affecting systemic Agt level (14). In animal models, changes in blood pressure induced by fasting and refeeding follow exactly the same pattern as those in adipocyte Agt production even though no changes were observed in hepatic Agt synthesis or plasma Agt concentration (14).

Several studies have reported a linkage between hypertension and angiotensinogen gene (AGT) markers (15–17) and a common variant of AGT [threonine at codon 235 (T allele) instead of methionine (M allele)] has been found more frequently among hypertensives than controls (18). The same allele is also associated with higher plasma Agt levels (17,19). However, the data on the associations between body fatness and AGT polymorphisms are scarce. In fact, to our knowledge, only one study has reported an association between waist-to-hip ratio (WHR) and the AGT T174M polymorphism (20). In that study, the difference in body mass index (BMI) between the T174 homozygotes and T174M heterozygotes, or the associations between WHR and BMI and the AGT M235T marker, were not statistically significant. However, both WHR and BMI are only estimates of body fatness and more refined adiposity phenotypes should be used to investigate associations with candidate genes. In addition to assessment under resting conditions, blood pressure measured during submaximal exercise provides further information on the cardiovascular function under physiological stress, especially in normotensive subjects. Thus, the purpose of this study was to investigate 1) the association of resting and exercise blood pressure and body fat mass with the AGT M235T polymorphism, and 2) the interactions between body fatness and the AGT M235T polymorphism with regard to blood pressure in healthy, sedentary subjects.

Subjects and Methods

Subjects

The HERITAGE Family Study is a multicenter study designed to investigate the role of the genotype in cardiovascular, metabolic, and hormonal responses to aerobic exercise training. The centers are located at the Indiana University, Laval University, University of Minnesota, Texas A&M University, and Washington University. The study cohort consisted of 191 parents (97 males and 94 females, aged 42 to 65 years) and 331 offspring (160 males, 171 females, aged 17 to 41 years) from 99 families, measured at baseline in the HERITAGE Family Study. All subjects were Caucasians. The study design and inclusion criteria have been described previously (21). To be eligible, the individuals were required to be in good health, i.e., free of diabetes, cardiovascular diseases, or other chronic diseases that would prevent their participation in an exercise training program. Subjects were also required to be sedentary, defined as not having engaged in regular physical activity over the previous 6 months. Individuals with resting systolic blood pressure (SBP) greater than 159 mmHg and/or diastolic blood pressure (DBP) more than 99 mmHg were excluded. In parents, the prevalences of overweight (BMI 25.0–29.9 kg/m^2) and obesity (BMI>30.0 kg/m^2) were 45% and 35% in men and 38% and 29% in women. In offspring, the respective values were 28% and 17% in men and 21% and 9% in women. The study protocol had been approved by each of the Institutional Review Boards of the HERITAGE Family Study research consortium. Written informed consent was obtained from each participant.

Body Composition

Stature was measured to the nearest 0.1 cm with the subject standing erect on a flat surface, heels, buttocks, and back pressed against the stadiometer, and the head positioned in the Frankfort horizontal plane. Body mass was recorded to the nearest 100 grams using a balance scale with subjects clothed only in a light-weight bathing suit. Body density was assessed by underwater weighing (22). Body density was converted to fat mass (FM) using the Siri equation (23) for men and the Lohman equation (24) for women. Separate equations were selected to optimize the sex-specificity of the fat mass estimates (24). Pulmonary residual volume was measured using the oxygen dilution technique (25) in the Indiana, Minnesota, and Texas clinical centers and by the helium dilution method (26,27) in the Québec clinical center. Reproducibility of the body density, fat mass, and pulmonary residual volume assessments were very high with intraclass correlations for repeated measures ranging between 0.97 and 1.00 without significant differences between the four clinical centers (28).

Blood Pressure

Both resting and exercise blood pressures were measured using Colin STBP-780 automated units and the re-

cordings were confirmed by technicians wearing ear phones. Resting blood pressure was measured on two separate days before 11:00 a.m. in the post-absorptive state. Subjects were asked not to use any caffeine-containing or tobacco products 2 hours before measurements. Measurements were done in a quiet room at neutral ambient temperature (24–25°C) with the lights dimmed. Subjects rested for 5 minutes before the initial measurement in a reclining chair with legs slightly elevated and back support reclined at about 45° from the ground. Following the rest period, four blood pressure readings were taken at 2-minute intervals between measurements. The first recording was discarded automatically, and up to three valid measurements were made. SBP and DBP were defined as the mean of all valid readings taken on both days, i.e., up to a maximum of six. Exercise blood pressure was measured during a submaximal cycle ergometer test in relative steady state for 8–12 minutes at a constant power output (50 W). The mean of four readings was used for analyses.

Angiotensinogen M235T Polymorphism

Genomic DNA was isolated from lymphoblastoid cell lines following a standard protocol (29). The M235T polymorphism of the AGT gene was typed with the polymerase chain reaction (PCR) followed by digestion with Tth 111 I. The PCR conditions were slightly modified from those previously described (30). In order to lower the PCR temperatures, the upstream and downstream primers were shortened by seven and five base pairs, respectively. The primers used were 5′-CCG-TTT-GTG-CAG-GGC-CTG-3′ (upstream) and 5′-TGC-TGT-CCA-CAC-TGG-ACC-CC-3′ (downsteam). The PCR was performed in standard buffer (Perkin Elmer Co., Norwalk, CT) and each 20-μL PCR reaction contained 100 ng genomic DNA, 0.3 μmol/L each primer, 200 μM each dNTPs, and 0.5 unit Taq polymerase (Perkin Elmer Co., Norwalk, CT). The reactions were incubated at 95°C for 3 minutes, 62°C for 15 seconds, and 70°C for 1.0 minutes, followed by 40 cycles of 95°C for 15 seconds, annealing at 62°C for 15 seconds, and extension at 70°C for 1.0 minutes, and finally one cycle of 72°C for 10 minutes using a Model 9600 Perkin Elmer thermal cycler (Perkin Elmer Co., Norwalk, CT).

The PCR product was digested with 5 U of Tth 111 I (New England BioLabs Inc., Missisauga, ON, Canada) at 65°C for 5 hours. The resulting fragments were separated on 8% acrylamide gel and visualized under UV light after ethidium bromide staining. The absence and presence of the restriction site for Tth 111 I generated fragments of 165 and 141 base pairs (bp), respectively. When the restriction site was not present, the nucleotide 704 in the exon 2 is T and the codon 235 encodes methionine (M allele), but when the restriction site was present the nucleotide is C and the ensuing amino acid is threonine (T allele).

Smoking

Information concerning current smoking habits were collected with questionnaires. Those who were smoking cigarettes, cigars, or pipe at the time of the study were classified as smokers, whereas never-smokers and ex-smokers were classified as non-smokers.

Statistical Analyses

A chi-square test was used to confirm that the observed genotype frequencies follow a Hardy–Weinberg equilibrium and to test whether genotype frequencies varied across the FM tertiles. Differences in blood pressure and fat mass between smokers and non-smokers were tested with a Student's *t*-test. The associations of fat mass (adjusted for age, age^2, age^3) and blood pressure (adjusted for age, age^2, age^3, FM and smoking) with the angiotensinogen M235T polymorphism were analyzed with analysis of covariance using the MIXED and GLM procedures of the SAS statistical software. The selection of covariates was based on the significant univariate associations between the dependent variable and covariates. First, the analyses were done in the whole cohort with AGT polymorphism, sex and generation as main effects and age (up to cubic polynomial) as covariates using MIXED procedure. Because all phenotypes tested (except exercise SBP) showed significant sex difference, further analyses were done separately in men and women. Next, a generation by AGT polymorphism interaction term was added into the model, and in several cases either generation or generation by AGT interaction term were significant. Thus, the final analyses were done separately in four sex-by-generation subgroups. Finally, in parents the interactions between FM and the AGT polymorphism with regard to blood pressure were tested using two-way analysis of variance (PROC GLM) with FM [dichotomized using the median (24 kg for both women and men) as a cut-off point], AGT polymorphism and FM by AGT interaction term as main effects and age and smoking as covariates.

Although the number of phenotypes tested tends to be high, we did not make any adjustment for multiple testing because the purpose of these analyses is to explore the possible associations between the candidate gene marker and phenotypes. If strict corrections for multiple testing had been applied, the risk of type II error would have increased and valuable information could have been lost.

Results

Basic characteristics of the 522 subjects are given in Table 1. Subjects were mainly normotensive, and only three women and nine men of the parent generation had a resting SBP between 140 and 153 mmHg. None of the women and five of the men had a resting DBP between 90 and 96 mmHg. In offspring, only one man had a resting SBP above 140 mmHg. The frequencies of the MM, MT, and TT geno-

Table 1. Characteristics of the subjects (mean±standard deviation)

	Parents		Offspring	
	Men (n=97)	Women (n=94)	Men (n=160)	Women (n=171)
Age (years)	53.5 (5.2)	52.0 (5.0)	25.2 (6.0)	25.4 (6.3)
Height (cm)	176 (6)	162 (6)	179 (6)	165 (6)
Weight (kg)	87.8 (15.0)	72.9 (14.1)	82.3 (16.6)	64.4 (13.0)
Body mass index (kg/m^2)	28.4 (4.4)	27.6 (5.0)	25.7 (4.9)	23.7 (4.4)
Fat mass (kg)	24.6 (9.0)	27.0 (10.4)	17.0 (11.0)	18.0 (9.8)
Fat-free mass (kg)	62.3 (7.5)	44.5 (5.0)	64.2 (8.1)	46.1 (5.2)
Resting SBP (mmHg)	122.1 (13.1)	116.9 (11.9)	119.2 (8.7)	110.5 (7.8)
Resting DBP (mmHg)	72.9 (8.6)	67.7 (6.8)	65.5 (8.4)	61.8 (6.4)
SBP at 50 W (mmHg)	154.4 (21.3)	157.7 (24.8)	141.0 (13.4)	134.5 (12.0)
DBP at 50 W (mmHg)	78.1 (11.5)	78.6 (9.7)	68.7 (10.0)	65.6 (8.7)

types were 0.40, 0.42, and 0.18 in women and 0.37, 0.46, and 0.16 in men. The genotype frequencies were in Hardy–Weinberg equilibrium in both genders. The prevalence of smoking was 10.6% in mothers, 28.4% in fathers, 20.4% in daughters and 20.8% in sons. In mothers, smokers had lower resting SBP ($p=0.047$) and DBP ($p=0.017$) whereas the difference in exercise blood pressure did not reach statistical significance. In fathers, resting blood pressure was similar in smokers and non-smokers but DBP during exercise was higher in smokers ($p=0.030$). Body weight, body fatness, and the AGT genotype frequencies were similar in smokers and non-smokers in both genders.

There were no differences in resting or exercise blood pressure across the AGT M235T genotypes (Tables 2 and 3). In mothers, the homozygotes for the T allele showed a 8.8 kg and 7.1 kg greater ($p=0.017$) body FM (adjusted for age) than the MM homozygotes and the heterozygotes, respectively. When the genotype frequencies were analyzed across tertiles of FM, 69% of all TT homozygotes were found in the highest tertile whereas only 16% of the MM homozygotes fell in the same tertile ($p=0.008$, Table 4). Body mass index and fat-free mass did not differ between the genotypes in mothers. In fathers and offspring, body mass index and body fatness did not differ between AGT genotypes.

A two-way analysis of variance revealed a statistically significant ($p=0.01$) interaction between fat mass and AGT M235T genotypes with regard to resting DBP. Among women with FM≥24.0 kg, resting DBP was 63.3 (1.9), 69.2 (1.5), and 70.2 (1.8) mmHg in the MM, MT, and TT genotypes, respectively, whereas the corresponding values in women with FM<24.0 kg were 68.6 (1.4), 65.7 (1.5), and 64.0 (3.7) mmHg. However, because there were only three TT homozygotes in the lower FM group, the analyses were repeated by combining the MT and TT genotypes (T allele carrier status) to increase statistical power. Using the carrier status classification, the interaction between AGT and FM became stronger ($p=0.002$). Although statistically less significant, interactions were nonetheless evident with regard to resting SBP ($p=0.011$) and exercise DBP ($p=0.012$). In men, no interaction was found with resting blood pressure (p-values from 0.575 to 0.927), whereas for exercise DBP a trend toward an interaction effect ($p=0.091$) as in women was noted.

Discussion

The results suggest that sedentary middle-aged women with the TT genotype of the AGT M235T polymorphism have higher FM than those with the MM and MT genotypes. The role of the AGT gene on hypertension has been extensively investigated whereas data on the AGT polymorphism and body fatness are scarce. Hegele et al. (20) reported a significant association between the waist-to-hip ratio and an AGT codon 174 polymorphism in the male North American Hutterites. However, body mass index was similar across the AGT genotypes. Despite the limited data on AGT polymorphisms and body fat mass, both in vitro and in vivo studies support a role for Agt and Ang II in the development of adipose tissue. Adipocytes produce physiologically significant amounts of Agt (5,7,14) and adipose tissue is able to convert Agt to Ang II (7,9). In adipocytes, Ang II enhances the production of prostacyclin (PGI_2), which is a powerful stimulator of differentiation of preadipocytes to adipocytes (11). The PGI_2 production enhancing capacity of Ang II has been documented both in cell cultures (11) and in vivo using the microdialysis technique (31). Thus, the prostacyclin seems to offer a physiologically plausible mechanism by which Agt/Ang II may influence adipose tissue growth. Moreover, Ang II promotes growth in vascular smooth muscle cells and in renal tissue. A common feature in all tissues is that Ang II per se cannot stimulate growth but requires another component. In kidneys, Ang II

Table 2. Resting and exercise blood pressure (BP), body mass index, fat mass, and fat-free mass across the angiotensinogen M235T genotypes in parents of the HERITAGE Family Study

	MM	MT	TT	*p*-value
Women				
Systolic BP (mmHg)				
Resting	115.5 (1.8)	116.0 (1.7)	120.0 (2.7)	0.365
50 W	156.7 (3.5)	156.1 (3.3)	153.4 (5.2)	0.871
Diastolic BP (mmHg)				
Resting	66.8 (1.2)	67.4 (1.1)	69.1 (1.8)	0.586
50 W	77.3 (1.6)	79.6 (1.5)	77.4 (2.4)	0.524
Body mass index (kg/m^2)	26.6 (0.8)	27.4 (0.8)	29.4 (1.2)	0.143
Fat mass (kg)	24.4 (1.8)	26.1 (1.7)	33.2 (2.5)	0.017
Fat-free mass (kg)	44.0 (0.9)	43.9 (0.8)	46.3 (1.2)	0.246
Men				
Systolic BP (mmHg)				
Resting	123.5 (2.3)	122.1 (2.0)	120.2 (3.4)	0.706
50 W	155.5 (3.4)	156.4 (3.1)	148.2 (5.2)	0.397
Diastolic BP (mmHg)				
Resting	71.6 (1.5)	74.4 (1.3)	72.1 (2.2)	0.337
50 W	77.6 (2.0)	78.8 (1.8)	77.5 (3.0)	0.883
Body mass index (kg/m^2)	27.7 (0.7)	29.0 (0.7)	28.1 (1.1)	0.437
Fat mass (kg)	23.8 (1.5)	25.3 (1.4)	24.4 (2.4)	0.771
Fat-free mass (kg)	63.4 (1.3)	62.7 (1.2)	58.9 (2.0)	0.160

Values are means±S$\bar{x}$(standard error of the mean). Blood pressure phenotypes are adjusted for age, fat mass, and smoking, whereas body composition phenotypes are adjusted for age.

induced fibrosis develops in interaction with transforming growth factor beta (32) and, in vascular smooth muscle cells, the mitogenic effect of Ang II is seen in the presence of oleic acid (33). In adipose tissue, Ang II promotes differentiation only when fully differentiated adipocytes are present (11).

In addition to its growth promoting properties, Ang II may also influence fatty acid balance in adipocytes by regulating blood flow in adipose tissue. Frederich et al. (14) have proposed that a reduction in Agt production in adipocytes during fasting promotes local vasodilation and thereby increases blood flow. Increased circulation transports fatty acids released from adipocytes more efficiently to be used as a metabolic fuel in other tissues. Likewise, increased Agt production induces vasoconstriction and decreases adipose tissue blood flow. These conditions should favor whole body glucose metabolism and fatty acid deposition into adipocytes. However, it is not known whether this hypothesis is applicable to humans. In order to explain our results, both of the mechanisms described above require that Agt production is higher in women with the TT genotype. Previous studies have shown that plasma Agt levels increase gradually from MM to TT genotypes (17). Although adipose tissue derived Agt is likely to contribute also to plasma Agt levels, most of the Agt in systemic circulation is of hepatic origin. Thus, it is unclear to what extent plasma concentration reflects Agt produced in adipose tissue. In animal models, plasma Agt levels and hepatic Agt production remained unchanged despite marked changes in adipocyte Agt production in response to changes in feeding pattern (14). It has been shown that angiotensinases are capable of cleaving the excess Agt produced by local renin-angiotensin systems that may thus limit the action to the target tissue (34).

In the present study, blood pressure measured at rest and during exercise was not associated with the AGT polymorphism. This is not surprising, since our subjects were mainly normotensives and the associations reported so far have been restricted mainly to hypertensive populations, especially to those with positive family history or more severe forms of the disease (18). However, our results suggest that in sedentary normotensive women, the level of body fat mass may modify the association between blood pressure, especially DBP, and the AGT M235T polymorphism. The higher blood pressure associated with the T-

Table 3. Resting and exercise blood pressure (BP), body mass index, fat mass, and fat-free mass across the angiotensinogen M235T genotypes in offspring of the HERITAGE Family Study

	MM	MT	TT	*p*-value
Women				
Systolic BP (mmHg)				
Resting	110.6 (1.1)	110.2 (0.9)	110.3 (1.4)	0.965
50 W	134.7 (1.7)	133.7 (1.3)	135.1 (2.1)	0.816
Diastolic BP (mmHg)				
Resting	60.9 (0.9)	62.0 (0.7)	62.7 (1.1)	0.405
50 W	64.5 (1.1)	65.8 (0.8)	65.0 (1.4)	0.641
Body mass index (kg/m^2)	24.5 (0.6)	23.4 (0.5)	23.1 (0.8)	0.268
Fat mass (kg)	19.8 (1.3)	17.7 (1.0)	16.3 (1.7)	0.226
Fat-free mass (kg)	47.1 (0.7)	45.3 (0.6)	46.0 (0.9)	0.156
Men				
Systolic BP (mmHg)				
Resting	119.5 (1.2)	119.3 (1.1)	118.3 (2.1)	0.881
50 W	141.8 (1.8)	139.9 (1.7)	139.7 (3.4)	0.719
Diastolic BP (mmHg)				
Resting	65.4 (1.1)	65.6 (1.0)	65.6 (1.9)	0.987
50 W	69.5 (1.2)	67.7 (1.1)	69.2 (2.3)	0.541
Body mass index (kg/m^2)	24.9 (0.6)	26.4 (0.5)	25.0 (1.1)	0.162
Fat mass (kg)	16.2 (1.4)	18.2 (1.2)	16.0 (2.4)	0.486
Fat-free mass (kg)	64.5 (1.1)	64.3 (1.0)	64.4 (1.8)	0.992

Values are means±S$\bar{x}$. Blood pressure phenotypes are adjusted for age, fat mass, and smoking, whereas body composition phenotypes are adjusted for age.

allele was seen in women in the upper half of the FM distribution, whereas in lean women no association was evident. This observation is in line with results from a previous study showing a significant interaction between body mass index and the AGT M235T genotypes with regard to renal plasma flow response to infused Ang II (35). Both a high BMI and the TT genotype were associated with blunted responses and, in the TT homozygotes, obesity had a greater blunting effect. These observations suggest that in normotensive subjects the combination of a high FM and the presence of the T-allele at the AGT M235T locus may predispose to higher blood pressure than when only one of these factors is present. Concordant with this observation, it has been suggested that adipocyte-derived Agt induces a local vasoconstriction of the arteries, since the Agt produced locally in the vascular wall is mainly derived from the adipocytes around or within the vessel wall (5).

The present data suggest that there are sex differences in the associations between body fat, blood pressure and the AGT polymorphism. First, the association between body fat and the AGT polymorphism was evident only in women, and second, the interaction between body fat and the AGT polymorphism was strongest in women. A sex difference in renal vasoconstriction response to angiotensin I and II infusion has been reported (35,36). Previous studies have reported a higher plasma Agt concentration in women, especially in the homozygotes for the T allele at the AGT M235T locus (17). The promoter region of the AGT gene contains an estrogen responsive element (17) and estrogen has been shown to enhance Agt production both in humans (37,38) and animals (39). Thus, the gender differences observed in the present study may have a real physiological basis rather than being simply the result of spurious statistical observations. Our results also suggest a generation difference in the association between the AGT M235T polymorphism and fat mass in women. It is possible that an exposure of several decades to a relevant condition is required for the association to become detectable. In addition, as recently suggested (40), there may exist robust counterregulatory mechanisms in younger subjects that compensate for the effects of a given mutation. However, as a result of aging these counterregulatory mechanisms may not be sufficient and could be overcome.

The present data were derived from a cohort of healthy

Table 4. Frequencies of the Angiotensinogen M235T genotypes in the tertiles of fat mass in women

	MM	MT	TT
Tertile I	13 (0.46)	13 (0.46)	2 (0.07)
	(0.41)	(0.36)	(0.12)
Tertile II	14 (0.50)	11 (0.39)	3 (0.11)
	(0.44)	(0.31)	(0.19)
Tertile III	5 (0.18)	12 (0.43)	11 (0.39)
	(0.16)	(0.33)	(0.69)

$\chi^2 = 13.85$, df = 4, p = 0.008.
Values given are number of subjects followed by the genotype frequencies within the fat mass tertile. The frequencies below the number of subjects shows the proportion of the subjects with given genotype found in each fat mass tertile (e.g., 69% of all TT homozygotes are found in the highest tertile).

sedentary subjects. Both physical activity and chronic metabolic disorders, such as diabetes and lipid metabolism disorders, are potential confounders in studies on adiposity and blood pressure. Therefore, our results were not affected by variation due to differences in these two confounders. Fat mass was measured using the underwater weighing technique, which is widely accepted as a valid and reliable method. If the Agt and AGT polymorphism play a role in adipose tissue growth, it is particularly important to have a valid and reliable measurement of total body fat mass and not rely solely on the body mass index. Resting blood pressure was measured on two separate days, and the mean of six measurements was used for analysis. With this protocol, it was possible to decrease the error variance and attenuate the effect of day-to-day variation in the blood pressure phenotypes. However, it must be remembered that due to the exclusion criteria, our results can be generalized only to healthy, sedentary Caucasian populations.

In summary, the present data suggest that body fat mass is associated with the AGT M235T polymorphism in healthy sedentary women. Moreover, fat mass may influence the association between resting systolic and diastolic and exercise diastolic blood pressure and the AGT M235T polymorphism.

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