

# Genetic Variation in the Renin–Angiotensin System and Abdominal Adiposity in Men: The Olivetti Prospective Heart Study

Pasquale Strazzullo, MD; Roberto Iacone, MD; Licia Iacoviello, MD, PhD; Ornella Russo, Gianvincenzo Barba, MD; Paola Russo, BS; Andria D’Orazio; Antonio Barbato, MD; Francesco Paolo Cappuccio, MD, FRCP; Eduardo Farinaro, MD; and Alfonso Siani, MD

**Background:** The renin–angiotensin system is involved in adipocyte growth and differentiation and possibly in adipose tissue metabolism.

**Objective:** To investigate the association of polymorphism in the angiotensin-converting enzyme (ACE) *I/D* gene, angiotensinogen *M235T* gene, and angiotensin II type 1 receptor *A1166C* gene with body mass index, body fat pattern, and obesity-associated hypertension.

**Design:** Cross-sectional longitudinal study.

**Setting:** The Olivetti factories in Marcianise and Pozzuoli, suburbs of Naples, Italy.

**Participants:** 959 adult men, 25 to 75 years of age.

**Measurements:** Renin–angiotensin system polymorphism, anthropometric indexes, blood pressure, and serum glucose and insulin levels.

**Results:** No association was detected between angiotensinogen or angiotensin II type 1 receptor gene polymorphism and anthropometric indexes or blood pressure. For ACE *I/D* polymorphism, significant age–genotype interaction was detected on cross-

sectional observation; the relation of body mass index, waist circumference, and diastolic blood pressure to age was significantly greater in persons with the *DD* genotype than in those with the *ID* or *II* genotype. Overweight and abdominal adiposity were more common in men with the *DD* genotype, particularly among older participants (51.1% vs. 36.5% and 33.1% vs. 22.0%, respectively). Odds ratios were 1.82 (95% CI, 1.16 to 2.87) for overweight and 1.76 (CI, 1.06 to 2.90) for abdominal adiposity. Among 314 untreated men first examined 20 years earlier, those with the *DD* genotype had greater age-adjusted weight gain (1.45 kg [CI, 0.12 to 2.78 kg]) and change in diastolic blood pressure (2.83 mm Hg [CI, 0.39 to 5.28 mm Hg]). The relative risk for overweight was 2.34 (CI, 1.32 to 4.15) among participants with the *DD* genotype versus those with the *ID* or *II* genotype.

**Conclusions:** The ACE *I/D* polymorphism was a significant predictor of overweight and abdominal adiposity in men. *DD* homozygosity was associated with larger increases in body weight and blood pressure in aging persons, as well as with higher incidence of overweight.

*Ann Intern Med.* 2003;138:17-23.

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For author affiliations, see end of text.

**H**uman obesity is caused by the interaction of genetic predisposition and many environmental and lifestyle factors (1). Although the chromosomal location of a few putative major genes for human obesity has been identified (2–5), the presence of a greater number of minor genes involved in the process of adipogenesis or in the regulation of adipocyte metabolism probably engenders susceptibility to obesity (6). Polymorphism in several obesity candidate genes has been the subject of intensive investigation, but little attention has been paid to the genes encoding for components of the renin–angiotensin system. The products of these genes (angiotensinogen [AGT], angiotensin-converting enzyme [ACE], and angiotensin II type 1 [AT2R1] and type II [AT2R2] receptors) are expressed in the adipose tissue in animal models as well as in humans (7–10). Recent experimental studies suggest that adipose tissue in the renin–angiotensin system plays a role in adipocyte growth and differentiation through angiotensin II (11, 12). In addition, epidemiologic studies have reported associations between AGT plasma levels (13, 14), plasma renin activity (15, 16), plasma ACE activity (17), and body mass index (BMI).

We investigated the relationship of overweight, obesity, and body fat distribution to three common polymorphisms of the renin–angiotensin system: intron 16 of the ACE gene on chromosome 17 (18), the *M235T* polymor-

phism of the AGT gene in exon 2 on chromosome 1 (19), and the A-to-C polymorphism in the 3'-untranslated region at nucleotide 1166 of the AT2R1 gene on chromosome 3 (20). Because the renin–angiotensin system plays a fundamental role in blood pressure regulation and in vascular and cardiac modifications, these polymorphisms have been studied with regard to hypertension and cardiovascular disease. Associations have been reported between the AGT *M235T* and the AT2R1 *A1166C* variants and hypertension (19–24), as well as among ACE *I/D* polymorphism, insulin sensitivity (25–27), and the risk for coronary heart and cerebrovascular disease (28, 29). In adult men who attended the 1994–1995 follow-up examination of the Olivetti Prospective Heart Study, we examined associations between these polymorphic variants and overweight or obesity, body fat distribution, and related metabolic and hemodynamic variables. We also reported longitudinal findings for a subset of participants who were first examined in 1975 and had been followed for 20 years.

## METHODS

### Study Sample and Procedures

We used the DNA bank and the database of the Olivetti Prospective Heart Study, an epidemiologic investigation of cardiovascular risk factors in men working at the

**Context**

Angiotensinogen (AGT), angiotensin-converting enzyme (ACE), and angiotensin II receptor type I (AT2R1) are expressed in adipose tissue, but their role in obesity is unknown. Common polymorphisms involve the ACE gene on chromosome 17 (ACE *I/D*), the AGT gene on chromosome 1, and the AT2R1 receptor gene on chromosome 3.

**Contribution**

Among 959 adult Italian men, *DD* homozygosity in the ACE gene was associated with overweight (odds ratio, 1.82 [95% CI, 1.16 to 2.87]) and abdominal obesity (odds ratio, 1.76 [CI, 1.06 to 2.90]) compared with the genotypes *I/D* and *II*. It was also associated with increases in weight over 20 years. Polymorphisms of AGT and AT2R1 were unrelated to measures of body size.

**Implications**

These results suggest that the renin–angiotensin system plays a role in the development of obesity.

*—The Editors*

Olivetti factories in southern Italy. The procedures of the Olivetti Prospective Heart Study, which began in 1975, have been described in detail elsewhere (30). Between May 1994 and December 1995, we examined 1075 men 25 to 75 years of age. The participants were seen in the morning, after fasting, in a quiet room at the medical center of the Olivetti factories in Pozzuoli and Marcianise, suburbs of Naples, Italy. We obtained anthropometric measurements, performed blood tests, and administered a fixed-sequence questionnaire that assessed demographic information and medical history. Genotyping of the three polymorphisms of the renin–angiotensin system was possible in 959 participants. A group of 457 men seen at the 1994–1995 follow-up visit of the Olivetti Prospective Heart Study had also been examined in 1975; of these, 143 reported being under dietary restriction for various reasons at follow-up examination. Since the Olivetti Prospective Heart Study aims to evaluate spontaneous changes in body mass and blood pressure, this subgroup was excluded from the main

**Table 1. Characteristics of Participants in the Olivetti Prospective Heart Study at the 1994–1995 Examination (*n* = 959)\***

Characteristic	Mean Value ± SD (Range)
Age, y	51.9 ± 7.4 (25–79)
BMI, kg/m <sup>2</sup>	26.9 ± 3.1 (18.8–37.0)
Waist circumference, cm	94.6 ± 8.2 (70–127)
Arm circumference, cm	30.1 ± 2.8 (21–54)
Systolic blood pressure, mm Hg	130.2 ± 17.5 (89–225)
Diastolic blood pressure, mm Hg	84.1 ± 10.0 (44–130)
Serum insulin level, pmol/L	56.7 ± 35.4 (6.8–577.9)
HOMA index	2.4 ± 1.8 (0.2–22.8)

\* BMI = body mass index; HOMA = homeostatic model assessment.

analysis. The local ethics committee approved the study protocol, and participants gave informed consent.

**Anthropometric Measurements**

Body weight and height were measured on a standard beam-balance scale with an attached ruler. Body weight was measured to the nearest 0.1 kg, and height was measured to the nearest 1 cm; participants wore light indoor clothing and no shoes. Body mass index was calculated as weight in kilograms divided by the square of the height in meters.

At the 1994–1995 examination, but not at the 1975 examination, waist and arm circumferences were also measured. Waist circumference was measured at the umbilicus level as participants stood erect with abdomens relaxed, arms at their sides, and feet together. After the acromion was marked with each participant's arm flexed at a 90-degree angle, arm circumference was measured at the midpoint between the acromion and the olecranon with the arm relaxed and hanging just away from the side of the body. The measurements were obtained to the nearest 0.1 cm with a flexible plastic measuring tape. Overweight was defined as a BMI greater than or equal to 27 kg/m<sup>2</sup>, obesity was defined as a BMI greater than or equal to 30 kg/m<sup>2</sup>, and abdominal adiposity was defined as a waist circumference greater than 1.00 m.

**Blood Pressure Measurement**

Blood pressure was measured after the participant had been sitting upright for at least 10 minutes. Systolic and diastolic (phase V) blood pressure was measured three times 2 minutes apart with a random-zero sphygmomanometer (Gelman Hawksley Ltd., Sussex, United Kingdom). The first reading for each type of pressure was discarded, and the average of the second two readings was recorded. Hypertension was defined as a systolic blood pressure 140 mm Hg or greater, a diastolic blood pressure 90 mm Hg or greater, or both, or as current use of antihypertensive drugs.

**Biochemical Assays**

After blood pressure was measured, a fasting venous blood sample was taken in the seated position without stasis. The blood specimens were immediately subjected to centrifugation and stored at –70 °C until analyzed. Glucose levels were measured by using an automated method (Cobas-Mira, Roche, Italy), and serum insulin concentration was measured by using radioimmunoassay (Insuline Lisophase, Technogenetics, Milan, Italy). Insulin resistance was estimated by homeostasis model assessment using the following formula, as described by Matthews and colleagues (31): fasting serum insulin level (μU/mL) × fasting serum glucose level (mmol/L)/22.5.

**Gene Polymorphisms in the Renin–Angiotensin System**

Genomic DNA was isolated from leukocytes with a nonenzymatic, salting-out procedure (32). The ACE *I/D* polymorphism in intron 16 was typed by using the method

**Table 2.** Ten-Year Differences in Selected Variables, according to Angiotensin-Converting Enzyme *I/D* Polymorphism: 1994–1995 Cross-Sectional Examination\*

Dependent Variable	Participants with the <i>DD</i> Genotype (n = 385)	Participants with the <i>ID</i> or <i>II</i> Genotype (n = 574)	Between-Group Difference†
BMI, kg/m <sup>2</sup>	0.46 (0.06 to 0.85)	-0.19 (-0.54 to 0.17)	0.65 (0.11 to 1.19)
Waist circumference, cm	2.74 (1.66 to 3.81)	0.91 (-0.01 to 1.83)	1.83 (0.40 to 3.25)
Arm circumference, cm	0.01 (-0.30 to 0.48)	-0.04 (-0.68 to -0.07)	0.05 (-0.45 to 0.55)
Systolic blood pressure, mm Hg	7.34 (5.19 to 9.48)	5.15 (3.23 to 7.08)	2.19 (-0.73 to 5.11)
Diastolic blood pressure, mm Hg	2.79 (1.53 to 4.05)	0.55 (-0.64 to 1.75)	2.24 (0.46 to 4.02)
Serum insulin, pmol/L	4.58 (-1.13 to 10.28)	1.24 (-2.21 to 4.69)	3.34 (-2.87 to 9.55)
HOMA index	0.32 (0.04 to 0.61)	0.24 (0.05 to 0.43)	0.08 (-0.25 to 0.41)

\* The figures are the slope of the regression of each variable by age according to angiotensin-converting enzyme *I/D* polymorphism. Values in parentheses are 95% CIs. BMI = body mass index; HOMA = homeostatic model assessment.

† Estimates the size of the interaction between age and genotype.

of Rigat and associates (33). To address the possibility of mistyping *ID* heterozygotes as *DD* homozygotes because of the preferential amplification of the smaller *D* allele, all samples typed as *DD* homozygotes were subjected to a second, independent polymerase chain reaction with a primer pair that permits amplification only in the presence of the *I* allele; this was done by using the method described by Lindpaintner and coworkers (34). The *T235* allele of the ATG gene was detected by using the method of Russ and colleagues (35), and the *A1166C* polymorphism of the AT2R1 gene was tested as described elsewhere (36). Allelic frequencies were estimated by using gene counting, and genotype distribution was tested for Hardy–Weinberg equilibrium by using chi-square analysis.

### Statistical Analysis

Analysis of variance was used to evaluate differences in quantitative variables according to genotype; analysis of covariance was performed to account for confounders. A nonparametric test (Kruskal–Wallis) was used for variables that were not normally distributed. The interaction between the effects of gene polymorphism and age on the anthropometric indexes and blood pressure was tested by using multiple linear regression analysis. The association of categorical variables with gene polymorphisms was tested by using logistic regression analysis and is expressed as odds ratios and 95% CIs. Results are expressed as the

mean  $\pm$  SD or as the mean  $\pm$  SE, as specified. Two-sided *P* values and 95% CIs were used to test the statistical significance of between-group differences. Statistical analysis was performed by using SPSS statistical software, version 10.0 (SPSS, Inc., Chicago, Illinois).

### Role of the Funding Source

The funding source had no role in the collection, analysis, or interpretation of the data or in the decision to submit the paper for publication.

## RESULTS

**Table 1** summarizes the main characteristics of the participants of the Olivetti Prospective Heart Study at the 1994–1995 examination. Nine hundred fifty-nine participants were tested for ACE, AGT, and AT2R1 polymorphism. For the ACE *I/D* polymorphism, 40% (n = 385) had the *DD* genotype, 45% (n = 431) had the *ID* genotype, and 15% (n = 143) had the *II* genotype. For the AGT polymorphism, 31% (n = 297) had the *M235M* genotype, 48% (n = 460) had the *M235T* genotype, and 21% (n = 202) had the *T235T* genotype. For the AT2R1 polymorphism, 54% (n = 518) had the *A1166A* genotype, 39% (n = 377) had the *A1166C* genotype, and 7% (n = 64) had the *C1166C* genotype. All three polymorphisms were in Hardy–Weinberg equilibrium, showing that the

**Table 3.** Prevalence of Overweight, Obesity, Abdominal Adiposity, and Hypertension in the Entire Study Sample and in Older Participants, according to Angiotensin-Converting Enzyme *I/D* Polymorphism\*

Condition	All Participants			Participants $\geq 54$ Years of Age		
	<i>DD</i> Genotype (n = 385)	<i>II</i> or <i>ID</i> Genotype (n = 574)	<i>P</i> Value	<i>DD</i> Genotype (n = 136)	<i>II</i> or <i>ID</i> Genotype (n = 182)	<i>P</i> Value
Overweight	52.1	43.9	0.014	51.1	36.5	0.009
Obesity	18.1	15.5	>0.2	19.3	15.5	>0.2
Abdominal adiposity	24.4	18.3	0.023	33.1	22.0	0.027
Hypertension	41.5	41.7	>0.2	55.6	59.0	>0.2

\* Overweight = body mass index  $\geq 27$  kg/m<sup>2</sup>; obesity = body mass index  $\geq 30$  kg/m<sup>2</sup>; abdominal adiposity = waist circumference  $> 1.00$  m; and hypertension = systolic blood pressure  $\geq 140$  mm Hg, diastolic blood pressure  $\geq 90$  mm Hg, or both, or current antihypertensive treatment.

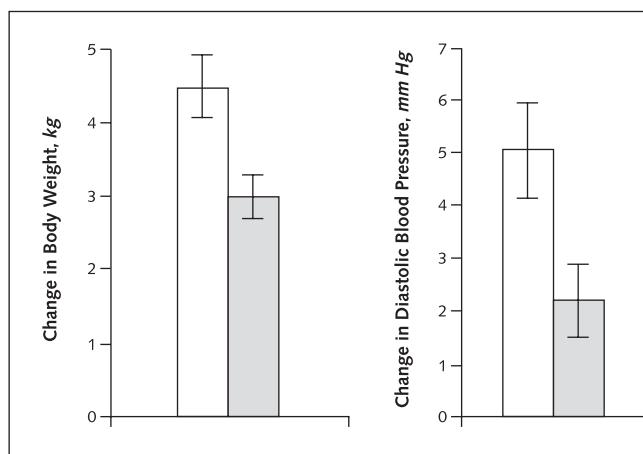
**Table 4.** Baseline Characteristics of Participants Undergoing 20-Year Follow-up Observation, according to Angiotensin-Converting Enzyme *I/D* Polymorphism (*n* = 314)

Variable	Participants with the <i>DD</i> Genotype ( <i>n</i> = 129)	Participants with the <i>ID</i> or <i>II</i> Genotype ( <i>n</i> = 185)	Between-Group Difference (95% CI)
Age, y	37.1 ± 0.6	35.8 ± 0.4	1.3 (-0.1 to 2.7)
Body mass index, kg/m <sup>2</sup>	25.2 ± 0.2	25.1 ± 0.2	0.1 (-0.5 to 0.7)
Systolic blood pressure, mm Hg	123.2 ± 1.3	122.6 ± 1.1	0.6 (-2.7 to 4.0)
Diastolic blood pressure, mm Hg	79.1 ± 0.8	81.9 ± 0.8	-2.8 (-5.0 to -0.6)

study sample excluded selection pressure for the genotypes under investigation.

No difference was detected in any of the variables examined for AGT and AT2R1 polymorphism. However, participants with the *DD* genotype had higher BMI and arm circumference than participants with either the *ID* or *II* genotype. Mean BMI and arm circumference ( $\pm$ SE), respectively, were  $27.2 \pm 0.1$  kg/m<sup>2</sup> and  $30.4 \pm 0.1$  cm for those with the *DD* genotype,  $26.8 \pm 0.1$  kg/m<sup>2</sup> and  $29.9 \pm 0.1$  cm for those with the *ID* genotype, and  $26.7 \pm 0.3$  kg/m<sup>2</sup> and  $29.7 \pm 0.2$  cm for those with the *II* genotype ( $P = 0.050$  and  $P = 0.009$  for each variable, respectively). Since no difference was detected between *ID* and *II* genotypes, the effect of the *D* allele on these variables was analyzed according to a recessive model in which the *DD* genotype was compared with the *ID* and *II* genotypes combined. In this model, the differences between the two groups for BMI and arm circumference were  $0.4$  kg/m<sup>2</sup> (95% CI,  $-0.04$  to  $0.76$  kg/m<sup>2</sup>) and  $0.52$  cm (CI,  $0.16$  to  $0.88$  cm), respectively. A minor difference in waist circumference was also noted ( $0.79$  cm [CI,  $-0.28$  to  $1.86$ ]).

**Figure.** Age-adjusted time changes in body weight (left) and diastolic blood pressure (right) over 20 years of follow-up, according to the *D* recessive model of the angiotensin-converting enzyme *I/D* gene polymorphism.



White bars indicate participants with the *DD* genotype (*n* = 129); shaded bars indicate participants with the *ID* or *II* genotype (*n* = 185). For between-group difference in body weight,  $P = 0.033$ ; for between-group difference in diastolic blood pressure,  $P = 0.029$ .

Because the tendency toward fat accumulation, insulin resistance, and high blood pressure increases with age, we tested the possible interaction between ACE *I/D* polymorphism and age with respect to these variables. Table 2 shows the slope and the size of the interaction term, calculated by multiple linear regression, of the different variables and age, according to ACE *I/D* polymorphism. For blood pressure, this analysis was performed after 173 participants taking antihypertensive drugs were excluded. We observed an increase in systolic blood pressure and in the homeostasis model assessment index with age in both genotype groups. In contrast, BMI, waist circumference, and diastolic blood pressure increased significantly with age only in men carrying the *DD* genotype. As indicated by the interaction term (age  $\times$  genotype interaction), there were statistically significant differences in the slopes of these variables by age according to genotype.

The prevalence of overweight, obesity, abdominal adiposity, and hypertension was analyzed in the *DD* group and in the *ID* plus *II* group for the whole study sample and after stratification by age (<49 years, 49 to 53 years, and >53 years). Overall, both overweight and abdominal adiposity were significantly more prevalent in *DD* homozygotes than in participants carrying the *I* allele (Table 3), especially among older participants (51.1% vs. 36.5% and 33.1% vs. 22.0%, respectively). Odds ratios were 1.82 (CI, 1.16 to 2.87) for overweight and 1.76 (CI, 1.06 to 2.90) for abdominal adiposity.

No such differences were observed for participants in the two lower tertiles of age. The difference in the prevalence of obesity did not reach statistical significance in the whole sample or in participants in the highest tertile of age, and the prevalence of hypertension was similar in the two groups in the entire sample as well as among older participants (Table 3). The population attributable risk for overweight and abdominal adiposity associated with the *DD* genotype, calculated as [whole population risk – (*ID* plus *II* risk)]/whole population risk, was 6.8% for overweight and 11.6% for abdominal adiposity.

Table 4 shows the baseline characteristics of the cohort undergoing 20-year follow-up observation (*n* = 314) according to ACE *I/D* polymorphism. A *D* allele recessive model was used, as in the cross-sectional analysis. The frequency of *DD* homozygotes (41%) in this group was similar to that observed in the entire sample. The two geno-

typic groups had similar age, BMI, and systolic blood pressure at study entry. Diastolic blood pressure was lower in *DD* homozygotes.

The Figure shows age-adjusted changes in body weight and blood pressure over 20 years according to ACE *I/D* polymorphism. There was a significantly greater increase in body weight in *DD* group compared with the *ID* plus *II* group (1.45 kg [CI, 0.12 to 2.78]). Significant difference over time was detected for changes in diastolic blood pressure (2.83 mm Hg [CI, 0.39 to 5.28]) but not systolic blood pressure. However, when the changes in diastolic blood pressure were adjusted for the concomitant changes in body weight, the difference was no longer statistically significant ( $P = 0.089$ ).

Among 241 participants who had a BMI less than 27 kg/m<sup>2</sup> at baseline, the incidence of overweight (that is, a BMI  $\geq 27$  kg/m<sup>2</sup> at the 1994–1995 examination) was 37.5% for *DD* homozygotes and 20.4% for the *ID* plus *II* group ( $P = 0.003$ ). The relative risk for overweight associated with the *DD* genotype was 2.34 (CI, 1.32 to 4.15) compared with the *ID* plus *II* group. Among 293 participants who were not obese at study entry in 1975, the rate of obesity (BMI  $\geq 30$  kg/m<sup>2</sup>) was similar between *DD* participants and those in the *ID* plus *II* group (9.8% vs. 8.2%).

The incidence of hypertension among participants who were normotensive at baseline ( $n = 248$ ) was analyzed on the basis of blood pressure values greater than or equal to 140 mm Hg (for systolic blood pressure), greater than or equal to 90 mm Hg (for diastolic blood pressure), or both, or on the basis of current use of antihypertensive drugs. No significant difference was detected in the incidence of hypertension according to ACE *I/D* polymorphism (33.0% for the *DD* group and 37.3% for the *ID* plus *II* group). This was also true if participants who reported dietary restrictions were included in the analysis (36.8% for the *DD* group and 42.3% for the *ID* or *II* group).

Over 20 years of follow-up, we observed no associations between changes in body weight and blood pressure and the other two gene polymorphisms of the renin–angiotensin system examined in our study. Likewise, no differences in the incidence of overweight and hypertension were detected as a function of the different polymorphic variants.

## DISCUSSION

Obesity is a complex disorder caused by the interaction of environmental and genetic (polygenic) susceptibility. The detection of functional mutations in genes involved in the process of fat accumulation may help identify specific targets for pharmacologic intervention. We analyzed the relationships of three common polymorphic variants of genes in the renin–angiotensin system to body fat accumulation and body fat distribution in a large sample of adult men. In our cross-sectional analysis, we observed that

BMI and abdominal adiposity were significantly related to age in persons with the *DD* genotype but not in those with the *ID* or *II* genotype. In addition, the prevalence of overweight or abdominal adiposity was significantly greater in the *DD* group than in the *ID* plus *II* group. This finding, however, applied only to participants 54 years of age and older.

We found no evidence of association between the polymorphic variants of the AGT and the AT2R1 genes and the tendency to overweight or to abdominal adiposity, nor did we find interaction among the three polymorphisms examined and body fat accumulation. In aggregate, these findings indicate an association between the *DD* genotype and a tendency to accumulate body fat, particularly abdominal fat, with age. Although arm circumference, a measure of peripheral fat deposition, was also larger in participants with the *DD* genotype than in those with the *ID* or *II* genotype, it did not increase with age, and change with age did not differ between groups. Systolic blood pressure was associated with age in both the *DD* and the *ID* plus *II* groups, but no significant between-group difference was seen. An association of diastolic pressure with age was also detected, but only in participants with the *DD* genotype.

We could not show any significant association between fasting serum insulin level or the homeostasis model assessment index, taken as a marker of insulin resistance, and polymorphic variants of the renin–angiotensin system. This differs from the expected finding of reduced insulin sensitivity in *DD* homozygotes, given their increased tendency toward overweight. A recent study reported that *DD* genotype was associated with the insulin resistance syndrome in patients with premature coronary heart disease (25). However, previous studies reported that the *I* allele occurred more frequently among obese patients (26) and those with type 2 diabetes mellitus (27). These differing results could be related to obvious differences of sex and ethnicity in the samples studied. Yet, given the lack of a direct assessment of insulin sensitivity in our study, relatively subtle differences that occurred as a function of the ACE *I/D* genotype may have gone undetected.

Of importance, our study provides data on the relationship between the three renin–angiotensin system polymorphisms and body weight over 20 years of follow-up. These data confirm the modulating effect of genotype on the relationship between age and body weight, since overweight developed more frequently in the *DD* group than in the *ID* plus *II* group. Among participants with the *DD* genotype, there was also a trend toward a larger increase in diastolic blood pressure over time. However, differences in body weight largely explained this trend. This finding provides additional evidence of the lack of association between ACE *I/D* polymorphism and blood pressure (37).

Our study is limited by lack of measures of fat distribution at the first examination in 1975, which made it impossible to analyze differences in the pattern of fat accu-

mulation over time according to genotype. In addition, we studied only white men, so caution is needed in generalizing our conclusions to other populations. We recently reported a similar interaction between the *Trp64Arg* polymorphism in the  $\beta 3$ -adrenergic receptor gene and age with regard to body fat accumulation and the prevalence of abdominal adiposity (38). Both in that study and in our present analysis of the ACE polymorphism, it is conceivable that a relatively small effect of the genetic variant became apparent only later in life through cooperation with other concurrent factors. The relatively small effect associated with genetic variation in the ACE gene may explain why we detected a significant difference in the incidence of overweight but not in the rate of obesity.

Although statistical associations do not prove relationships of cause and effect, there are reasons to suggest that ACE *I/D* genetic variation might involve a functional alteration in adipose tissue metabolism that leads to quantitative and qualitative differences in body fat accumulation. First, in our cross-sectional analysis, the consistency in the age-related greater prevalence of overweight and abdominal adiposity in *DD* homozygotes was matched by the higher incidence of overweight among *DD* participants over 20 years of follow-up. Second, there is a well-known association between the ACE *I/D* polymorphism and plasma ACE activity; persons with the *DD* genotype show approximately twice as much activity as *II* homozygotes, and persons with the *ID* genotype have a level of activity that is between that of the other groups (18, 38). Cooper and associates (17) found a positive correlation between plasma ACE activity and BMI, and similar associations have been described for plasma renin activity (15, 16) and plasma AGT levels (13, 14, 39). Third, increasing evidence from human and experimental studies shows that the renin–angiotensin system is represented with all its components within the adipose tissue and probably plays a role in the development of adipose tissue as well as its metabolic activities (40).

Expression of ACE has been demonstrated in isolated human adipocytes and cultured adipose cells (8–10, 41, 42). In rats, ACE inhibition was associated with substantial weight loss (43), and age-related hypertrophy of white adipose tissue was prevented by the AT2R1 antagonist losartan (44). Increased activity in the renin–angiotensin system was associated with greater insulin-resistant lipolysis in obese hypertensive patients, and ACE inhibitors were able to counteract this metabolic alteration (45). These data may be relevant to our finding of increased fat accumulation with age in persons with the *DD* genotype, assuming that the increased plasma ACE activity reported in these persons is actually matched by similarly increased enzyme activity within the adipose tissue. Of note, in association with locally enhanced angiotensin II production, increased bradykinin inactivation could also play a role in adipocyte metabolism.

In summary, we found that, in white adult men, ho-

mozygosity for the *D* allele at intron 16 of the ACE gene was associated with an age-related greater prevalence of abdominal adiposity. It was also associated with a greater tendency to develop overweight and a related increase in diastolic pressure during 20 years of follow-up. Although these effects were relatively small, they support the possible role of the local renin–angiotensin system in adipose tissue metabolism and, in general, the role of genetic influence on the control of fat deposition (46, 47).

From Federico II University of Naples Medical School, Naples, Consorzio Mario Negri Sud, Santa Maria Imbaro, and Institute of Food Sciences, CNR, Avellino, Italy; and St. Georges' Hospital Medical School, London, United Kingdom.

**Acknowledgments:** The authors thank Dr. A. Scottoni, Dr. U. Candura, and Ms. M. Bartolomei for organizing and coordinating the fieldwork and the workers of the Olivetti factories for their cooperation. They also thank Drs. E. Ragone, F. Stinga, and L. Russo for fieldwork; Dr. Antonella Venezia for laboratory support; and Mrs. Rosanna Scala and Grazia Fanara for editorial assistance.

**Grant Support:** By funds from MURST (Italian Ministry of University and of Scientific and Technological Research COFIN, 1998 and 2000).

**Potential Financial Conflicts of Interest:** None disclosed.

**Requests for Single Reprints:** Pasquale Strazzullo, MD, Department of Clinical and Experimental Medicine, Federico II University of Naples, via S. Pansini 5, 80131 Naples, Italy; e-mail, strazzul@unina.it.

Current author addresses and author contributions are available at [www.annals.org](http://www.annals.org).

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**Current Author Addresses:** Drs. Strazzullo, Barbato, and Farinaro; Mr. Iacone; and Ms. O. Russo: Department of Clinical and Experimental Medicine, Federico II University of Naples, via S. Pansini 5, 80131 Naples, Italy.

Dr. Iacoviello and Ms. D'Orazio: Department of Vascular Medicine and Pharmacology, Consorzio Mario Negri Sud, via Nazionale, Santa Maria Imbaro, 66030 Lanciano (Chieti), Italy.

Drs. Barba and Siani and Ms. P. Russo: Institute of Food Sciences, CNR, via Roma 52A/C, 83100 Avellino, Italy.

Dr. Cappuccio: St. Georges' Hospital Medical School, Cranmer Terrace, London SW17 ORE, United Kingdom.

**Author Contributions:** Conception and design: P. Strazzullo, R. Iacone, L. Iacoviello, F.P. Cappuccio, E. Farinaro, A. Siani.

Analysis and interpretation of the data: P. Strazzullo, R. Iacone, L. Iacoviello, O. Russo, G. Barba, P. Russo, F.P. Cappuccio.

Drafting of the article: P. Strazzullo, R. Iacone, O. Russo.

Critical revision of the article for important intellectual content: P. Strazzullo, L. Iacoviello, G. Barba, P. Russo, F.P. Cappuccio, E. Farinaro, A. Siani.

Final approval of the article: P. Strazzullo, L. Iacoviello, P. Russo, F.P. Cappuccio, E. Farinaro, A. Siani.

Provision of study materials or patients: P. Strazzullo, A. D'Orazio, A. Barbato.

Statistical expertise: G. Barba, F.P. Cappuccio, A. Siani.

Obtaining of funding: P. Strazzullo.

Administrative, technical, or logistic support: R. Iacone, L. Iacoviello, A. D'Orazio.

Collection and assembly of data: O. Russo, A. Barbato.