Associations between circulating components of the renin-angiotensin-aldosterone system and left ventricular mass

Heribert Schunkert, Hans-Werner Hense, Michael Muscholl, Andreas Luchner, Susanne Kurzinger, A H Jan Danser, Günter A J Riegger

Abstract

Objective—Cardiac growth may be modulated in part by the trophic effects of neurohormones. The aim of the present study was to investigate the relation between the basal activity of the renin-angiotensin-aldosterone system and left ventricular mass.

Design—A population based sample of 615 middle-aged subjects was studied by standardised echocardiography; anthropometric measurements; and biochemical quantification of renin, pro-renin, angiotensinogen, angiotensin converting enzyme (ACE), and aldosterone.

Results—Echocardiographic left ventricular mass index correlated significantly with arterial blood pressure, age, and body mass index. In addition, men ACE activity was significantly related to left ventricular mass index in univariate (P = 0·0007) and multivariate analyses (P = 0·008). Men with left ventricular hypertrophy presented with significantly higher serum ACE concentrations than those with normal left ventricular mass index (P = 0·002). In both men and women serum aldosterone was strongly related to septal and posterior wall thickness. Furthermore, in women serum aldosterone was positively and independently associated with left ventricular mass index (P = 0·0001). This effect was most prominent in hypertensive women. Finally, women with left ventricular hypertrophy presented with significantly higher serum aldosterone (P = 0·01). No significant associations with left ventricular mass index were observed for angiotensinogen, renin, or pro-renin.

Conclusions—The data suggest that the variability of serum ACE or aldosterone, as occurred in this large population based sample, may contribute to the modulation of left ventricular mass.

(Heart 1997;77:24–31)

Keywords: angiotensin converting enzyme; aldosterone; angiotensinogen; left ventricular hypertrophy.

Left ventricular hypertrophy is associated with a markedly increased incidence of myocardial infarction, heart failure, or premature death.4 5 Traditionally, chronic pressure overload of the heart, imposed by arterial hypertension or valvar heart disease, is considered to be the aetiological factor responsible for this major risk factor.6 In recent years, however, experimental investigations directed interest to the question whether neurohormonal stimulation may contribute to the development of left ventricular hypertrophy.6 7 In particular, numerous studies on isolated myocytes or isolated experimental hearts provided convincing evidence that angiotensin II or adrenergic agonists may accelerate myocardial growth even when haemodynamic load is low.7 8

Further attention was drawn to these results by the demonstration that a deletion polymorphism of the angiotensin converting enzyme (ACE) gene may be associated with left ventricular hypertrophy as determined by echocardiography10 or echocardiography.11 12

Genetically determined chronic increases in circulating or tissue concentrations of ACE activity found in subjects carrying the deletion allele13 14 offer a potential mechanism to account for the increased risk of left ventricular hypertrophy. However, a recent report failed to demonstrate any association between the ACE deletion polymorphism and echocardiographic left ventricular mass.15 The ACE deletion polymorphism, however, represents only about 20% of the interindividual variability of plasma and tissue ACE activities.16 17

In addition to the rather modest effect of the ACE deletion polymorphism on ACE protein concentrations, genetic variants may be present in linkage or association studies by definition represent a genomic locus rather than a specific gene. Therefore, the major aim of the present investigation was to address the question whether, in a population based sample, ACE activity in and of itself was related to left ventricular mass. Furthermore, the relations of left ventricular mass to renin, pro-renin, angiotensinogen, and aldosterone concentrations were systematically investigated.

Subjects and methods

STUDY POPULATION

The subjects of this study had initially participated in the MONICA (Multinational Monitoring of Trends and Determinants in Cardiovascular Disease), Augsburg, baseline survey of 1984/85 and its follow up examination in 1987/88.19 Subjects originate from a sex-age-stratified random sample of all German residents of the Augsburg study area. In 1994, a second follow up examination including electrocardiographic, echocardiographic, biochemical, and anthropometric measurements was offered to a total of 1010...
men and women, aged 52 to 65 years, of whom 615 (61%) attended.

All subjects responded to a questionnaire on medical history, physical activities, medication, and personal habits. Body height and weight were recorded in light clothing, and body mass index was computed as weight in kg divided by height in metre squared (kg/m²).

Resting blood pressures were measured after subjects had been sitting for at least 30 minutes. A mercury sphygmomanometer was used to measure blood pressure three times at the right arm by two investigators. The mean of three measurements was used for this study. Hypertension was defined as systolic blood pressure $\geq 160$ mm Hg, or diastolic blood pressure $\geq 95$ mm Hg or when subjects were taking long term antihypertensive medication, borderline hypertension was defined as a blood pressure equal or above $140/90$ mm Hg and below $160/95$ mm Hg, and normotension was defined as blood pressure values below $140/90$ mm Hg. Subjects with valvar heart disease ($n = 2$) and those taking ACE inhibitors ($n = 24$) were excluded from further analysis.

**ECHOCARDIOGRAPHIC MEASUREMENTS**

A two dimensionally guided M mode echocardiogram was performed on each subject by one of two expert sonographers (Sonos 1500, Hewlett Packard). M mode tracings were recorded on stripchart paper at 50 mm/s. Only tracings that demonstrated optimal visualisation of left ventricular interfaces were used, a requirement that resulted in exclusion of 17% of potential subjects. To reduce interobserver variability, all M mode tracings were analysed by one cardiologist. The cardiologist was blinded to the clinical and biochemical data. Measurements for M mode guided calculation of left ventricular mass were taken just below the tip of the mitral valve. Left ventricular internal end diastolic (EDD) and end systolic dimensions (ESD) and septal (SWth) and posterior wall thickness (PWh) were measured according to the guidelines of the American Society of Echocardiography.

Left ventricular mass (LVM) was calculated from M mode echocardiograms according to the formula described by Devereux et al:\textsuperscript{21}

$$\text{LVM} = 0.8(1.04 \times \text{EDD} + \text{SWth} + \text{PWh})^3 - \text{EDD}^3 + 0.6g$$

Left ventricular mass was indexed to body surface area as left ventricular mass index in g/m² body surface area. Left ventricular hypertrophy by M mode criteria was considered when left ventricular mass index $> 134$ g/m² body surface area in men or $> 110$ g/m² body surface area in women.\textsuperscript{22} Patterns of left ventricular geometry were defined as previously proposed by Ganau et al—that is, normal geometry when left ventricular mass index was normal and relative wall thickness $(2 \times \text{PWh/EDD}) < 0.45$, concentric remodelling when normal left ventricular mass index was combined with relative wall thickness $> 0.45$, concentric hypertrophy when left ventricular hypertrophy occurred with a relative wall thickness $> 0.45$, and eccentric left ventricular hypertrophy when left ventricular hypertrophy and a relative wall thickness $< 0.45$ were combined.\textsuperscript{23}

**BIOCHEMICAL MEASUREMENTS**

Blood was drawn from non-fasting subjects who were in a supine resting position for at least 30 minutes. All determinations of circulating components of the renin-angiotensin system were carried out in duplicate. Immunoreactive renin was measured in a 200 µl plasma sample by means of an immunoradiometric assay kit (Nichols Institute, Wychen, The Netherlands), following the methods proposed by Derkx et al.\textsuperscript{24,25} The concentration of pro-renin was calculated by subtracting the results obtained before activation of pro-renin (that is active renin) from those obtained after activation (that is total renin). Pro-renin was activated non-proteolytically, using the renin inhibitor remikiren. Aldosterone was quantified in 100 µl serum by standard radioimmunoassay (Peninsula, Belmont, CA). For determination of angiotensinogen in 10 µl serum, 50 ng recombinant human renin (a generous gift of Dr Fischli, Hoffmann-LaRoche, Basel, Switzerland) was used to generate angiotensin I as previously described.\textsuperscript{26} Angiotensin I was measured by standard radioimmunoassay (Peninsula, Belmont, CA). Angiotensin converting enzyme activity was determined by a fluorimetric assay as described in detail elsewhere.\textsuperscript{27}

**STATISTICAL ANALYSIS**

Values measured for serum aldosterone, angiotensinogen, renin, and pro-renin were not normally distributed and were skewed to the right. Therefore, the correlation and regression analyses were performed on logarithmically transformed values to take account of the deviation from normal distribution. However, the use of non-transformed values confirmed the principal observations with respect to these components of the renin angiotensin system. ACE activity was almost normally distributed in our study sample and the use of logarithmically transformed values did not result in perceivable differences in results. Therefore, all analyses that include ACE activity are presented as an untransformed variable.

To assess the statistical significance of differences in mean values between subjects with normal and raised left ventricular mass indices we used $t$ tests to compare independent samples. Univariate linear associations between measures of left ventricular mass and circulating components of the renin-angiotensin system were assessed by calculation of Pearson's and Spearman's rank coefficients of correlation. After log-transformation of all skewed components of the renin-angiotensin-aldosterone system there were no marked quantitative differences between the two types of coefficients and here we report results for Pearson's correlation coefficient only. Multivariate analyses were run separately for men and women by computation of partial correlation coefficients. Each component of
the renin-angiotensin system was assessed after controlling for systolic blood pressure, body mass index, and age. Covariance-adjusted means of the left ventricular mass across quartiles of ACE and logarithmically transformed (ln) aldosterone concentrations were calculated, after controlling for systolic blood pressure, body mass index, and age, by linear regression techniques and assessed for linearity by modelling the median values of each quartile as a continuous variable. Likewise, adjusted mean values of ACE and ln aldosterone concentrations were calculated in groups of subjects with differing types of left ventricular geometry. Stratification of the analyses for the potentially modifying effects of age and hypertension produced no evidence for statistical interaction and is therefore not included in this report.

### Results

**BLOOD PRESSURE, BODY MASS INDEX, AND AGE**

Anthropometric data on subjects with normal left ventricular mass index (M mode echocardiography) and those with left ventricular hypertrophy evaluated in the present investigation are shown in table 1. In both men and women, systolic or diastolic arterial blood pressures were significantly related to left ventricular mass index (table 2). Closest associations were detected for systolic blood pressure (table 2). Furthermore, the prevalence of left ventricular hypertrophy increased from 3.3% in normotensive individuals, to 6.5% in those with borderline hypertension, and to 23.2% in those with manifest hypertension. Likewise, body mass index and age were found to be related to echocardiographic left ventricular mass index (table 2). Since systolic blood pressure, body mass index, and age in women revealed close associations with left ventricular mass index by echocardiography these variables were included as control variables in subsequent multivariate analyses.

### Table 1  Anthropometric and biochemical data of participants according to the presence or absence of left ventricular hypertrophy (LVH) (mean) (SEM)

<table>
<thead>
<tr>
<th>variable</th>
<th>Normal LVMI (n = 179)</th>
<th>LVH (n = 32)</th>
<th>Normal LVMI (n = 227)</th>
<th>LVH (n = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>57.5 (5.3)</td>
<td>57.8 (4.8)</td>
<td>57.4 (0.2)</td>
<td>59.8 (0.7)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.2 (2.2)</td>
<td>27.6 (0.6)</td>
<td>26.5 (0.03)</td>
<td>29.6 (0.9)</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>143 (1)</td>
<td>153 (3)*</td>
<td>142 (1)</td>
<td>162 (2)*</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>91 (0.2)</td>
<td>96 (2)*</td>
<td>87 (0.6)</td>
<td>97 (1.4)*</td>
</tr>
<tr>
<td>LVMI (g/m²)</td>
<td>95.3 (4.4)</td>
<td>158.6 (4.9)*</td>
<td>78.1 (1.0)</td>
<td>128.0-1.5*</td>
</tr>
<tr>
<td>Sepal wall (mm)</td>
<td>10.8 (0.1)</td>
<td>15.1 (0.4)*</td>
<td>9.3 (0.1)</td>
<td>13.9 (0.3)*</td>
</tr>
<tr>
<td>Posterior wall (mm)</td>
<td>9.1 (0.1)</td>
<td>12.3 (0.3)*</td>
<td>7.8 (0.1)</td>
<td>11.0 (0.3)*</td>
</tr>
<tr>
<td>ACE (U/L)</td>
<td>247.5 (0.5)</td>
<td>39.4 (1.4)*</td>
<td>253.4 (0.4)</td>
<td>264 (1.1)</td>
</tr>
<tr>
<td>Aldosterone (pg/ml)</td>
<td>137 (7)</td>
<td>142 (14)</td>
<td>123 (5)</td>
<td>252 (61)</td>
</tr>
<tr>
<td>Angiotensinogen (µg/ml)</td>
<td>0.95 (0.02)</td>
<td>1.01 (0.05)</td>
<td>1.18 (0.04)</td>
<td>1.22 (0.07)</td>
</tr>
<tr>
<td>Renin (pg/ml)</td>
<td>132 (1.7)</td>
<td>144 (2.7)</td>
<td>142 (7)</td>
<td>147 (12)</td>
</tr>
<tr>
<td>Pro-renin (pg/ml)</td>
<td>266 (13.5)</td>
<td>270 (40.5)</td>
<td>179 (6.2)</td>
<td>176 (14.2)</td>
</tr>
</tbody>
</table>

LVMI, left ventricular mass index by M mode echocardiography (ASE formula); LVH, left ventricular hypertrophy (LVMI > 134 g/m² in men or > 110 g/m² in women; BMI, body mass index; BP, blood pressure; ACE, serum angiotensin converting enzyme activity.

*P < 0.05 v subjects with normal LVMI; *P < 0.01 v subjects with normal LVMI; *P < 0.05, a non-parametric test (Kruskal-Wallis) was used to calculate significance because aldosterone concentrations were not normally distributed.

### Table 2  Pearson correlation coefficients of left ventricular mass indices and clinical variables

<table>
<thead>
<tr>
<th>variable</th>
<th>Men (n = 211)</th>
<th>Women (n = 277)</th>
<th>r</th>
<th>p</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>0.08</td>
<td>NS</td>
<td>0.29</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m² BSA)</td>
<td>0.23</td>
<td>0.0007</td>
<td>0.32</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>0.30</td>
<td>0.0001</td>
<td>0.41</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>0.27</td>
<td>0.0001</td>
<td>0.39</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE (U/L)</td>
<td>0.23</td>
<td>0.0007</td>
<td>0.05</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ln aldosterone (pg/ml)</td>
<td>0.09</td>
<td>NS</td>
<td>0.25</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ln angiotensinogen (µg/ml)</td>
<td>0.05</td>
<td>NS</td>
<td>0.01</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ln renin (µg/ml)</td>
<td>-0.09</td>
<td>NS</td>
<td>-0.13</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ln pro-renin (pg/ml)</td>
<td>-0.04</td>
<td>NS</td>
<td>-0.05</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LVMI, left ventricular mass index (g/m² BSA) by M mode echocardiography (ASE formula); BMI, body mass index; BP, blood pressure; ACE, serum angiotensin converting enzyme activity.

### Table 3  Pearson correlation coefficients of left ventricular wall thickness or end diastolic dimension and serum angiotensin converting enzyme activity or aldosterone

<table>
<thead>
<tr>
<th>variable</th>
<th>Men (n = 211)</th>
<th>Women (n = 277)</th>
<th>r</th>
<th>p</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWth</td>
<td>0.14</td>
<td>0.04</td>
<td>0.20</td>
<td>0.004</td>
<td>0.004</td>
<td>NS</td>
</tr>
<tr>
<td>PWth</td>
<td>0.17</td>
<td>0.02</td>
<td>0.16</td>
<td>0.02</td>
<td>-0.17</td>
<td>0.02</td>
</tr>
<tr>
<td>LVEDD</td>
<td>0.19</td>
<td>0.07</td>
<td>0.27</td>
<td>0.001</td>
<td>0.28</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

SWth and PWth, septal and posterior wall thickness by M mode echocardiography, respectively; LVEDD, left ventricular end diastolic diameter; ACE, serum angiotensin converting enzyme activity.
Table 4  Partial correlation coefficients of left ventricular mass index, left ventricular wall thickness, or end diastolic dimension and serum angiotensin converting enzyme activity or aldosterone

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men (n = 211)</th>
<th>Women (n = 277)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LVMI</td>
<td>SWth</td>
</tr>
<tr>
<td>ACE (U/L)</td>
<td>0.21</td>
<td>0.008</td>
</tr>
<tr>
<td>In aldosterone (pg/ml)</td>
<td>NS</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

LVMI, left ventricular mass index (g/m² BSA); SWth, septal wall thickness (mm); PWth, posterior wall thickness (mm); LVEDD, and left ventricular end diastolic diameter (mm) have been adjusted for systolic blood pressure, age, and body mass index. ACE, serum angiotensin converting enzyme activity.

Index persisted in multivariate analyses that accounted for systolic blood pressure, body mass index, and age (table 4). Furthermore, after adjustment for co-variables, the data allowed us to calculate that an increase of 10 U/L in ACE activity was associated with an increase of left ventricular mass index of 8.6 g/m² (95% confidence interval 3.2 to 13.9, P = 0.0021). When men were divided in quartiles of ACE activity (<20.5, 20.5–25.0, 25.1–29.0, >29 U/L) the co-variable-adjusted mean of left ventricular mass index (M mode echo) increased progressively (test for linear trend P < 0.02) (fig 1A). Men with left ventricular hypertrophy by ASE criteria presented with significantly higher serum ACE activity (table 1, P = 0.002). The highest levels of ACE activity were found in those men with an eccentric pattern of left ventricular hypertrophy (fig 2A).

In women, no association between serum ACE activity and any of the indices of left ventricular mass was observed (tables 2 and 4).

ALDOSTERONE

In men univariate analysis showed significant associations between logarithmically transformed serum aldosterone concentration (in

![Figure 1](A) Left ventricular mass index adjusted for systolic blood pressure, body mass index, and age in men according to serum ACE activity levels. Men were stratified by quartiles of serum ACE activity. LVMI represents left ventricular mass index. (B) Left ventricular mass index adjusted for systolic blood pressure, body mass index, and age in women according to serum aldosterone concentrations. Women were stratified by quartiles of serum aldosterone. LVMI, left ventricular mass index.

![Figure 2](A) Serum ACE activity in men according to left ventricular geometry. ACE activity was highest in men with eccentric left ventricular hypertrophy. LVMI, left ventricular mass index; LVH, left ventricular hypertrophy. (B) Serum aldosterone concentrations in women according to left ventricular geometry. Concentrations of aldosterone were raised in women with concentric and eccentric left ventricular hypertrophy. Geometric patterns are defined in the methods section. LVMI, left ventricular mass index; LVH, left ventricular hypertrophy.
Interestingly, there was a tendency towards smaller left ventricular end diastolic dimensions (table 3) that abolished significant associations with left ventricular mass index (table 2). Controlling for systolic blood pressure, body mass index, and age blunted the association of ln aldosterone with left ventricular wall thickness in men. However, a significant inverse correlation between ln aldosterone and end diastolic dimensions persisted ($\beta = -1.51$, $P = 0.006$). In men with left ventricular hypertrophy, ln aldosterone was not different from that in men with normal left ventricular mass (table 1).

In women significant univariate associations were observed between ln aldosterone and both septal or posterior wall thickness (table 3). In addition, significant associations were found between ln aldosterone and left ventricular mass index despite a slight but non-significant inverse association between ln aldosterone and end diastolic dimensions (tables 2 and 3). Significant correlations between ln aldosterone and left ventricular mass index were also found when we excluded women who were on any form of antihypertensive medication, including diuretics, or who displayed evidence of myocardial infarction ($n = 219$, $r = 0.21$, $P = 0.0015$). However, when only non-hypertensive women were considered, the association between ln aldosterone and left ventricular mass index was no longer detectable ($n = 161$, $r = 0.07$, $P = NS$).

Multivariate analyses that accounted for systolic blood pressure, body mass index, and age, confirmed a significant association between ln aldosterone and echocardiographic estimates of left ventricular mass index (table 4). After adjustment for covariables, the increase of aldosterone from 75 to 165 pg/l (equal to the interquartile difference in this sample) was associated with an increase of 4.1 g/m² in left ventricular mass index (95% confidence interval 1.7 to 7.3, $P = 0.002$). When women were divided in quartiles of aldosterone concentration ($<60.3$, $60.4-110$, $111-164$, $>164$ pg/ml aldosterone; or $<4.1$, $4.1-4.7$, $4.8-5.0$, $>5.1$ ln aldosterone) the adjusted mean of left ventricular mass index increased progressively (test for linear trend ($P < 0.03$) (fig 1B). Furthermore, aldosterone in women with left ventricular hypertrophy by ASE criteria was significantly higher than that in women with normal left ventricular mass ($P = 0.0001$; table 1). Raised concentrations of ln aldosterone were found in women with concentric and eccentric patterns of left ventricular hypertrophy (fig 2B).

**ANGIOTENSIN CONVERSION ENZYME**

In both men and women no associations were observed between serum angiotensinogen concentration and any of indices of left ventricular mass (table 2). Furthermore, angiotensinogen concentrations in individuals with left ventricular hypertrophy by ASE criteria (table 1) did not differ significantly from those with normal left ventricular mass.

**RENIN AND PRO-RENIN**

No significant positive associations between left ventricular mass index and plasma renin or plasma pro-renin concentrations were detectable in either men or women (table 2). In fact, left ventricular mass index by $M$ mode echocardiography in women was inversely associated with ln renin. However, significant inverse associations between systolic blood pressure and both ln renin and ln pro-renin concentrations ($r = -0.14$; $P = 0.05$; $r = -0.17$; $P = 0.001$, respectively) may have confounded this observation.

**Discussion**

The results of this population-based study suggest that the basal activity of the renin-angiotensin-aldosterone system, in addition to blood pressure,$^1$ body mass index,$^{19}$ and age,$^2$ is related to left ventricular mass. In particular, serum aldosterone concentrations in both women and men were found to be associated with septal and posterior wall thickness. In addition, in women there was a strong association between serum aldosterone concentrations and echocardiographic indices of left ventricular mass. Finally, serum ACE activity displayed a strong and independent association with left ventricular mass and hypertrophy, an effect that was, however, restricted to men.

**ANGIOTENSIN CONVERTING ENZYME**

Growing evidence suggests that serum ACE activity may be higher in subjects with various cardiovascular disorders. In addition to the present finding, myocardial infarction, diabetic nephropathy, and carotid artery thickening have been associated with raised serum ACE concentrations.$^{18-20}$ Furthermore, in children, serum ACE activity was found to be related to the level of arterial blood pressure.$^16$

Serum and tissue ACE activities are largely determined by genetic disposition and vary little in a given individual.$^{10}$ Thus it may be hypothesised that genetically determined ACE concentrations may contribute to the genetic risk of developing such cardiovascular disorders.$^{15,22,23}$ However, vascular$^{24,25}$ or cardiac pressure overload$^{26}$ or treatment with ACE inhibitors$^{27}$ can also induce ACE gene expression. Therefore, analyses that associate serum ACE activity with cardiovascular disease such as left ventricular hypertrophy cannot be used to establish causal relations.

Interestingly, a deletion polymorphism of the ACE gene that accounts for about 20% of serum$^{16,18}$ and tissue$^{17,26}$ ACE activities has been related to myocardial infarction, diabetic nephropathy, carotid wall thickness, and left ventricular hypertrophy.$^{15,20-22}$ Such molecular genetic association studies, however, fall short of establishing a link with a specific gene. Furthermore, a second, at present not yet identified, variant of the ACE gene has been predicted to account for most of the inherited variability of ACE activity.$^{16,18}$ Thus at present molecular genetic tools may be of limited sensitivity and specificity in identifying subjects
with inherited high ACE concentrations. Indeed, the ACE deletion polymorphism may not be a strong predictor of the cardiovascular disorders mentioned above. Though numerous reports have supported the original findings, some recent reports were negative. Nevertheless, the conjuction of both molecular genetic and epidemiological methods may overcome some of the limitations of either method and strongly suggest a significant role of ACE in the pathophysiology of left ventricular hypertrophy.

In the present study, an increase of 10 U/l in ACE activity was associated with an estimated increase in left ventricular mass of 8.6 g/m². This resembles the estimated increase in left ventricular mass that occurs when systolic blood pressure increases by 20 mm Hg or body mass index increases by 5.2 kg/m². Thus given the multifactorial and potentially polygenetic aetiology of left ventricular hypertrophy ACE activity in men accords with other important predictors of this condition.

As in our previous molecular genetic study, we did not see any association between ACE activity and left ventricular mass in women. We cannot explain the mechanism of this apparent gender-related difference. There are, however, analogous situations in ACE “knock-out” mice and genetically hypertensive rats. In particular, in male mice blood pressure seems to be regulated by the level of ACE activity because male transgenic mice that carry only one copy of the ACE gene (and display decreased serum ACE activity) are characterised by significant reduction of systolic blood pressure. In contrast, no changes in blood pressure were detectable in heterozygote female mice missing one copy of the ACE gene. Even more strikingly, Harris et al using molecular linkage analysis in male New Zealand hypertensive rats have shown that a gene at the ACE gene locus may increase cardiac mass without affecting blood pressure. In female rats, this locus was related to left ventricular mass as well but only in conjunction with higher blood pressure levels. Given the analogies found in human investigations, these experimental models may offer a key to explore the mechanisms that account for the effects of ACE on cardiac growth.

**ANGIOTENSINOGEN**

In women serum angiotensinogen has been found to be largely related to oestrogen status, a finding confirmed in the present cohort (data not shown). Most women studied here were postmenopausal and 30% were taking oestrogen replacement therapy. Therefore, this regulatory mechanism may complicate studies like the present that aim to associate slowly progressing structural alterations such as left ventricular hypertrophy with serum angiotensinogen concentrations.

In men serum angiotensinogen concentration, like ACE activity, is partially determined by genetic variants. Some of these variants have been linked to hypertension, an effect that was particularly strong in certain subgroups such as men with normal body mass index. In the present study we found no association between angiotensinogen and left ventricular mass and M mode estimates of left ventricular mass index. However, we found that serum angiotensinogen was related to two dimensional echocardiographic estimates of left ventricular mass index (data not shown).
Subjects in whom both M mode and two dimensional echocardiograms were technically feasible were less obese (body mass index 26.7 v 28.6 in M mode group, P = 0.001); this may in part account for the observed discrepancy. Nevertheless, our data fall short of identifying a consistent relation between angiotensinogen and left ventricular mass index and further studies in conjunction with molecular genetic approaches are needed to clarify this issue.

RENIN AND PRO-RENIN
In the present population, no significant positive associations between left ventricular mass and either renin or pro-renin concentrations were detectable. It is noteworthy, however, that in middle-aged subjects renin and prorenin are regulated by several factors. In particular, renin and pro-renin concentrations have been found to correlate inversely with blood pressure, sodium intake, or angiotensin II, all factors that are known to induce left ventricular hypertrophy.65 Unfortunately, measurements of sodium excretion or angiotensin II are not available in this cohort. Therefore, we cannot exclude the likelihood that renin or pro-renin has independent effects on left ventricular mass, as has been suggested by some but not all previous investigators.49 50 56

LIMITATIONS
The statistical regression analyses presented here are based on the means of three measurements of resting blood pressure and a single blood sampling for determination of renin, pro-renin, ACE, angiotensinogen, and aldosterone concentrations. Integration of repeated measurements of either blood pressure (such as 24 hour ambulatory recordings) or circulating components of the renin-angiotensin-aldosterone system may allow better estimation of the haemodynamic load or neurohormonal stimulation that is chronically imposed on the left ventricle.57 59 Furthermore, we studied the circulating renin-angiotensin-aldosterone system and we cannot estimate the local tissue concentrations of its components. Thus the present study may underestimate the impact of dynamic and local effects of various factors on left ventricular mass. Nevertheless, this epidemiological study may help to extend the substantial experimental evidence on the effects of the renin-angiotensin-aldosterone system on cardiac growth.

We thank Mr RJA de Brun for the excellent technical support. This study was supported by the Deutsche Forschungsgemeinschaft (DFG Schu 617/5-1, 9-1 and 10-1), an Astra Award for Cardiovascular Research (HS), and the Bundesministerium für Forschung und Technologie (HS, HWH).

 Associations between circulating components of the renin-angiotensin-aldosterone system and left ventricular mass

45 Devereux RB, Savage DD, Sachs I, Laragh JH. Relation of hemodynamic load to left ventricular hypertrophy and function. Am J Cardiol 1983;51: 171-6.