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The association between circulating angiotensin-converting enzyme and cardiovascular risk in the elderly – a cross-sectional study

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Abstract

Introduction. A polymorphism in the angiotensin-converting enzyme gene (ACE I/D polymorphism) has been associated with increased risk for cardiovascular disease. This polymorphism affects the level of circulating ACE, but there is great individual variation, even between those with the same genotype. Few earlier studies have investigated the link between circulating ACE and cardiovascular risk. The aim of this study was to investigate this association, and to examine the relationship between ACE level, ACE genotype and cardiovascular disease.

Materials and Methods. The study population consisted of 322 men and 350 women aged 69-87. Plasma ACE level was determined using ELISA, and ACE genotype was analysed using PCR followed by gel electrophoresis.

Results. In men, ACE levels increased with increasing number of cardiovascular risk factors (p=0.003). There was a significant association in men between increased ACE level and both diabetes (p=0.007) and smoking (p=0.037).

Conclusions. This study shows that cardiovascular risk factors (such as smoking and diabetes) are associated with higher levels of circulating ACE in men. High ACE levels may represent one of the cellular mechanisms involved in producing the vascular damage associated with cardiovascular risk factors.

Keywords: endothelium, smoking, genetics, diabetes, cardiovascular risk factors

Introduction

The renin-angiotensin-aldosterone system is one of the most important systems in cardiovascular homeostasis and blood pressure regulation. Angiotensin-converting enzyme (ACE) is a key enzyme in the renin-angiotensin-aldosterone system, converting angiotensin I to angiotensin II and degrading bradykinin. A genetic variation in the gene encoding ACE, ACE I/D polymorphism, affects circulating ACE levels. I/I, I/D and D/D are associated with low, intermediate and high levels, respectively.[1,2] In 1992, it was reported that the D/D genotype was associated with increased risk for myocardial infarction.[3] Since then, the influence of the ACE I/D polymorphism on cardiovascular risk has been extensively researched. Some studies have shown an association between the D/D genotype and coronary artery disease,[4] hypertension[5] and left ventricular hypertrophy[6]. Other studies, however, have failed to confirm this.[7-9] This inconsistency may be due to differences between studies in terms of in ethnicity,[10,11] gender[10,12] or publication bias (positive results being easier to publish). Although ACE levels are influenced by the ACE I/D polymorphism, there are large variations between carriers of the same genotype. It has been shown that ACE I/D polymorphism accounts for 20-50% of the variance in plasma ACE levels,[1,2] implying that 50-80% of the variation is due to other factors. Apart from ACE I/D polymorphism, a number of other genetic variants, some outside the ACE coding region, have been shown to influence levels of circulating ACE.[13-15] In addition, several endogenous and exogenous factors (such as estradiol,[16] vascular endothelial growth factor,[17] salt-intake[18] etc.) have also been shown to influence ACE in vivo or in vitro. Despite this, surprisingly few studies have investigated the association between ACE level and cardiovascular risk. The aims of this study were to investigate the association between circulating ACE and cardiovascular risk as well as examine the connection between ACE level, ACE genotype and cardiovascular disease (CVD).

Materials and Methods

Study population

The study population consisted of 672 individuals (322 men, 350 women) aged 69-87. In 1999, all inhabitants aged 64-82 in a rural municipality in South East Sweden were invited to take part in a longitudinal study. Of the 1130 originally invited, 876 individuals agreed to participate. When a follow-up study was conducted between 2003 and 2005 we had the opportunity to perform a cross-sectional study in this population to investigate the association between ACE and cardiovascular risk. All subjects participating in the original study was invited to take part. A total of 123 individuals died before the follow-up study started. 675 of the remaining subjects agreed to participate. Two individuals were excluded from the study due to difficulties in obtaining a blood sample, and one individual was excluded due to hepatitis infection. All the data presented in this report come from the follow-up study. The study was approved by the Regional Ethical Review board in Linkoping, Sweden. All participants gave their informed consent. The study was conducted in accordance with principles stated in the Declaration of Helsinki and all procedures complied with institutional guidelines.

Data sampling

History of cardiovascular risk factors, CVD, and medications were recorded by an experienced cardiologist, who also performed a physical examination of all participants. Blood pressure was determined with subjects in the supine position after at least 30 min rest, using a sphygmomanometer. The mean of three consecutive measurements was calculated, and following standard clinical practice, adjusted to the nearest 5 mmHg. The measurements were performed by an experienced cardiologist. Height and weight were recorded and body mass index (BMI) calculated. Blood samples were taken following overnight fasting and

collected in pre-chilled plastic Vacutainer tubes (Terumo EDTA K-3). Plasma was prepared by centrifugation at 3000 *g* for 10 min at 4°C and transferred to new tubes. Blood and plasma were stored at -70°C pending analysis. Fasting plasma glucose concentrations were determined.

Morbidity

Diabetes mellitus was defined as a fasting blood glucose ≥ 7 mmol/L or a previous diagnosis of diabetes with ongoing treatment. Individuals diagnosed and treated for hypertension, or with a blood pressure ≥ 140/90 mmHg were defined as hypertensive. Ischaemic heart disease (IHD) was defined as history of angina pectoris or ECG-verified myocardial infarction. Heredity for CVD was defined as mother, father, or siblings with a history of hypertension, stroke, or myocardial infarction before the age of 65. Individuals who stated that they smoked were classified as smokers. Hyperlipidaemia was defined as known hyperlipidaemia and/or treatment with lipid-lowering drugs.

Determination of ACE genotype

Genomic DNA was isolated from peripheral blood using QIAmp DNA Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. ACE genotype was determined by PCR using a triple primer approach, which has been shown to be a reliable PCR strategy to avoid mistyping of I/D carriers[19]. The method has been described in detail elsewhere[20]. In brief, DNA was amplified with PCR using three primers; 5´-CTGCAGGTGTCTGCAGCATGTGC-3´,

5´-GATTACAGGCGTGATACAGTCACTTTT-3´ and

5´-GCCATCACATTCGTCAGATCTGGTAG-3´ (Invitrogen, Paisley, UK). Each primer was used at a concentration of 0.4 µM. DNA amplification started with an initial denaturation at

94°C for 5 min, followed by 30 cycles of the following thermal profile: 94°C for 45 sec, 65°C for 45 sec and 72°C for 45 sec, followed by a final extension at 72°C for 5 min. Amplified DNA was separated by gel electrophoresis using a 1.5% agarose gel stained with ethidium bromide and visualised by UV-light. The determination of ACE genotype was based on the length of the DNA fragments. Samples were re-analysed if there were the least uncertainty about the result.

Angiotensin-converting enzyme assay

Plasma ACE level was analysed using Quantikine, Human ACE Immunoassay (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions. All samples were analysed in duplicate and mean values were calculated. Samples with a coefficient of variation of >20% were re-analysed. Intra-assay variation was 6.5 % and inter-assay variation was 12%.

Validation of ACE assay

Validation of the ACE assay was done to verify that ACE level (ng/ml) correlated with ACE activity (U). Blood samples were taken from twenty-three men, 70 years of age. Whole blood was collected in silicone-coated tubes (Venoject, Terumo, Somerset, NJ, USA) and left at room temperature for 2 h. Serum was prepared by centrifugation at 210 g for 10 min at 22°C, transferred to new tubes and stored at -70°C pending analysis. ACE activity was analysed using a commercial radioenzymatic assay (ACE-direct REA, Bühlmann Laboratories, Schönenbuch, Switzerland) following the manufacturer's instructions. Each sample was counted for 5 min in a beta counter (LKB Wallac, 1217 Rackbeta, Turku, Finland). Preparation of plasma and measurement of ACE level using Quantikine were done as described above.

Statistical analyses

As cardiovascular manifestations are known to be expressed differently between genders, data are presented in men and women separately. Student's *t*-test was used to compare continuous data between men and women. One-way ANOVA followed by Bonferroni's post hoc test were used to compare mean values for continuous data between genotypes. Pearson's χ^2 test was used to compare the frequency of cardiovascular risk factors, CVD and medications. General linear models were used to study the association between plasma ACE level and cardiovascular risk factors and CVD. Main effects and 2-factor interactions were analysed, and potentially confounding factors were included. In order to address if any of the significant associations were gender-specific, logistic regression or general linear models including gender-interactions, were performed in the overall population. The impact of cardiovascular medications on plasma ACE was analysed in a separate model, in which only the main effects were analysed. Correlation analysis was used to study the relationship between ACE level and ACE activity. The relationship between ACE level and age was analysed using linear regression. Hardy Weinberg equilibrium was assessed by χ^2 analysis. The results are presented as mean values ± SD, unless otherwise stated. P-values <0.05 were considered statistically significant. Statistical analyses were carried out using SPSS 17.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results

The characteristics of the study population are shown in Table 1. Compared to men, women had higher BMI, higher systolic blood pressure, greater heredity for CVD, and more were on diuretics. History of IHD and smoking were more frequent among men.

ACE I/D polymorphism

Characteristics of the study population according to ACE I/D polymorphism are shown in Table 2. The prevalence of I/I, I/D and D/D genotypes was not significantly different from values predicted by Hardy Weinberg equilibrium. In women, carriers of the D/D genotype were slightly younger than carriers of the I/I genotype. A history of IHD was commoner in women carrying the I/I genotype than in those with I/D or D/D ($p=0.002$), while no difference was seen among men ($p=0.736$). Logistic regression analysis showed a significant interaction between IDH and gender, indicating that the association between the ACE I/D polymorphism and IHD is gender-dependent. No significant differences between genotypes were observed for any other clinical variable. As shown in Figure 1, plasma ACE levels were influenced by ACE genotype (ANOVA $p<0.001$ for men and women), but there were large variations within each genotype. ACE I/D polymorphism accounted for 10% of the variance in plasma ACE level in men, and 17% in women. There were no differences in mean plasma ACE level between men and women carrying the same genotype.

ACE activity vs. ACE level

In order to evaluate the correlation between ACE activity and ACE level, serum and plasma from twenty-three elderly men were analysed. There was a strong correlation between ACE activity (U) and ACE level (ng/ml); between serum ACE activity and serum ACE level

8

$(r^2=0.6408, p<0.0001)$ and between serum ACE activity and plasma ACE level $(r^2=0.5980,$
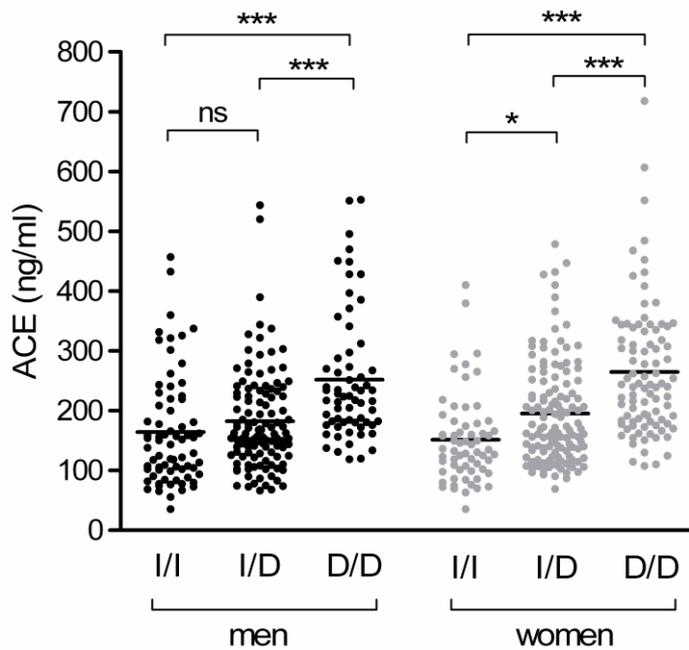
$p<0.0001)$ (data not shown).

Figure 1. ACE level (ng/ml) in plasma from men ● and women ● carrying the ACE I/I, I/D or D/D genotype. Individuals on ACE inhibitor treatment are excluded. n=531 (Men: I/I 71, I/D 115, D/D 64. Women: I/I 63, I/D 128, D/D 90) * p<0.05, *** p<0.001 (ONE-way ANOVA followed by Bonferroni's post hoc test).

ACE level in plasma

General linear models were used to investigate the impact of cardiovascular medications on

circulating ACE level. Treatment with ACE inhibitors (ACEi) was associated with higher

levels of ACE in plasma. In men treated with ACEi, the mean plasma ACE level was 299.9

ng/ml compared to 195.0 ng/ml in men who were not on ACEi treatment (p<0.001). In

women, the treated group had a mean plasma ACE level of 343.0 ng/ml compared to 207.9

ng/ml for those not on ACEi treatment (p<0.001). All individuals treated with ACEi (n= 72

men and 69 women) were therefore excluded from the following statistical analyses of

plasma ACE level. Although, there were no significant effects seen in treatment with beta

receptor blockers, angiotensin II receptor blocker, diuretics or statins, adjustment for

antihypertensive treatment was made, when appropriate, to ensure that this was not a confounding factor. In addition, adjustment for ACE genotype was made to compensate for uneven distribution between genotypes. Furthermore, a linear regression analysis showed that there was a significant relationship between ACE level and age ($p<0.001$, $r^2=0.024$). Age was therefore considered a confounding factor and adjusted for in all analyses.

To examine the relationship between ACE level and cardiovascular risk factors, subjects were divided into 4 groups based on number of risk factors (Figure 2). Risk factors were diabetes, heredity for CVD, hyperlipidaemia, hypertension and smoking. In men, the level of ACE increased with increasing numbers of cardiovascular risk factors (ANOVA p=0.003), and bonferroni's post hoc test showed significant differences between 0 and ≥ 3 risk factors (p=0.034) and between 1 and ≥ 3 risk factors (p=0.007). Adjustment for antihypertensive treatment had only a slight effect on this association (ANOVA p=0.008). No significant association was found in women (ANOVA p=0.573) and the interaction between gender and number of cardiovascular risk factors was not significant (p=0.365).

Association between ACE level in plasma and cardiovascular risk factors and CVD were analysed using general linear models (Table 3). In the model including all subjects, ACE levels were higher in subjects with IHD (p=0.025), however this association did not remain after adjustment for age (p=0.135). A higher ACE level was found in men with diabetes (p=0.007), while no difference was seen in women (p=0.967). In addition, there was a significant interaction between gender and diabetes (p=0.042), indicating that the association between ACE level and diabetes is gender-dependent. Furthermore, a higher ACE level was found in male smokers (p= 0.007) while a non-significant tendency towards increased ACE levels was seen in women (p=0.135). The interaction between gender and smoking was non-

significant (p=0.797). In addition, in the overall group, there was a significant interaction between smoking and IHD.
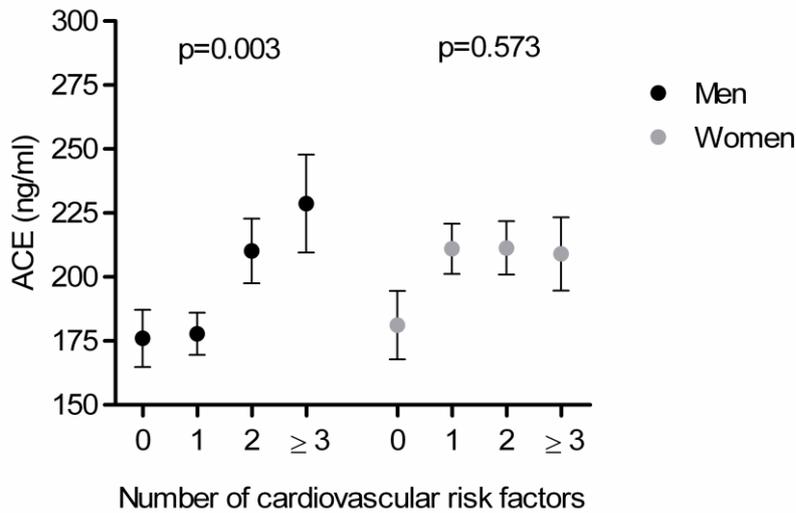

Figure 2. Plasma ACE levels (ng/ml) according to number of cardiovascular risk factors. Risk factors are diabetes, heredity for cardiovascular disease, hyperlipidaemia, hypertension and smoking. Subjects on ACE inhibitor treatment are excluded. n=531 (250 men, 281 women). Values are mean ± SEM in men ● and in women ●. P-values are ANOVA adjusted for age and ACE-genotype. Bonferroni's post hoc test showed significant differences in men between 0 and ≥ 3 risk factors (p=0.034) and between 1 and ≥ 3 risk factors (p=0.007).

There was no difference in ACE level between hypertensive and non-hypertensive subjects (Table 3), and there was no correlation between ACE level and systolic (p=0.070), diastolic (p=0.663) or pulse pressure (p=0.071). However, ACE levels were higher in women with known hypertension before entering the study than in those without (p=0.002). This difference remained after adjustment for antihypertensive treatment (p=0.004). The same analysis in men showed significantly higher ACE levels in men with known hypertension only after adjustment for antihypertensive treatment (p=0.042).

Discussion

Our study shows that, in men, circulating ACE level increases with increasing number of cardiovascular risk factors. In addition, men with diabetes and male smokers have a higher level of circulating ACE than non-diabetic and non-smoking men. Numerous studies have explored the association between ACE I/D polymorphism and various diseases.[3-6] Although a relationship between I/D polymorphism and plasma ACE level has been repeatedly confirmed,[1,2] other factors have been shown to influence circulating ACE levels.[13-18] Despite this, earlier studies have mainly focused on the ACE I/D polymorphism and its possible connection with CVD, disregarding the fact that the role of circulating ACE in cardiovascular disease is largely unknown. Therefore, in addition to investigate the relationship between ACE genotype and CVD, this study also focuses on the link between ACE level, cardiovascular risk factors and CVD.

Little is known about the regulation of ACE in the circulation. Possible mechanisms involved in this regulation might be the synthesis and secretion of ACE into the circulation, the clearance of ACE from the circulation as well as the shedding of the enzyme from ACE containing cells.[21,22] We found that ACE level increases with increasing number of cardiovascular risk factors in men (Figure 2). Although no significant association was found in women, there was no evidence for a gender-specific association. The correlation between number of cardiovascular risk factors and risk for CVD has been reported previously.[23] However, to the best of our knowledge, a correlation between number of risk factors and ACE level has not previously been reported. Raised ACE levels may represent one cellular mechanism involved in the vascular damage associated with cardiovascular risk factors, possibly mediated by increased production of angiotensin II, increased degradation of bradykinin and altered levels of other angiotensin peptides (e.g. angiotensin 1-7). However,

we can not exclude the possibility that a raised ACE level is a non-pathological phenomenon resulting from e.g. increased shedding of ACE from vascular endothelium. When we examined the relationship between ACE level and smoking, higher ACE levels were found in male smokers than in male non-smokers (Table 3). This is consistent with the observation that nicotine and its metabolites increase the expression and activity of ACE in human endothelial cells.[20] A few *in vivo* studies examining the effect of smoking on serum ACE activity have indicated that the acute effects of smoking include increased serum ACE,[24,25] whereas results regarding the long-term effects are inconsistent.[26,27] Although no significant association was found in women, there was no evidence for a gender-specific association. The lack of association in women might be due to the low proportion of female smokers. Furthermore, we reported an increase in plasma ACE level in male diabetic subjects, while no association was found in women. There was a significant interaction between gender and diabetes, indicating that the association between ACE level and diabetes is gender-dependent. Such gender-dependent effect has previously not been shown, and needs to be confirmed in future studies. Our data do not however, lend support to the previously reported association between D/D genotype and diabetes,[28] although our study might be to small to detect moderate effects. Diabetic nephropathy, a common complication among diabetics, is associated with activation of the renin-angiotensin-aldosterone system.[29] As adjustment for glomerular filtration rate had no effect on the association between diabetes and increased ACE level, it seems unlikely that this effect was due to impaired renal function. A few early studies have shown that diabetic patients have higher levels of circulating ACE than healthy controls.[30,31] Although none of the previous studies considered the influence of ACE genotype on ACE level, and few evaluated gender differences, their results are similar to our findings. These findings are particularly interesting as ACEi treatment has been shown to lower the onset of diabetes in high-risk individuals and to reduce the vascular complications of diabetes.[32]

Angiotensin II, the effector peptide of ACE, is a powerful vasoconstrictor with well-described effects on blood pressure. As angiotensin II raises blood pressure, one might expect to find a correlation between ACE level and blood pressure. Previous studies that have investigated this association have, however, produced conflicting results.[33,34] We found no difference in ACE level between hypertensive subjects, defined as diagnosis with ongoing treatment or a blood pressure ≥ 140/90 mmHg, and non-hypertensive subjects. Nor were there correlations between ACE level and systolic, diastolic and pulse pressure. However, subjects with known hypertension before entering the study had higher ACE level than subject without a prior diagnosis. This effect was seen in women independently of potentially confounding factors and in men following adjustment for antihypertensive treatment. It might be speculated that the adjustment of blood pressure values to the nearest 5 mmHg is a confounder, possibly masking a correlation between ACE level and blood pressure.

Furthermore, in the model that includes all participants, subjects with IHD had higher ACE levels than subjects without IHD. This is probably because there is an association between ACE and age, as this difference disappeared when age adjustments were made. Such age-dependent effect has, to the best of our knowledge, previously not been shown in adults.[35,36] Whether this effect is due to a higher prevalence of diseases in older subjects or to age *per se* remains unclear. However, age obviously needs to be taken into consideration when studying circulating ACE levels, at least in the elderly.

In agreement with previous studies[1,2] ACE levels were influenced by ACE I/D polymorphism. I/I, I/D and D/D carriers had low, medium and high levels respectively (Figure 1). However, there were large variations in plasma ACE level within each group, and

ACE I/D polymorphism accounted for only 10% of the variation in men and 17% in women. Several individuals within the I/I group had considerably higher plasma ACE level than most D/D carriers. The low correlation between ACE-genotype and ACE level lends support to the view that other factors are involved, and this emphasises the need for studies that are looking at the role of ACE-genotype in cardiovascular disease to also include estimations of circulating ACE levels.

A history of IHD was commoner in women carrying the I/I genotype than in women with I/D and D/D (Table 2), while no difference was not seen in men. This association seems to be gender-dependent, as a significant interaction between ACE genotype and gender was found. Data from previous studies are conflicting,[8,9] but a number of studies have, contrary to our findings, suggested that the D/D genotype is associated with increased cardiovascular risk.[3-6] These inconsistencies may have several explanations. Firstly, the higher incidence of IHD in I/I carriers in this study may be related to age, as female carriers of the I/I genotype were older than D/D carriers. Secondly, if the D-allele is in fact associated with increased cardiovascular risk, carriers of this allele are more likely to die from e.g. myocardial infarction than carriers of the I/I genotype, introducing selection bias in an elderly population, although genotypes were in accordance with Hardy Weinberg equilibrium. Thirdly, a previous large scale study that included 5000 cases and 6000 controls and a meta-analysis suggest that carriers of the D/D genotype may have only a 12% increase in risk of myocardial infarction.[37] Demonstrating difference of this scale requires far larger sample populations than we used in our study.

In conclusion, our study shows that cardiovascular risk factors (such as smoking and diabetes) are associated with higher levels of circulating ACE in men. We could not,

however, confirm the previously reported association between the D/D genotype and CVD.[3,4,6] Increased ACE level may represent one of the cellular mechanisms involved in producing the vascular damage associated with cardiovascular risk factors. Further studies are needed to fully establish the role of ACE in the pathophysiology of cardiovascular disease. These studies need to look at both the genotype and phenotype of ACE.

Limitations

The relationship between ACE and CVD was investigated using circulating ACE level rather than ACE activity. We were, however, able to show a strong correlation between the two (see results). We studied circulating ACE levels, although it has been suggested that tissue ACE has a more important role in CVD. However, although not proven, it is likely that increase in tissue ACE also would be reflected in the circulation, as circulating ACE originates from tissue ACE. As in all population based studies on elderly there could be a survival bias as a number of subjects obviously have died before the initiation of the study. Moreover, a longitudinal study design would probably have resulted in additional data. In addition, a larger sample would possibly have strengthened our findings.

Acknowledgements

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Table 1 Characteristics of study population

	All	Men	Women	P
Number of subjects n	672	322	350	0.28
Age years (SD)	78.2 (3.5)	77.9 (3.2)	78.3 (3.6)	0.111
Body mass index kg/m^2 (SD)	27.2 (4.2)	26.8 (3.3)	27.6 (4.9)	**0.007**
Plasma glucose mmol/L (SD)	5.8 (2.2)	5.9 (2.2)	5.7 (2.1)	0.158
Blood pressure mmHg (SD)				
Systolic pressure	150 (19)	148 (19)	152 (19)	**0.005**
Diastolic pressure	76 (10)	76 (10)	75 (10)	0.482
History n (%)				
Ischaemic heart disease	158 (24)	92 (29)	66 (19)	**0.003**
Hypertension	564 (84)	263 (82)	301 (86)	0.127
Diabetes	159 (24)	78 (24)	81 (23)	0.742
Hyperlipidaemia	154 (23)	73 (23)	81 (23)	0.884
Heredity	140 (21)	54 (17)	86 (25)	**0.013**
Smoking	63 (9)	47 (15)	16 (5)	**<0.001**
Medications n (%)				
ACE inhibitors	141 (21)	72 (22)	69 (20)	0.400
Angiotensin II receptor blockers	28 (4)	13 (4)	15 (4)	0.872
Beta receptor blockers	245 (37)	125 (39)	120 (34)	0.222
Diuretics	234 (35)	100 (31)	134 (38)	**0.049**
Statins	141 (21)	71 (22)	70 (20)	0.514

Values are mean (SD) or number of subjects (%).

Student's t-test was used for continuous data and Pearson's χ^2 test for categorical data.

P-values are men vs. women

Table 2 Characteristics according to ACE I/D polymorphism

Characteristics	Men				Women			
	I/I	I/D	D/D	P	I/I	I/D	D/D	P
Number of subjects n (%)	95 (30)	148 (46)	79 (25)		80 (23)	163 (47)	107 (31)	
Age years (SD)	78.2 (3.2)	78.1 (3.5)	77.4 (2.7)	0.197	78.6 (3.6)	78.7 (3.6)	77.6 (3.6)	**0.042**
Body mass index kg/m^2 (SD)	26.6 (3.4)	26.7 (3.2)	27.1 (3.2)	0.517	27.6 (5.1)	27.5 (5.1)	27.9 (4.4)	0.766
Laboratory data (SD)								
Plasma glucose mmol/L	6.2 (2.6)	5.8 (2.0)	5.8 (2.0)	0.318	5.4 (1.2)	5.9 (2.4)	5.6 (2.3)	0.225
ACE ng/ml (SD)	182 (105)	216 (109)	267 (106)	**<0.001**	175 (95)	231 (128)	285 (131)	**<0.001**
Blood pressure mmHg (SD)								
Systolic pressure	146 (20)	149 (19)	148 (18)	0.396	153 (21)	151 (18)	152 (20)	0.827
Diastolic pressure	75 (10)	76 (10)	76 (10)	0.738	77 (10)	75 (10)	75 (10)	0.452
History n (%)								
Ischaemic heart disease	30 (32)	40 (27)	22 (28)	0.736	26 (33)	23 (14)	17 (16)	**0.002**
Hypertension	77 (81)	123 (83)	63 (80)	0.809	70 (88)	140 (86)	91 (85)	0.891
Diabetes	28 (30)	34 (23)	16 (20)	0.328	17 (21)	45 (28)	19 (18)	0.155
Hyperlipidaemia	25 (26)	32 (22)	16 (20)	0.584	20 (25)	39 (24)	22 (21)	0.736
Heredity	17 (18)	23 (16)	14 (18)	0.862	20 (25)	40 (25)	26 (24)	0.994
Smoking	16 (17)	15 (10)	16 (20)	0.092	3 (4)	8 (5)	5 (5)	0.919
Medications n (%)								
ACE inhibitors	24 (25)	33 (22)	15 (19)	0.613	17 (21)	35 (22)	17 (16)	0.490
Angiotensin II receptor blockers	7 (7)	5 (8)	1 (1)	0.108	2 (3)	7 (4)	6 (6)	0.583
Beta receptor blockers	39 (41)	56 (38)	30 (38)	0.868	27 (34)	58 (36)	35 (33)	0.883
Diuretics	35 (37)	43 (29)	22 (28)	0.343	23 (29)	64 (39)	47 (44)	0.101
Statins	26 (27)	32 (22)	13 (17)	0.221	14 (18)	33 (20)	23 (22)	0.791

Values are mean (SD) or number of subjects (%)

P-values for continuous data are from ONE-way ANOVA, and categorical data are from Pearson's χ^2 test.

Table 3 Plasma ACE level in subjects with or without cardiovascular risk factors and cardiovascular disease (subjects treated with ACEi are excluded)

CVD/CV risk factors	N yes/no	ACE ng/ml		
		Yes	No	P
All subjects				
Ischaemic heart disease	109/422	212 (105)	199 (98)	0.135
Hypertension	439/92	205 (104)	188 (77)	0.200
Diabetes	107/424	218 (102)	198 (99)	**0.030**
Hyperlipidaemia	100/431	197 (95)	203 (101)	0.799
Heredity	109/422	221 (110)	197 (97)	0.113
Smoking	44/487	239 (110)	199 (99)	**0.026**
Men				
Ischaemic heart disease	64/186	215 (119)	188 (89)	0.387
Hypertension	203/47	198 (104)	181 (66)	0.270
Diabetes	51/199	227 (114)	187 (92)	**0.007**
Hyperlipidaemia	50/200	198 (110)	194 (95)	0.643
Heredity	44/206	220 (114)	190 (94)	0.474
Smoking	34/216	235 (116)	189 (94)	**0.037**
Women				
Ischaemic heart disease	45/236	208 (84)	208 (104)	0.148
Hypertension	236/45	210 (103)	196 (88)	0.402
Diabetes	56/225	205 (91)	207 (104)	0.967
Hyperlipidaemia	50/231	196 (78)	210 (105)	0.407
Heredity	65/216	221 (109)	204 (99)	0.162
Smoking	10/271	228 (90)	207 (102)	0.206

ACE levels are mean (SD)

P-values for the association between ACE level and cardiovascular disease and cardiovascular risk factors are from general linear models including adjustment for ACE-genotype and age.