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# Fetuin-A and arterial stiffness in patients with normal kidney function

Marcel Roos [a,*], Tom Richart [b,c], Tatiana Kouznetsova [b], Maximilian von Eynatten [a], Jens Lutz [a], Uwe Heemann [a], Marcus Baumann [a], Jan A. Staessen [b,c]

[a] Department of Nephrology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany
[b] Genetic Epidemiology Unit, Department of Epidemiology, University of Maastricht, Maastricht, The Netherlands
[c] Division of Hypertension and Cardiac Rehabilitation, Department of Cardiovascular Diseases, University of Leuven, Leuven, Belgium

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ABSTRACT

*Aim:* To evaluate the association between fetuin-A level (AHSG), its encoding gene (Thr256Ser) and arterial function in subjects with normal kidney function.
*Introduction:* The aortic pulse wave velocity (aPWV) is a predictor for cardiovascular mortality. Fetuin-A is a calcification inhibitor and correlates negatively with increased vascular stiffness in dialysis patients. The fetuin-A polymorphism (Thr256Ser) is associated with reduced fetuin levels and accelerated vascular calcification in dialysis patients. Little is known about the role of fetuin-A as an independent predictor for the development of arterial stiffness in healthy subjects.
*Materials and methods:* We studied 116 subjects with normal kidney function (age 47 ± 12 years, 50 females and 66 males) of the FLEMENGHO study. Calcium measurements, plasma fetuin-A, its encoding gene (Thr256Ser) and indexes of arterial stiffness, such as aPWV and arterial distensibility, were determined.
*Results:* Fetuin-A levels were negatively correlated with aPWV ($r = -0.21$, $p = 0.029$). After an adjustment for multiple covariables, fetuin-A levels were independently associated with aPWV ($r = -0.30$, $p = 0.022$) in males but not in females. Male fetuin-A SerSer carrier had lower fetuin-A levels and higher aPWV (fetuin-A: 61.9 ± 29.0 μg/ml; aPWV: 14.3 ± 0.9 m/s) as compared to ThrThr (fetuin-A: 109.9 ± 54.9 μg/ml; aPWV: 6.4 ± 1.3 m/s) and ThrSer carrier (fetuin-A: 100.8 ± 52.5 μg/ml; aPWV: 6.6 ± 1.3 m/s). Other calcium variables were not significantly associated with arterial stiffness.
*Conclusion:* With respect to common calcium variables, only fetuin-A level showed an inverse relation with aPWV in men with normal renal function. Male fetuin-A SerSer carriers demonstrate particularly high aortic stiffness, possibly implying a status of increased cardiovascular risk.



## 1. Introduction

During a person's lifetime, as a consequence of atherosclerosis or other pathological processes, the aorta stiffens. The aortic pulse wave velocity (aPWV) reflects central arterial stiffness and represents a predictor of cardiovascular outcomes above and beyond traditional cardiovascular risk factors [1], such as oxidative stress, accumulation of glycosylation endproducts and inflammation [2]. Furthermore, abnormalities in calcium metabolism and loss of bone matrix proteins, such as fetuin-A, are important risk factors for arterial wall calcification and mortality [3,4].

Fetuin-A (α2-Heremans Schmid glycoprotein AHSG) acts as an inhibitor of spontaneous calcium-phosphate precipitation. Absence of fetuin in fetuin-A knockout mice results in massive extraosseous calcification [4]. Fetuin-A levels are reduced in individuals with renal failure and are associated with an increased risk of vascular calcified plaque, inflammation and all-cause and cardiovascular disease (CVD) mortality [5]. Fetuin-A accumulates in the mineralized bone matrix, in atherosclerotic plaques, and in pathologically mineralized tissues [6]. These findings suggest, that the levels of calcified artherosclerotic plaques may vary based on the fetuin-A concentration and the related calcium-phosphate-homeostasis, including several hormones, such as parathyroid hormone and 1,25$(OH)_2$ vitamin D.

Fetuin-A gene polymorphisms have been associated with the serum levels of the associated fetuin-A protein [7]. Dialysis patients carrying the fetuin-A single nucleotide polymorphism (SNP) on position Thr256Ser in the AHSG gene had reduced serum levels of fetuin-A protein and an increased risk of accelerated arterial calcification [8].

Whereas fetuin-A showed a negative correlation with the aortic PWV in children and adults on dialysis [9,10], little is known of the role of fetuin-A as an independent predictor for the development of arterial stiffness as a consequence of vascular calcification in healthy subjects. The objective of this study was to evaluate the association between parameters of the calcium homeostasis with a focus on

* Corresponding author. Department of Nephrology, Klinikum rechts der Isar, Ismaninger Str. 22, D-81675 München, Germany. Tel.: +49 89 4140 2231; fax: +49 89 4140 4878.
*E-mail address:* marphiro@hotmail.com (M. Roos).

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fetuin-A in correlation with aortic pulse wave velocity in women and men with normal renal function. In addition, we examined the relationship between the fetuin-A Thr256Ser polymorphism and aPWV in these subjects.

## 2. Materials and methods

### 2.1. Study population

We randomly selected 116 members of the FLEMish study of ENvironment, Genes and Health Outcomes (FLEMENGHO) [11], which involves a random sample of families living in a defined geographical area in northern Belgium. Eligible offspring were at least 10 years old. The Ethics Committee of the University of Leuven approved the study. All participants or their parents gave informed written consent. The participation rate averaged 64.3%. Normal renal function was defined as a creatinine clearance >60 ml/min.

### 2.2. Clinical measurements

For at least 3 h before being examined, the participants refrained from heavy exercise, smoking, alcohol or caffeine-containing beverages. Trained nurses measured blood pressure and anthropometric characteristics in the morning. They administered a questionnaire to collect information about each subject's medical history, smoking and drinking habits, and intake of medications. Each participant's office blood pressure was the average of five consecutive readings. Body mass index (BMI) was defined as weight in kilograms divided by the square of height in metres. We measured parathyroid-hormone (PTH), 25-OH vitamin D3, calcitonin, serum calcium on a venous blood sample and 24-h urine calcium by standard biochemical methods. Fetuin-A was measured in duplicates using an ELISA kit (BioVendor Laboratory Medicine, GmbH, Heidelberg, Germany), following the instructions of the manufacturer. Coefficient of intraassay variations of plasma fetuin-A levels was 7.1%.

Each participant was characterized with respect to the components of the metabolic syndrome (MetS). Diagnosis was based on a modified version of the definition of MetS by the National Cholesterol Education Program Adult Treatment Panel (NCEP ATP III). In the modification, the waist circumference is replaced with body mass index, as was done in the West of Scotland Coronary Prevention Study (WOSCOPS) [12] and Women's Health Study [13]. Data of menopause status of the female subjects, to evaluate a possible relation with fetuin-A levels or aPWV, were not available. We therefore decided to split the women in two groups: women $\leq$50 years and women $\geq$50 years, with women $\geq$50 years to be expected in menopause, as previously described [14].

### 2.3. Vascular measurements

By means of a pulsed ultrasound wall-tracking system (Wall Track System; Pie Medical, Maastricht, the Netherlands), arterial measurements were obtained at the common carotid artery 2 cm proximal of the carotid bulb, at the femoral artery 1 cm proximal of the bifurcation into the profound and superficial branches, and at the right brachial artery 2 cm proximal of the antecubital fossa [11]. The observers also determined carotid–femoral pulse wave velocity from the length of the carotid–femoral segment and the transit time of the pulse wave. The carotid–femoral segment was the difference of the distances between the site of the carotid ultrasound measurement and the suprasternal notch and between the suprasternal notch and the site of the femoral measurement. During the ultrasound examination, an automated oscillometric device (Dinamap 845; Critikon Inc., Tampa, Florida, USA) recorded blood pressure at the upper arm at 5-min intervals. As for the conventional auscultatory measurements, cuff size was adjusted to the circumference of the upper arm [15]. Standard cuffs had an inflatable bladder of 12 cm–24 cm. If the arm circumference exceeded 31 cm, larger cuffs with a bladder of 15 cm–35 cm were utilized. As described elsewhere [16], the observers applied applanation tonometry with a pencilshaped probe (Millar Instruments Inc., Houston, Texas, USA) and calibration to mean and diastolic pressure at the brachial artery to derive the local pulse pressure at the other arteries. We computed cross-sectional compliance (CC) and the distensibility coefficient (DC) from the diastolic cross-sectional area ($A$), the systolic increase in cross-sectional area ($\Delta A$) and the local pulse pressure ($\Delta P$) [17]: $CC = \Delta A / \Delta P$ and $DC = (\Delta A / A) / \Delta P$. $A$ and $\Delta A$ were calculated as $A = \pi \times (D/2)^2$ and $DA = \pi \times [(D + \Delta D)/2]^2 - \pi - (D/2)^2$.

### 2.4. Thr256Ser SNP-genotyping

For genotyping, the MassARRAY system (Sequenom, San Diego, USA) was used as previously described in detail [18]. From a 5 ml EDTA sample of peripheral blood DNA was extracted using QIAamp DNA kits (Qiagen, Valencia, CA, USA). Sequence amplification was performed by the polymerase chain reaction (PCR) on a real time PCR-cycler (Perkin Elmer PCR 9700, Applied Biosystems). The PCR reaction volume was 50 µl. The PCR-protocol and selection of fetuin-A Thr256Ser polymorphism was performed and detected respectively, as described by Stenvinkel et al. [8]. Briefly, the Thr256Ser polymorphism was amplified in a single fragment, using forward primer 5′-TCCTTTTTCCAGCCCGTGA-3′ and reverse primer 5′-TGCACCACTGTGCGTGTTTT-3′. Sequencing primers were placed adjacent to the SNP and were 5′-GGTTGGGGCTGTGAG-3′; for fetuin-A Thr256Ser. All nucleotides were synthesized by Apara-Bioscience GmbH — Synthetische Biomoleküle, Denzlingen, Germany). Genotyping calls were made in real time with MASSARRAY RT software (Sequenom, San Diego, USA).

### 2.5. Statistical analysis

For database management and statistical analysis SPSS software version 14.0 (SPSS Inc., Chicago, IL, USA) was used. Women and men

**Table 1**
Characteristics of demographic and clinical data of participants by gender.

| Variable name | Women ($n=50$) | Men ($n=66$) |
|---|---|---|
| *Demographic characteristics* | | |
| Age (years) | 47.9 ± 9.9 | 46.7 ± 12.9 |
| Body mass index (kg/m$^2$) | 25.0 ± 4.2* | 26.1 ± 2.9* |
| Systolic blood pressure (mm Hg) | 126.1 ± 17.3 | 129.7 ± 12.5 |
| Diastolic blood pressure (mm Hg) | 81.1 ± 11.0 | 83.7 ± 10.0 |
| Pulse rate (bpm) | 62.8 ± 7.2 | 61.9 ± 8.0 |
| *Numbers with characteristics (%)* | | |
| Smokers | 18 (36%) | 19 (29%) |
| Drinkers | 6 (12%)* | 26 (39%)* |
| Antihypertensive therapy | 2 (4%) | 5 (8%) |
| *Biochemical measurements* | | |
| Serum glucose (fasting) (mmol/l) | 4.7 ± 0.4* | 4.9 ± 0.5* |
| Serum calcium (mmol/l) | 2.3 ± 0.1 | 2.3 ± 0.1 |
| Serum creatinine (µmol/l) | 81.8 ± 16.4* | 100.5 ± 13.6* |
| Creatinine clearance (ml/min) | 77.5 ± 23.2* | 88.7 ± 21.9* |
| Serum albumin (g/l) | 45.0 ± 2.4* | 46.2 ± 2.8* |
| Plasma renin activity (ng/ml) | 0.84 ± 0.57 | 0.95 ± 0.73 |
| Parathyroid-hormone (pmol/l) | 1.1 ± 0.9 | 1.0 ± 0.8 |
| 25-OH-vitamin-D (ng/ml) | 72.7 ± 31.3 | 70.9 ± 25.2 |
| Plasma fetuin-A (µg/ml) | 113.4 ± 51.8 | 108.2 ± 60.4 |
| Urinary 24-h calcium (mmol/l) | 4.2 ± 2.4 | 4.2 ± 2.1 |
| Triglycerides (mg/dl) | 180.2 ± 91.2 | 247.6 ± 136.4* |
| Total cholesterol (mg/dl) | 218.8 ± 37.5 | 217.4 ± 38.1 |
| High-density cholesterol (mg/dl) | 57.2 ± 14.9* | 48.3 ± 11.2 |
| Low-density cholesterol (mg/dl) | 136.6 ± 33* | 132.9 ± 36 |
| Calcitonin (pg/ml) | 5.8 ± 2.6 | 6.4 ± 2.2 |

Unless indicated otherwise, values are means ± SD. [a]Average of five auscultatory readings at the brachial artery obtained at the examination centre. An asterisk (*), indicates a significant ($p<0.05$) sex difference.

were considered separately in the statistical analyses. Distribution of quantitative measurements and proportions was compared by means of the Kruskal–Wallis- or Mann–Whitney-*U* test and the $\chi^2$ test, respectively. Median and/or mean values were reported with standard deviation or percentiles. Spearman correlation coefficient was used to assess bivariate relationship between quantitative measurements. The statistical methods also included single and multiple linear regression. Covariates considered for entry into the model were age, body mass index, pulse rate, systolic blood pressure, current smoking and the use of antihypertensive drugs. In all statistical analyses a *p*-value less than 0.05 was considered statistically significant.

## 3. Results

### 3.1. Characteristics of participants

Demographic and clinical data of the subjects are presented in Table 1. The median age of the 116 participants, 66 men and 50 women, was 47.2 years (26 to 72 years). Median plasma fetuin-A levels were 113 ± 51.8 μg/ml in women and 108.2 ± 60.4 in men. Overall, 7 patients were taking antihypertensive drugs, (ACE-inhibitor $n=1$; β-blocker $n=4$; central acting hypertensive drug $n=2$; diuretics $n=2$). In smokers (18 women, 19 men), median tobacco use was 17 cigarettes per day (10th to 90th percentile interval, 5 to 29). In drinkers (6 women, 26 men), median alcohol consumption was 19 g per day (1 to 59 g). One woman used oral contraceptives.

### 3.2. Vessel wall properties in relation to calcium homeostasis

The vessel wall properties by gender and vascular territory are given in Table 2. Fetuin-A levels of the whole study population correlated significantly to aPWV ($r=-0.21$, $p=0.029$), whereas after adjustments for age, gender, systolic blood pressure, body mass index, current smoking and serum albumin, this relation lost statistical significance (partial $r=-0.16$, $p=0.094$).

With respect to gender distribution only men demonstrated an inverse relationship to aPWV before ($r=-0.28$, $p=0.025$) and after multivariable adjustment (partial $r=-0.3$, $p=0.022$), whereas in women no significant correlation was observed ($r=-0.1$, $p=0.475$), see Table 3. Neither women, nor men showed a relationship between arterial distensibility of the femoral, brachial and carotid artery and fetuin-A (Table 3). Arterial diameter and distensibility were significantly ($p<0.01$) associated with age and body mass index. Furthermore, aPWV was significantly ($p<0.01$) associated with age and body mass index. Apart from fetuin-A no association was found between parameters of the calcium homeostasis and aPWV (data not shown).

**Table 2**
Vessel wall properties by gender and vascular territory.

| Variable name | Women ($n=50$) | Men ($n=66$) |
|---|---|---|
| Carotid–femoral pulse wave velocity (m/s) | 6.61 ± 2.21 | 6.87 ± 2.05 |
| *Common carotid artery* | | |
| Pulse pressure (mm Hg) | 43.0 ± 7.8* | 46.2 ± 9.2* |
| Distensibility coefficient ($10^{-3}$/kPa) | 23.4 ± 8.0 | 22.7 ± 8.8 |
| Compliance coefficient ($mm^2$/kPa) | 0.83 ± 0.26* | 1.01 ± 0.37* |
| Intima–media thickness (mm) | 0.78 ± 0.13* | 0.84 ± 0.19* |
| *Brachial artery* | | |
| Pulse pressure (mm Hg) | 43.8 ± 6.8* | 48.5 ± 8.0* |
| Distensibility coefficient ($10^{-3}$/kPa) | 25.4 ± 11.2* | 21.3 ± 9.6* |
| Compliance coefficient ($mm^2$/kPa) | 0.25 ± 0.10* | 0.35 ± 0.13* |
| *Femoral artery* | | |
| Pulse pressure (mm Hg) | 48.0 ± 8.4* | 52.3 ± 10.4* |
| Distensibility coefficient ($10^{-3}$/kPa) | 9.1 ± 4.5* | 7.6 ± 3.4* |
| Cross-sectional compliance ($mm^2$/kPa) | 0.51 ± 0.25* | 0.62 ± 0.28* |

Unless indicated otherwise, values are means ± SD. An asterisk (*) indicates a significant ($p<0.05$) sex difference.

**Table 3**
Regression coefficient between vessel wall properties and plasma fetuin-A levels by gender.

| | Women ($n=50$) | | Men ($n=66$) | |
|---|---|---|---|---|
| Measurement | Coefficient ± SE | *p* | Coefficient ± SE | *p* |
| Carotid–femoral pulse wave velocity (m/s) | −0.033 ± 0.039 | 0.83 | −0.3 ± 0.039 | 0.022* |
| Common carotid artery Distensibility ($10^{-3}$/kPa) | 0.022 ± 0.018 | 0.22 | 0.018 ± 0.015 | 0.25 |
| Brachial artery Distensibility ($10^{-3}$/kPa) | 0.060 ± 0.032 | 0.069 | 0.004 ± 0.021 | 0.85 |
| Femoral artery Distensibility ($10^{-3}$/kPa) | 0.003 ± 0.011 | 0.83 | 0.005 ± 0.008 | 0.53 |

Apart from the aortic pulse wave velocity in men ($p=0.022$) before and after adjustments for age, gender, systolic blood pressure, body mass index, current state of smoking and serum albumin (all patients $p=0.029$ ($p=0.094$), women $p=0.475$ ($p=0.83$)), the arterial distensibility of the femoral, brachial and carotid artery was not affected by plasma fetuin-A levels.
Results were adjusted for age, gender, systolic blood pressure, body mass index, current smoking and serum albumin. An asterisk (*) indicates a significant ($p<0.05$) sex difference.

### 3.3. Genotype and allele frequencies

The Thr256Ser (C→G) SNP of the fetuin-A gene was investigated in 107 patients (8 patients went missing due to technical difficulties). The genotype distribution is given in Table 4. The SerSer genotype, when men and women were grouped together, ($n=7$) was associated with lower fetuin-A plasma levels ($p=0.007$) and a higher aPWV ($p=0.028$). In sex-specific analysis, women ($n=4$) with the SerSer genotype had lower fetuin-A levels (59.66 ± 22.38 μg/ml, $p=0.019$) and men ($n=3$) an increased aPWV (14.3 ± 0.9 m/s, $p=0.01$) compared to subjects with the homozygous wildtype (ThrThr), *n* (w/m) = 27/34, and the heterozygous (ThrSer) $n=(15/24)$ form.

### 3.4. Metabolic syndrome, fetuin-A levels and aPWV

According to the NCEP ATP III criteria, 28 of 116 patients were diagnosed with a prevalent metabolic syndrome (MetS) (10 females vs. 18 males). We did not find a significant difference in fetuin-A levels between MetS and non-MetS patients ($p=0.69$). In addition when males and females were analyzed separately, no significant difference in fetuin-A levels was obvious (men $p=0.60$, women $p=1.0$). On the other hand, there was a significant correlation between prevalent MetS and aPWV ($p=0.046$). In multivariable linear regression analysis after adjustment for sex, RR systole, serum albumin, current state of smoking, BMI and age, MetS was still independently associated with aPWV ($\beta=-0.227$; $p=0.016$). The test of an interaction between gender and MetS regarding aPWV was not significant ($p=0.082$). We could not detect a significant correlation

**Table 4**
Fetuin-A polymorphisms of the Ser256Thr gene.

| Polymorphism | PWV (m/s) | | Fetuin-A (μg/ml) | |
|---|---|---|---|---|
| Thr256Ser (w/m) | Women | Men | Women | Men |
| Homozygous Thr/Thr (27/34) | 6.08 ± 1.26 | 6.36 ± 1.31 | 130.9 ± 53.8 | 109.9 ± 54.9 |
| Heterozygous Ser/Thr (15/24) | 6.97 ± 1.47 | 6.66 ± 1.27 | 98.31 ± 42.6 | 100.9 ± 52.5 |
| Homozygous Ser/Ser (4/3) | 6.29 ± 0.81 | 14.26 ± 0.89* | 59.7 ± 22.4 | 61.9 ± 29.1 * |

Patients ($n=7$) carrying the SerSer genotype had significantly lower fetuin-A plasma levels ($p=0.07$) and a higher aPWV ($p=0.028$). Women with the SerSer genotype had lower plasma fetuin-A levels ($p=0.019$) and men with the SerSer genotype had higher aPWV ($p=0.01$) compared to ThrThr and ThrSer genotype carriers.
Values are means ± SD. (F/m) reflects the number of subjects and gender (f = female, m = male) for the gene status. An asterisk (*) indicates a significance difference ($p<0.05$) as compared to the Ser256Thr genotype within sex groups. Results were adjusted for age, gender, systolic blood pressure, body mass index, current smoking and serum albumin.

between the MetS and the SNPs, neither in the total study group ($p = 0.198$), nor in the sex-adjusted analyses (women $p = 0.293$, men $p = 0.446$).

### 3.5. Menopause, fetuin-A, aPWV and SNP

To investigate whether women ≥50 years (likely to be postmenopausal) show similar results as our male subjects, we compared the two groups for fetuin-A levels, aPWV, presence of MetS and SNP distribution. Overall, 18 female subjects were ≥50 years. Compared with males, fetuin-A levels ($p = 0.72$) and aPWV ($p = 0.88$) were not significantly different in postmenopausal females. Moreover we did not find a significant correlation between fetuin-A levels and aPWV in women ≥50 years ($r = -0.028$, $p = 0.91$).

## 4. Discussion

Our data from a group of 116 patients with normal kidney function suggest a role for fetuin-A as an independent risk factor for increased arterial stiffness in men with normal kidney function. Other investigated measurements of calcium homeostasis were not associated with any large artery function in our study group. Furthermore, homozygous male with the SerSer genotype for the fetuin-A gene (Thr256Ser) had significantly higher aPWV as compared with other fetuin-A genetic variabilities.

Thus, our results extend previous observations demonstrating that an inverse relationship exists between fetuin-A, aPWV and cardiovascular mortality in patients with end-stage renal disease [5,9,10].

The stiffness of the vascular wall depends on the relative contribution of its two predominant scaffolding proteins: collagen and elastin [19,20]. Possibly, fetuin-A modulates vascular collagen deposition by influencing profibrotic pathways. TGF-β is responsible for a wide range of biological processes, such as cell proliferation, differentiation and extracellular matrix (ECM) production [21]. It has been demonstrated that fetuin-A competitively blocks the binding of TGF-β to the extracellular domain of RII [22]. Moreover it was demonstrated that fetuin-A is able to inhibit collagen synthesis [23] and that fetuin-A knock-out mice show severe cardiac diastolic dysfunction and enhanced cardiac fibrosis [24]. Thus, as a consequence of low fetuin-A levels, TGF-β may continuously stimulate the synthesis of collagen (ECM), subsequently, resulting in enhanced arterial stiffness. On the other hand, increased fetuin-A was shown to attenuate apoptosis and dystrophic calcification in atherosclerosis [25], inhibit bone morphogenic protein (BMP) and downregulate vascular profibrotic activities [26].

Recently, Ix et al. demonstrated, that increased fetuin-A levels were positively associated with the presence of the MetS and an atherogenic lipid profile [27]. Further studies have demonstrated fetuin-A as an independent risk factor for type 2 diabetes mellitus [28], thus linking fetuin-A with metabolic disorders, known to influence endothelial function and arterial stiffness [29]. Convincingly, we found lower levels of fetuin-A to be associated with a higher aPWV in men. However, we did not see a significant relation between the MetS and fetuin-A levels. A possible explanation for this may be the lower study number compared with the study by Ix et al. On the other hand there was a significant correlation between the MetS and aPWV in our study, which is in line to previous publications [29]. Analysis of the Thr256Ser SNP did not reveal a significant relation between fetuin levels, aPWV, metabolic parameters and the MetS itself. Thus, we believe, that the negative correlation between fetuin-A and aPWV in males may be reflected by the antifibrotic effect of this protein.

Our study is in accordance with a previous study, in which low fetuin-A was an important predictor of death at 6 months in ST-elevation acute myocardial infarction (STEMI) in male patients [30]. By contrast, Mori et al. reported a positive association between fetuin-A levels with arterial stiffness independent of known atherogenic factors [31]. However, this study mainly comprised female patients and focused on the carotid artery measuring the stiffness parameter β, which may account for the discordant results [31].

In our study, no significant association between fetuin-A and aPWV was seen in females. Generally, pre-menopausal females are a lower risk for cardiovascular events [32]. As we have not evaluated the menopause status of our female patients, it is difficult to judge, whether women in the pre-menopausal state may be protected from arterial stiffness independent of circulating fetuin-A levels. Studies investigating possible effects between fetuin-A, sex hormones, or estrogen levels are lacking. In subgroup analyses we compared females ≥50 years (likely to be postmenopausal) with males. Interestingly, neither fetuin-A levels nor aPWV were significantly different among these two groups. Again there was no significant correlation between fetuin-A levels and aPWV in this female subgroup. Yet, the underlying cause for the lack of an association between fetuin-A and aPWV in postmenopausal women remains unsolved.

Our third finding, although only based on 7 patients is that carriers with the SerSer genotype had lower plasma fetuin-A levels in both genders, suggesting a connection between these factors as recently shown [8]. Moreover, the 3 male SerSer genotype carriers had a markedly increased aPWV, which represents a high cardiovascular risk. These findings are in line with Stenvinkel et al. who demonstrated that CKD patients with fetuin-A SerSer allele had a higher cardiovascular mortality [8]. As a higher aPWV predicts a composite of cardiovascular outcomes above and beyond traditional cardiovascular risk factors [33] it is tempting to believe, that men carrying the SerSer genotype are at risk for cardiovascular disease, either as HD patients [10] or as healthy subjects [34]. Certainly, also our study is limited by the small population of 116 subjects and the fact that we could only evaluate data of 7 SerSer carriers.

In conclusion, we demonstrate fetuin-A as an independent risk factor for increased arterial stiffness in men with normal kidney function. Moreover, male SerSer genotype carriers of the Thr256Ser polymorphism in the fetuin-A gene confirm the association between fetuin-A and arterial stiffness. Further studies in larger healthy cohorts are needed to confirm our findings, in particular the potential cardiovascular risk of SerSer genotype carriers.

## References

[1] Hansen TW, Staessen JA, Torp-Pedersen C, Rasmussen S, Thijs L, Ibsen H, Jeppesen J. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. Circulation Feb. 7 2006;113:664–70.
[2] London GM, Marchais SJ, Guerin AP, Metivier F, Adda H, Pannier B. Inflammation, arteriosclerosis, and cardiovascular therapy in hemodialysis patients. Kidney Int Suppl 2003:S88–93.
[3] Schinke T, Amendt C, Trindl A, Poschke O, Muller-Esterl W, Jahnen-Dechent W. The serum protein alpha2-HS glycoprotein/fetuin inhibits apatite formation in vitro and in mineralizing calvaria cells. A possible role in mineralization and calcium homeostasis. J Biol Chem 1996;271:20789–96.
[4] Schafer C, Heiss A, Schwarz A, Westenfeld R, Ketteler M, Floege J, Muller-Esterl W, Schinke T, Jahnen-Dechent W. The serum protein alpha 2-Heremans-Schmid glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. J Clin Invest Aug 2003;112(3):357–66.
[5] Ketteler M, Bongartz P, Westenfeld R, Wildberger JE, Mahnken AH, Bohm R, Metzger T, Wanner C, Jahnen-Dechent W, Floege J. Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. Lancet 2003;361:827–33.
[6] Keeley KW, Sitarz EE. Identification and quantitation of alpha 2-HS-glycoprotein in the mineralized matrix of calcified plaques of atherosclerotic human aorta. Atherosclerosis 1985;55:63–9.
[7] Lehtinen AB, Burdon KP, Lewis JP, Langefeld CD, Ziegler JT, Rich SS, Register TC, Carr JJ, Freedman BI, Bowden DW. Association of alpha2-Heremans-Schmid glycoprotein polymorphisms with subclinical atherosclerosis. J Clin Endocrinol Metab Jan 2007;92(1):345–52 [Electronic publication 2006 Oct 24].
[8] Stenvinkel P, Wang K, Qureshi AR, Axelsson J, Pecoits-Filho R, Gao P, Barany P, Lindholm B, Jogestrand T, Heimburger O, Holmes C, Schalling M, Nordfors L. Low fetuin-A levels are associated with cardiovascular death: impact of variations of gene encoding fetuin. Kidney Int 2005;67:2283–392.
[9] Hermans MM, Brandenburg V, Ketteler M, Kooman JP, van der Sande FM, Gladziwa U, Rensma PL, Bartelet K, Konings CJ, Hoeks AP, Floege J, Leunissen KM. Study on the relationship of serum fetuin-A concentration with aortic stiffness in patients on

dialysis. Nephrol Dial Transplant May 2006;21(5):1293–9 [Electronic publication 2006 Jan 5].
[10] Shroff RC, Shah V, Hiorns MP, Schoppet M, Hofbauer LC, Hawa G, Schurgers LJ, Singhal A, Merryweather I, Brogan P, Shanahan C, Deanfield J, Rees L. The circulating calcification inhibitors, fetuin-A and osteoprotegerin, but not Matrix Gla protein, are associated with vascular stiffness and calcification in children on dialysis. Nephrol Dial Transplant May 9 2008 [Electronic publication ahead of print].
[11] Balkestein EJ, Staessen JA, Wang JG, van Der Heijden-Spek JJ, Van Bortel LM, Barlassina C, Bianchi G, Brand E, Herrmann SM, Struijker-Boudier HA. Carotid and femoral artery stiffness in relation to three candidate genes in a white population. Hypertension Nov 2001;38(5):1190–7.
[12] Sattar N, Gaw A, Scherbakova O, Ford I, O'Reilly DS, Haffner SM, Isles C, Macfarlane PW, Packard CJ, Cobbe SM, Shepherd J. Metabolic syndrome with and without C-protein as a predictor of coronary heart disease and diabetes in the West of Scotland Coronary prevention study. Circulation 2003;108:414–9.
[13] Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow up of 14719 initially healthy American women. Circulation 2003;107:391–7.
[14] Gold EB, Bromberger J, Crawford S, Samuels S, Greendale GA, Harlow SD, Skurnick J. Factors associated with age at natural menopause in a multiethnic sample of midlife women. Am J Epidemiol May 1 2001;153(9):865–74.
[15] Kuznetsova T, Staessen JA, Kawecka-Jaszcz K, Babeanu S, Casiglia E, Filipovsky J, Nachev C, Nikitin Y, Peleska J, O'Brien E. Quality control of the blood pressure phenotype in the European Project on Genes in Hypertension. Blood Press Monit 2002;7:215–24.
[16] Van Bortel LM, Balkestein EJ, van der Heijden-Spek JJ, Vanmolkot FH, Staessen JA, Kragten JA, Vredeveld JW, Safar ME, Struijker Boudier HA, Hoeks AP. Non-invasive assessment of local arterial pulse pressure: comparison of applanation tonometry and echo-tracking. J Hypertens 2001;19:1037–44.
[17] Hoeks APG, Reneman RS. Biophysical principles of vascular diagnosis. J Clin Ultrasound 1995;23:71–9.
[18] Schedel M, Carr D, Klopp N, Woitsch B, Illig T, Stachel D, Schmid I, Fritzsch C, Weiland SK, Von Mutius E, Kabesch M. A signal transducer and activator of transcription 6 haplotype influences the regulation of serum IgE levels. J Allergy Clin Immunol 2004;114:1100–5.
[19] Laurent S, Boutouyrie P, Lacolley P. Structural and genetic bases of arterial stiffness. Hypertension Jun 2005;45(6):1050–5 [Electronic publication 2005 Apr 25, Review].
[20] Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: part I: aging arteries: a “set up” for vascular disease. Circulation Jan 7 2003;107(1):139–46 [Review, No abstract available].
[21] Ruiz-Ortega M, Rodríguez-Vita J, Sanchez-Lopez E, Carvajal G, Egido J. TGF-beta signaling in vascular fibrosis. Cardiovasc Res May 1 2007;74(2):196–206 [Electronic publication 2007 Feb 12, Review].
[22] Demetriou M, Binkert C, Sukhu B, Tenenbaum HC, Dennis JW. Fetuin/alpha2-HS glycoprotein is a transforming growth factor-beta type II receptor mimic and cytokine antagonist. J Biol Chem 1996;271:12755–61.
[23] Verma.Gandhu M, Peterson MR, Peterson TC. Effect of fetuin, a TGF-beta antagonist and pentoxifylline, a cytokine antagonist on hepatic stellate cell function and fibrotic parameters in fibrosis. Eur J Pharmacol Oct 31 2007;572(2–3):220–7.
[24] Merx MW, Schäfer C, Westenfeld R, Brandenburg V, Hidajat S, Weber C, Ketteler M, Jahnen-Dechent W. Myocardial stiffness, cardiac remodeling, and diastolic dysfunction in calcification-prone fetuin-A-deficient mice. J Am Soc Nephrol Nov 2005;16(11):3357–64 [Electronic publication 2005 Sep 21].
[25] Reynolds JL, Skepper JN, McNair R, Kasama T, Gupta K, Weissberg PL, Jahnen-Dechent W, Shanahan CM. Multifunctional roles for serum protein fetuin-A in inhibition of human vascular smooth muscle cell calcification. J Am Soc Nephrol 2005;16:2920–30.
[26] Coen G, Ballanti P, Balducci A, Grandi F, Manni M, Mantella D, Pierantozzi A, Ruggeri M, Sardella D, Sorbo G, Bonucci E. Renal osteodystrophy: alpha-Heremans Schmid glycoprotein/fetuin-A, matrix GLA protein serum levels, and bone histomorphometry. Am J Kidney Dis Jul 2006;48(1):106–13.
[27] Ix JH, Shlipak MG, Brandenburg VM, Ali S, Ketteler M, Whooley MA. Association between human fetuin-A and the metabolic syndrome: data from the Heart and Soul Study. Circulation Apr 11 2006;113(14):1760–7 [Electronic publication 2006 Mar 27].
[28] Stefan N, Fritsche A, Weikert C, Boeing H, Jost HG, Häring HU, Schulze MB. Plasma fetuin-A levels and the risk of type 2 diabetes. Diabetes 2008 Oct;57(10):2762–7 [Electronic publication 2008 Jul 15].
[29] Sipilä K, Koivistoinen T, Moilanen L, Nieminen T, Reunanen A, Jula A, Salomaa V, Kaaja R, Kööbi T, Kukkonen-Harjula K, Majahalme S, Kähönen M. Metabolic syndrome and arterial stiffness: the Health 2000 Survey. Metabolism Mar 2007;56(3):320–6.
[30] Lim P, Collet JP, Moutereau S, Guigui N, Mitchell-Heggs L, Loric S, Bernard M, Benhamed S, Montalescot G, Dubois Rande JL, Gue'ret P. Fetuin-A is an independent predictor of death after ST-elevation myocardial infarction. Clin Chem 2007;53:10,1835–40.
[31] Mori K, Emoto M, Araki T, Yokoyama H, Teramura M, Lee E, Motoyama K, Koyama H, Shoji T, Inaba M, Nishizawa Y. Association of serum fetuin-A with carotid arterial stiffness. Clin Endocrinol (Oxf) 2007;66:246–50.
[32] Rosano GM, Panina G. Oestrogens and the heart. Therapie May–Jun 1999;54(3):381–5.
[33] Willum-Hansen T, Staessen JA, Torp-Pedersen C, Rasmussen S, Thijs L, Ibsen H, Jeppesen J. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. Circulation Feb 7 2006;113(5):664–70.
[34] Cozzolino Mario, Biondi Maria Luisa, Galassi Andrea, Gallieni Maurizio, Melzi d'Eril Gian Vico, Brancaccio Diego. Gene polymorphism and serum alpha-2-Heremans-Schmid levels in Italian haemodialysis patients. Am J Nephrol 2007;27:639–42.