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Is Fetuin-A/α2-Heremans-Schmid Glycoprotein Associated with the Metabolic Syndrome in Patients with Chronic Kidney Disease?

Jonas Axelsson[a, b] Xin Wang[a, c] Markus Ketteler[d] Abdul Rashid Qureshi[a] Olof Heimbürger[a] Peter Bárány[a] Bengt Lindholm[a] Louise Nordfors[b] Peter Stenvinkel[a, b]

[a]Divisions of Renal Medicine and Baxter Novum, Department of Clinical Science, Intervention and Technology, and [b]Department of Molecular Medicine, Karolinska Institutet, Stockholm, Sweden; [c]Institute of Nephrology, First Hospital, Peking University, Beijing, PR China; [d]Klinikum Coburg GmbH, Coburg, Germany

Key Words
Dyslipidemia · Insulin resistance · Obesity · Chronic kidney disease

Abstract
Introduction: Components of the metabolic syndrome are highly prevalent in chronic kidney disease (CKD) patients – some of which paradoxically appear to predict an improved outcome in this population. We hypothesized that the circulating calcification inhibitor fetuin-A/AHSG, which is also a natural inhibitor of the tyrosine kinase insulin receptor, could be one factor explaining the association between increased fat mass and a survival advantage in CKD and thus conducted an explorational study to provide preliminary data to support further research into this hypothesis. ***Patients and Methods:*** In a cross-sectional study, we evaluated 198 CKD stage 5 patients (GFR 6.8 ± 0.2 ml/min; 62% males, mean age 52 ± 1 years) close to the start of renal replacement therapy. We studied circulating AHSG (ELISA) and two common functional *AHSG* gene polymorphisms (at amino acids Thr248Met (C-T) and Thr256Ser (C-G) using Pyrosequencing®) and related these to multiple components of the metabolic syndrome. ***Results:*** Median circulating AHSG was lower ($p < 0.01$) in type-2 (0.22 g/l) and type-1 (0.16 g/l) diabetics as compared to non-diabetic CKD-5 patients (0.24 g/l). AHSG correlated with both total and truncal fat mass in type-2 diabetics (rho 0.37 and 0.39; $p < 0.001$, respectively), but not in type-1 diabetics or non-diabetics. Both SNPs significantly influenced circulating levels of AHSG, and were also associated with significant differences in serum triglycerides and HDL cholesterol. Furthermore, there were significant differences in the prevalence of metabolic syndrome criteria between the *AHSG* Thr256Ser (C-G) genotype groups, with a more atherogenic lipid profile in AHSG high producers (Thr/Thr homozygotes). In multivariate analysis, the association between circulating AHSG and fat mass remained significant also after adjustment for age, gender, inflammation (CRP >10 mg/l), and *AHSG* genotype. ***Conclusions:*** The present, explorational, study supports further, mechanistic, studies into a physiological link between AHSG and body fat mass in patients with CKD. As we observed an association between higher fat mass and elevated AHSG levels, these preliminary results may form the basis of further study to establish if the observed associations may be one reason why obesity has been reported to constitute a survival advantage in CKD.

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Jonas Axelsson, MD, PhD
Department of Renal Medicine
K56 Karolinska University Hospital Huddinge
SE–141 86 Stockholm (Sweden)
Tel. +46 8 5858 39 81, Fax +46 8 5858 39 25, E-Mail jonas.axelsson@ki.se

Introduction

We [1, 2] have previously described the link between decreased circulating levels of α2-Heremans-Schmid glycoprotein/human fetuin-A (henceforth AHSG) and increased cardiovascular morbidity in patients with advanced chronic kidney disease (CKD). There is compelling evidence that AHSG is an important regulator of calcium and phosphate solubility and a disruptor of ectopic tissue calcification [3–5], a process that is impaired in patients with advanced CKD – who suffer from rapid vascular ossification, an important predictor of death [6]. However, in diabetic patients with mild and moderate CKD, Mehrotra et al. [7] recently reported that patients with a high serum AHSG had higher triglycerides and were at increased risk of coronary artery calcification.

Consequently, the pathophysiological role of AHSG in CKD may be more intricate than just modulation of the calcification process. Indeed, AHSG was first described as an acute phase reactant and early research focused mainly on the reported ability of AHSG to interfere with insulin receptor phosphorylation [8]. Thus, it is interesting that several recent clinical studies in non-renal patients [9–11] have shown significant associations between increased circulating AHSG with insulin resistance and other components of the metabolic syndrome.

Recently, Lavebratt et al. [12, 13] demonstrated that a common genetic variant of the *AHSG* gene that leads to lower AHSG protein levels is more common among lean than among obese and overweight men. Thus, it could be speculated that a genetic trait associated with obesity may be coupled with elevated production of AHSG. Indeed, components of the metabolic syndrome [14], including atherogenic dyslipidemia and insulin resistance, are also highly prevalent in CKD patients [15] – but conversely appear to predict an improved outcome in this population [16]. Thus, we hypothesized that AHSG may be one link between the conflicting traditional and non-traditional risk factors associated with cardiovascular disease (CVD) in CKD, offering novel insights into its complex pathophysiology. In an exploratory, cross-sectional study to generate a more detailed hypothesis, we evaluated both circulating AHSG, a common *AHSG* gene polymorphism previously shown to influence circulating plasma levels [2], and multiple components of the metabolic syndrome in a cohort of incident CKD stage 5 patients close to the start of renal replacement therapy. The present study was thus aimed to generate preliminary results that may form the basis of further study to establish a physiological link between AHSG levels and adipose tissue mass, as well as an explanation for the reported survival advantage of obesity in dialysis patients.

Patients and Methods

Patients

The study comprised 198 CKD stage 5 patients (GFR 6.8 ± 0.2 ml/min; 62% males, mean age 52 ± 1 years) analyzed post-hoc as participants of a prospective cohort study of incident patients starting dialysis in the Karolinska University Hospital at Huddinge. The original study started in 1994 and is still ongoing. The exclusion criteria were age <18 years or >70 years, clinical signs of acute infection, active vasculitis or liver disease at the time of evaluation, and unwillingness to participate in the study. The causes of CKD were chronic glomerulonephritis in 59 patients, diabetic nephropathy in 65 patients, polycystic kidney disease in 19 patients and other, or unknown, etiologies in 45 patients. Applying WHO diagnostic criteria [17], 33 (17%) patients were diagnosed as type-1 diabetics, while 46 patients (23%) were diagnosed as type-2 diabetics. In accordance with current therapy recommendations, all of the patients initially taking oral antiglycemic agents or on restricted diets had been switched to insulin therapy at the time of inclusion in the study. The majority of patients were on antihypertensive medications (angiotensin-converting enzyme inhibitors and/or angiotensin II receptor antagonists, n = 121; β-blockers, n = 137; calcium-channel blockers, n = 94) and other commonly used drugs in CKD, such as phosphate and potassium binders, diuretics, erythropoiesis-stimulating agents, iron substitution and vitamin B, C, and D supplementation. Only 36 patients were on lipid-lowering HMG-CoA-reductase inhibitors (statins). The Ethics Committee of the Karolinska Institute approved the study at the Karolinska University Hospital, Huddinge, and informed consent was obtained from each patient.

Metabolic Syndrome Characterization

Each participating patient was characterized with respect to the components of the metabolic syndrome as defined by the International Diabetes Federation (IDF) 2005 guidelines [14]. However, data on waist circumference were not available in the cohort. Thus, patients were given a 'metabolic syndrome score' of between 0 and 4 depending on the presence or absence of one or more of raised serum triglycerides (>1.7 mmol/l), reduced high-density lipoprotein (HDL) cholesterol (<1.03 mmol/l in males and <1.29 mmol/l in females, or treatment with statins), raised blood pressure (BP; systolic BP >130 or diastolic BP >85 mm Hg, or antihypertensive treatment) or raised fasting plasma glucose (>5.6 mmol/l, or previously diagnosed type-2 diabetes).

Measurement Methods

After an overnight fast, venous blood samples were drawn and stored at –70°C for biochemical analyses. Glomerular filtration rate (GFR) was estimated by the mean of creatinine and urea clearance, calculated from 24-hour urinary samples. Serum AHSG levels were measured by a sandwich immunoenzymometric assay using two polyclonal human AHSG-specific antibodies (Epitope Diagnostics, Inc., San Diego, Calif., USA). The lowest level of human AHSG in prediluted serum samples detected by

this assay is 0.025 g/l, and the assay linear measurement range of human AHSG in prediluted serum sample is up to 7 g/l. The intra-assay variation is less than 5.5% and inter-assay variation less than 6.8%, depending on the sample concentration. Plasma analysis for insulin was performed on an Immulite® system (DPC Corp., Los Angeles, Calif., USA) using commercially available assays (from DPC Corp). The levels of serum cholesterol and triacylglycerols were analyzed by standard enzymatic procedures (Roche Diagnostics GmbH). HDL cholesterol was determined after precipitation of apolipoprotein (Apo) B-containing lipoproteins by phosphotungsten acid. Plasma interleukin (IL)-6 levels were measured by a commercially available high-sensitivity photometric enzyme-linked immunosorbent assay (ELISA) (Linco Research, St Charles, Miss., USA). The plates were read using ELISA VERSAmax Reader™ (Molecular Devices Corp., Sunnyvale, Calif., USA) and the data were analyzed with the Softmax-PRO® software (Molecular Devices Corp., Sunnyvale, Calif., USA). The remaining biochemical analyses were done using routine methods at the Department of Clinical Chemistry at Karolinska University Hospital at Huddinge.

Clinical signs of CVD were obtained by the recruiting physician using patient's medical history. Body mass index was calculated as weight (in kg)/(height [in m])^2. In non-diabetic patients, insulin resistance was calculated by the homeostasis model assessment for insulin resistance (HOMA-IR: fasting serum insulin [μU/ml] · fasting plasma glucose [mmol/l]/22.5). Insulin resistance was not assessed in diabetic subjects. Lean body mass (LBM), total fat mass and truncal fat mass were estimated by dual-energy x-ray absorptiometry (DXA) using the DPX-L device (Lunar Corp., Madison, Wisc., USA). With this technique, fat and LBM distribution are directly estimated without making assumptions about the two-compartment model. DXA has proved superior to other simple non-invasive methods for determining body composition in renal failure, especially if repeated measurements are made [18]. However, it must be kept in mind that, although the state of hydration does not affect the estimate of fat mass with DXA, it does affect that of LBM.

Genotyping

As previously reported in this cohort [2], genotyping of *AHSG* was performed in the 170 patients were informed consent and DNA was available. From a 5-ml EDTA sample of peripheral blood, DNA was extracted using QIAamp® DNA kits (Qiagen, Valencia, Calif., USA). Samples were stored at –20°C. Sequence amplification was performed by the polymerase chain reaction (PCR) on a PTC-225 Thermocycler (MJ Research, Inc., Cambridge, Mass., USA). The PCR reaction volume was 50 μl, containing 20–50 ng of DNA, 10 pmol of each forward and reverse primer, 0.2 μl of each dNTP, 0.3 U of DyNAzymeTM II (DNA Polymerase, Finnzymes, Espoo, Finland), 10 mmol/l of Tris-HCl, 1.5 μl of $MgCl_2$, 50 μl of KCl, and 0.1% Triton X-100. PCR primers were designed using the software Primer Designer 4 for Windows, version 4.1 (Scientific and Educational Software, Cary, N.C., USA), and one primer in each primer pair was biotinylated. The exon 7 SNP at position Thr248Met (C-T) (Thr248Met) was amplified using forward primer 5′ biotin-GGCTTTTGTAAGGCAACACT-3′ and reverse primer 5′-CAAGAATATTCACGGAGCT-3′, while the exon 8 SNP at position Thr256Ser (C-G) (Thr256Ser) was amplified using forward primer 5′ biotin-TCCTTTTTCCAGCCCGTGA-3′ and reverse primer 5′-TGCACCACTGTGCGTGTTTT-3′. Sequencing primers were placed adjacent to the single nucleotide polymorphisms and were 5′-CCTGTGTTTGGAACACC-3′ for *AHSG* Thr248Met and 5′-GGTTGGGGCTGTGAG-3′ for *AHSG* Thr256Ser, respectively. All oligonucleotides were synthesized by Thermo Electron Corp. (Ulm, Germany). The pyrosequencing reaction was performed on a PSQ96™ Instrument from Biotage AB (Uppsala, Sweden). Both analyzed SNPs attained Hardy-Weinberg equilibrium.

Statistical Analysis

Results are expressed as mean and standard error of mean (normally distributed variables) or median and interquartile (25–75%) range (non-normal distribution) unless otherwise indicated, with $p < 0.05$ indicating significance. Normality of distribution for each variable was assessed using the Shapiro-Wilk test [19]. Comparisons of continuous variables between groups were then made using ANOVA (for normally distributed variables) or for non-normally distributed variables Wilcoxon (2 groups) or Kruskal-Wallis (>2 groups) tests. Comparisons between groups for nominal variables were made using the χ^2 test. As many values were not normally distributed, correlations between variables were calculated according to Spearman rank. The relative independence of observed correlations were tested in two linear regression models, incorporating all factors significantly associated with AHSG in univariate analysis, as well as sex. Based upon previous studies [2, 4, 12, 13, 20, 21] showing an effect of the Ser allele on AHSG transcription, we used a dominant model for analyzing genotype data. All analyses were performed using statistical software JMP IN® (SAS Inc., Cary, N.C., USA).

Results

Differences between Groups

Clinical characteristics of the patient material, grouped according to diabetic status, are given in table 1. As expected, truncal fat mass (13.1 ± 0.8 kg vs. 11.4 ± 0.5 kg and 10.1 ± 0.9 kg; $p < 0.01$) and serum IL-6 (median 8.0 [3.8–15.4] pg/ml vs. 5.5 [2.9–9.5] pg/ml and 6.1 [4.2–10.8] pg/ml; $p < 0.05$) were both higher in patients with type-2 diabetes mellitus when compared to non-diabetics and type-1 diabetics respectively. Median circulating AHSG was lower ($p < 0.01$) in type-2 (0.22 g/l) and type-1 (0.16 g/l) diabetics as compared to non-diabetic CKD stage 5 patients (0.24 g/l). There was no significant difference in circulating AHSG between patients with and without clinical CVD.

Univariate Correlations with AHSG

Spearman rank correlations for serum AHSG with clinical and biochemical parameters, divided according to diabetic status and the use of insulin therapy, are given in table 2. Notably, AHSG correlated with both total and truncal fat mass in type-2 diabetics receiving insulin

Table 1. Baseline characteristics of the 198 patients recruited for the study; grouped according to diabetic status

	Non-diabetics (n = 119)	Type-1 diabetes mellitus (n = 33)	Type-2 diabetes mellitus (n = 46)	Significance[a] p
Age, years	52 ± 1	45 ± 2	59 ± 2	<0.001
Male gender, %	59	58	70	n.s.
Cardiovascular disease, %	21	36	57	<0.001
Malnutrition (%, SGA >1)	29	47	42	n.s.
GFR, ml/min	6.8 ± 0.2	7.5 ± 0.4	6.7 ± 0.4	n.s.
AHSG Thr256Ser (% non-Ser)	49	33	42	n.s.
Metabolic syndrome score[b]	2.3 ± 0.1	2.8 ± 0.2	2.9 ± 0.1	<0.001
Body composition				
Body mass index, kg/m^2	24.1 ± 0.4	24.4 ± 0.7	26.4 ± 0.6	<0.001
Lean body mass, kg	48.4 ± 1.0	49.8 ± 1.8	50.5 ± 1.5	n.s.
Total fat mass, kg	21.2 ± 0.9	19.7 ± 1.7	23.8 ± 1.4	n.s.
Truncal fat mass, kg	11.4 ± 0.5	10.1 ± 0.9	13.1 ± 0.8	<0.01
Markers of glucose homeostasis and blood lipids				
HbA1c, %	4.9 ± 0.1	7.7 ± 0.2	6.0 ± 0.2	<0.001
HOMA-IR	4.0 ± 2.4	n/a	n/a	n/a
S-triglycerides, mmol/l	2.1 ± 0.1	2.5 ± 0.2	2.3 ± 0.2	n.s.
S-cholesterol, mmol/l	5.5 ± 0.1	6.0 ± 0.2	5.7 ± 0.2	n.s.
S-HDL cholesterol, mmol/l	1.2 (0.9–1.5)	1.3 (0.8–1.3)	1.0 (0.8–1.3)	<0.05
Inflammatory biomarkers				
S-AHSG, g/l	0.24 (0.18–0.38)	0.16 (0.13–0.39)	0.22 (0.12–0.29)	<0.01
S-albumin, g/l	34.5 ± 0.6	29.5 ± 1.0	30.9 ± 0.9	<0.001
S-interleukin-6, pg/ml	5.5 (2.9–9.5)	6.1 (4.2–10.8)	8.0 (3.8–15.4)	<0.05
S-hsC-reactive protein, mg/l	4.5 (1.8–15.0)	3.5 (1.7–12.5)	9.0 (2.9–25.0)	<0.01

n.s. = Not significant; n/a = not applicable.
[a] ANOVA, Kruskal-Wallis or χ^2 test. [b] Number of IDF 2005 diagnostic criteria fulfilled.

(rho = 0.37 and 0.39; $p < 0.001$, respectively), but not in type-1 diabetics.

Impact of Genotype

Circulating AHSG levels in the three *AHSG* genotypes are shown in figure 1. As AHSG Thr248Met (C-T) and Thr256Ser (C-G) were in total linkage disequilibrium (data not shown), only data from the Thr256Ser (C-G) SNP is shown. When compared to the other two possible alleles, the Ser/Ser genotype was associated with lower AHSG. Percent body fat mass, but not total fat mass or truncal fat mass, by DXA were significantly different between non-serine vs. serine allele carriers (fig. 1).

Independent Correlations

Table 3 shows the output from two linear regression models investigating independent predictors of (A) serum AHSG and (B) total fat mass in the whole study population. Briefly, the association between circulating AHSG and truncal fat mass remained also after adjustment for age, sex, serum albumin, inflammation, triglycerides and *AHSG* genotype.

Discussion

The present study shows that obesity in CKD-5 is associated with elevated levels of the tissue-ossification inhibitor protein human fetuin-A/AHSG. AHSG is a liver-derived protein with diverse biological functions that include the regulation of calcium homeostasis [1, 2, 5, 22]. Several studies have shown that low circulating AHSG is associated with increased ectopic tissue ossification and an increased mortality in patients with CKD [1, 2, 23, 24]. We hypothesize that an increased fat mass in CKD is somehow linked to elevated circulating AHSG, which was previously shown to interfere with insulin receptor phosphorylation [8]. Should this prove to be the case, it may partly explain the protective effect of obesity report-

Table 2. Spearman rank correlations and partial correlations showing associations between serum AHSG levels and selected variables in 198 CKD patients grouped according to diabetic status

	Spearman rho	p value
Non-diabetics (n = 119)		
Age, years	–0.23	<0.001
S-GFR, ml/min	0.27	<0.001
Metabolic syndrome score	0.14	n.s.
BMI, kg/m^2	0.21	<0.01
Total fat mass, kg	0.17	<0.05
Truncal fat mass, kg	0.17	<0.05
B-HbA1c, %	0.04	n.s.
S-Ca·PO_4, mmol/l	0.18	<0.05
S-triglycerides, mmol/l	0.15	n.s.
S-cholesterol, mmol/l	0.15	n.s.
S-HDL cholesterol, mmol/l	0.01	n.s.
S-hsC-reactive protein, mg/l	–0.15	<0.05
S-albumin, g/l	0.27	<0.001
Type-1 diabetics (n = 33)		
No significant correlations with any of the variables listed above		
Type-2 diabetics with insulin (n = 46)		
Age, years	0.01	n.s.
S-GFR, ml/min	0.01	n.s.
Metabolic syndrome score	–0.10	n.s.
BMI, kg/m^2	0.16	n.s.
Total fat mass, kg	0.37	<0.01
Truncal fat mass, kg	0.39	<0.01
B-HbA1c, %	0.01	n.s.
S-Ca·PO_4, mmol/l	0.23	<0.01
S-triglycerides, mmol/l	0.18	n.s.
S-cholesterol, mmol/l	0.18	n.s.
S-HDL-cholesterol, mmol/l	0.04	n.s.
S-hsC-reactive protein, mg/l	–0.13	n.s.
S-albumin, g/l	0.34	<0.01

n.s. = Not significant.

Table 3. The output from two linear regression models investigating predictors of serum levels of log-transformed AHSG (g/l; by ELISA) and total fat mass (kg; by DEXA) in 198 CKD patients. The models include all studied factors significantly associated with the dependent variable in univariate analysis, as well as age and gender

Factor	β estimate	SE	p value
Circulating AHSG[1]			
Age, years	–0.01	0.004	<0.05
S-triglycerides, mmol/l	0.07	0.04	0.06
S-albumin, g/l	0.03	0.001	<0.001
AHSG 256 (Thr/Thr vs. others)	–0.12	0.05	<0.01
Total fat mass[2]			
Age, years	0.08	0.03	<0.01
Sex (male vs. female)	–1.68	0.41	<0.001
S-AHSG, g/l[a]	1.22	0.67	<0.05

The initial model included age, sex, CRP < or ≥ 10 mg/dl, S-albumin, S-triglycerides, S-AHSG (for total fat mass) and AHSG 256.

[1] Whole model adjusted $r^2 = 0.17$.

[2] Whole model adjusted $r^2 = 0.10$.

[a] Non-normally distributed variables were log-transformed prior to analysis.

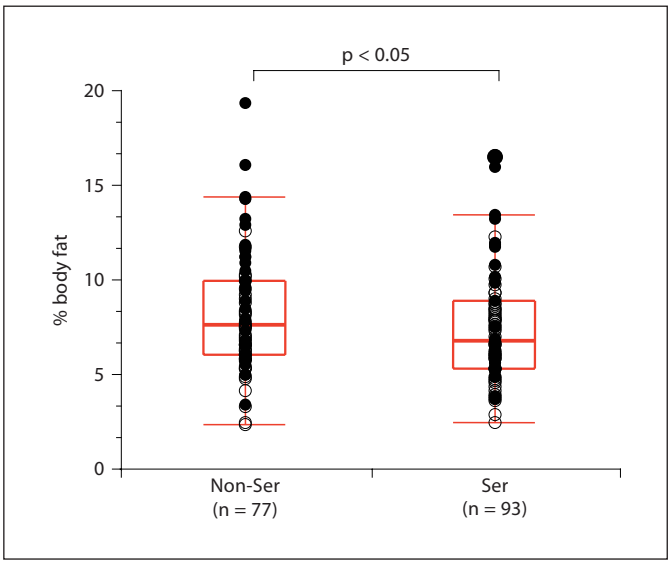

Fig. 1. The significant impact of a well-characterized and common single nucleotide polymorphism of *AHSG* on % body fat mass (by DXA) in 170 CKD patients with genotype. As the Ser allele has previously been associated with lower levels of S-AHSG [2, 4, 12, 13, 20, 21], we have used a dominant model grouping Ser and non-Ser alleles.

ed in this patient group [16, 25]. Supporting such a hypothesis, several recent clinical studies in non-renal patients have shown significant associations between increased circulating AHSG with insulin resistance and other components of the metabolic syndrome [9–11].

In a recent study of 711 non-diabetic outpatients with coronary artery disease participating in the Heart and Soul Study, it was found that a high circulating AHSG concentration was associated with the metabolic syndrome, even after adjustment for potential confounding

variables [9]. In the same study, higher AHSG was also associated with higher LDL cholesterol and triglyceride concentrations, and lower HDL cholesterol concentrations [9]. In the present study, circulating AHSG was linked to body fat content, itself an important predictor of dyslipidemia (table 2). Similarly to what we previously reported [2], we found associations between both elevated circulating AHSG and the *AHSG 256* Thr/Thr genotype, previously shown to give higher circulating AHSG [2], with body fat (fig. 1).

A possible role for AHSG in the metabolic syndrome was first suggested by in vitro studies demonstrating that AHSG inhibits, in a dose-dependent manner, the insulin-stimulated tyrosine kinase activity of the insulin receptor, insulin receptor autophosphorylation, and insulin substrate-1 phosphorylation [26]. These effects were corroborated in vivo in rat liver and skeletal muscle following acute injection of human recombinant AHSG [20] and in AHSG-null mice, which exhibit significantly enhanced insulin sensitivity and are resistant to weight gain on a high-fat diet [11]. In humans, serum AHSG levels have been reported to be associated with insulin resistance and fat accumulation in the liver [10], as well as with regulation of weight gain through modulation of adipocyte β_2-adrenoceptor function [12, 13]. Furthermore, Lavebratt et al. [12, 13] recently demonstrated that a common SNP in the *AHSG* gene associated with lower circulating AHSG is more common among lean than obese and overweight men. Moreover, a SNP in the promoter region of AHSG was recently associated with insulin-mediated inhibition of lipolysis and the stimulation of lipogenesis in adipocytes [27]. Together, these observations suggest that the weak associations observed in the present study may have a physiological basis. Clearly, further studies must now be performed to evaluate these and other pathways linking AHSG with adipose tissue biology in CKD.

In vivo, mechanistic studies suggest a negative regulation of AHSG synthesis in the liver by IL-6, IL-1β [28] and TNF-α [29]. Together with several well-studied functional *AHSG* polymorphisms [2, 4], this is thought to be the main reason for decreased circulating AHSG and the link to an increased mortality risk in CKD patients [2]. Indeed, in the present study, type-2 diabetics had lower AHSG than non-diabetics, despite exhibiting a higher fat mass, putatively due to an elevated systemic inflammatory status (table 1). Here, it should however be noted that in a recent report by Hermans et al. [30], evaluating dialysis patients with low levels of inflammation, AHSG was not a predictor of aortic stiffness. Indeed, we found a strong correlation between AHSG and multiple markers of inflammation (table 2), suggesting that AHSG is regulated by many of the same mechanisms as albumin. However, while albumin is known to be strongly associated with GFR, a recent study by Ix et al. [31] found no such association for AHSG investigated in 1,024 patients with CVD and mild to moderate renal dysfunction (GFR 45–90 ml/min/1.73 m^2). As we saw no differences in serum albumin concentrations between fat mass groups, it appears less likely that differences in inflammation or GFR can explain the significant differences in AHSG levels between the groups. Indeed the observed association between AHSG and fat mass was present even when inflammation was included in the model (table 3).

While suggestive, the results of the present study are not conclusive. However, perhaps the most intriguing implication of the present study is the antithetical role of AHSG in influencing outcomes both under normal conditions, where elevated circulating AHSG seems to engender metabolic derangements, and under extreme circumstances, such as CKD, where low circulating AHSG is hypothesize to contribute to increased ectopic tissue ossification. Indeed, it may be that AHSG is one explanation for why traditional cardiovascular risk factors, including obesity, actually predict an improved survival in CKD – the so-called 'reverse epidemiology' [25]. Thus, it may be that while indeed exposed to the cardiovascular consequences of a metabolic syndrome, obese CKD patients that reach ESRD may actually benefit from their obesity through a linked increase in circulating AHSG, which may act to reduce vascular calcification – likely a more important cause of mortality in the CKD population [32]. Clearly, after this hypothesis-generating study we and others will have to perform mechanistic studies to evaluate the physiological validity and relevance of the observed correlations.

The present study suffers from several weaknesses, which should be acknowledged. First of all, it is a cross-sectional study giving rather weak associations. Also, the large number of correlative tests run compared with the relatively large p value chosen for significance ($p < 0.05$) means that we cannot exclude that chance findings can have influenced our conclusions. Thus, a more focused and mechanistic study is necessary to corroborate our hypothesis and findings. Secondly, we did not measure waist circumference or visceral fat, and the measurement used for insulin resistance (HOMA-IR) was less accurate than an insulin clamp (the golden standard). Thirdly, the use of genetic association studies should preferably be done with haplotype blocks and verified in at least two

unrelated populations. None of these criteria were followed in the present study, precluding us from drawing any definite conclusions but bringing forth an intriguing hypothesis for further study. Finally, the circulating serum levels of AHSG are lower in this study compared to the serum levels previously reported [3–5]. Although differences in the study population may partly contribute to the observed difference, it is a fact that currently available AHSG assays are not standardized versus each other, and use different antibodies and standards. Supporting our findings, many previous reports have used proprietary ELISAs, Western blots, Rocket immunoassays and nephelometry, while we have used a commercial AHSG sandwich assay with two distinct antibodies specific for AHSG. While the difference in absolute AHSG levels remains unexplained, the relative intra-assay level range appears valid, and suggests that confounders such as matrix effects, impurities of the standards and of hidden epitopes among assays, do not compromise our data.

In conclusion, based upon the results of the present study, it could be hypothesized that increased circulating AHSG levels are linked to the metabolic syndrome and obesity in patients with advanced CKD. Further studies are needed to investigate whether this may be one reason why a high body mass index is associated with a better outcome in this patient group.

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Conflicts of Interest: B.L. is an employee of Baxter Healthcare Inc.

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