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Association between testosterone, estradiol and sex hormone binding globulin levels in men with type 1 diabetes with nephropathy

Christine Maric[a,b], **Carol Forsblom**[c,d], **Lena Thorn**[c,d], **Johan Wadén**[c,d], and **Per-Henrik Groop**[c,d] **on behalf of the FinnDiane Study Group**

[a] Department of Physiology and Biophysics, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS, 39216, USA [b] Women's Health Research Center, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS, 39216, USA [c] Folkhälsan Institute of Genetics, Folkhälsan Research Center, Biomedicum Helsinki, University of Helsinki, Helsinki, Finland [d] Division of Nephrology, Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland

Abstract

Male sex is a risk factor for development and progression of diabetic nephropathy; however, the relationship between sex hormone levels and diabetic nephropathy in type 1 diabetic men is unknown. This was a prospective follow-up study as part of the nationwide Finnish Diabetic Nephropathy (FinnDiane) Study; 297 patients were followed for 5.9±1.5 years. Serum total testosterone (Tt) and estradiol (Te), calculated free testosterone (cFt) and estradiol (cFe) and sex hormone binding globulin were measured at baseline and correlated with urinary albumin excretion rate, estimated glomerular filtration rate and markers of metabolic syndrome. Diabetes without renal disease was associated with decreased Tt ($p<0.001$), Te ($p<0.001$) and cFt ($p=0.001$) levels compared with healthy non-diabetic men. With progression of renal disease from micro- to macroalbuminuria, this decrease in serum Tt was even more pronounced. Cox regression showed that cFt and cFe were independent predictors of the progression from macroalbuminuria to end-stage renal disease. Our study shows that men with type 1 diabetes exhibit dysregulated sex hormone levels, which is most pronounced in men with progressive renal disease, suggesting that sex hormones may play a role in the pathogenesis of diabetic nephropathy associated with type 1 diabetes.

Keywords

type 1 diabetes; diabetic nephropathy; albuminuria; testosterone; SHBG; estradiol

Address for correspondence and requests for reprints to: Per-Henrik Groop, MD, Folkhälsan Institute of Genetics, Folkhälsan Research Center, Biomedicum Helsinki, University of Helsinki, Haartmaninkatu 8, P.O Box 63, FIN-00014 Helsinki, Finland. per-henrik.groop@helsinki.fi.

1. Introduction

The incidence and the rate of progression of non-diabetic renal disease are far greater in men compared to age-matched women [1,2]. In the setting of diabetes however, the female sex as a protective factor against the development of renal disease is diminished, such as that diabetes narrows the gap between the sexes in terms of development and progression of renal disease [1,2]. However, studies show that the male sex remains a risk factor, and that the rate of progression of diabetic renal disease is still greater in men compared with age-matched women. Specifically, in patients with type 1 diabetes male sex was shown to be a risk factor for the progression of albuminuria in adult men with initially normal renal function or mild renal insufficiency [3]. In patients with established diabetic nephropathy, male sex was associated with a more severe decline in glomerular filtration rate (GFR) over 5 years of patient follow-up [4]. Similarly, several studies have shown a higher incidence of diabetic nephropathy, as evidenced by micro- or macroalbuminuria, in type 2 diabetic adult males compared to age-matched females [5–7]. These observations strongly support the notion that the male sex is a risk factor for the development and progression of diabetic nephropathy, at least in adults.

Based on the fact that the male sex is a risk factor for the development and progression of diabetic nephropathy, one could presume that testosterone, being the predominant sex hormone in males, would be the underlying cause of this risk. Further supporting this concept is the fact that diabetic renal complications very rarely develop before puberty [8], while the onset of puberty, and thus the surge of androgens, greatly accelerates disease development [9,10]. Indeed, increased levels of testosterone have been observed during puberty in patients with type 1 diabetes developing microalbuminuria [11]. In addition, increased serum testosterone levels have been shown in young adult males (average age of subjects 23 years) with type 1 diabetes with proliferative retinopathy compared with diabetic males with minimal or no retinopathy [12]. These studies favor the hypothesis that high testosterone concentrations may be associated with the development of diabetic end-organ complications in younger type 1 diabetic men. In contrast, decreased testosterone levels have been observed in men with type 2 diabetes [13–15]. However, to date, no studies have examined the correlation between testosterone levels and diabetic nephropathy associated with type 1 diabetes in adult men.

Furthermore, emerging evidence suggests that increased estradiol levels are associated with complications, such as atherosclerosis in men with type 2 diabetes mellitus [14]. However, virtually nothing is known about the association between estradiol levels and diabetic nephropathy in men with type 1 diabetes. Thus, the aim of the present study was to examine the correlation between sex hormone levels and progression of diabetic nephropathy in adult men with type 1 diabetes.

2. Experimental

2.1 Data collection

This study is part of the ongoing prospective Finnish Diabetic Nephropathy Study (FinnDiane), which is a nationwide, comprehensive multicenter study, with the aim to identify genetic and environmental risk factors for diabetic complications, with special emphasis on diabetic nephropathy in patients with type 1 diabetes.

At baseline and at follow-up, all patients underwent a thorough clinical exam during a visit to the attending physician. For all patients in the present analysis, all available medical files, including laboratory data were reviewed and changes in renal status and new cardiovascular events were verified. Data on medication and diabetic complications were registered with a

standardized questionnaire which was completed by the patient's attending physician at regular patient visits based upon medical files. Blood pressure was measured twice in the sitting position after a 10 minute rest and the average of these measurements were used in the analysis. Height, weight and waist hip ratio (WHR) were recorded, and blood was drawn for the measurements of HbA_{1c}, lipids, creatinine and sex hormones. Estimated glucose disposal rate (eGDR) was calculated as earlier described as a measure of insulin sensitivity [16]. No patients were receiving testosterone replacement.

The ethics committees of all participating centers approved the study protocol. Written informed consent was obtained from each patient and the study was performed in accordance with the Declaration of Helsinki as revised in the year 2000. The protocol of patient recruitment has previously been published [17]. For this particular study, all data were collected between the years of 1998–2002.

2.2 Participants and definition of renal disease progression

A total of 297 men with type 1 diabetes were included in the present study and were followed for 5.9±1.5 years. Based on their urinary albumin excretion rate (AER) in three consecutive overnight or 24-h urine collections, at baseline, 101 men had normal AER (NORMO; AER<20 μg/min or <30 mg/24h), 96 men microalbuminuria (MICRO; 20≥AER<200 μg/min or 30≥AER<300 mg/24h) and 100 men macroalbuminuria (MACRO; AER≥200 μg/min or AER≥300mg/24h). Men with end-stage renal disease (ESRD), defined as men on dialysis or having received a kidney transplant, were excluded from the study.

Progression of renal disease was defined as follows: all urinary AER data between baseline and the follow-up visit (5.9±1.5 years) were reviewed and based on the AER in any two-out of three consecutive urine collections during the follow-up period, the patient's renal status was classified as in the baseline examination (Note: In patients without kidney disease (i.e. normal AER) measurements were performed at least once yearly. If patients had micro- or macroalbuminuria the measurements were more frequent, sometimes up to 4 times per year). Progression was defined as a change from one level to a higher level of albuminuria or the development of ESRD (Note: We used the second "positive" collection out of any three consecutive urine collections during the follow-up period as the time point for progression). Men without progression of renal disease were classified as non-progressors. Type 1 diabetes was defined as the onset of diabetes before the age of 35 years and permanent insulin treatment initiated within one year of diagnosis. The study also included a group of non-diabetic men (n=96) as a control for comparing changes in sex hormone levels between healthy, non-diabetic subjects and men with type 1 diabetes. The healthy controls were enrolled from the staff of the research center and their spouses. They underwent the same physical examination as well as the same blood and urine sampling as the diabetic patients. None of them had a family history of diabetes or kidney disease or reported any other serious medical conditions. The group of non-diabetic men were reproductively normal males without significant comorbidities.

2.3 Laboratory measures

HbA_{1c} was determined by standardized assays at each participating center. Serum lipid and lipoprotein concentrations were measured at the research laboratory of Helsinki University Central Hospital, Division of Cardiology, Helsinki, Finland, by automated enzymatic methods using the Cobas Mira analyzer (Hoffmann-LaRoche, Basel, Switzerland). Serum creatinine was measured by enzymatic methods at a central laboratory. Urinary AER was determined in one 24-h or overnight urine collection at a central laboratory by immunoturbidimetry. The glomerular filtration rate (eGFR) was calculated using the Cockroft-Gault formula.

Serum testosterone, estradiol and SHBG levels were measured in blood samples collected at baseline using commercially available enzyme immunoassay kits (Alpco Diagnostics, Salem, NH) according to the manufacturers' protocol. Duplicate measures for each hormone were performed for each sample. The sensitivity and intra- and inter-assay coefficients of variation, respectively, were as follows: testosterone, 0.02 ng/ml, 8.0 and 8.3%; estradiol, 10 pg/ml, 7.7 and 8.7%; SHBG, 0.1 nmol/L, 5.3 and 9.6%. Serum cFt and cFe levels were calculated from Tt, Te and SHBG and serum albumin based on mass action laws with Vermeulen's formula [18,19].

2.4 Data analysis

All data shown are baseline data and are expressed as means ± SD for normally distributed values and as medians with interquartile range for non-normally distributed values. Differences between groups for normally distributed variables were tested using ANOVA and variables that were not normally distributed with the Kruskal-Wallis test. Categorical variables were analyzed with a χ^2-test. Baseline risk factors to the progression of diabetic nephropathy were assessed using Cox regression analysis. All calculations were performed with SPSS 15.0.1 (SPSS Inc., Chicago, IL). Values $P<0.05$ were considered as statistically significant.

3. Results

The clinical characteristics at baseline of the men enrolled in the study are given in Table 1. The study included 96 non-diabetic, healthy men, 101 men with type 1 diabetes with normal uAER, 96 with microalbuminuria and 100 with macroalbuminuria.

Uncomplicated type 1 diabetes was associated with an overall reduction in serum Tt, cFt, Te and cFe, but not in SHBG levels compared with non-diabetic men (Table 2). When further corrected for age, BMI, WHR, eGDR and SHBG, serum Tt (p=0.003) and cfT (p=0.002) remained significantly reduced in men with type 1 diabetes in comparison with healthy men, while Te (0.088) and cFe (0.094) levels were only of borderline significance (data not shown). Interestingly, in men with established diabetic nephropathy (macroalbuminuria), all these variables were higher compared to men with either normal uAER or microalbuminuria; however, the values were still reduced in these men with macroalbuminuria compared with non-diabetic men.

When the data was analyzed according to disease progression (i.e. progressors vs non-progressors) by univariate analysis, we found that in men with incident (i.e. new onset) microalbuminuria (n=50), baseline Tt, cFt, Te or cFe levels were no different from those in men that did not develop microalbuminuria (n=51); however, SHBG levels were decreased with new onset microalbuminuria (Table 3). This decrease in SHBG levels was associated with WHR, serum triglyceride concentrations and HbA_{1c} and a trend in eGDR (Table 3).

In those men that progressed from microalbuminuria to macroalbuminuria, SHBG levels were reduced even further (Table 4). Tt was also recued and there was a trend towards increased cFe levels compared with men that remained microalbuminuric (Table 4). These changes were associated with a significant increase in serum triglyceride concentrations and HbA_{1c}, a decrease in eGDR and a trend towards an increase in WHR and systolic blood pressure as well as lower HDL-cholesterol (Table 4).

Interestingly, in men that progressed from macroalbuminuria to ESRD, a trend towards an increase in Tt and cFt, as opposed to a decrease that was observed in men that progressed from microalbuminuria to macroalbuminuria, was observed (Table 5). Te and cFe levels were twice as high in men that progressed from macroalbuminuria to ESRD compared with

non-progressors. No differences in SHBG levels were observed in these men. eGFR in he progressors was 39.8±25.3 compared with 68.5±21.7 in non-progressors (P=0.001). In these men, there was a significant correlation between eGFR and cFe (R=−0.332; P=0.001) and cFt (R=−0.258; P=0.005), but there was no correlation between eGFR and SHBG.

In Cox regression analyses, including the duration of diabetes, WHR, HbA_{1c}, triglycerides, eGDR, cFt, cFe and SHBG, only the increases in WHR (P=0.033) and HbA_{1c} (P=0.008) were predictors of incident microalbuminuria (Table 6). None of the sex steroids or the SHBG predicted new onset microalbuminuria. We replaced the duration of diabetes with systolic blood pressure in the Cox regression analyses for progression from microalbuminuria to macroalbuminuria and found that increased serum triglyceride concentrations (P=0.004) and cFt (P=0.036) were significant predictors from microalbuminuria to macroalbuminuria. Importantly, while cFe was not a predictor of progression from microalbuminuria to macroalbuminuria, it was an independent predictor of the progression from macroalbuminuria to ESRD (P<0.001), together with the decline in eGFR (P<0.001) and increase in cFt (P=0.038). This model included serum triglycerides, cFt, cFe, SHBG and eGFR.

4. Discussion

The present study highlights three major observations: 1. Type 1 diabetes is men is associated with an overall reduction in sex hormone levels; 2. A decrease in serum testosterone is predictive of the progression from microalbuminuria to macroalbuminuria alongside increased serum triglycerides, a marker of insulin resistance; 3. Increases in serum estradiol and testosterone are independent predictors of the progression from macroalbuminuria to ESRD. These data implicate sex hormones in the development and progression of diabetic nephropathy associated with type 1 diabetes.

While several studies have reported reduced Tt and/or cFt levels in men with type 2 diabetes [13–15], to our knowledge, this is the first report on differences in sex hormone levels in type 1 diabetic compared with normal, healthy adult men. A recent report [20] showed no differences in Tt and cFT levels in type 1 diabeteic men, while there was a reduction in Tt and cFt in patients with type 2 diabetes. However, this study did not include an age- and weight-matched non-diabetic control group and only compared testosterone levels to the reported physiological reference range for healthy men. The reduction in testosterone levels, at least in type 2 diabetic men has largely been ascribed to hypogonadism [14] associated with reduced gonadotropin secretion [21], reduced levels of both LH and FSH [21,22], cytokine-mediated inhibition of testosterone production [23] and reduced SHBG levels [24]. While similar mechanisms are likely to contribute to the reduced testosterone levels in men with type 1 diabetes as well, further studies are necessary to determine the precise mechanisms underlying the reduced testosterone levels and type 1 diabetes.

In addition to reduced Tt and cFt levels, our data show that men with type 1 diabetes also exhibit reduced Te and cFe levels compared with non-diabetic men. One of the possible mechanisms for this observation on estradiol levels may be reduced levels of testosterone and thus lack of substrate for aromatization to estradiol [25]. In addition, reduced aromatase activity may also contribute to reduced estradiol synthesis [25]. Indeed, a mutation in the aromatase gene, resulting in aromatase deficiency, was first identified in a man with type 2 diabetes [26]; however, it is unknown whether changes in aromatization contributes to reduction in estradiol levels in type 1 diabetics. Interestingly, no differences in SHBG levels compared with normal, healthy controls were observed in our study. Although SHBG levels have been reported to be decreased during puberty in boys, as well as in young men with type 1 diabetes [27], SHBG levels have shown to be increased in adult type 1 diabetic men

[28]. Although portal insulin levels have been shown to regulate SHBG levels [29], the study by van Dam and colleagues showing increased SHBG levels in type 1 diabetic men concluded that estimated portal insulin levels did not influence SHBG [28]. Future studies are needed to determine whether no changes in SHBG found in our patient population is related to portal insulin levels.

While our study does not show that incipient microalbuminuria is associated with any changes in sex hormone levels, increased testosterone levels have been observed in patients with type 1 diabetes developing microalbuminuria during puberty [11], but this effect is more pronounced in women than men. This study suggested that increased testosterone levels during puberty could be related to reduced insulin-like growth factor levels, poor glycemic control and also growth hormone levels [11]. Lower growth hormone levels in the adult population may potentially explain the absence of an association between incipient microalbuminuria and testosterone levels observed in our study.

Our data show that the progression from microalbuminuria to macroalbuminuria is associated with a further reduction in testosterone levels. This reduction appears to be associated with components of the metabolic syndrome and insulin resistance. These observations are consistent with the previous reports that low testosterone is associated with insulin resistance [30,31] and is predictive of development of metabolic syndrome and type 2 diabetes in middle-aged men [13,32]. In addition to reduced testosterone levels, there was a concomitant decrease in SHBG levels. Reduced levels of SHBG have been shown to predict the metabolic syndrome and type 2 diabetes in middle-aged men [13,24,32]. Indeed, SHBG production is known to be regulated by insulin concentration or insulin resistance (higher insulin levels or insulin resistance are associated with lower levels of SHBG) [29,33].

The present study shows that men that progressed from macroalbuminuria to ESRD exhibit an 86% increase in Te, 119% increase in Fe and 31% in cFt compared with the men that did not progress. In contrast, Grossmann *et al* showed a reduction in cFT levels in men with type 1 diabetes [30]. The discrepancy in these findings may partly be explained by the fact that their study included a mix of men with and without chronic kidney disease and the results were not compared to a non-diabetic control group, but rather to a published reference range of testosterone in healthy men. In our study, the Cox model showed that cFe was an independent predictor of the progression from macroalbuminuria to ESRD together with the decline in renal function and increase in cFt. Part of the reason for the relative increase in sex hormone levels in the progresses (note: the overall hormone levels in these men were still decreased compared with non-diabetic men) can possibly be explained by their reduced clearance due to the decrease in GFR. However, given the fact that cFe and cFt were both predictors of the disease progression independently of eGFR suggests that the increase in cFe and cFt observed with the progression from macroalbuminuria to ESRD may be unrelated to the decline in eGFR.

The increased production of sex hormones may be a result of either increased hormone biosynthesis and/or decreased degradation. It is conceivable that increased levels of cFe observed with progression from macroalbuminuria to ESRD may occur due to increased testosterone aromatization at tissue and peripheral levels. Similarly, the increased levels of cFt may potentially also be explained by increased testosterone production in response to progressive target organ injury. However, further studies are needed to test these hypotheses.

The strength of our study is that it included a larger number of men with type 1 diabetes than previous studies. Furthermore, the men included in the study were part of the nationwide, multicenter FinnDiane study, as opposed to studies in which patients were recruited from a

single center. In addition, our study included an age and weight-matched non-diabetic control group. While previous studies have reported limitations in comparing sex hormone levels in blood drawn at varying times of the day, in our study, the samples were collected in the early morning, according to a standardized protocol. Moreover, sex hormones were measured in all samples at the same time. The study is limited by the fact that we did not include measures of testosterone secretion, such as levels of luteinizing and follicle stimulating hormones, so we can not explain the mechanisms of reduced hormone levels. In addition, we did not examine the relationship between insulin and sex hormone levels in our patients. One of the caveats of the study is that sex hormones were measured by commercial ELISA without extrication by chromatography [34]. While extraction followed by mass spectrometry is emerging as the gold standard for measurement of sex hormones, the cost associated with this methodology make it virtually impossible to apply in studies that include a large number of samples.

In conclusion, our study shows that men with type 1 diabetes exhibit dysregulated sex hormone levels. This dysregulation is most pronounced in men with progressive renal disease, suggesting that sex hormones may play a role in the pathogenesis of diabetic kidney disease.

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TABLE 1

Baseline clinical characteristics of male patients with type 1 diabetes and healthy controls

	NORMO	MICRO	MACRO	CONTROL	P-value Within DM	P-value DM vs CONTROL
N	101	96	100	96		
Age (yrs)	35.5 ± 10.3	36.6 ± 9.8	41.2 ± 9.0	34.3 ± 10.2	<0.001	0.386
Duration of diabetes (yrs)	21.3 ± 9.1	25.2 ± 9.3	27.7 ± 7.0	NA	<0.001	NA
BMI (kg/m^2)	25.3 ± 3.3	25.4 ± 3.4	26.0 ± 4.3	24.5 ± 2.9	0.275	0.087
WHR	0.90 ± 0.07	0.92 ± 0.06	0.94 ± 0.07	0.92 ± 0.07	<0.001	0.033
Systolic BP (mmHg)	129 ± 13	136 ± 15	145 ± 20	131 ± 11	<0.001	0.263
Diastolic BP (mmHg)	80 ± 10	82 ± 10	85 ± 9	78 ± 9	0.002	0.168
AHT (%)	14.0	69.8	93.9	3.1	<0.001	0.007
HbA_{1c} (%)	8.7 ± 1.5	9.0 ± 1.7	9.1 ± 1.7	5.5 ± 0.3	0.223	<0.001
Triglycerides (mmol/l)	1.00 (0.33 – 7.04)	1.22 (0.57 – 8.82)	1.71 (0.55 – 8.38)	0.98 (0.31 – 11.75)	<0.001	0.250
Total cholesterol (mmol/l)	4.80 ± 1.03	4.96 ± 0.91	5.44 ± 1.06	4.70 ± 0.93	<0.001	0.478
HDL-cholesterol (mmol/l)	1.23 ± 0.35	1.22 ± 0.34	1.04 ± 0.33	1.39 ± 0.25	<0.001	0.001
eGFR ($ml/min/1.73m^2$)	102.7 ± 19.6	98.0 ± 22.9	56.2 ± 27.2	109.1 ± 19.0	<0.001	0.022
uAER (mg/24h)*	12 (2 – 76)	79 (6 – 483)	861 (10 – 5088)	7 (2 – 25)	<0.001	<0.001
eGDR (mg/kg/min)	6.41 ± 2.29	4.09 ± 1.63	3.54 ± 1.45	8.38 ± 1.84	<0.001	<0.001

Data are presented as means ± SD, medians with ranges or percentages where appropriate.

Abbreviations: BMI, body mass index; WHR, waist-hip ratio; AHT, antihypertensive therapy; eGFR, estimated glomerular filtration rate; uAER, urinary albumin excretion rate; eGDR, estimated glucose disposal rate;

*NOTE: The urinary AER presented is the last measurement of each patient. Because the classification was based on two out of three consecutive samples, a single value may be higher. A low value may be due to the effect of treatment.

TABLE 2

Baseline sex hormone levels in male patients with type 1 diabetes and healthy controls

	NORMO	MICRO	MACRO	CONTROL	P-value Within DM	P-value DM vs CONTROL
Tt (ng/ml)	5.26 (1.68 – 14.19)	4.86 (0.91 – 11.42)	6.40 (2.02 – 32.92)	7.20 (2.60 – 23.56)	<0.001	<0.001
cFt (ng/ml)	0.050 (0.018 – 0.190)	0.047 (0.016 – 0.229)	0.057 (0.023 – 0.749)	0.063 (0.021 – 0.465)	0.003	0.001
Te (pg/ml)	47.0 (9.3 – 1416)	51.5 (11.3 – 2096)	63.2 (25.2 – 1228)	69.5 (25.1 – 741)	<0.001	<0.001
cFe (pg/ml)	0.429 (0.122 – 15.600)	0.446 (0.115 – 19.650)	0.489 (0.201 – 7.935)	0.553 (0.139 – 4.895)	0.043	0.088
SHBG (nmol/l)	108.2 ± 49.2	102.0 ± 50.5	106.9 ± 44.7	119.3 ± 49.9	0.638	0.116

Data are presented as means ± SD, medians with ranges or percentages where appropriate.

Abbreviations: Tt, total testosterone; cFt, calculated free testosterone; Te, total estradiol; cFe, calculated free estradiol.

TABLE 3

Baseline clinical characteristics of male patients with type 1 diabetes and normal AER with and without progression to microalbuminuria

	Non-progressors	Progressors	P-value
N	51	50	
Age (yrs)	35.6 ± 10.5	35.4 ± 10.2	0.915
Duration of diabetes (yrs)	23.4 ± 7.0	19.1 ± 10.4	0.015
BMI (kg/m^2)	25.0 ± 3.2	25.5 ± 3.5	0.518
WHR	0.88 ± 0.08	0.91 ± 0.06	0.022
Systolic BP (mmHg)	130 ± 12	129 ± 14	0.517
Diastolic BP (mmHg)	81 ± 9	78 ± 10	0.257
AHT (%)	13.7	14.3	0.936
HbA_{1c} (%)	8.1 ± 1.0	9.4 ± 1.6	<0.001
Triglycerides (mmol/l)	0.91 (0.33 – 5.47)	1.15 (0.55 – 7.04)	0.004
Total cholesterol (mmol/l)	4.65 ± 0.86	4.97 ± 1.16	0.118
HDL-cholesterol (mmol/l)	1.22 ± 0.33	1.24 ± 0.37	0.839
eGFR (ml/min/1.73m^2)	99.9 ± 18.7	105.4 ± 20.2	0.158
uAER (mg/24h)	7 (3 – 32)	19 (2 – 76)	<0.001
eGDR (mg/kg/min)	6.83 ± 2.64	5.99 ± 1.82	0.069
Tt (ng/ml)	5.26 (1.98 – 14.19)	5.39 (1.68 – 14.11)	0.537
cFt (ng/ml)	0.047 (0.020 – 0.190)	0.053 (0.018 – 0.162)	0.166
Te (pg/ml)	47.1 (18.6 – 1416)	47.0 (9.3 – 1028)	0.817
cFe (pg/ml)	0.384 (0.122 – 15.600)	0.589 (0.123 – 8.485)	0.141
SHBG (nmol/l)	118.9 ± 48.3	97.2 ± 48.1	0.025

Data are presented as means ± SD, medians with range or percentages when appropriate.

TABLE 4

Baseline clinical characteristics of male patients with type 1 diabetes and microalbuminuria with and without progression to macroalbuminuria

	Non-progressors	Progressors	P-value
N	59	37	
Age (yrs)	37.0 ± 9.5	36.0 ± 10.4	0.629
Duration of diabetes (yrs)	24.5 ± 8.4	26.4 ± 10.5	0.350
BMI (kg/m^2)	25.3 ± 3.3	25.5 ± 3.6	0.763
WHR	0.91 ± 0.06	0.93 ± 0.06	0.070
Systolic BP (mmHg)	133 ± 15	139 ± 14	0.063
Diastolic BP (mmHg)	81 ± 10	84 ± 10	0.135
AHT (%)	71.2	67.6	0.707
HbA_{1c} (%)	8.6 ± 1.4	9.7 ± 2.0	0.003
Triglycerides (mmol/l)	1.05 (0.57 – 4.10)	1.72 (0.60 – 8.82)	<0.001
Total cholesterol (mmol/l)	4.73 ± 0.79	5.34 ± 0.96	0.001
HDL-cholesterol (mmol/l)	1.27 ± 0.34	1.14 ± 0.32	0.083
eGFR (ml/min/1.73m^2)	96.7 ± 18.4	100.0 ± 28.8	0.496
uAER (mg/24h)	46 (6 – 184)	152 (59 – 483)	<0.001
eGDR (mg/kg/min)	4.41 ± 1.51	3.55 ± 1.69	0.012
Tt (ng/ml)	5.30 (0.91 – 11.42)	4.26 (1.45 – 9.23)	0.043
cFt (ng/ml)	0.047 (0.020 – 0.229)	0.049 (0.016 – 0.175)	0.625
Te (pg/ml)	52.1 (11.3 – 2096)	48.3 (21.2 – 943)	0.530
cFe (pg/ml)	0.344 (0.115 – 19.650)	0.504 (0.193 – 10.010)	0.062
SHBG (nmol/l)	112.9 ± 52.2	84.6 ± 43.0	0.007

Data are presented as means ± SD, medians with range or percentages when appropriate.

TABLE 5

Baseline clinical characteristics of male patients with type 1 diabetes and macroalbuminuria with and without progression to end-stage renal disease

	Non-progressors	Progressors	P-value
N	57	43	
Age (yrs)	41.5 ± 9.2	40.8 ± 8.9	0.706
Duration of diabetes (yrs)	27.7 ± 6.6	27.6 ± 7.7	0.960
BMI (kg/m^2)	26.0 ± 3.3	26.1 ± 5.3	0.853
WHR	0.93 ± 0.07	0.94 ± 0.08	0.601
Systolic BP (mmHg)	143 ± 19	148 ± 21	0.260
Diastolic BP (mmHg)	83 ± 9	86 ± 9	0.166
AHT (%)	94.7	92.9	0.698
HbA_{1c} (%)	8.9 ± 1.6	9.4 ± 2.0	0.219
Triglycerides (mmol/l)	1.64 (0.55 – 4.51)	1.93 (0.76 – 8.38)	0.011
Total cholesterol (mmol/l)	5.32 ± 0.85	5.60 ± 1.28	0.187
HDL-cholesterol (mmol/l)	1.07 ± 0.31	0.99 ± 0.35	0.192
eGFR ($ml/min/1.73m^2$)	68.5 ± 21.7	39.8 ± 25.3	<0.001
uAER (mg/24h)	619 (10 – 4777)	1561 (70 – 5088)	<0.001
eGDR (mg/kg/min)	3.70 ± 1.51	3.32 ± 1.36	0.209
Tt (ng/ml)	6.19 (2.02 – 17.05)	7.77 (2.82 – 32.92)	0.171
cFt (ng/ml)	0.051 (0.023 – 0.211)	0.067 (0.026 – 0.749)	0.066
Te (pg/ml)	52.1 (25.2 – 1228)	96.9 (33.4 – 594)	<0.001
cFe (pg/ml)	0.417 (0.201 – 7.935)	0.913 (0.252 – 5.130)	<0.001
SHBG (nmol/l)	110.3 ± 43.4	102.3 ± 46.5	0.377

Data are presented as means ± SD, medians with range or percentages when appropriate.

TABLE 6

Cox regression models for progression in renal status: A) from normal AER to microalbuminuria, B) from microalbuminuria to macroalbuminuria, and C) from macroalbuminuria to end-stage renal disease (ESRD).

Model A		
Variable	**Hazard ratio (95%CI)**	**P-value**
Duration of diabetes (yrs)	0.97 (0.93 – 1.02)	0.213
WHR	557.96 (1.65 – 188561.26)	0.033
HbA_{1c} (%)	1.37 (1.09 – 1.72)	0.008
ln(Triglycerides)	0.95 (0.61 – 1.71)	0.982
eGDR (mg/kg/min)	1.15 (0.92 – 1.44)	0.214
ln(cFt)	0.89 (0.41 – 1.96)	0.776
ln(cFe)	1.01 (0.68 – 1.49)	0.945
SHBG (nmol/l)	1.00 (0.99 – 1.01)	0.666

Model B		
Variable	**Hazard ratio (95%CI)**	**P-value**
Systolic blood pressure (mmHg)	1.02 (1.00 – 1.05)	0.096
WHR	0.04 (0.01 – 726.67)	0.529
HbA_{1c} (%)	1.17 (0.91 – 1.51)	0.230
ln(Triglycerides)	3.44 (1.48 – 8.04)	0.004
eGDR (mg/kg/min)	0.93 (0.65 – 1.33)	0.690
ln(cFt)	0.43 (0.19 – 0.95)	0.036
ln(cFe)	1.18 (0.77 – 1.81)	0.445
SHBG (nmol/l)	1.00 (0.99 – 1.01)	0.822

Model C		
Variable	**Hazard ratio (95%CI)**	**P-value**
ln(cFe)	3.03 (1.67 – 5.50)	<0.001
eGFR (ml/min/1.73m^2)	0.95 (0.93 – 0.97)	<0.001
ln(cFt)	0.46 (0.22 – 0.96)	0.038
ln(Triglycerides)	1.68 (0.93 – 3.02)	0.085
SHBG (nmol/l)	1.01 (1.00 – 1.01)	0.221