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Androgens and Development of Posttransplantation Diabetes Mellitus in Male Kidney Transplant Recipients: A Post Hoc Analysis of a Prospective Study

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OBJECTIVE

Posttransplantation diabetes mellitus (PTDM) effects up to 30% of all kidney transplant recipients (KTR). Recent studies in mice found that sufficient androgen levels are necessary for β-cell health and adequate insulin secretion. This raises the question whether a similar relationship might be present in KTR. Hence, we hypothesized that dihydrotestosterone and testosterone are associated with the development of PTDM in male KTR.

RESEARCH DESIGN AND METHODS

We conducted a post hoc analyses of a prospective single-center cohort study including adult male KTR with a functioning graft ≥1 year posttransplantation. Androgen levels were assessed by liquid chromatography–tandem mass spectrometry. Development of PTDM was defined according to the American Diabetes Association's criteria.

RESULTS

We included 243 male KTR (aged 51 ± 14 years), with a median dihydrotestosterone 0.9 (0.7–1.3) nmol/L and testosterone of 12.1 (9.4–15.8) nmol/L. During 5.3 (3.7–5.8) years of follow-up, 28 KTR (11.5%) developed PTDM. A clear association was observed, as 15 (19%), 10 (12%), and 3 (4%) male KTR developed PTDM in the respective tertiles of dihydrotestosterone ($P = 0.008$). In Cox regression analyses, both dihydrotestosterone and testosterone as continuous variables were inversely associated with the risk to development PTDM, independent of glucose and HbA_{1c} (hazard ratio [HR] 0.31 [95%CI 0.16–0.59], $P < 0.001$; and HR 0.32 [95%CI 0.15–0.68], $P = 0.003$, respectively).

CONCLUSIONS

Our results suggest that low androgen levels are a novel potential modifiable risk factor for the development of PTDM in male KTR.

Kidney transplantation is a well-established treatment for patients suffering from end-stage kidney disease (1). Yet, non optimal long-term survival rates after kidney transplantation still warrant a necessity for improvement (2). Posttransplantation

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diabetes mellitus (PTDM) is associated with mortality in kidney transplant recipients (KTR) and has therefore been deemed an important contributor to adverse outcomes after kidney transplantation (3,4). In addition, PTDM has also been linked with other important comorbidities, including infections and diabetic complications (5,6). In order to halt the development of PTDM, numerous studies have aimed to identify modifiable risk factors (7). Subsequently, transplantation-specific risk factors, such as the use of immunosuppressive drugs, and traditional risk factors including BMI were discovered as important determinants (7,8). Yet, despite all efforts, the current incidence level of PTDM still ranges from 10 to 30% 3 years posttransplantation, necessitating more and novel research to prevent the development of PTDM in KTR (3,9).

Failure of pancreatic β-cells to produce and secrete enough insulin is an important pathophysiological component in the development of PTDM (10). A recent animal study, complemented with studies in human cell cultures, uncovered that in males, dihydrotestosterone (DHT) acts on the androgen receptor in pancreatic β-cells and thereby enhances insulin secretion (11). In line with this, previous observational studies observed that testosterone deficiency plays a role in the development of type 2 diabetes in aging males and in men receiving androgen-deprivation therapy (12,13). These findings could be of particular importance for KTR, as a recent study observed that a substantial number of male KTR suffer from testosterone deficiency after kidney transplantation (14). We therefore aimed to investigate whether androgens are linked to the development of PTDM in male KTR. To address this hypothesis, we investigated whether levels of DHT and testosterone, the two most potent androgens, are associated with the development of PTDM in male KTR.

RESEARCH DESIGN AND METHODS

Study Design and Population

For this post hoc analysis of a prospective single-center cohort study, all KTR who were ≥18 years of age, visited the outpatient clinic of the University Medical Center Groningen between 2008 and 2011, and had a functioning graft ≥1 year were approached for participation. Written informed consent was acquired from 401 male KTR. Out of the KTR who gave informed consent, 60 KTR had a missing androgen profile on baseline and were therefore excluded from analyses. Furthermore, the 14 KTR who suffered from diabetic nephropathy due to type 1 diabetes as primary renal disease were excluded from analyses. Moreover, seven KTR whose primary renal disease was diabetic nephropathy due to type 2 diabetes were also excluded. An additional 72 KTR were not deemed eligible for analyses due to a high fasting plasma glucose (FPG), a high HbA_{1c} level, and/or use of glucose-lowering drugs on baseline. Moreover, two recipients were excluded due to treatment with either androgen deprivation of supplementation therapy. Lastly, three recipients were excluded due to having DHT levels that were evident outliers in the association of DHT with total testosterone. No KTR were lost to follow-up, resulting in 243 KTR eligible for analyses. A STROBE flow diagram detailing the study population size and number of male KTR eligible for analyses can be found in Supplementary Fig. 1. In addition to the study protocol, which has previously been described, all KTR received care according to the Kidney Disease: Improving Global Outcomes Clinical Practice Guideline for the Care of Kidney Transplant Recipients. As recommended in this guideline, this included a serum creatinine and estimated glomerular filtration rate (eGFR) every 3 months and annual glucose and/or HbA_{1c} measurements (15,16).

The current study protocol was approved by the institutional review board (METc 2008/186) and is part of the TransplantLines Food and Nutrition Biobank and Cohort Study, which is registered at ClinicalTrials.gov under number NCT02811835. The current study adheres to the principles of the Declaration of Helsinki and Declaration of Istanbul.

Baseline Data Collection and Measurements

All clinical data were collected during a morning visit to the outpatient clinic of the University Medical Center Groningen. Blood pressure was measured using a semiautomatic device (Dinamap 1846; Critikon, Tampa, FL). Waist circumference was measured on bare skin halfway between the 10th rib and the iliac crest. BMI was defined as weight divided by height squared. A positive cardiovascular history was defined as a clinically diagnosed myocardial infarction, stroke, and/or peripheral arterial disease. Transplantation vintage was defined as the time between transplantation and baseline (years).

Venous blood samples were collected between ∼08:00 and 10:00 h in fasting state after an 8–12-h fast. The collection of 24-h urine was done according to a strict protocol, which was performed as follows: at the start of collection, all patients were instructed to start by discarding a urine void and to subsequently collect all urine for the next 24 h, including a void at exactly 24 h after the collection start. All samples were stored in −80°C (−112°F) freezers (Panasonic, 's-Hertogenbosch, the Netherlands). FPG was measured using a Roche P Analyzer, and HbA_{1c} was measured by turbidimetric inhibition immunoassay (Roche Diagnostics, Basel, Switzerland). Serum creatinine was measured using an enzymatic isotope dilution assay, traceable on mass spectrometry, on a Roche P-Modulator automated analyzer (Roche Diagnostics). The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (17).

Laboratory Measurements

All DHT and testosterone measurements were collected at baseline and performed using stored EDTA plasma samples with no freeze-thaw cycles. The median time between collection and measurement of DHT and total testosterone was 7.7 (7.3–8.5) years. DHT and total testosterone were measured using liquid chromatography–tandem mass spectrometry (LC/MS-MS) (TQ-S Xevo, Waters, Milford, MA; and Kinetex C18 column, Phenomenex, Torrance, CA) (18). To allow for the measurement of total testosterone levels, a pepsin solution (Labor Diagnostika Nord, Nordhorn, Germany) was added. Intra-assay and interassay coefficients of variation of DHT were ≤5.3% and 9.6%, respectively, whereas the intra-assay and interassay coefficients of variation for total testosterone measurement were ≤3.1% and ≤2.9%, respectively (18).

Immunosuppressive Regimens

As previously described, immunosuppressive medication was regulated according

to standardized protocols. Changes made to the standard regimes were due to side effects or due to treatment of allograft rejection (19).

The cumulative prednisolone dose was calculated by multiplying the prescribed dose of prednisolone on baseline by the time since transplantation and adding the dose of methylprednisolone and/or prednisolone needed for treatment of acute allograft rejection. A conversion factor of 1.25 was used to convert methylprednisolone doses to prednisolone doses.

Outcome Measures

The development of PTDM was defined in accordance with the American Diabetes Association's diagnostic criteria. This entails: *1*) an FPG ≥7.0 mmol/L (126 mg/dL), *2*) a plasma glucose level of ≥11.1 mmol/L (≥200 mg/dL) during an oral glucose tolerance test (OGTT), *3*) HbA_{1c} ≥48 mmol/mol (6.5%), or *4*) a random glucose plasma level of ≥11.1 mmol/L (≥200 mg/dL) combined with classic symptoms of hyperglycemia (20). Furthermore, we deemed the requirement to start glucose-lowering medication an important hallmark of disease progression and therefore added the start of this medication as a criterion. To confirm the diagnosis of PTDM, KTR had to meet at least one of the criteria above. Follow-up was recorded until 30 September 2015. KTRs were censored for PTDM at time of death or graft failure. For the purpose of this study, graft failure was defined as return to dialyses or when a retransplantation was performed.

Statistical Analysis

Normally distributed variables are presented as mean ± SD, and skewed distributed variables are presented as median (interquartile range). Categorical variables are presented as a number (percentage). To test for differences across tertiles, one-way ANOVA tests were used for normally distributed variables, Kruskal-Wallis tests when variables were skewed, and χ^2 tests for categorical variables. All reported *P* values are two-tailed, and *P* values of ≤0.05 were considered to be statistically significant.

Kaplan-Meier curves combined with the log-rank test were first used to determine the effect of DHT and testosterone on the development of PTDM. Cox regression analyses were used to assess the prospective association of DHT and testosterone with PTDM. The proportional hazards assumption was checked using the Schoenfeld residuals. Multiple models were composed to adjust for various potential confounders. First, a crude analysis was performed (model 1). To prevent the inclusion of too many variables for the number of events, further models were composed additive to model 1. The first multivariable model, model 2, was adjusted for age. In model 3, adjustments for renal function and transplantation vintage were performed, and model 4 was adjusted for BMI and levels of hs-CRP. To take medication use into account, model 5 was adjusted for cumulative prednisolone dose and tacrolimus use. Lastly, adjustments for FPG levels and HbA_{1c} were performed in model 6. In continuous Cox proportional hazards regression models, DHT and testosterone were log-base 2 transformed to allow for expression of the hazard ratios (HRs) per doubling of DHT and testosterone, respec-tively. In addition, both DHT and testosterone were used as categorical variables for analyses by tertiles.

We also performed sensitivity analyses to investigate effect modification of age, eGFR, and BMI on the association of DHT and testosterone with the development of PTDM by using the product of the potential interaction and DHT or testosterone and added this product to the statistical model. Data were presented as HR with 95% CI. Statistical analyses were performed using SPSS version 23.0 (IBM Inc., Chicago, IL) and STATA version 14.2 (StataCorp LP, College Station, TX). Visual depiction of the associations was done using Prism 8.4.2 (GraphPad, San Diego, CA).

RESULTS

Baseline Characteristics

Baseline characteristics according to tertiles of DHT are presented in Table 1. KTR in the lowest tertile had a higher BMI and a lower eGFR compared with male KTR in the other tertiles. Men in the highest tertile had the highest transplantation vintage and the highest cumulative prednisolone dose. There were no differences in age or ethnicity across the tertiles of DHT. Additional characteristics on KTR who developed PTDM versus nondevelopers can be found in Supplementary Table 4.

Development of PTDM

The censored median follow-up time was 5.3 (4.5–6.0) years, whereas the overall median follow-up was 5.3 (3.7–5.8) years, in which time 28 male KTR (11.5%) developed PTDM. The number of men who developed PTDM gradually decreased over the tertiles of DHT, as 15 (19%), 10 (12%), and 3 (4%) men, in the respective first, second, and third tertile of DHT developed PTDM ($P = 0.008$ for log-rank test) (Fig. 1). A similar trend was observed for the number of PTDM diagnoses across the tertiles of testosterone, with 14 (17%), 11 (14%), and 3 (4%) men developing PTDM in the respective first, second, and third tertile of testosterone ($P = 0.01$) (Fig. 1).

Primary Analysis: DHT and the Development of PTDM

The proportional hazards assumption using the Schoenfeld residuals was met ($P = 0.54$). The prospective analyses of DHT as log-transformed continuous variable and tertiles of DHT with the development of PTDM are shown in Table 2. In a crude Cox regression analysis, DHT was significantly associated with the development of PTDM (HR 0.27 per doubling of DHT [95% CI 0.14–0.51]; $P < 0.001$, model 1). In multivariable analyses, the association of DHT with PTDM was independent of age (HR 0.27 [95% CI 0.14–0.51]; $P < 0.001$, model 2). When additively adjusted for relevant covariates, including eGFR and transplantation vintage in model 3, BMI and levels of hs-CRP in model 4, and medication use in model 5, the association was not materially changed (HR 0.27 [95% CI 0.15–0.52]; $P < 0.001$, model 5). Lastly, when adjusted for baseline FPG levels and baseline HbA_{1c}, DHT remained significantly associated with PTDM (HR 0.31 [95% CI 0.16–0.59]; $P < 0.001$, model 6).

In additional Cox regression analyses for tertiles of DHT, male KTR in the lowest tertile of DHT had an ∼4.7-fold higher risk to develop PTDM compared with male KTR in the highest tertile, independent of baseline FPG and HbA_{1c} levels (HR 4.74 [95% CI 1.36–16.53]; $P = 0.02$) (Table 2, model 6, and Supplementary Fig. 2).

Table 1—Baseline characteristics of 243 male kidney transplant recipients (KTR) according to tertiles of dihydrotestosterone (DHT)

	Tertiles of DHT			
	Tertile 1	Tertile 2	Tertile 3	*P* value
Men (*n*)	81	81	81	
DHT, nmol/L	0.6 (0.5–0.7)	0.9 (0.8–1.0)	1.4 (1.3–1.7)	
Testosterone, nmol/L	8.9 (7.8–10.5)	11.6 (10.2–14.7)	16.3 (14.2–21.0)	<0.001
Demographics				
Age, years	49.4 ± 14.3	51.2 ± 12.6	51.7 ± 13.9	0.52
Caucasian, *n* (%)	79 (97.5)	81 (100.0)	81 (100.0)	0.13
Clinical characteristics				
Weight, kg	85.2 ± 16.0	84.8 ± 12.2	79.4 ± 13.1	0.01
BMI, kg/m^2	26.6 ± 4.1	25.8 ± 3.2	24.8 ± 3.6	0.009
Waist circumference, cm	100.7 ± 11.6	100.1 ± 10.8	95.5 ± 13.3	0.15
Creatinine excretion, mmol/24 h	13.3 ± 3.4	13.9 ± 3.1	13.5 ± 3.0	0.46
Cardiovascular history, *n* (%)	8 (9.9)	7 (8.6)	7 (8.6)	0.95
SBP, mmHg	135.3 ± 15.2	137.4 ± 15.8	135.8 ± 16.3	0.67
Hypertension, *n* (%)	79 (97.5)	77 (95.1)	75 (92.6)	0.35
Smoking status, *n* (%)				0.54
Current smoker	9 (11.8)	16 (20.5)	10 (13.0)	
Former smoker	37 (48.7)	31 (39.7)	34 (44.2)	
Never smoker	30 (39.5)	31 (39.7)	33 (42.9)	
Renal function				
eGFR, mL/min/1.73 m^2	49.1 ± 19.4	51.5 ± 19.3	57.5 ± 19.7	0.02
Proteinuria, *n* (%)	23 (28.4)	13 (16.0)	19 (23.5)	0.17
Laboratory parameters				
Glucose, mmol/L	5.4 ± 0.6	5.3 ± 0.6	5.1 ± 0.6	0.07
HbA_{1c}, %	5.7 (5.4–6.0)	5.6 (5.5–6.0)	5.6 (5.4–5.8)	0.35
HbA_{1c}, mmol/mol	39.0 (36.0–42.0)	38.0 (37.0–42.0)	38.0 (36.0–40.0)	0.35
hs-CRP, mg/L	1.5 (0.5–6.1)	1.3 (0.5–2.2)	1.0 (0.5–2.7)	0.25
Triglycerides, mmol/L	1.9 (1.5–2.5)	1.6 (1.2–2.2)	1.4 (1.0–1.9)	<0.001
Total cholesterol, mmol/L	4.9 ± 1.1	4.9 ± 1.1	5.2 ± 1.1	0.18
HDL cholesterol, mmol/L	1.2 ± 0.3	1.2 ± 0.3	1.4 ± 0.4	<0.001
LDL cholesterol, mmol/L	2.9 ± 0.9	3.0 ± 0.9	3.1 ± 1.0	0.35
Transplant characteristics				
Transplantation vintage, years	4.6 (1.2–14.3)	4.4 (1.3–9.1)	8.1 (3.2–14.7)	0.01
Steroid-treated acute rejection, *n* (%)	18 (22.8)	19 (23.2)	20 (24.2)	0.97
Living donor, *n* (%)	33 (40.7)	33 (40.7)	25 (30.9)	0.33
Medication				
Calcineurin inhibitor, *n* (%)	51 (63.0)	53 (65.4)	39 (51.9)	0.05
Tacrolimus, *n* (%)	14 (17.3)	17 (21.0)	15 (18.5)	0.83
Proliferation inhibitor, *n* (%)	63 (77.8)	68 (84.0)	69 (85.2)	0.42
Cumulative prednisolone dose, g	16.9 (5.8–41.8)	13.8 (4.7–32.0)	22.6 (12.5–44.2)	0.007

Data are mean ± SD, median (interquartile range), or *n* (%). Differences were tested by ANOVA for normally distributed variables, Kruskal-Wallis test for skewed variables, and χ^2 test for categorical variables. Cardiovascular history was defined as a history of cerebrovascular accident, myocardial infarction, and/or peripheral arterial disease. Transplantation vintage was defined as the time between transplantation and baseline. Hypertension was defined as an SBP ≥140 mmHg and/or DBP ≥90 mmHg and/or the use of antihypertensive medication; proteinuria was defined as total protein excretion ≥0.5 g/24 h. DBP, diastolic blood pressure; SBP, systolic blood pressure eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive Protein.

Secondary Analysis: Testosterone and the Development of PTDM

The proportional hazards assumption using the Schoenfeld residuals was met ($P = 0.32$). The prospective analyses of testosterone and tertiles of total testosterone with the development of PTDM are shown in Table 3. In crude Cox regression analysis, total testosterone as log-transformed continuous variable was significantly associated with the development of PTDM (HR 0.26 per doubling of total testosterone [95% CI 0.12–0.54]; $P < 0.001$, model 1). In multivariable analyses, the association of total testosterone with PTDM was independent of age (HR 0.25 [95% CI 0.12–0.54]; $P < 0.001$, model 2). When additively adjusted for relevant covariates, the association was not materially changed (HR 0.24 [95% CI 0.11–0.51]; $P < 0.001$, model 5). Lastly, when adjusted for baseline FPG and HbA_{1c} levels, testosterone remained significantly association with PTDM (HR

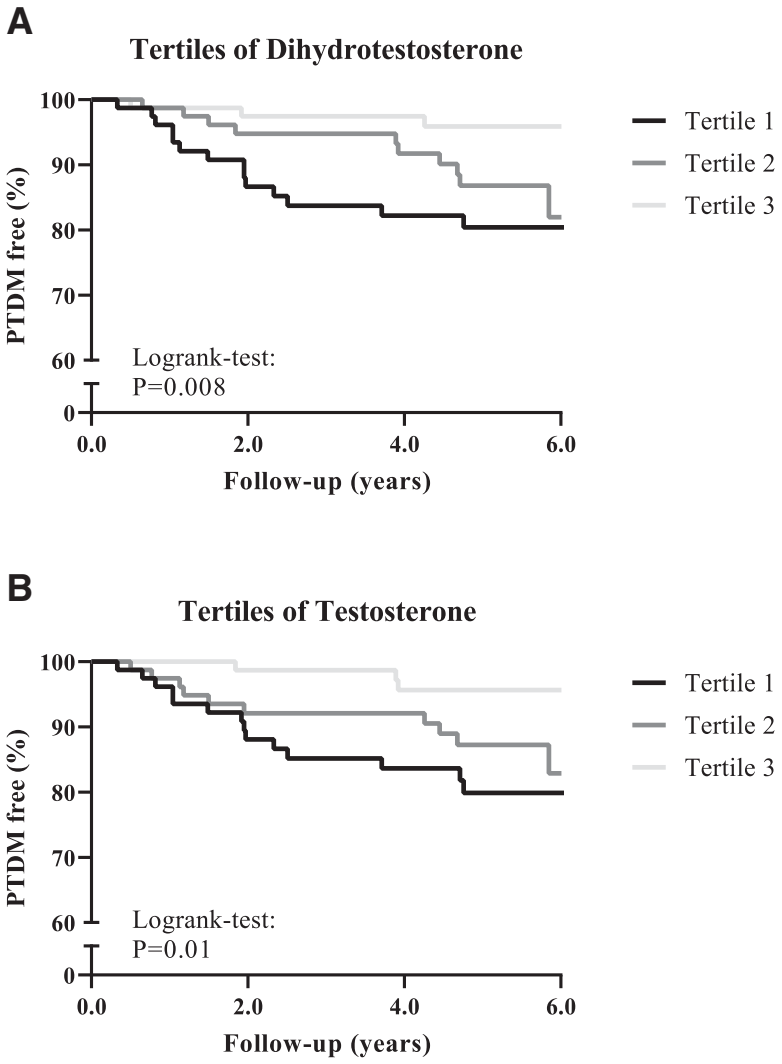

Figure 1—Kaplan-Meier curves for the development of PTDM according to the tertiles of DHT (*A*) and testosterone (*B*) in male KTR.

0.32 [95% CI 0.15–0.68]; $P = 0.003$, model 6).

In additional Cox regression analyses for tertiles of total testosterone, male KTR in the lowest tertile of total testosterone had an ∼4.2-fold higher risk to develop PTDM compared with male KTR in the highest tertile, independent of baseline FPG and HbA_{1c} levels (HR 4.15 [95% CI 1.16–14.84]; $P = 0.03$) (Table 3, model 6, and Supplementary Fig. 2).

Sensitivity Analyses

In sensitivity analyses, we tested for interaction by age, eGFR, and BMI and found no effect modification (all $P \geq 0.05$). Additionally, we tested for interaction by prediabetes as defined according to the American Diabetes Association and observed effect modification for both DHT ($P = 0.04$) and testosterone ($P = 0.004$). Subgroup analyses showed that in KTR with and without pre-diabetes, DHT levels were associated with the development of PTDM ($P = 0.01$ and $P = 0.005$, respectively) (Supplementary Table 5). Yet, levels of testosterone were only associated with the development of PTDM in KTR who had no prediabetes on baseline ($P = 0.17$ and $P = 0.001$, respectively) (Supplementary Table 6). As one of the subgroups had only five events, only univariable analysis could be performed.

In further sensitivity analyses, we aimed to investigate whether visceral adiposity could be a driving factor for the association of DHT with the development of PTDM and therefore performed additional analyses in which BMI was substituted for waist circumference and triglyceride levels, as both are strongly related to visceral adiposity. Both additional analyses did not reveal a meaningful difference (HR 0.28 [95% CI 0.15–0.54], $P < 0.001$ vs. HR 0.28 [95% CI 0.14–0.55], $P < 0.001$ and HR 0.30 [95% CI 0.15–0.58], $P < 0.001$, respectively).

CONCLUSIONS

In this prospective cohort of stable male KTR, we demonstrated that DHT and testosterone are associated with the development of PTDM. More specifically, we showed that male KTR in the lowest tertile of DHT and testosterone have an independent 4.7-fold and 4.2-fold increased risk to develop PTDM, respectively. These results suggest that androgen insufficiency could play a role in the frequent deterioration of the glucose metabolism after kidney transplantation.

Although the biological plausibility of androgens impacting glycemic control has been firmly established, the current study is the first longitudinal epidemiological study to link androgens inversely to risk of PTDM in KTR. Furthermore, to our knowledge, no studies have demonstrated a prospective association between levels of DHT and testosterone and the development of type 2 diabetes in patients with chronic kidney disease. Nevertheless, multiple systematic reviews that included aging subjects and people from the general population have demonstrated an evident lower risk of type 2 diabetes for men with higher total testosterone levels compared with men with lower levels of testosterone (21,22). Similarly, multiple randomized controlled trials have demonstrated a favorable effect of testosterone replacement therapy on several metabolic factors, including HbA_{1c} in hypogonadal men with type 2 diabetes (23–25). This evidence from clinical studies shows an evident link between testosterone and diabetes. In concordance, experimental studies even demonstrated a direct effect of testosterone on the pancreatic β-cell function and health (26,27). For example, Muthusamy et al. (26) observed that testosterone deficiency reduced serum insulin levels and inc-

Table 2—Association of log2 DHT and tertiles of DHT with PTDM

	Continuous		Tertile 1		Tertile 2		Tertile 3
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	Reference
Events, *n*	28		15		10		3
Model 1	0.27 (0.14–0.51)	<0.001	5.82 (1.68–20.12)	0.005	3.54 (0.97–12.86)	0.06	1.00
Model 2	0.27 (0.14–0.51)	<0.001	5.95 (1.72–20.60)	0.005	3.61 (0.99–13.13)	0.05	1.00
Model 3	0.28 (0.15–0.54)	<0.001	5.32 (1.52–18.62)	0.009	3.25 (0.88–11.97)	0.08	1.00
Model 4	0.28 (0.14–0.53)	<0.001	5.37 (1.53–18.88)	0.009	3.30 (0.90–12.10)	0.07	1.00
Model 5	0.27 (0.15–0.52)	<0.001	5.93 (1.70–20.65)	0.005	3.45 (0.94–12.71)	0.06	1.00
Model 6	0.31 (0.16–0.59)	<0.001	4.74 (1.36–16.53)	0.02	2.54 (0.67–9.62)	0.17	1.00

Model 1: crude analysis; model 2: model 1 plus adjustment for age; model 3: model 1 plus adjustment for eGFR and transplantation vintage; model 4: model 1 plus adjustment for BMI and hs-CRP; model 5: model 1 plus adjustment for cumulative prednisolone dose and tacrolimus use; and model 6: model 1 plus adjustment for glucose levels and HbA_{1c}.

reased glucose levels in male rats and that testosterone supplementation reversed all alterations. It also has been suggested that androgens act via the androgen receptor on β-cells to improve glucose-stimulated insulin secretion, once again implicating androgens in β-cell function (11,28). Interestingly, low androgen levels have also long since been linked to the presence of insulin resistance and visceral adiposity (29). In our population, DHT and testosterone were associated with the development of PTDM independent of the waist circumference and triglyceride levels, which are strongly related to visceral adiposity. It is, however, important to realize that without β-cell dysfunction, neither visceral adiposity nor insulin resistance can induce hyperglycemia (30–32). This indicates that β-cell failure has an influential role in the development of hyperglycemia. Fascinatingly, patients who developed PTDM had significantly lower insulin levels in fasting state and after an OGTT, indicating that the pathophysiological mechanism of β-cell dysfunction indeed plays a role in the development of PTDM in KTR (33). A recent study showed that DHT is necessary for normal glucose-stimulated insulin secretion in cultured pancreatic islets from male human donors (11). Furthermore, Navarro et al. (11) discovered that the insulinotropic effect of DHT via the androgen receptor in β-cells is dependent on activation of the glucagon-like peptide 1 (GLP-1), indicating that DHT can upregulate insulin secretion by enhancing the insulinotropic effects of GLP-1. We therefore hypothesize that male KTR with low androgen levels are not able to adequately upregulate insulin secretion in response to insulin resistance, predisposing these patients to develop PTDM. This mechanism may potentially also provide an opportunity for the treatment of PTDM. As the problem is at least partially due to insufficient insulin secretion, GLP-1 receptor agonists could be used to stimulate glucose-dependent insulin sec-retion by binding to the GLP-1 receptor on the β-cell surface. GLP-1 receptor agonists have recently been deemed safe and effective for KTR and may simultaneously also provide other benefits, such as weight loss (34). Although larger epidemiological studies and clinical trials investigating long-term efficacy of GLP-1 receptor agonists in KTR are still warranted, their usage, possibly in combination with testosterone supplementation to maximize the efficacy of the GLP-1 receptor pathway, has the potential to

Table 3—Association of log2 total testosterone and tertiles of total testosterone with PTDM

	Continuous		Tertile 1		Tertile 2		Tertile 3
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	Reference
Events, *n*	28		14		11		3
Model 1	0.26 (0.12–0.54)	<0.001	5.52 (1.58–19.23)	0.007	4.02 (1.12–4.41)	0.03	1.00
Model 2	0.25 (0.12–0.54)	<0.001	5.40 (1.53–19.01)	0.009	4.02 (1.12–14.41)	0.03	1.00
Model 3	0.27 (0.13–0.58)	0.001	5.00 (1.41–17.71)	0.01	3.61 (0.99–13.18)	0.05	1.00
Model 4	0.27 (0.13–0.58)	0.001	5.06 (1.43–17.96)	0.01	3.69 (1.01–13.52)	0.05	1.00
Model 5	0.24 (0.11–0.51)	<0.001	5.81 (1.65–20.44)	0.006	4.01 (1.11–14.52)	0.03	1.00
Model 6	0.32 (0.15–0.68)	0.003	4.15 (1.16–14.84)	0.03	3.88 (1.08–13.92)	0.04	1.00

Model 1: crude analysis; model 2: model 1 plus adjustment for age; model 3: model 1 plus adjustment for eGFR and transplantation vintage; model 4: model 1 plus adjustment for BMI and hs-CRP; model 5: model 1 plus adjustment for cumulative prednisolone dose and tacrolimus use; and model 6: model 1 plus adjustment for glucose levels and HbA_{1c}.

improve glycemic control in hypogonadal male KTR. Another potential opportunity may lie in replacing the diabetogenic T-cell inhibitor tacrolimus as part of the immunosuppressive regimen for the nondiabetogenic T-cell inhibitors belatacept or abatacept (35).

The correct method of androgen measurement and the presence of androgen deficiency in male KTR remains the topic of an ongoing debate (14,36). One of the drivers of this discussion is the recent switch from the utilization of immunochemical assays to LC/MS-MS as the new gold standard (37,38). As a consequence, reference values for androgen levels of male KTR determined by LC/MS-MS are not yet available. As these reference values are of importance for both clinical practice and scientific research, we would encourage future studies to determine population specific reference values. Future research may also want to focus on the responsible mechanisms that cause low androgen levels posttransplantation.

The current study has multiple strengths, including measurement of DHT and testosterone with the gold-standard method LC/MS-MS, and has a substantial follow-up of median 5.3 years. Additionally, the current study was based on a cohort of stable male KTR who were all >1 year posttransplantation. This is important as transient posttransplantation hyperglycemia is very common shortly after kidney transplantation and can be due to many temporary complications (39). A formal diagnosis of PTDM should therefore only be made once KTR are on maintenance immunosuppression, have a stable allograft function, and have no acute infections (39). Furthermore, by assessing both DHT and testosterone, we were able to focus on proposed mechanism of enhanced insulinotropic effects by DHT through the androgen receptor, while also providing information on testosterone, which is used more often in clinical practice. This division is essential, as testosterone can undergo aromatization to form estradiol, which has been shown to amplify insulin synthesis (40). Moreover, the current study is the first longitudinal epidemiological study to link androgens inversely to risk of development of PTDM in KTR. Lastly, there was no loss of follow-up, limiting potential selection bias.

The current study also has several limitations. As OGTT were not part of standard clinical care, this test did not serve as a diagnostic tool to evaluate PTDM development. Because annual HbA_{1c} testing was not yet part of clinical routine during a part of the study follow-up, it is likely that some cases of diabetes were missed given that fasting glucose might be normal or not frankly elevated in early diabetes. This could have resulted into underestimation of the number of KTR suffering from PTDM, which, in turn, could have reduced the power of the current study due to the relatively low number of men whom developed PTDM. The percentage of men with PTDM, as found in the current study, is, however, in concordance within the percentage observed by Conte and Secchi (9). Furthermore, as androgen data were only available in male KTR, no insights have been gained on the potential association of DHT and testosterone with PTDM in female KTR. Similarly, as the current study predominantly included Caucasian KTR, extrapolation to different populations might be limited. Additionally, the development of prediabetes could not adequately be assessed due to the design of the study and, as a result, the potential association of androgens with the development of prediabetes could not be investigated in this study. Lastly, as with any observational study, potential residual confounding cannot be excluded despite adjustment for a relatively large number of potential confounders.

In conclusion, in stable male KTR, DHT and total testosterone are associated with the development of PTDM. Men in the lowest tertiles of DHT and total testosterone have an $\sim$4.7-fold and 4.2-fold increased risk to develop PTDM, respectively. The testosterone metabolism may be a novel modifiable risk factor and mechanism to intervene upon in order to lower the risk of PTDM after kidney transplantation.

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Author Contributions. S.P.S., A.v.d.V., M.J.V., and I.P.K. were responsible for the laboratory measurements of DHT and testosterone levels. S.P.S. and A.W.G.-N. collaborated in clinical data collection. S.P.S. analyzed the data and wrote the first draft of the paper. S.P.S., S.S., M.F.E., A.W.G.-N., P.R.v.D., J.J.v.Z., M.J.V., I.P.K., A.P.v.B., and S.J.L.B. contributed to the interpretation of results and provided important advice and intellectual content. All authors had access to the data, contributed to critical revision of the manuscript, and approved the final version of the manuscript. A.P.v.B. and S.J.L.B. are the guarantors of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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APPENDIX

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