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Testosterone Concentrations and Cardiovascular Autonomic Neuropathy in Men with Type 1 Diabetes in the Epidemiology of Diabetes Interventions and Complications Study (EDIC)

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Abstract

Introduction—Previous studies have reported that lower testosterone concentrations are associated with cardiovascular autonomic neuropathy (CAN), a risk factor for cardiovascular events. However, no studies have examined this relationship in men with type 1 diabetes, who are at high risk for CAN.

Aim—To examine the associations between testosterone concentrations and measures of CAN in a large, well-characterized cohort of men with type 1 diabetes.

Methods—We conducted an analysis of men in the Diabetes Control and Complications Trial (DCCT), a randomized trial of intensive glucose control, and its observational follow-up the Epidemiology of Diabetes Intervention and Complications (EDIC) Study. Testosterone was measured by liquid chromatography mass spectrometry in stored samples from EDIC follow-up years 10 and 17. Regression models were used to assess the cross-sectional relationships between testosterone and CAN measures.

Main Outcome Measures—The main CAN measure from EDIC follow-up year 17 was a standardized composite of R-R variation with paced breathing < 15, or R-R variation 15-20 combined with either a Valsalva ratio < 1.5 or a decrease in diastolic blood pressure > 10 mm Hg upon standing. Continuous R-R variation and Valsalva ratio were secondary outcome measures.

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Results—Lower total and bioavailable testosterone concentrations at follow-up years 10 and 17 were not associated with the presence of CAN at year 17. In analyses using Valsalva ratio as a continuous measure, higher total ($p=0.01$) and bioavailable testosterone concentrations ($p=0.005$) were associated with a higher (more favorable) Valsalva ratio after adjustment for covariates including age, body mass index, smoking status, hypertension, and glycemic control.

Conclusions—Testosterone levels are not associated with CAN among men with type 1 diabetes. Although testosterone is associated with a higher Valsalva ratio, a more favorable indicator, the clinical significance of this association is not known.

Keywords

testosterone; type 1 diabetes; cardiovascular reflex tests; cardiovascular autonomic neuropathy

Introduction

Cardiovascular autonomic neuropathy (CAN) has been shown to be an independent risk factor for cardiovascular disease (CVD) and CVD mortality.^{1, 2} While mechanisms are not fully elucidated, CAN is associated with left ventricular dysfunction³ and increased pulse pressure.⁴ Low testosterone concentrations have also been shown to increase the risk of cardiovascular events in men.⁵⁻⁹ Although men with type 1 diabetes have a prevalence of CAN as high as 20%,¹⁰ no studies have examined whether testosterone concentrations are related to CAN in this population. It is possible that the insulin requirements and subsequent increases in weight and waist circumference are associated with low testosterone concentrations,¹¹ and that low testosterone concentrations subsequently increase the risk of CAN.

Aims

We examined the association between endogenous testosterone levels and CAN in the Diabetes Control and Complications Trial/Epidemiology of Interventions and Complications Study (DCCT/EDIC). This large, well-characterized cohort of adults with type 1 diabetes has almost decades of follow-up and has collected testosterone concentrations and detailed CAN measurements.³ We hypothesized that lower concentrations of endogenous testosterone would be associated with a higher prevalence of CAN and abnormal CAN markers.

Methods

Population and Setting

The DCCT has been described in detail.¹² Briefly, the DCCT was a randomized clinical trial designed to compare the impact of intensive and conventional diabetes treatment on the development and progression of early microvascular complications of type 1 diabetes. The DCCT included a primary prevention cohort with no retinopathy or nephropathy detectable at baseline and a secondary intervention cohort with evidence of minimal complications at baseline.¹² Individuals were excluded from the trial if they had hypertension, were taking any blood pressure or lipid-lowering medications, or had a history of symptomatic ischemic

heart disease or symptomatic peripheral neuropathy that required medication. Participants were followed for 3 to 9 years (mean 6.5 years). At the end of the trial, all participants were instructed in intensive therapy. All procedures were approved by institutional review boards of all participating centers. Written informed consent was provided by all participants.

EDIC, the follow-up observational study of the DCCT cohort began in 1994, one year after completion of the DCCT. A detailed description of EDIC study procedures and baseline characteristics has been published.¹³ Clinical and biochemical endpoints were obtained annually by history, exam, and laboratory testing; blood was drawn in the morning after an overnight fast.¹³ Blood pressure, BMI (kg/m²), insulin dosage (unit/kg/day), and hemoglobin A1c (HbA1c) were assessed annually.¹⁴ Hypertension was defined as sitting systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mmHg or the use of antihypertensive medication. Peripheral neuropathy was assessed annually using the Michigan Neuropathy Screening Instrument;¹⁵ for the purposes of this cross-sectional analysis, peripheral neuropathy measurements from year 10 (the time of the initial testosterone measurement) were used.

CAN testing protocols have also been described previously.¹⁶ CAN was performed in EDIC years 16/17 using a standardized protocol. Participants who experienced hypoglycemia after midnight or with acute illnesses were excluded. Participants with proliferative retinopathy, recent history of laser therapy or vitrectomy, no eye exam in the last 4 years, or who could not perform the required forced expiration were excluded from the Valsalva maneuver, although they were defined as having CAN if they met the other CAN criteria. R-R variation measures the magnitude of cardiac sinus arrhythmia, predominantly a function of the parasympathetic nervous system, and is computed as a dimensionless circular mean vector of R-R intervals. The Valsalva ratio evaluates cardiovagal function in response to a standardized increase in intrathoracic pressure, and is influenced by parasympathetic and sympathetic activity. This battery of tests is well suited to explore long-term changes in CAN function and has been recommended as the gold standard for assessing CAN.¹⁷ The main CAN measure was a standard composite measure of R-R variation with paced breathing < 15 , or R-R variation 15-20 combined with either a Valsalva ratio ≥ 1.5 or a decrease in diastolic blood pressure > 10 mm Hg upon standing.² CAN testing was performed with Hokanson ANS2000 devices (Hokanson, Bellevue, Washington). Results were analyzed at a central reading center by a single masked investigator, and R-R variation and the Valsalva maneuver had high test-retest correlations ($\kappa=0.78$ and 0.80 , respectively, $p<0.0001$ for both).

An ancillary study, Uro-EDIC, was designed to examine urologic complications of diabetes. Uro-EDIC included the testosterone evaluations in 2003 (EDIC Year 10), which were repeated in 2010 (EDIC year 17). The 615 men who had testosterone measurements at both time points and CAN measures comprise the cohort for this analysis. Total testosterone was measured at the University of Minnesota using a rapid liquid chromatography mass spectrometry platform. The lower and upper limits of detection of the assay are 7 ng/dl and 1154 ng/dl, respectively. The assay was certified by the CDC Hormone Standardization Program (HoST, <http://www.cdc.gov/labstandards/hs.html>) and passed the required intra-assay performance criterion of 6.4%. Specifically, intra-assay mean bias was 4.6% at 199

ng/dl and 1.8% at 1007 ng/dl. Calculated bioavailable testosterone was determined using the Vermeulen formula with an association constant of testosterone binding to albumin of 3.6×10^4 L/mol and testosterone binding to SHBG of 10^9 L/mol.¹⁸ Androgen deficiency was defined as serum total testosterone concentrations of <300 ng/dl.^{19, 20}

Statistical Analyses

For the purposes of this analysis, participants with CAN at DCCT close-out were excluded (n=45 men). Participant characteristics at the time of the first testosterone measurement were compared by CAN status using t-tests or χ^2 tests (Table 1). Multivariable linear regression was used to estimate the association between testosterone (independent variable) and bioavailable testosterone (independent variable) with continuous CAN measures at years 16/17. Multivariable logistic regression was used to estimate the association between testosterone (independent variable) with the presence or absence of CAN at years 16/17 (Tables 2 through 4). Covariates in both linear and logistic models included variables previously found to be associated with CAN markers in EDIC, including age (years), smoking status, BMI (kg/m²), DCCT treatment arm (conventional vs. intensive treatment), HbA1c level, hypertension, and presence of microvascular complications at randomization.^{15, 17, 21} Quadratic terms evaluated the linearity of the relationships. Due to previous reports noting associations between low testosterone and insulin and waist circumference, we also evaluated whether waist circumference at year 10 or time-weighted insulin dosage altered the associations observed between testosterone and measures of CAN.¹¹ All analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

Main Outcome Measures

The main outcome measure was CAN as defined above;¹⁶ continuous R-R variation and Valsalva ratio were secondary outcome measures. For the purposes of this analysis, we used CAN measures from year 16/17.

Results

Table 1 shows participant characteristics at year 10 by the presence of CAN at year 17. Men with CAN were slightly older than men without CAN and more likely to be smokers and have hypertension. Men with CAN were also more likely to have had longer diabetes duration higher HbA1c levels over time, microvascular complications at DCCT baseline, and peripheral neuropathy. Total testosterone and bioavailable testosterone concentrations were slightly lower among men with CAN and testosterone concentrations < 300 ng/dl were more common among men with CAN, but these associations were only of borderline statistical significance.

Continuous measures of total and bioavailable testosterone at year 10 were not associated with R-R variation or Valsalva ratios (Table 2). Although measures of total and bioavailable testosterone at year 17 were associated with higher Valsalva ratios, low testosterone concentrations (as indicated by a cutpoint of <300 ng/dl) were not.

Continuous measures of total and bioavailable testosterone at year 10 were not associated with increased odds of CAN, abnormal R-R variation, or Valsalva ratios at year 16/17.

Testosterone concentrations < 300 ng/dl at year 10 were associated with a slightly higher odds of abnormal R-R variation. However, associations were not observed between testosterone levels at year 17 and CAN outcomes, with the exception that higher bioavailable testosterone concentrations were associated with a slightly lower odds of abnormal Valsalva ratios. These associations were not appreciably altered with the addition of waist circumference or insulin dosage to the models (results not shown), possibly due to inclusion of BMI and randomization to intensive insulin therapy in the models.

Discussion

Testosterone prescriptions in men have increased over the past decade, totaling \$1.8 billion in sales in 2011²² and are predicted to reach \$3.8 billion per year in the U.S. by 2018.²³ Promotion in the popular press²³ may have contributed to the wide use of testosterone in the U.S, and reports have noted that supplementation may relieve fatigue and possibly reduce risk of cardiovascular disease events.^{20, 24, 25} However, in a cohort of well-characterized men with type 1 diabetes, we did not find an association between testosterone concentrations and CAN. Greater testosterone concentrations corresponded with more favorable Valsalva ratios. However, consistent associations were not observed between testosterone and R-R variation or between testosterone and CAN. Although previous studies have reported associations between exogenous testosterone administration and CVD risk²⁶ and between low endogenous testosterone concentrations and CVD risk,²⁷ testosterone is unlikely to influence CVD outcomes through CAN.

The peripheral nervous system expresses both classic and non-classic steroid receptors, and rat studies suggest that androgens can affect neuron proliferation and myelin protein expression.²⁸ However, the majority of such studies are limited to streptozotocin-induced diabetes animal models.²⁸ Few studies have examined the relationship between testosterone and CAN in humans. Such studies have focused on men with known CVD. Rydlewska and colleagues noted that among men with mild systolic congestive heart failure, men with lower testosterone concentrations had decreases in parasympathetic tone as indicated by baroreflex sensitivity.²⁹ Similarly, Wranicz and colleagues noted that in 88 middle-aged men who had experienced a myocardial infarction, lower testosterone concentrations (<530 ng/dl) were associated with decreased (less favorable) heart rate variability.³⁰ Caminiti and colleagues noted that elderly men with congestive heart failure who were randomized to testosterone therapy had subsequent improvement in exercise capacity and baroreflex sensitivity, but the study size was small.³¹

Our results may have differed from these studies for several reasons. It is possible that the relationship between testosterone and CAN is significant primarily in men with a greater degree of impairment in other types of cardiac disease. The DCCT/EDIC population was relatively young (mean age approximately 45 years) and with a low prevalence of clinical coronary artery disease at the time of these analyses. Other markers of cardiovascular impairment including myocardial ischemia and abnormal cardiac structure were also relatively rare.³² The participants described in previous studies had congestive heart failure, which can entail impaired sympathetic vs. parasympathetic balance as part of compensatory mechanisms. We also examined relatively young men with type 1 diabetes.²¹ The

relationship between testosterone and CAN may be present primarily at extremely low concentrations, and thus we may have been underpowered to detect an association in this regard. Finally, it is possible that testosterone has relatively little impact upon CAN measures in the type 1 population compared with other risk factors for autonomic disease, particularly hypertension and glycemic control.³

Strengths of this report include the large sample size, examination of a carefully characterized population for multiple risk factors, standardized CAN measurements, and mass spectrometric testosterone assays. Limitations are that hypoandrogenism was relatively uncommon; however, assessment of both outcomes and testosterone as continuous measures also failed to suggest strong or consistent relationships. This absence of a dose-response relationship suggests that testosterone may not be related to CAN. Even though extremely low testosterone concentrations were uncommon, CAN was not. This suggests that among men with type 1 diabetes, hypertension and glycemic control are more important in the pathophysiology of CAN. It is possible that testosterone has a greater impact in populations with a greater degree of myocardial dysfunction. Our results suggest that the previously documented associations of testosterone and CAN may not be important in younger, healthier populations, despite the high prevalence of CAN in EDIC.

Conclusions

We conclude that among men with type 1 diabetes who have a high prevalence of CAN, testosterone concentrations are not associated with the presence of CAN and have weak associations with continuous markers of CAN. Future studies should examine whether testosterone is associated with other pathophysiologic mechanisms of heart disease, particularly atherosclerosis and cardiac structure and function.

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Table 1
Sociodemographic/Clinical and Diabetes Characteristics in Men at EDIC year 10 by
Cardiovascular Autonomic Neuropathy Status at EDIC Year 16/17 (n=615)

Characteristics at EDIC Year 10	Overall N=615	CAN* N=231	No CAN N=384	p-value
Age (years)	44.5±6.6	46.7±6.2	43.2±6.5	<0.0001
Current cigarette smoker (n,%)	83 (14)	43 (19)	40 (11)	0.004
Body mass index (BMI) (kg/m ²)	28.1±4.1	28.5±4.6	27.9±3.8	0.4
BMI category (n,%)				0.3
BMI<25 kg/m ²	137 (22)	52 (23)	85 (22)	
BMI 25-30 kg/m ²	310 (51)	109 (47)	201 (53)	
BMI ≥30 kg/m ²	163 (27)	69 (30)	94 (25)	
BMI change since EDIC baseline (kg/m ²)	1.8±2.2	1.6±2.2	1.9±2.2	0.07
Waist circumference (cm)	95.4±10.9	96.9±12.4	94.4±9.8	0.02
Randomization to intensive treatment (n,%)	305 (50)	106 (46)	199 (52)	0.2
Primary prevention cohort (n,%)	305 (50)	96 (42)	209 (54)	0.003
Duration of diabetes (years)	22.4±4.8	23.4±4.9	21.9±4.7	0.0001
Time weighted DCCT/EDIC HbA1c (%)	8.0±1.0	8.3±1.1	7.9±0.9	<0.0001
Time-weighted DCCT/EDIC insulin dosage (units/kg/day)	0.66±0.18	0.66±0.18	0.65±0.18	0.8
Peripheral neuropathy (n,%) [‡]	217 (36)	103 (45)	114 (30)	0.0001
Hypertension (n,%) [‡]	349 (57)	155 (68)	194 (51)	<0.0001
Total testosterone (ng/dL)	595.5±210.3	578.1±216.1	606.0±206.3	0.07
Bioavailable testosterone (ng/dL)	9.7±2.5	9.4±2.5	9.9±2.4	0.06
Total testosterone <300 ng/dl (n,%)	39 (6)	20 (9)	19 (5)	0.07

* Defined using autonomic testing completed in EDIC year 16/17 and abnormal finding defined as R-R variation<15 or RR variation 15-20 in combination with Valsalva ratio <1.5 or a decrease of >10 mmHg in diastolic blood pressure.

[‡] Defined at EDIC year 10 by the Michigan Neuropathy Screening Instrument >6 responses on the questionnaire or a score of >2 on the exam.

[‡] Hypertension is defined as sitting systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg or the use of antihypertensive medication.

Table 2

Multivariable linear regression models with continuous CAN measures as the dependent variables and testosterone measures as the independent variables. Data are regression coefficients (95% confidence intervals) from separate multivariable linear regression models.*

	R-R Variation β-coefficient (standard error), p-value	Valsalva Ratio β-coefficient (standard error), p-value
Total testosterone		
At EDIC year 10	0.0015 (0.0033), p=0.65	0.00010 (0.00007), p=0.15
At EDIC year 17	0.0012 (0.0032), p=0.71	0.00017 (0.00007), p=0.01
Bioavailable Testosterone		
At EDIC year 10	0.15 (0.28), p=0.60	0.0062 (0.0061), p=0.32
At EDIC year 17	0.028 (0.27), p=0.92	0.0162 (0.0058), p=0.005
Total testosterone < 300 ng/dl		
At EDIC year 10	-0.31 (2.70), p=0.90	-0.075 (0.056), p=0.19
At EDIC year 17	1.42 (2.22), p=0.52	-0.088 (0.048), p=0.067

* Models adjust for primary vs. secondary cohort, DCCT/EDIC time-weighted HbA1c, and the following EDIC year 10 characteristics: age, BMI, smoking status, and hypertensive status.

Table 3

Multivariable logistic regression models with CAN measures as the dependent variables and testosterone measures as the independent variables. Data are odds ratios (95% confidence intervals) from separate multivariable logistic regression models.*

	R-R Variation < 15 Odds Ratio (95% CI)	Valsalva Ratio 1.5 Odds Ratio (95% CI)	Presence of CAN Odds Ratio (95% CI)
Total testosterone			
At EDIC year 10	0.99 (0.99, 1.00)	0.99 (0.99, 1.00)	0.99 (0.998, 1.00)
At EDIC year 17	1.00 (0.99, 1.00)	0.99 (0.99, 1.00)	0.99 (0.99, 1.00)
Bioavailable testosterone			
At EDIC year 10	0.98 (0.90, 1.07)	0.95 (0.87, 1.04)	0.98 (0.91, 1.07)
At EDIC year 17	0.98 (0.90, 1.06)	0.92 (0.84, 0.99)	0.96 (0.89, 1.03)
Total testosterone < 300 ng/dl			
At EDIC year 10	2.38 (1.09, 5.19)	1.49 (0.71-3.15)	1.83 (0.87-3.84)
At EDIC year 17	1.15 (0.60, 2.18)	2.11 (1.13-3.97)	1.63 (0.88-3.02)

* Models adjust for primary vs. secondary cohort, DCCT/EDIC time-weighted HbA1c, and the following EDIC year 10 characteristics: age, BMI, smoking status, and hypertensive status.