Endocrinology & Metabolic Syndrome

Research Article **Open Access**

Relationship between Testosterone and Sex Hormone Binding Globulin Concentrations with Cardiometabolic Parameters and Macrovascular Disease in Afro-Caribbean Men with Type 2 Diabetes

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Abstract

Introduction: Low testosterone concentrations have been reported in type 2 diabetes (T2D) and Coronary Artery Disease (CAD). Contrary to diabetes, the prevalence of CAD is lower in Afro-Caribbean (AC) from French West Indies islands than in people from mainland France. However, CAD is a frequent complication of T2D. To evaluate this paradoxical situation, we investigated the prevalence of sex steroids deficiency that might be associated with CAD in AC men with T2D and the relationships with metabolic parameters.

Methods: We performed a cross-sectional study in the Department of Diabetology of the University Hospital of Guadeloupe. Clinical and biological data were collected from men with T2D. Total Testosterone (TT) and Sex Hormone Binding Globulin (SHBG) concentrations were measured using electrochemiluminescence and immunoradiometric assays. Results are expressed as mean ± SD or percentages. The data were analyzed using the Mann–Whitney and chi-squared tests, Pearson correlations and logistic regression. P values<0.05 were considered significant. Results: One hundred thirty-three AC men with T2D were included. Prevalence of testosterone deficiency was 43.5%. The mean TT and SHBG concentrations were 12.06 ± 6.24 and 35.9 ± 17.54 nmol/l respectively. Low TT concentrations were more frequently found in obese subjects compared to subjects with normal weight and low SHBG concentrations were high rather in subjects with overweight. Both sex steroids levels were higher in subjects with dyslipidemia than subjects without dyslipidemia. TT deficiency was more frequent in subjects with macrovascular disease including CAD, peripheral artery disease or stroke than subjects without macrovascular disease.

Conclusion: Testosterone deficiency and low SHBG concentrations occur frequently in AC men with T2D and are associated with metabolic profile that may promote the development of macrovascular disease. In this population, it may be necessary to systematically apply a testosterone replacement in case of deficiency, to improve cardiometabolic profiles without forget the risk of prostate cancer.

Introduction

Testosterone and Sex Hormone Binding Globulin (SHBG), the specific binding protein of testosterone, are classically measured to evaluate hypogonadism in men [1]. Testosterone concentrations decrease with age, and this leads to an increase in SHBG concentrations [2]. Thus, the interpretation of sex steroid hormone concentrations depends on different parameters. In the past 20 years, the sex steroid hormones (testosterone and SHBG) have been studied as biological parameters that could be involved in cardiometabolic diseases. Several cross-sectional studies have reported an association between sex steroid hormone concentrations and cardiovascular diseases, the metabolic syndrome, and diabetes [3-5]. In Afro-Caribbean (AC) people from the French West Indies islands (FWI), the prevalence of diabetes exceeds 8% of the 400,000 inhabitants in comparison with 4.4% of the population in the mainland France [6]. Conversely, data from the regional health observatory of FWI indicate a lower mortality rate from Coronary Artery Disease (CAD)—by 50% in men and 40% in women—compared with mainland France, while CAD is one of the main complications of T2D [7]. These ethnic differences have also been reported in AC people in England [8]. Although the relationships between sex steroids levels and CAD are reported in diabetic subjects in the literature, we have no data on their assessment in AC diabetic subjects from FWI. The aim of this study was to first evaluate Total Testosterone (TT) and SHBG concentrations in AC men with T2D, then to analyze the relationships between these concentrations and the metabolic and cardiovascular profile.

Materials and Methods

Patient selection

This study was initially designed as a cross-sectional survey of men with T2D hospitalized in the Department of Diabetology of the University Hospital of Guadeloupe for annual evaluation of their disease. One inclusion criterion was the absence of androgen therapy, and any testosterone-replacement therapy needed was provided during the study. Prospective inclusion was performed for 9 months. All of the included patients had given their informed consent to participate

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in this study of atypical biological parameters for the evaluation of cardiovascular risk. The approval of the interregional ethics board "Sud-Ouest Outre-Mer" for studies on diabetes and cardiovascular risk was received.

Clinical data

The diagnosis of T2D was consistent with the "expert committee on the diagnosis and classification of diabetes mellitus criteria for diabetes" published in 2003 [9]. For analysis, we considered that diabetes was inadequately controlled when HBA1C was >8%. We recorded age and anthropometric parameters including weight, height, and waist circumference using standard tools. Body Mass Index (BMI) was calculated as weight (kg) divided by height squared (m^2). Overweight was defined as BMI ≥ 25 kg/m^2. Individuals with a BMI ≥30 kg/m^2 were defined as obese and abdominal obesity was confirmed as a Waist Circumference (WC) ≥94 cm (International Diabetes Federation criteria). Blood Pressure (BP) was measured with a standard electronic sphygmomanometer after a 10 min rest in a supine position. Arterial hypertension was defined as the value of systolic BP (SBP) ≥140 mm Hg and/or Diastolic BP (DBP) ≥90 mm Hg on at least 2 previous visits in the previous year [10]. We also included subjects known to have hypertension and receiving pharmacological treatment for high BP. CAD was defined as a documented history of acute coronary syndrome, coronary stenosis, coronary angioplasty, or coronary artery bypass surgery. Analysis of the cardiometabolic profile included anthropometric data, dyslipidemia, glycemic control, hypertension, and the presence of CAD. Information about the presence of Macrovascular Disease (MVD) was collected. MVD was defined as a history of myocardial infarction, acute coronary syndrome, peripheral artery disease, or stroke.

Biochemical analyses

Blood samples were drawn with the subject in the supine position in the early morning after an overnight fast. Fasting Plasma Glucose (FPG), serum total cholesterol, High-Density Lipoprotein (HDL) cholesterol, Low-Density Lipoprotein (LDL) cholesterol, and Triglyceride (TG) levels were measured in fresh blood samples by the University Hospital Biochemistry Laboratory of Pointe-à-Pitre. Dyslipidemia was defined as the use of a hypolipidemic agent, serum HDL level<0.4 g/l, LDL level>1 g/l, or TG level>1.5 g/l. Serum TT level was measured using an electrochemiluminescence method with a minimum detection limit of 0.087 nmol/l. The intra- and interassay coefficients of variation were assessed as 2% and <4%, respectively. The reference range for this TT assay was 10.4-41.6 nmol/l. An immunoradiometric assay was used to measure SHBG concentrations (SHBG-RIACT kit, Cisbio Bioassays, Gif sur Yvette, France). The reference range for SHBG was 14-48 nmol/l and the mean intra- and interassay coefficients of variation were both <6%. Hypogonadism was defined as a TT level<10.4 nmol/l, and a low SHBG level was defined as an SHBG level<14 nmol/l.

Statistical analysis

The data are presented as mean ± standard deviation for continuous variables and as percentages for categorical variables. The Mann–Whitney U test was used to compare means, and the chi-squared test was used to compare proportions. Pearson's correlation (for parametric data) was used to establish correlations. The association between sex steroids levels and cardiometabolic parameters was evaluated by a logistic regression. We first performed a univariate analysis and retained only the results with p value<0.1 for multivariate logistic regression analysis after adjustment for age and BMI. p values<0.05 were considered significant. IBM SPSS Statistics for Windows, version 19.0 (IBM Corp., Armonk, NY) was used to analyze the data.

Results

Description of the population

Data from 133 consecutive AC men with type T2D were analyzed. The clinical and biological characteristics of the population studied are described in Table 1. In addition to their diabetes, more than half of the patients had a metabolic profile with a high risk of cardiovascular disease: 71.4% had abdominal obesity, 67.7% had hypertension, and 56.5% had dyslipidemia. The prevalence rates of CAD and MVD were 27.4% and 33%, respectively.

The mean TT level in the population studied was 12.06 ± 6.24 nmol/l and the mean SHBG level was 35.9 ± 17.54 nmol/l. The prevalence of hypogonadism was evaluated at 43.5%. All patients with a low SHBG level also had a low TT level.

TT concentrations and cardiometabolic profile

We analysed TT concentrations according to the metabolic profile of the studied subjects. In subjects with overweight (BMI ≥ 25 and <30 kg/m^2) compared to subjects with normal BMI (<25 kg/m^2), we found lower TT concentrations (11.42 ± 6.09 vs. 14.08 ± 6.47 nmol/l, p=0.04). Lower TT concentrations were found in obese subjects compared to non-obese subjects (9.2 ± 5.5 vs. 13.1 ± 6.2 nmol/l, p=0.002). Subjects with dyslipidemia had lower TT concentrations compared with those without dyslipidemia (11 ± 5.8 vs. 13.7 ± 6.4 nmol/l, p=0.01). TT concentrations did not differ according to the FPG and BP profile. After analysis of correlations, we found an inverse correlation between TT level and BMI (r=-0.26, p=0.003) and SBP (r=-0.13, p=0.028). In subjects with low TT concentrations compared to those with normal TT levels, the frequencies of obesity, dyslipidemia, and MVD were higher (Table 2). After multivariate logistic regression, a significant association was found between TT and BMI but only a trend was found with dyslipidemia and MVD (Table 3). Subjects with CAD were older than subjects without CAD but metabolic profile and TT concentrations were not different. Subjects with MVD were older and had more frequently hypogonadism than subjects without MVD (Table 4).

	Mean ± SD or %
Age (years)	61.7 ± 10.8
BMI (Kg/m^2)	28 ± 4.7
Obesity (%)	26.6
Waist circumference (cm)	99.6 ± 13.9
Abdominal obesity (%)	71.4
Systolic blood pressure (mm Hg)	130.8 ± 15.7
Diastolic blood pressure (mm Hg)	76.1 ± 11.5
Hypertension (%)	67.7
FPG (mmol/l)	7.35 ± 2.9
HBA1C>8% (%)	31.5
HBA1C levels (%)	7.9 ± 2.6
Total Cholesterol (mmol/l)	4.22 ± 1.14
LDL Cholesterol (mmol/l)	2.4 ± 0.9
HDL Cholesterol (mmol/l)	1.22 ± 0.39
Triglycerides (mmol/l)	1.23 ± 0.84
TT levels (nmol/l)	12.06 ± 6.24
SHBG levels (nmol/l)	35.9 ± 17.5
Low TT levels<10.4 nmol/l	43.5%
Low SHBG levels<14 nmol/l	4%

Table 1: Characteristics of the population.

	Low TT levels N=54	TT levels in normal ranges N=70	p
Age<60 yrs (%)	51.9	62.9	-
Overweight (%)	11.1	34.3	0.001
Obesity (%)	42.6	14.3	0.001
Abdominal obesity (%)	74.5	69.1	-
Dyslipidemia (%)	68.5	47.1	0.02
HBA1C>8% (%)	38	30.3	-
Hypertension (%)	77 .8	61.8	-
CAD (%)	33.3	22.9	-
MVD (%)	42.6	25.7	0.04
Low SHBG (%)	9.3	0	0.02

CAD: Coronary Artery Disease; MVD: Macrovascular Disease.

Table 2: Cardiometabolic profile and Total Testosterone (TT) Concentrations (complete data were available for 124 subjects).

Parameters	OR (95% CI)	p values
Age	1.03 (0.98-1.08)	0.17
BMI	1.21 (1.07-1.37)	0.002
Dyslipidemia	2.25 (0.88-5.73)	0.087
High BP	1.87 (0.69-5.03)	0.21
SHBG	0.93 (0.89-0.96)	0.001
MVD	1.96 (0.74-5.19)	0.17

OR: Odds Ratio; CI: Confidence Interval; BMI: Body Mass Index; BP: Blood Pressure; SHBG: Sex Hormone Binding Globulin; MVD: Macrovascular Disease.

Table 3: Results of the multivariate logistic regression for the association between total testosterone concentrations and cardio metabolic parameters.

SHBG level and cardiometabolic profile

SHBG concentrations were lower in overweight subjects compared to subjects with normal BMI (32.92 ± 14.65 vs. 45.21 ± 22.21 nmol/l, p=0.001) but were not different in obese subjects compared to non-obese subjects (31.64 ± 12.63 vs. 37.44 ± 18.73 nmol/l, p=0.099). SHBG levels did not differ according to glucose profile. SHBG concentrations were lower in subjects with hypertension or dyslipidemia compared with those with normal BP or without dyslipidemia (34.7 ± 16.1 vs. 41.8 ± 21.1 nmol/l, p=0.03, and 33.8 ± 15.7 vs. 40.4 ± 19.8, p=0.03, respectively). SHBG concentrations were non-significantly lower in subjects with CAD or MVD compared with those without a history of a vascular event (data not shown). Analysis of correlations shown TT and SHBG concentrations correlated significantly (r=0.48, p<0.001). There was an inverse correlation between SHBG level and BMI (r=-0.22, p=0.013) and a trend for an inverse correlation between SHBG concentrations and WC (r=-0.17, p=0.06). SHBG concentrations correlated inversely with SBP (r=-0.26, p=0.003).

After logistic regression, we only found a significant association between low SHBG and FPG (OR 1.42 [CI 95% 1.08-1.86, p=0.01]). CAD seemed to be more frequent in subjects with a low SHBG concentration, but this relationship was not significant (60% vs. 26%, p=0.13). We also performed a logistic regression analysis to evaluate the association between MVD and metabolic parameters and sex steroids hormones. MVD was significantly associated with age (OR 1.04 [CI 95% 1-1.08, p=0.02]) and a tendency with low TT concentrations was noted (OR 2.28 [CI 95% 0.99-5.26, p=0.05]).

Discussion

In this cross-sectional study, we report for the first time a high prevalence of 43.5% of low TT concentrations in AC men with T2D regardless of their glycemic control. This result is similar to the prevalence from the study by Grossman et al. for a population of >500 Australian men with T2D [11] and to the results of Dhinsa et al showing a 43.7% prevalence of low testosterone levels in 103 men with T2D [12]. We found that the mean TT concentration was low but similar to that reported in previous studies for men with T2D from different ethnic groups [13-15]. However, it has been reported that TT concentrations are higher in black men compared with white men from the CARDIA study, and that the higher TT level could protect black people against cardiovascular disease [16,17]. A recent study of sex steroid concentrations reported a mean testosterone level of 16.82nmol/l in healthy European subjects and a mean testosterone level of 18.27 nmol/l in healthy non-diabetic AC subjects which is higher than the concentrations measured in our AC population with TD2 and confirms the relationship between sex steroid level and T2D [18]. However, no longitudinal controlled study has included a long-term analysis of these variables. Comparison with previous studies is difficult because of the heterogeneity of the populations studied. Analysis of testosterone concentrations must consider the variability resulting from the use of different kits for measurement of testosterone concentrations in different laboratories.

In our study, sex steroid levels did not differ according to the glucose profile. Previous studies have reported that the sex steroid concentrations are associated with the incidence of insulin resistance and T2D [3,19]. This result may reflect the small sample size of subjects with an HbA1c level>8% and the glucose control obtained because of their hospital follow-up. The recent literature has described associations between sex steroid concentrations and cardiovascular risk factors, including T2D, dyslipidemia, hypertension, and obesity [20-22]. We found an inverse correlation between TT level and BMI with a significant association between low TT concentrations and BMI after a logistic regression, as reported in other studies [11,12,22,23]. Testosterone level is inversely related to the amount of visceral fat and decreases with increasing BMI [24,25]. In the context of sex hormone deficiency, testosterone therapy improves BMI and fat mass [19,20,26]; the mechanisms remain unknown but may be related to the promotion of lipolysis by testosterone [20-22].

In our study, the frequency of dyslipidemia was higher in men with a low TT level compared with those with a normal level. Previous observational studies have reported that low testosterone level is associated with dyslipidemia and particularly with high LDL cholesterol and low HDL cholesterol levels, which confer a high risk for cardiovascular disease [27-29]. In this context, testosterone therapy might improve the lipid profile and reduce cardiovascular risk in hypogonadal men [4,20,26]. Dyslipidemia in association with low TT level is related to T2D and has also been described in non-diabetic men, which suggests that the glucose level is not involved in this relationship [30].

We also found an inverse correlation between sex steroid level and SBP. This relationship has been described in healthy men [31-33]. Different mechanisms have been linked to the effect of testosterone on the blood vessel wall, membrane ion channels, and aldosterone production. Testosterone deficiency may also increase sympathetic activity [34,35].

We found that the metabolic profile of men with a low SHBG level involved overweight, dyslipidemia, and hypertension, the latter of which occurs frequently in people of African descent.

We observed low TT levels when BMI exceeded 30 Kg/m² (definition of obesity) while low SHBG levels were observed once an overweight was occurred, with BMI ≥ 25 Kg/m² but this relation was not anymore at the stage of obesity. We wanted to highlight that variation

	CAD N=34 (27.4%)*	Non CAD	p	MVD N=41 (33%)*	Non MVD	p
Mean Age (yrs)	65.4 ± 10.2	60.3 ± 10.7	0.02	64.92 ± 10.43	60.0 ± 10.69	0.02
Mean FPG (mmol/l)	7.28 ± 2.99	7.38 ± 2.87		7.33 ± 3.03	7.36 ± 2.85	
Mean HBA1C (%)	7.64 ± 2.28	8.02 ± 2.67	-	7.95 ± 2.43	7.91 ± 2.65	-
HBA1C>8% (%)	23.3	37.2	-	30.6	35	-
Dyslipidemia (%)	64.7	53.3	-	63.4	53	-
Hypertension (%)	63.6	70.8	-	67.5	69.5	-
BMI (Kg/m^2)	28.65 ± 4.77	27.87 ± 4.79	-	28.45 ± 4.58	27.9 ± 4.88	-
Obesity (%)	32.4	24.4	-	31.7	24.1	-
Overweight (%)	23.5	24.4	-	24.4	24.1	-
Abdominal obesity (%)	63.6	74.4	-	66.7	73.8	-
Mean WC (cm)	100 ± 17.8	99.43 ± 12.2	-	99.69 ± 16.5	99.56 ± 12.62	-
Mean TT levels (nmol/l)	11.14 ± 6.02	12.41 ± 6.31	-	10.6 ± 5.83		-
Mean SHBG levels (nmol/l)	33.31 ± 21.18	36.87 ± 16.01	-	32.5 ± 21.26	12.79 ± 6.33	0.07
Low TT<10.4nmol/l (%)			-	56.1	37.57 ± 15.35	
Low SHBG<14 nmol/l (%)	52.9	40	-	7.3		-
	8.8	2.2	0.09		37.3	0.04
					2.4	

*Calculated from124 available data.

Table 4: Metabolic and sex steroid profiles according to Coronary Artery Disease (CAD) and Macrovascular Disease (MVD) status (after adjusting for age).

of SHBG would not linked to strong weight changes but probably to metabolic variations early observed in the presence of overweight such as metabolic syndrome, variations of fat mass or fat liver. In this study, the association between sex steroids and WC was not significant (data not shown) that may suggest that this clinical parameter would not be suitable. In a previous study of dysmetabolic subjects, we found that SHBG level correlated inversely with glucose and lipids levels but that this relationship depended on the intrahepatic fat content, which we did not measure in the present study [36]. Moreover, in our current study, SHBG concentrations correlated inversely with BMI and SBP, and this confirmed the data of previous studies of different ethnic populations [32,37]. SHBG concentrations could modulate BP through a direct effect in endothelial cells that express the SHBG receptor or interactions with the renin system [32].

We did not find differences in sex steroid concentrations according to CAD status or significant association between metabolic parameters, sex steroids and CAD after logistic regression (data non-shown), although previous observational studies have reported low TT concentrations in men with CAD [38-43]. The results of the logistic regression could be explained by the multiple confounding factors in the studied population (pharmacological treatment for dyslipidemia or for high blood pressure). We did not find difference in metabolic profiles between men with and without CAD, which suggests that sex steroid concentrations may not be an independent risk factor for CAD. Conversely, we found that low TT concentrations were more frequent in AC men with MVD compared with those without. This result suggests that TT may play an important role in the development of MVD. The literature includes reports of an association between low TT levels and atherosclerosis parameters; for example, the intima-media thickness of the carotid artery is inversely associated with TT levels [44]. The same results have been described in diabetic men [22], and our results are consistent with these earlier findings. However, the association between TT levels and cardiovascular diseases seems to be complex because some studies have found no association [45-47] or that both lower and higher endogenous testosterone concentrations are associated with an increased risk of arterial ischemic events, thereby indicating a J-shaped relationship between testosterone level and cardiovascular diseases [48]. Some limitations of our study must be mentioned such as the small sample size that could explain particularly the results of the logistic regression analysis, and the lack of information regarding the smoking status, the plasma insulin levels values or the intrahepatic fat evaluation that could be related to sex steroids concentrations.

Conclusion

This study assessed for the first time the prevalence of low TT level in a population of AC men with T2D. We found that TT and SHBG concentrations were lower in men with a metabolic profile at high cardiovascular risk or with vascular disease. Interventional and randomized studies with long-term follow-up are needed to analyze the effects of increasing sex steroid concentrations on the cardiometabolic profile and particularly on the incidence of MVD in this population.

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