

Steroids in Adult Men With Type 1 Diabetes

A tendency to hypogonadism

EVELINE W.C.M. VAN DAM, MD^{1,2}
 JACQUELINE M. DEKKER, PHD³
 EEF G.W.M. LENTJES, MD, PHD⁴
 FRED P.T.H.M. ROMIJN⁴

YVO M. SMULDERS, MD, PHD²
 WENDY J. POST⁵
 JOHANNES A. ROMIJN, MD, PHD¹
 H. MICHEL J. KRANS, MD, PHD¹

OBJECTIVE — To compare steroids and their associations in men with type 1 diabetes and healthy control subjects.

RESEARCH DESIGN AND METHODS — We studied 52 adult men with type 1 diabetes without microvascular complications, compared with 53 control subjects matched for age and BMI. Steroids and their binding globulins were assessed in a single venous blood sample and a 24-h urine sample.

RESULTS — In adult men with type 1 diabetes, total testosterone did not differ from healthy control subjects, but sex hormone-binding globulin (SHBG) (42 [14–83] vs. 26 [9–117] nmol/l, $P < 0.001$), cortisol-binding globulin (CBG; 0.87 ± 0.17 vs. 0.73 ± 0.10 nmol/l, $P < 0.001$), and cortisol levels (0.46 ± 0.16 vs. 0.39 ± 0.14 nmol/l, $P < 0.01$) were higher. The free testosterone index was lower (60 [17–139] vs. 82 [24–200], $P < 0.001$), and the calculated free testosterone was slightly lower (497 [115] vs. 542 [130], $P < 0.064$), but the pituitary-gonadal axis was not obviously affected in type 1 diabetes. The calculated free serum cortisol was not different, and 24-h urinary free cortisol excretion was lower in type 1 diabetes (121 [42–365] vs. 161 [55–284] nmol/24 h, $P < 0.009$). Testosterone was mainly associated with SHBG. Estimated portal insulin was a contributor to SHBG in control subjects but not in type 1 diabetes. Cortisol was associated with CBG. HbA_{1c} contributed to CBG in men with diabetes but not in control subjects, whereas estimated portal insulin did not contribute.

CONCLUSIONS — Adult men with fairly controlled type 1 diabetes without complications who are treated with subcutaneous insulin have a tendency to hypogonadism, as reflected by lower free testosterone levels in the presence of similar total testosterone levels and higher SHBG levels.

Diabetes Care 26:1812–1818, 2003

There are several reports about abnormalities of steroids in type 1 diabetes. Besides the well-known risk factors such as diabetes duration, hyperglycemia, hypertension, and hyperlipidemia (1), steroids may also be involved in the development of micro- and macrovascular complications. However, the data

about steroid levels presented in the literature are not unequivocal (2–17). Several factors, such as insulin treatment, insulin resistance, glucose levels, presence of complications such as neuropathy or retinopathy, age, and sex, are likely to be involved in the explanation of the variation of the results between the different publications. In most studies, testosterone levels did not differ between type 1 diabetes and control subjects (2,3,5,6), except in patients with neuropathy (15) or erectile dysfunction (14) and in adolescents (4), who all had lower levels of total testosterone. Because sex hormone-binding globulin (SHBG) levels are often increased, free testosterone levels may be lower in type 1 diabetes (5,11,12,18). Also, for other steroids, differences between type 1 diabetes and control subjects may be present. However, none of these studies described the associations between steroids and their binding globulins in a single homogenous study population.

Therefore, the objective of the present study was to assess serum steroid levels in a well-defined group of adult men with type 1 diabetes, compared with control subjects matched for age, sex, and BMI. We hypothesized that differences in steroid levels between men with type 1 diabetes and control subjects could be determined by differences in steroid-binding globulins, which in turn may be related to differences in insulin and glucose levels.

RESEARCH DESIGN AND METHODS

Subjects

We studied 52 men aged 30–45 years with type 1 diabetes of between 2 and 15 years duration and requiring insulin within 1 year after onset of diabetes, and we studied 53 healthy control subjects matched for age and BMI. Men with type 1 diabetes were recruited from the outpatient department of the diabetes unit of the Leiden University Medical Center and

From the ¹Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Leiden, the Netherlands; the ²Department of Internal Medicine, VU Medical Centre, Amsterdam, the Netherlands; the ³Institute for Research and Medicine, VU Medical Centre, Amsterdam, the Netherlands; the ⁴Department of Clinical Chemistry, Leiden University Medical Center, Leiden, the Netherlands; and the ⁵Department of Medical Statistics, Leiden University Medical Center, Leiden, the Netherlands.

Address correspondence and reprint requests to Eveline W.C.M. van Dam, Department of Internal Medicine 4 a 19, Vrije Universiteit Medical Centre, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands. E-mail: ew.vandam@vumc.nl.

Received for publication 4 October 2002 and accepted in revised form 5 March 2002.

Abbreviations: CBG, cortisol-binding globulin; DHEA, dehydroepiandrosterone; DHEAS, DHEA-sulfate; FSH follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone-binding globulin; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

© 2003 by the American Diabetes Association.

from five associated clinics. Control subjects were recruited by advertising in local newspapers. None of the men with type 1 diabetes had nephropathy or clinical signs of neuropathy and/or proliferative retinopathy. None of the subjects was using any medication except insulin in type 1 diabetes. The control subjects had no family history of diabetes up to the 2nd degree, and none had a fasting venous blood glucose <5.7 mmol/l. The purpose, nature, and possible risks of the study were explained to all subjects before consent was obtained. The study protocol was approved by the ethical committee of the Leiden University Medical Center.

All participants collected urine during 24 h on the day before admission. On the morning of admission before 9:30 A.M., a single venous blood sample was drawn from all participants in a sitting or supine position after a 10-h overnight fast and 45 min after insertion of an intravenous canula. In men with type 1 diabetes, the blood sample was drawn before their usual morning insulin injection. The blood was centrifuged within 30 min, and the serum was stored at -20°C . Body height and body weight were measured. Waist (minimum value between the iliac crest and the lateral costal margin) and hip circumference (maximum value over the buttocks) were measured, and the waist-to-hip ratio (WHR) was calculated to obtain information on the pattern of body fat distribution. Blood pressure was measured in a sitting position.

Assays

Serum glucose, creatinine, and urinary creatinine were measured on a Hitachi 747 analyzer (Roche, Almere, the Netherlands). HbA_{1c} was measured by ion exchange high-performance liquid chromatography on a Diamat (Bio-Rad, Veenendaal, the Netherlands). Testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and progesterone were measured by immunoluminometric assay on an Elecsys (Roche). Cortisol was estimated by a fluorescence polarization immunoassay on a TDx (Abbott, Amstelveen, the Netherlands) and SHBG by an immunoradiometric assay (Orion, Amersfoort, the Netherlands). Radioimmunoassays were used for the measurement of insulin and cortisol-binding globulin (CBG; Medgenix, Etten-Leur, the Netherlands), C-peptide

(Serono, Milan, Italy), androstenedione (after extraction), dehydroepiandrosterone (DHEA; DPC, Apeldoorn, the Netherlands), DHEA-sulfate (DHEAS; in-house method), 17-hydroxyprogesterone (OHP; DPC), estrone (DSL, Webster, Texas), and estradiol (Orion).

In men, the interassay coefficients of variation at physiological concentrations were as follows: testosterone 7%, SHBG 7%, FSH 4.5%, LH 12%, cortisol 6%, CBG 6%, estradiol 6%, estrone 7%, androstenedione 12%, DHEA 15%, DHEAS 12%, 17-hydroxyprogesterone 10%, progesterone 15%, insulin 12%, and C-peptide 12%. In this study, the samples were analyzed batchwise, thereby reducing assay variability.

Insulin concentrations in the portal vein were calculated, assuming a fasting portal venous-systemic insulin gradient of 2.4 in healthy control subjects. This latter value represents the mean of the values reported in the literature, thus providing a reasonable estimate of portal vein insulin concentrations (19–21). In men with type 1 diabetes, a value of 1 was used, which may overestimate portal insulin levels (11). For C-peptide levels below the detection limit of 0.020 nmol/l, a value of 0.010 nmol/l was used.

Free cortisol in serum was calculated, based on a system of three binding proteins (CBG, SHBG, and albumin) and 21 steroids, the binding affinities, and free hormone concentrations for the other steroids, except cortisol (22). The ratios of androstenedione to 17-hydroxyprogesterone and androstenedione to progesterone were used as an indication for 17-hydroxylase and 17,20-lyase activity (23). The ratio of testosterone to SHBG was used as a free testosterone index (24). In addition, free testosterone concentrations were calculated according to the method described by Vermeulen et al. (25).

Statistical analysis

The results in men with type 1 diabetes and control subjects were compared using Student's *t* tests for two independent samples; for skewed variables we used the Mann-Whitney *U* test. We subsequently evaluated whether observed differences of steroid levels between men with type 1 diabetes and control subjects were explained by differences in the steroid binding globulin levels, using linear regression analysis. Possible differences in the asso-

ciations between men with type 1 diabetes and control subjects were tested by including an interaction term into the model. We further investigated which components of the diabetic state (i.e., glucose, HbA_{1c} , or estimated portal insulin levels) explained differences in steroid binding globulin levels. We also adjusted for smoking and alcohol consumption. $P < 0.05$ was considered to be statistically significant. Computations were performed by using SPSS 10.0 for Windows (SPSS, Chicago, IL).

RESULTS

Clinical characteristics and glucose metabolism-related parameters

The clinical characteristics of men with type 1 diabetes and control subjects are shown in Table 1. By protocol, age and BMI as well as blood pressure and WHR were similar. Men with type 1 diabetes smoked more and consumed more alcohol than control subjects. The daily insulin dose of diabetes patients was ~ 58 units per day, a dose at which insulin antibodies are unlikely to be present (26). The estimated portal insulin levels were lower in men with type 1 diabetes than control subjects, despite peripheral hyperinsulinemia. C-peptide levels were <0.20 nmol/l in subjects with type 1 diabetes (27), except in two subjects who had C-peptide levels of 0.47 and 0.31 nmol/l, respectively. In two men with diabetes, hypoglycemia was found. No hypoglycemia during the day and night before admission was reported by any of the men with type 1 diabetes. Both serum and 24-h urinary creatinine were lower in type 1 diabetes. Albuminuria was not different between men with type 1 diabetes and control subjects.

Steroids and their binding globulins

As shown in Table 2, men with type 1 diabetes had similar testosterone, higher SHBG, a 25% lower free testosterone index, and a 10% lower calculated free testosterone than control subjects. FSH and estrone, but not LH or estradiol, were higher in men with type 1 diabetes. Men with type 1 diabetes had higher androstenedione, DHEA, and ratios of androstenedione to 17-hydroxyprogesterone and androstenedione to progesterone, compared with control subjects, whereas DHEAS did not differ. Total cortisol and CBG were higher in diabetes patients,

Table 1—Characteristics of men with type 1 diabetes and control subjects

Variable	Type 1 diabetes	Control subjects	P
n	52	53	
Age (years)	36.6 (4.0)	36.1 (4.5)	0.591
BMI (kg/m ²)	24 (2)	24 (3)	0.992
WHR (cm/cm)	0.86 (0.13)	0.87 (0.05)	0.415
Systolic blood pressure (mmHg)	134 (13)	135 (14)	0.665
Diastolic blood pressure (mmHg)	85 (7)	86 (6)	0.288
Alcohol (units/week [% users])	6 (0–63) [94]	4 (0–18) [85]	0.013
Smoking (number/day [% users])	1 (0–25) [50]	0 (0–22) [28]	0.015
Serum glucose (mmol/l)	10.9 (2.0–23.0)	4.7 (4.0–5.7)	<0.001
HbA _{1c} (%)	8.0 (5.8–13.1)	5.1 (4.3–6.1)	<0.001
Diabetes duration (years)	11 (4)	—	—
Daily insulin doses (units/day)	58 (13)	—	—
Serum insulin (mU/l)	17 (2–36)	11 (1.5–37)	<0.001
Estimated portal insulin (mU/l)	17 (2–36)	26 (4–89)	<0.001
Serum C-peptide (nmol/l)	0.01 (0.01–0.47)	0.51 (0.22–1.30)	<0.001
Serum creatinine (μmol/l)	85 (9)	90 (10)	0.021
Urinary creatinine (mmol/24 h)	14 (3)	16 (4)	0.002
Albuminuria (μg/min)	5 (2–83)	5 (0–40)	0.974

Normally distributed data are means ± SD, and skewed data are median (range). Differences between groups were analyzed using Student's *t* tests, or, if skewed, using the Mann-Whitney *U* test. The detection limit for C-peptide was 0.020 nmol/l.

thus free cortisol did not differ. Free urinary cortisol excretion was lower.

Multiple linear regression analysis revealed that the difference in total testosterone was practically fully explained by SHBG (Table 3). Adjustment for alcohol use and smoking did not materially affect the results (data not shown). Also, diabetes was no longer a significant determinant of the free testosterone index and of androstenedione after adjustment for SHBG. In contrast, diabetes remained independently associated with estrone, DHEA, the androstenedione-to-17-hydroxyprogesterone ratio and the androstenedione-to-progesterone ratio (Table 3). Separate analysis of the various components of the diabetic state in both groups revealed that SHBG was significantly associated with estimated portal insulin in control subjects and with long-term glycemic control, as reflected in HbA_{1c} levels, in men with diabetes. *P* values for interaction between diabetes and glucose, HbA_{1c}, and estimated portal insulin were 0.071, 0.830, and 0.210, respectively. Further adjustment for alcohol use and smoking did not affect the results (data not shown).

The difference in cortisol was largely explained by CBG (Table 3). Adjustment for alcohol consumption and smoking did not affect the results again (data not

shown). CBG was not associated with estimated portal insulin in both men with diabetes and control subjects (Table 4). However, CBG was associated with

HbA_{1c} in men with diabetes but not in control subjects. *P* values for interaction between diabetes and glucose, HbA_{1c}, and estimated portal insulin were 0.319, 0.840, and 0.481, respectively. Again, further adjustment for alcohol use and smoking did not affect the results (data not shown).

CONCLUSIONS— Adult men with type 1 diabetes have similar testosterone, higher SHBG, and a tendency to lower free testosterone compared with control subjects. They have higher FSH and estrone but not higher LH or estradiol levels. Men with type 1 diabetes also have higher androstenedione, DHEA, and ratios of androstenedione to 17-hydroxyprogesterone and androstenedione to progesterone, whereas DHEAS is not different. They also have higher total cortisol and CBG, whereas free cortisol is not different, and the urinary free cortisol excretion is lower. These differences could be partly explained by differences in steroid-binding globulin levels. Estimated portal insulin was a contributor to SHBG levels in control subjects but not in men with diabetes. CBG could be mostly explained by HbA_{1c} in men with diabetes but not in control subjects.

Table 2—Steroids and steroid-binding globulin concentrations in men with type 1 diabetes and in control subjects

Variable	Type 1 diabetes	Control subjects	P
n	52	53	
Testosterone (nmol/l)	24.7 (5.9)	22.9 (5.0)	0.096
SHBG (nmol/l)	42 (14–83)	26 (9–117)	<0.001
Free testosterone index (T*100/SHBG)	60 (17–139)	82 (24–200)	<0.001
Calculated free testosterone (pmol/l)	497 (115)	542 (130)	0.064
LH (units/l)	2.5 (0.9–8.0)	2.2 (0.8–9.2)	0.178
FSH (units/l)	4.7 (0.05–31.0)	3.5 (1.4–19.0)	0.036
Estradiol (pmol/l)	100 (20–311)	98 (20–203)	0.722
Estrone (pmol/l)	165 (53–353)	118 (46–197)	0.001
Androstenedione (nmol/l)	5.9 (1.8)	5.1 (1.7)	0.018
DHEA (nmol/l)	13.0 (3.9–44.0)	9.5 (3.7–21.0)	0.003
DHEAS (μmol/l)	6.9 (2.1)	7.3 (2.3)	0.399
DHEA/cortisol	31 (9–71)	24 (11–62)	0.113
Androstenedione/17-hydroxyprogesterone	1.3 (0.3–38.0)	1.0 (0.2–0.9)	0.003
Androstenedione/progesterone	20.6 (2.2–38.0)	16.0 (0.9–36.8)	0.026
Cortisol (μmol/l)	0.46 (0.16)	0.39 (0.14)	0.014
CBG (μmol/l)	0.87 (0.17)	0.73 (0.10)	0.001
Calculated free cortisol (nmol/l)	13 (3–80)	14 (3–77)	0.763
Urinary free cortisol (nmol/24 h)	121 (42–365)	161 (55–284)	0.009

Normal distributed data are means ± SD, and skewed data are median (range). Differences between groups were analyzed using Student's *t* tests, or, if skewed, using the Mann-Whitney *U* test.

Table 3—Steroid hormone concentrations, the contribution of SHBGs

	Type 1 diabetes	P	SHBG (nmol/l)	CBG (nmol/l)	P
Testosterone (nmol/l)					
Model I	1.79 ('-0.32 to 3.91)	0.096	—	—	—
Model II	-0.02 ('-2.14 to 2.09)	0.984	0.14 (0.08-0.20)	—	<0.001
Free testosterone index (T*100/SHBG)					
Model I	-23.8 ('-35.4 to -12.2)	<0.001	—	—	—
Model II	-6.9 ('-16.1 to 2.4)	0.144	-1.3 ('-1.6 to '-1.0)	—	<0.001
Calculated free testosterone (pmol/l)					
Model I	-45 ('-92 to '-3)	0.064	—	—	—
Model II	-15 ('-64 to 35)	0.552	-2 ('-4 to '-1)	—	0.002
Estrone (pmol/l)					
Model I	41.3 (20.5-62.2)	<0.001	—	—	—
Model II	39.1 (16.4-61.7)	0.001	0.2 ('-0.5 to 0.8)	—	0.606
Androstenedione (nmol/l)					
Model I	0.8 (0.1-1.5)	0.018	—	—	—
Model II	0.5 ('-0.2 to 1.2)	0.183	0.03 (0.004-0.047)	—	0.019
DHEA (nmol/l)					
Model I	4.5 (1.9-7.2)	0.001	—	—	—
Model II	4.2 (1.4-7.1)	0.004	0.02 ('-0.06 to 0.11)	—	0.595
Androstenedione/ 17-hydroxyprogesterone					
Model I	0.35 (0.15-0.55)	0.001	—	—	—
Model II	0.32 (0.10-0.54)	0.005	0.003 ('-0.004 to 0.009)	—	0.449
Androstenedione/progesterone					
Model I	3.7 (0.3-7.2)	0.035	—	—	—
Model II	4.3 (0.6-8.0)	0.025	-0.04 ('-0.16 to 0.07)	—	0.426
Cortisol (μ mol/l)					
Model I	0.074 (0.016-0.132)	0.014	—	—	—
Model II	0.044 ('-0.020 to 0.108)	0.176	—	0.214 (0.009-0.420)	0.041
Urinary free cortisol (nmol/ 24 h)					
Model I	-23.9 ('-49.1 to 1.4)	0.064	—	—	—
Model II	-22.4 ('-50.74 to 6.0)	0.121	—	-10.8 ('-101.7 to 80.0)	0.814

Linear regression analysis was used in men with type 1 diabetes and in control subjects. Data are regression coefficients (95% CIs). Model I is a crude model, and the results can be interpreted as the mean difference between type 1 diabetic and control subjects. Model II includes diabetes and SHBG or CBG.

Gonadal steroids and gonadotropins

This study confirms previously reported similar total testosterone levels in both type 1 diabetes and control subjects (2,3,5,6). Thus, chronic hyperinsulinemia, which is assumed to be present in type 1 diabetes, does not seem to affect total testosterone levels, which is in contrast to short-term hyperinsulinemia (10,23). In this study, total testosterone levels were explained by SHBG. SHBG is the transport protein for testosterone, and it binds ~44% of testosterone. In HepG2 cells, insulin inhibits the production and secretion of SHBG (28). In healthy men, low insulin levels induced by the administration of diazoxide increase SHBG levels (29). Indeed, in this study SHBG was

inversely related to estimated portal insulin in control subjects. Yki-Jarvinen et al. (11) previously showed that portal insulin and not insulin sensitivity determines SHBG in type 1 diabetes and healthy control subjects. Although in this study the free testosterone was lower in type 1 diabetes, LH was not different and FSH was slightly higher and not related to the lower free testosterone, not even in the four men with type 1 diabetes who had a lower calculated free testosterone level than any of the control subjects. It possibly indicates a tendency toward hypogonadism, under the assumption that biological activity of testosterone in non-hepatic tissues is likely to be a function of free hormone concentration (30,31). The

free testosterone index was used because of its widespread use. However, calculated free testosterone may be a better indicator of the free fraction than the free testosterone index, and calculated free testosterone indicates that the difference with nondiabetic men is ~10% (24). Hypogonadism was previously shown to be associated with neuropathy (15) and with erectile dysfunction in men with type 1 diabetes (14).

Estrone levels were higher in men with diabetes than in control subjects. Because both groups had a similar BMI, the lower serum and 24-h urinary creatinine level in men with type 1 diabetes may indicate the presence of lower muscle tissue mass and thus more body fat in these

Table 4—Steroid binding globulin concentrations, the contribution of components of diabetes (i.e., glucose levels, HbA_{1c}, and estimated portal insulin)

	Type 1 diabetes	Glucose (mmol/l)	P	HbA _{1c} (%)	P	Portal insulin (mU/l)	P
SHBG (nmol/l)							
Model I (all)	13.1 (7.0–19.2)	—	<0.001	—	—	—	—
Model II							
All	—	0.03 (–0.5 to 1.0)	0.472	2.5 (0.4–4.7)	0.019	–0.3 (–0.5 to –0.1)	0.014
Type 1 diabetes	—	0.4 (–0.5 to 1.3)	0.407	1.3 (–2.2 to 4.7)	0.462	–0.01 (–0.45 to 0.42)	0.947
Control subjects	—	–7.0 (–17.6 to 3.5)	0.186	6.2 (–0.5 to 17.9)	0.288	–0.4 (–0.7 to –0.1)	0.009
CBG (nmol/l)							
Model I	138 (84–193)	—	<0.001	—	—	—	—
Model II							
All	—	–3.9 (–10.3 to 2.4)	0.223	57 (39.3–74.8)	<0.001	–0.3 (–2.2 to 1.7)	0.789
Type 1 diabetes	—	–2.8 (–11.6 to 6.0)	0.526	58.1 (2.2–91.0)	0.001	1.1 (–3.1 to 5.2)	0.611
Control subjects	—	38.2 (–36.5 to 112.8)	0.309	62.5 (–20.3 to 145.2)	0.136	–1.3 (–3.2 to 0.70)	0.202

Data are regression coefficients (95% CI). Linear regression analysis in all participants and, separately, in men with type 1 diabetes and control subjects. Model I is a crude model, and the results can be interpreted as the mean difference between type 1 diabetes and control subjects. Model II includes glucose, HbA_{1c}, and (calculated) portal insulin levels. Portal insulin, estimated portal insulin.

men. Therefore, the higher estrone levels may be caused by higher androstenedione or substrate levels, higher SHBG levels, and possibly by a higher body fat mass in type 1 diabetes. These hypotheses were not supported by regression analysis. Unfortunately, we did not include any measure for body composition.

Adrenal steroids

Higher DHEA and androstenedione were found in men with type 1 diabetes. Gluud et al. (2) also reported this in the first year after onset of type 1 diabetes. In healthy men, short-term hyperinsulinemia is assumed to inhibit 17,20-lyase or oxidative cleavage of C17–20 bond activity (23). However, the higher androstenedione-to-17-hydroxyprogesterone ratio may suggest higher 17,20-lyase activity in type 1 diabetes, despite peripheral hyperinsulinemia. In this study, androstenedione levels were not explained by insulin or glucose levels (data not shown) but at least partly by SHBG levels.

We also found higher total cortisol in type 1 diabetes, which could be explained by the higher CBG. CBG is the major transport protein for cortisol and binds almost 90% of cortisol (22). CBG may regulate availability of steroid to tissues (32). In HepG2 cells, insulin seems to inhibit CBG (33,34) secretion. However, the mechanisms involved in the regulation of CBG levels are complex (34,35). Some clinical data (36) suggest suppressive effects of portal insulin on CBG release by the liver. However, we found no association of CBG with estimated portal

insulin. We did, however, find a positive association of CBG with HbA_{1c} in men with diabetes. A comparable positive association of CBG and HbA_{1c} was found in obese (glucose-intolerant) otherwise healthy humans (37), in whom CBG was negatively associated with the insulin response to a glucose challenge. The higher HbA_{1c} might reflect a condition of more severe insulin deprivation of tissues, including the liver. The similar calculated free cortisol, as measured in a single sample, and the lower 24-h urinary free cortisol excretion suggest a lower diurnal cortisol secretion in these selected men with type 1 diabetes. This is in contrast to the higher daytime urinary free cortisol excretion found in adolescents with type 1 diabetes (38). The difference may be caused by the frequently occurring fluctuations in glucose levels in adolescents. Hypoglycemia (for example nightly) can also increase cortisol levels, but in the present study, the patients did not report hypoglycemia. The ratio of DHEA to cortisol was not different in type 1 diabetes compared with control subjects, despite significant hyperglycemia in diabetes. Thus, the diversion of steroid synthesis from adrenal androgens to glucocorticoids, as shown previously in poorly controlled type 1 diabetes (6,7) and several other disease states (39,40), may be caused by underlying disease rather than elevated glucose levels.

Levels of DHEAS and other adrenal steroids (e.g., 17-hydroxyprogesterone and progesterone) were not different. DHEAS shows an age-related decline

(41). Low DHEAS, even adjusted for age, is associated with cardiovascular disease, insulin resistance, and concomitant long-term hyperinsulinemia (42). Short-term (4–6 h) hyperinsulinemia is reported to decrease DHEAS levels in normal men (43) and men with type 1 diabetes (10) or type 2 diabetes (44). Nevertheless, we found no lower DHEAS levels, despite chronic peripheral hyperinsulinemia in these selected men with type 1 diabetes. This finding does not support an inhibiting effect of chronic peripheral hyperinsulinemia on DHEAS levels.

Compared with previous studies, this study has the advantage of studying a spectrum of steroids in a relatively large homogenous group of men with type 1 diabetes and control subjects under standardized conditions. Limitations of this study are that we studied the subjects using only a single sample in the morning, whereas cortisol varies considerably over the day and testosterone varies from week to week (45). Free cortisol levels have not been measured, which may have obscured possible differences. However, the calculated free cortisol and 24-h urinary free cortisol levels did not suggest cortisol excess. We used the morning insulin level as an estimate of whole-day insulin exposure under the assumption that periprandial higher insulin levels contribute little to the daily insulin exposure of steroid-producing tissues and the steroid-binding globulin-producing liver. This may be reasonable in control subjects, but it may be more complex in subjects with type 1 diabetes, in whom serum insulin may be

determined predominantly by the insulin dose from the evening before the study. This may have obscured possible relations between estimated portal insulin and SHBG or CBG. Daily insulin dose was not associated with steroids and/or steroid-binding globulins. Despite these limitations, the data add considerably to the knowledge about steroids and steroid-binding globulins in type 1 diabetes.

In conclusion, adult men with type 1 diabetes treated with subcutaneous insulin have a tendency to hypogonadism. Hypogonadism was reflected in slightly lower free testosterone index levels attributable to similar total testosterone levels combined with higher SHBG levels. Although not confirmed in this study, the higher SHBG levels may be related to lower portal insulin levels.

Acknowledgments—This study was supported in part by the Dutch Diabetes Fund (grant no. 92.702).

We thank K. Bakker, H. van Houten, J.W.F. Elte, P.H.L.M. Geelhoed-Duijvestijn, and A.B. Arntzenius, who allowed us to study their patients.

References

- Ebeling P, Koivisto VA: Occurrence and interrelationships of complications in insulin-dependent diabetes in Finland. *Acta Diabetol* 34:33–38, 1997
- Gluud C, Madsbad S, Krarup T, Bennett P: Plasma testosterone and androstenedione in insulin-dependent patients at time of diagnosis and during the first year of insulin treatment. *Acta Endocrinol (Copenh)* 100:406–409, 1982
- Madsbad S, Gluud C, Bennett P, Krarup T: Rapid changes in plasma androgens during insulin withdrawal in male type 1 (insulin-dependent) diabetics. *J Endocrinol Invest* 9:21–25, 1986
- Arreola F, Paniagua R, Herrera J, Diaz-Bensussen S, Mondragon L, Bermudez JA, Perez PE, Villalpando S: Low plasma zinc and androgen in insulin-dependent diabetes mellitus. *Arch Androl* 16:151–154, 1986
- Small M, MacRury S, Beastall GH, MacCuish AC: Oestradiol levels in diabetic men with and without a previous myocardial infarction. *Q J Med* 64:617–623, 1987
- Cohen HN, Paterson KR, Wallace AM, Beastall GH, Manderson WG, MacCuish AC: Dissociation of adrenarche and gonadarche in diabetes mellitus. *Clin Endocrinol (Oxf)* 20:717–724, 1984
- Couch RM: Dissociation of cortisol and adrenal androgen secretion in poorly controlled insulin-dependent diabetes mellitus. *Acta Endocrinol (Copenh)* 127:115–117, 1992
8. Ghizzoni L, Vanelli M, Virdis R, Alberini A, Volta C, Bernasconi S: Adrenal steroid and adrenocorticotropin responses to human corticotropin-releasing hormone stimulation test in adolescents with type 1 diabetes mellitus. *Metabolism* 42:1141–1145, 1993
9. Almqvist EG, Groop LC, Manhem PJ: Hypothalamic-pituitary-adrenal response to different tests in type 1 diabetes mellitus. *Scand J Clin Lab Invest* 61:557–565, 2001
10. Ebeling P, Stenman UH, Seppala M, Koivisto VA: Androgens and insulin resistance in type 1 diabetic men. *Clin Endocrinol (Oxf)* 43:601–607, 1995
11. Yki-Jarvinen H, Makimattila S, Utriainen T, Rutanen EM: Portal insulin concentrations rather than insulin sensitivity regulate serum sex hormone-binding globulin and insulin-like growth factor binding protein 1 in vivo. *J Clin Endocrinol Metab* 80:3227–3232, 1995
12. Christensen L, Hagen C, Henriksen JE, Haug E: Elevated levels of sex hormones and sex hormone binding globulin in male patients with insulin dependent diabetes mellitus: effect of improved blood glucose regulation. *Dan Med Bull* 44:547–550, 1997
13. Valimaki M, Liewendahl K, Nikkanen P, Pelkonen R: Hormonal changes in severely uncontrolled type 1 (insulin-dependent) diabetes mellitus. *Scand J Clin Lab Invest* 51:385–393, 1991
14. Alexopoulou O, Jamart J, Maiter D, Hermans MP, De Hertogh R, De Nayer P, Buysschaert M: Erectile dysfunction and lower androgenicity in type 1 diabetic patients. *Diabetes Metab* 27:329–336, 2001
15. Ali ST, Shaikh RN, Ashfaqiddiqi N, Siddiqi PQ: Serum and urinary levels of pituitary-gonadal hormones in insulin-dependent and non-insulin-dependent diabetic males with and without neuropathy. *Arch Androl* 30:117–123, 1993
16. Haffner SM, Klein R, Moss SE, Klein BE: Sex hormones and the incidence of severe retinopathy in male subjects with type 1 diabetes. *Ophthalmology* 100:1782–1786, 1993
17. Meyer K, Deutscher J, Anil M, Berthold A, Bartsch M, Kiess W: Serum androgen levels in adolescents with type 1 diabetes: relationship to pubertal stage and metabolic control. *J Endocrinol Invest* 23:362–368, 2000
18. Holly JM, Dunger DB, al Othman SA, Savage MO, Wass JA: Sex hormone binding globulin levels in adolescent subjects with diabetes mellitus. *Diabet Med* 9:371–374, 1992
19. Blackard WG, Nelson NC: Portal and peripheral vein immunoreactive insulin concentrations before and after glucose infusion. *Diabetes* 19:302–306, 1970
20. Pelkonen R, Kallio H, Suoranta H, Karonen SL: Plasma insulin, C-peptide, and blood glucose in portal, hepatic and peripheral veins in liver cirrhosis. Effect of intravenous tolbutamide. *Acta Endocrinol (Copenh)* 97:496–502, 1981
21. Porksen N, Grofte T, Greisen J, Mengel A, Juhl C, Veldhuis JD, Schmitz O, Rossle M, Vilstrup H: Human insulin release processes measured by intraportal sampling. *Am J Physiol Endocrinol Metab* 282:E695–E702, 2002
22. Dunn JF, Nisula BC, Rodbard D: Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. *J Clin Endocrinol Metab* 53:58–68, 1981
23. Nestler JE, McClanahan MA, Clore JN, Blackard WG: Insulin inhibits adrenal 17,20-lyase activity in man. *J Clin Endocrinol Metab* 74:362–367, 1992
24. Nanjee MN, Wheeler MJ: Plasma free testosterone—is an index sufficient? *Ann Clin Biochem* 22:387–390, 1985
25. Vermeulen A, Verdonck L, Kaufman JM: A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab* 84:3666–3672, 1999
26. Kurtz AB, Nabarro JD: Circulating insulin-binding antibodies. *Diabetologia* 19:329–334, 1980
27. Bloomgarden ZT: Immunologic issues in type 1 diabetes. *Diabetes Care* 24:2143–2149, 2001
28. Plymate SR, Matej LA, Jones RE, Friedl KE: Inhibition of sex hormone-binding globulin production in the human hepatoma (Hep G2) cell line by insulin and prolactin. *J Clin Endocrinol Metab* 67:460–464, 1988
29. Pasquali R, Casimirri F, de lasio R, Mesini P, Boschi S, Chierici R, Flaminia R, Biscotti M, Vicennati V: Insulin regulates testosterone and sex hormone-binding globulin concentrations in adult normal weight and obese men. *J Clin Endocrinol Metab* 80:654–658, 1995
30. Vermeulen A, Stoica T, Verdonck L: The apparent free testosterone concentration, an index of androgenicity. *J Clin Endocrinol Metab* 33:759–767, 1971
31. Mendel CM: The free hormone hypothesis: a physiologically based mathematical model. *Endocr Rev* 10:232–274, 1989
32. Breuner CW, Orchinik M: Plasma binding proteins as mediators of corticosteroid action in vertebrates. *J Endocrinol* 175:99–112, 2002
33. Crave JC, Lejeune H, Brebant C, Baret C, Pugeat M: Differential effects of insulin and insulin-like growth factor I on the

- production of plasma steroid-binding globulins by human hepatoblastoma-derived (Hep G2) cells. *J Clin Endocrinol Metab* 80:1283–1289, 1995
34. Rosner W: The functions of corticosteroid-binding globulin and sex hormone-binding globulin: recent advances. *Endocr Rev* 11:80–91, 1990
35. Heyns W, Coolens JL: Physiology of corticosteroid-binding globulin in humans. *Ann N Y Acad Sci* 538:122–129, 1988
36. Anderson KE, Rosner W, Khan MS, New MI, Pang SY, Wissel PS, Kappas A: Diet-hormone interactions: protein/carbohydrate ratio alters reciprocally the plasma levels of testosterone and cortisol and their respective binding globulins in man. *Life Sci* 40:1761–1768, 1987
37. Fernandez-Real JM, Grasa M, Casamitjana R, Pugeat M, Barret C, Ricart W: Plasma total and glycosylated corticosteroid-binding globulin levels are associated with insulin secretion. *J Clin Endocrinol Metab* 84:3192–3196, 1999
38. Dacou-Voutetakis C, Peppas-Patrikiou M, Dracopoulou M: Urinary free cortisol and its nyctohemeral variation in adolescents and young adults with IDDM: relation to endothelin 1 and indices of diabetic angiopathy. *J Pediatr Endocrinol Metab* 11:437–445, 1998
39. Semple CG, Gray CE, Beastall GH: Adrenal androgens and illness. *Acta Endocrinol (Copenh)* 116:155–160, 1987
40. Zumoff B, Walsh BT, Katz JL, Levin J, Rosenfeld RS, Kream J, Weiner H: Subnormal plasma dehydroisoandrosterone to cortisol ratio in anorexia nervosa: a second hormonal parameter of ontogenic regression. *J Clin Endocrinol Metab* 56:668–672, 1983
41. Orentreich N, Brind JL, Vogelman JH, Andres R, Baldwin H: Long-term longitudinal measurements of plasma dehydroepiandrosterone sulfate in normal men. *J Clin Endocrinol Metab* 75:1002–1004, 1992
42. Barrett-Connor E, Khaw KT, Yen SS: A prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease. *N Engl J Med* 315:1519–1524, 1986
43. Ebeling P, Stenman UH, Seppala M, Koivisto VA: Acute hyperinsulinemia, androgen homeostasis and insulin sensitivity in healthy man. *J Endocrinol* 146:63–69, 1995
44. Katsuki A, Sumida Y, Murashima S, Fujii M, Ito K, Tsuchihashi K, Murata K, Yano Y, Shima T: Acute and chronic regulation of serum sex hormone-binding globulin levels by plasma insulin concentrations in male noninsulin-dependent diabetes mellitus patients. *J Clin Endocrinol Metab* 81:2515–2519, 1996
45. Morley JE, Patrick P, Perry HM III: Evaluation of assays available to measure free testosterone. *Metabolism* 51:554–559, 2002