Partial Androgen Deficiency in Aging Type 2 Diabetic Men and Its Relationship to Glycemic Control

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Aging in the male is associated with both a higher incidence of type 2 diabetes and hypogonadism. However, little information is available about the complex of symptoms and hormonal changes related to partial androgen deficiency in aging (called andropause) in type 2 diabetic men. Here, for the first time, we used a combination of clinical and hormonal criteria to define andropause and to analyze the relationships between the androgen environment and glucose metabolism in 55 type 2 diabetic men (63.6 ± 7.9 years, mean ± SD). Low plasma levels of total testosterone (≤3.4 ng/mL) and free testosterone (≤11 pg/mL) were found in 20% and 54.5%, respectively, of the diabetic men. The fraction of diabetic men with subnormal levels of total testosterone increased with aging: 14.2% (50 to 59 years), 17.4% (60 to 69 years) and 36% (> 70 years). The corresponding figures for subnormal values of free testosterone were 38%, 69.6%, and 54.5%, respectively. In the whole group of type 2 diabetic men, no significant linear correlations between total or free testosterone with fasting plasma glucose, insulin, C-peptide, or fructosamine values could be established. Total testosterone was positively correlated with glycosylated haemoglobin (HbA_{1c}) levels ($r = .322$, $P = .01$). Although fasting plasma glucose was marginally higher in aging type 2 diabetic patients with andropause than in those without andropause (162 ± 6.9 *v* 139 ± 8.9, mean ± SEM, $P = .05$), there were no differences between both subgroups for plasma fasting insulin, C-peptide, fructosamine, or HbA_{1c} levels. Replacement therapy (150 mg intramuscular [IM] of enanthate of testosterone every 14 days for 6 months) was applied in 10 type 2 diabetic men with clinical features of andropause associated with subnormal concentrations of serum testosterone. The treatment induced significant increases in total plasma testosterone (baseline: 3.9 ± 0.3; at 6 months: 7.1 ± 0.9 ng/mL, mean ± SEM, $P = .003$) and free testosterone (baseline: 9.3 ± 0.6; at 6 months 17.6 ± 2.4 pg/mL, $P = .003$), but had a neutral effect on overall glycemic control. These data show a high prevalence of andropause in aging type 2 diabetic men and suggest that the endogenous androgen environment, as well as correction of the partial androgen deficiency, do not have a meaningful effect on glycemic control.

AGING IN THE male is associated with a higher prevalence of diabetes mellitus[1] and exerts a longitudinal effect on serum total and free testosterone levels in healthy men, thus increasing the prevalence of hypogonadism.[2]

Although elderly patients with diabetes are now living longer and diabetes in aged adults may prove to be the most important epidemic of the 21st century,[3] little is known about "andropause" in aging diabetic patients, understanding with the term andropause a syndrome of somatic, psychologic, and hormonal changes related to the aged-associated decline in testosterone levels.[4] We believe the issue to be of potential interest considering various facts. Men with type 2 diabetes mellitus exhibit lower circulating testosterone levels than age-matched controls,[5] and low plasma levels of total testosterone in men predict insulin resistance and the development of type 2 diabetes in older adults.[6] Total and free testosterone serum values are significantly and negatively correlated with the waist-to-hip ratio, and low testosterone levels are strongly associated with a decrease in total and nonoxidative whole-body glucose disposal in men.[7] Large population-based studies have confirmed that men with type 2 diabetes have significantly lower levels of total testosterone and show that total testosterone and fasting blood glucose are inversely correlated in men.[8] On the other hand, treatment with testosterone in men over 65 years of age with low-normal serum testosterone levels decreases the body fat mass and increases lean body mass,[9] a change that theoretically improves insulin sensitivity. Some previous studies have shown that testosterone administration to middle-aged, obese men with relative hypogonadism improves insulin sensitivity[10] and that other forms of androgen therapy (19-nortestosterone) enhances insulin-independent glucose uptake in men.[11] Thus, it is possible that testosterone replacement therapy may have some influence on glycemic control in type 2 diabetic men. However, to our knowledge, the effect of testosterone replacement therapy in type 2 diabetic men has never been tested.

To gain further insight into the influence of androgenic status on the parameters of glucose metabolism in aging type 2 diabetic men, we performed a prospective study using several approaches. First, we studied the clinical and hormonal characteristics that define andropause in aging diabetic patients. Second, we explored the effect of the relative deficit of androgens on some parameters of glucose metabolism in such patients. Finally, some type 2 diabetic patients meeting both clinical and hormonal criteria of andropause were treated with testosterone to assess, for the first time, its possible influence on glycemic control.

PATIENTS AND METHODS

Patients and Control Subjects

Fifty-five men older than 50 (range, 51 to 86 years, mean ± SEM: 63.6 ± 1 years) with type 2 diabetes entered the study. Their body mass

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Table 1. Questionnaire of Androgen Deficiency in Aging Men

1. Do you have a decrease in libido (sexual drive)?
2. Do you have a decrease in strength and/or endurance?
3. Are you sad and/or grumpy?
4. Are your erections less strong?
5. Do you have a decrease in sexual activity?
6. Do you have less body hair?
7. Do you have a decrease in memory for recent events?
8. Do you have hot flushes?

index (BMI) was 27.6 ± 0.45 kg/m^2 (range, 19.1 to 34.4). Diagnosis of diabetes mellitus was established by the history or by the American Diabetes Association (ADA) criteria[12] in patients with recent onset of diabetes. Type 2 diabetes was diagnosed on the basis of a history of initial successful treatment with oral hypoglycemic agents, as well as by assessment of residual β-cell function, measuring C peptide serum levels. All the patients had C-peptide serum levels above the limit of detection of the radioimmunoassay (RIA), and in 50 of 52 patients, the concentration was within the range of normality (0.20 to 1.46 pmol/mL, confidence interval [CI] 95%). The remaining 2 had C-peptide values of 0.13 and 0.19 pmol/mL. Inclusion criteria included a positive history of fertility (indicating previous testicular health), a healthy partner (to avoid that the answers to the questionnaire used to obtain the clinical criteria of andropause could be influenced by exogenous factors), and the absence of clinically overt hypogonadism, the absence of hepatic, cardiac, or renal insufficiency, and a lack of ingestion of drugs that could induce hypogonadism. Exclusion criteria included diseases in which treatment with testosterone should be contraindicated, such as prostate cancer, active benign prostatic hyperplasia, breast carcinoma, sleep apnea, or chronic lung disease. All the patients were ambulatory and were seen in the outpatient clinic. Informed written consent was obtained after the purpose and the procedures of the study had been described. The Local Research Ethics Committee of the Hospital Universitario of Salamanca approved the study protocol.

The duration of diabetes ranged from 2 months to 38 years. Fifteen patients were treated only with insulin, 14 with oral hypoglycemic agents plus insulin, and 26 with 1 or a combination of oral hypoglycemic agents. Eleven patients had clinically evident atherosclerotic complications.

To compare hormonal serum levels in aging diabetic men, we included 2 groups of control subjects. One comprised 32 healthy subjects, aged 33 ± 5 years, in whom fertility had been demonstrated in the previous 2 years. The second group included 8 healthy men without diabetes mellitus and who were of similar age as the diabetic patients (range, 52 to 76 years; mean ± SEM: 64.4 ± 3) and with a similar BMI (mean ± SEM: 26.8 ± 1.2 kg/m^2).

Clinical and Hormonal Criteria of Andropause

To obtain the clinical criteria of andropause, we used a questionnaire consisting of 8 items related to androgen deficiency (Table 1); this was given to the patient in the presence of his partner. The first 4 questions were the same as in the validated Androgen Deficiency in the Aging Male (ADAM) questionnaire of the University of Saint Louis.[13] They were included in our test because in the analysis of the items of the ADAM questionnaire they exhibited the highest statistical significance for differentiation between males with low versus normal bioavailable testosterone levels. Our questionnaire included another 4 items (questions 5 to 8) different from those of the ADAM. These 4 additional items were included because we believe that they better reflect clinical androgen deficiency than the remaining 6 questions of the ADAM questionnaire and may indeed improve its specificity (60%). Questions 5 to 8 of the questionnaire reflect the classic symptoms related to the decrease in androgen levels in elderly men,[14] which have also been included in an aging males's symptoms rating scale.[15] In 93% of the visits, the questionnaire was applied by the same person (J.J.C.) and in the remaining visits by R.M.B. We considered that the questionnaire was positive and hence the patient met the clinical criterion of andropause when an affirmative answer ("yes") to questions 1 or 4 or to at least another 4 of the remaining questions was obtained. A negative result of the questionnaire was defined as a negative answer ("no") to questions 1 or 4 or to at least another 4 of the remaining questions, thus employing stricter criteria than in the ADAM questionnaire. Our questionnaire had a sensitivity of 90% and a specificity of 74% when applied to type 2 diabetic patients.[16]

We used several hormonal criteria to define partial or relative androgen deficiency in aging men. Criterion A is a total testosterone level in serum of ≤3.4 ng/mL, which represents the mean minus 1 SD of our healthy young controls. This figure is similar or identical to the level used to define biochemical andropause by authorities in the field[17] or in recent epidemiologic studies.[2] Criterion B was a total serum testosterone level of ≤4.2, which represents the 25th percentile of our young healthy control population. A lower percentile was not chosen to avoid the inclusion of overtly hypogonadal subjects. Criterion C was a free testosterone level of ≤11pg/mL, which represents the lower end of the normal range in young adults. According to this definition, we considered that andropause exists when the patient presents both the biochemical and clinical criteria.

Hormone and Other Measurements

Blood samples were obtained simultaneously for all the measurements between 8 AM to 9 AM. Plasma glucose levels were assayed by a glucose oxidase method. The techniques used for hormone measurements have been described in detail elsewhere.[18,19] Total and free testosterone concentrations were measured by solid-phase ^{125}I RIA (Coat-a-Count, DPL, Los Angeles, CA). Luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin (PRL) levels were determined by RIA (RIA-gnost; CIS Bio International, Gif-sur-Yvette, France). Dehydroepiandrosterone sulphate (DHEAS) was determined using a commercial RIA (Immunotech, Marseille, France). Prostate-specific antigen (PSA) serum levels were measured by RIA (RIA-React; CIS Bio International). Insulin was measured using an immunoradiometric assay (INS-Irma; Biosource, Nivelles, Belgium). C-peptide levels were measured using a commercial RIA (C-PEP; Biosource, Nivelles, Belgium). Glycosylated hemoglobin (HbA_{1c}) was measured using a commercial kit (Menarini Diagnostics, Firenze, Italy). Fructosamine was measured using a commercial kit (Fruc; Roche Diagnostics, Mannheim, Germany).

The sensitivity and the intra- and interassay variation coefficients of the methods were: 0.04 ng/mL, 5.7% and 6.7%, respectively, for total testosterone; 0.15 pg/mL, 5.4% and 8.5% for free testosterone; 0.1 mIU/mL, 2.9% and 3% for LH; 0.1 mIU/mL, 3.4% and 3% for FSH; 2 μIU/mL, 6.9% and 7.8% for PRL; 0.04 ng/mL, 2.2% and 4.2% for PSA; 1.5 μIU/mL, 6.9% and 8.1% for insulin; 0.04 pmol/mL, 8.2% and 9.3% for C-peptide; 2.5%, 5.2%, and 5.2% for HbA_{1c}; 10 μmol/L, 0.9%, and 2.9% for fructosamine.

Treatment With Testosterone

Ten patients, age 63.8 ± 1.9 years (mean ± SEM; range, 55 to 73 years) were treated with 150 mg intramuscular (IM) testosterone enanthate every 15 days for 6 months. They were selected because they had the clinical criteria of andropause associated with a total testosterone ≤3.4 ng/mL and/or a free testosterone serum level of ≤11 pg/mL and agreed to enter the protocol. The duration of their diabetes ranged between 2 and 21 years. Four were treated only with insulin, 3 with

Table 2. Mean (± SEM) Values of Hormone Serum Levels and Parameters of Glucose Metabolism

	Young Controls	Aging Controls	Diabetic Men
N	32	8	55
Total testosterone (ng/mL)	6.2 ± 0.5	5.2 ± 0.6*	4.5 ± 0.2*
Free testosterone (pg/mL)	16.6 ± 0.6	11.8 ± 1.5*	11.0 ± 0.3*
LH (mIU/mL)	3.2 ± 0.4	3.0 ± 0.7	5.8 ± 0.6*
FSH (mIU/mL)	4.5 ± 0.4	6.4 ± 1.2	8.5 ± 0.6*
PRL (μIU/mL)	272 ± 24	189 ± 24	225 ± 15
Fasting plasma glucose (mg/dL)	ND	85 ± 2.5	149 ± 5.9‡
Fasting insulin (μIU/mL)	ND	12.9 ± 3.7	13.7 ± 1.3
Fasting C-peptide (pmol/mL)	ND	0.95 ± 0.2	0.63 ± 0.1†
Fructosamine (μmol/L)	ND	ND	318 ± 8.9
HbA_{1c} (%)	ND	4.9 ± 0.73	7.3 ± 0.2‡

Abbreviation: ND, not determined.

*$P < .01$ *v.* young controls; †$P = .02$; ‡$P < .01$ *v.* aging controls.

insulin plus metformin, and 3 only with oral hypoglycemic agents. Their BMI was 27.1 ± 1.4 kg/m^2 (mean ± SEM; range, 19.1 to 30.2). No patient had been diagnosed with any type of atherosclerotic complication. They were studied before and at 1, 3, and 6 months after the initial injection of testosterone. On each of these visits, the following laboratory safety tests were performed: complete blood cell count, blood pressure, PSA levels and serum hepatic tests, as well as symptoms of urinary obstruction. Total and free testosterone levels in the follow-up were measured in the middle of the interval between injections. We were able to confirm that the treatment was being complied upon observation of the expected decrease in the serum LH levels induced by exogenously added testosterone.

Statistical Analysis

Data were analyzed using the Instat Statistical Software Program (GraphPad, San Diego, CA). The hormonal and other measurements in the different groups were compared using the nonparametric Mann-Whitney *U* test after analysis of the distribution of variables. When the distribution of variables was normal, Student's *t* test was used. Relationships between testosterone levels and the other variables were determined using the Spearman or Pearson correlation coefficients. Results were considered statistically significant at $P < .05$. All *P* values are 2-tailed. Values were expressed as means ± SEM.

RESULTS

The results of the hormone measurements in diabetic patients and their comparison with the corresponding values in healthy young men and healthy older men are shown in Table 2. In diabetic patients, the serum levels of total and free testosterone were significantly lower, while those of LH and FSH were significantly higher than in younger controls. In nondiabetic older men, total and free testosterone levels were also significantly lower than in the young controls, but no differences were detected for LH and FSH serum levels. No differences for sex hormone serum levels were observed between the diabetic and nondiabetic older men. As expected, fasting plasma glucose and HbA_{1c} values were significantly higher, while fasting C-peptide values were significantly lower in diabetic men as compared with nondiabetic aging controls.

The percentages of diabetic patients meeting the different hormonal criteria of andropause were 20% (criterion A), 43.6% (criterion B), and 54.5% (criterion C). Ten of 11 (90.9%) of the patients meeting criterion A, 21 of 24 meeting criterion B (87.5%), and 27 of 29 (93.1%) meeting criterion C had a positive questionnaire. As shown in Fig 1, aging was associated with an increase in the prevalence of diabetic patients presenting total testosterone serum levels ≤3.4 ng/mL. The percentage of patients presenting total testosterone ≤4.2 ng/mL or free testosterone ≤11 pg/mL was higher in patients 60 to 69 years old than in those aged 50 to 59 years, but the tendency to increase was maintained or disappeared in the oldest group.

In the whole group of diabetic patients, no significant correlations could be established between total or free testosterone serum levels with the BMI, time of evolution since the diagnosis of diabetes, or fasting glucose, fructosamine, insulin, and C-peptide blood levels. Total testosterone (but not free testosterone) was significantly correlated with HbA_{1c} ($r = .322$, $P = .01$).

To further explore the links between the serum levels of testosterone and the clinical parameters of the diabetic patients, we subdivided the patients according to 3 BMI categories (normal, BMI < 27; overweight, BMI 27 to 29.9; and obese, BMI > 30 kg/m^2). Total testosterone serum levels tended to be lower in the overweight men than in the patients with normal weight (4.2 ± 0.2 *v* 4.8 ± 0.3 ng/mL, $P = .06$), but no differences were found between either obese versus non-obese or between obese versus normal-weight patients. Free testosterone levels tended to be lower in obese than in normal-weight patients (9.9 ± 0.7 *v* 11.4 ± 0.6 pg/mL, $P = .09$) and also in obese than in overweight patients (9.9 ± 0.7 *v* 11.3 ± 0.5 pg/mL, $P = .09$). Free testosterone levels were lower in obese than in non-obese (normal plus overweight patients, 9.9 ± 0.7 *v* 11.4 ± 0.4, $P = .05$).

We also analyzed serum testosterone levels in patients with different HbA_{1c} values. Interestingly, the patients with serum HbA_{1c} levels between 6% and 6.9% had lower total testosterone serum levels than those with values higher than 9% (4.2 ± 0.2 *v* 5.4 ± 0.4 ng/mL, $P = .01$). No other differences in total or free testosterone levels were observed for the other subgroups of patients according to their HbA_{1c} levels.

The diabetic patients meeting both the clinical and hormonal criteria of andropause were compared with respect to the diabetic patients without andropause (results not shown). To this end, we performed the comparisons using the hormonal criterion of a total testosterone level of ≤3.4 ng/mL, as well as using the criterion of a free testosterone level of ≤11 pg/mL. Using the criterion of total testosterone, the patients with an-

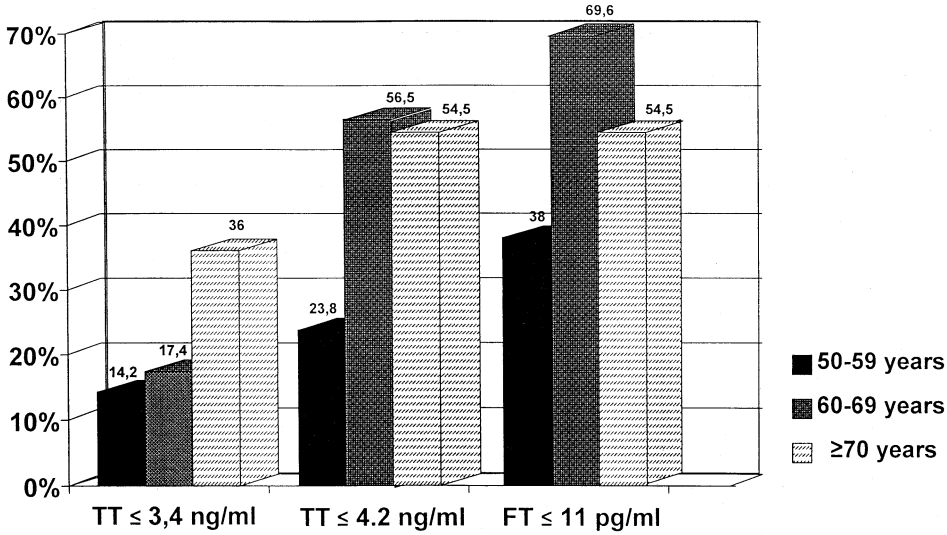

Fig 1. Percentage of type 2 diabetic men meeting the different hormonal criteria of partial androgen deficiency.

dropause had significantly lower levels of total testosterone (3.0 ± 0.09 *v* 4.9 ± 0.15 ng/mL, $P < .0001$), but there were no differences with regard to age, BMI, LH, FSH, PRL, fasting blood glucose, HbA_{1c}, fructosamine, insulin, and C-peptide levels nor in the time of evolution of diabetes between those with and without andropause. Using the criteria of free testosterone levels, patients with andropause had significantly lower concentrations of free testosterone (9.4 ± 0.2 *v* 12.7 ± 0.5 pg/mL, $P < .0001$), but again, no differences were detected for these parameters with the exception of fasting blood glucose, which was marginally higher in patients with andropause (162 ± 6.9 *v* 139 ± 8.9, $P = .05$).

The serum concentrations of testosterone and gonadotropins in the testosterone-treated group are shown in Fig 2. As expected, testosterone values increased by approximately 2-fold 1 month after starting the treatment, thereafter maintaining their values. It should be noted that the serum testosterone values obtained with treatment were in the midnormal range for young men, as suggested in consensus documents.[20] Serum concentrations of LH and FSH decreased, as expected, after therapy. No changes were observed for prolactin along the therapy. The corresponding values for fasting blood glucose, fructosamine, HbA_{1c}, insulin, and C-peptide are shown in Table 3.We did not observe that testosterone substitution therapy produced any changes in any of the parameters concerning glucose metabolism or glycemic control.

Six months after treatment with testosterone had commenced, the daily dose of insulin was lower than before treatment (by 2 and 3 U) in 2 patients (both treated with a mixture of insulin and metformin); it was maintained in 1 patient treated only with insulin, and it was increased (by 2, 4, 4, and 12 U) in 4 patients (3 treated only with insulin and 1 treated with insulin and metformin). In the patients receiving only oral hypoglycemic agents, the daily dose was maintained in 2 and increased in 1. Thus, half of our patients had their hypoglycemic treatments increased after starting testosterone therapy.

With respect to the side effects of testosterone treatment, no patient had any adverse cardiovascular or prostate effects. In 1 patient, testosterone therapy was withdrawn after completing the 6-month period due to an increase in his hematocrit value above 50%. Another patient had an increase in PSA values from 2.6 to 4.3 ng/mL, but no prostatic cancer was detected upon biopsy. The mean PSA level was 1.4 ng/mL before treatment and 1.8 ng/mL at the end. At the end of the 6-month period, the body weight of the treated patients was similar to that recorded at the beginning.

DISCUSSION

To date, andropause has mainly been studied in healthy, older men, and we do not know whether the conclusions drawn

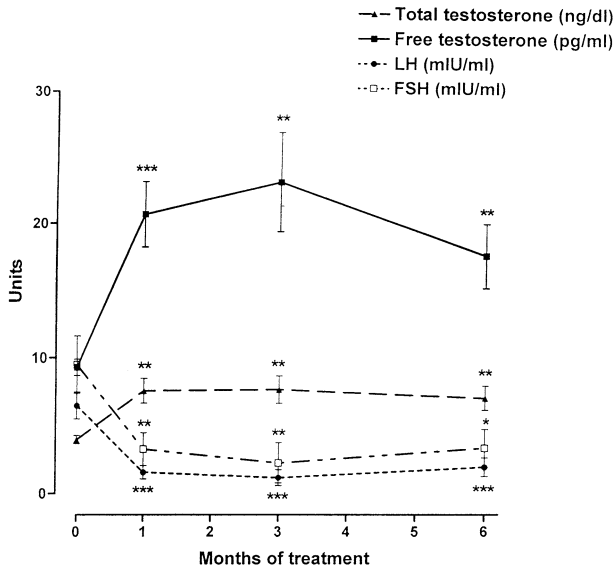

Fig 2. Testosterone and gonadotropin serum values before (0) and during 6 months of treatment with 150 mg testosterone enanthate IM in 10 type 2 diabetic men. Results are expressed as mean ± SEM. *$P < .05$, **$P < .01$, ***$P < .001$ *v* before treatment.

Table 3. Effects of Testosterone Therapy on Parameters of Glucose Metabolism in Type 2 Diabetic Patients (n = 10) With Partial Androgen Deficiency

	Before TX	1 Month TX	3 Months TX	6 Months TX
FBG (mg/dL)	154 ± 10.4	137 ± 14	137 ± 12	131 ± 8
Fructosamine (μmol/L)	308 ± 16	298 ± 8	281 ± 14	285 ± 9
HbA_{1c} (%)	6.9 ± 0.4	6.8 ± 0.4	6 ± 0.2	6.7 ± 0.3
Insulin (μIU/mL)	16.7 ± 4.4	16.3 ± 4.3	14.5 ± 5.6	18 ± 4.2
C-peptide (pmol/mL)	0.51 ± 0.1	0.44 ± 0.1	0.46 ± 0.1	0.41 ± 0.1

NOTE. Results are expressed as means ± SEM. TX: 150 mg testosterone enanthate was applied IM every 2 weeks for 6 months.
Abbreviation: TX, treatment.

could be applicable to chronically ill patients. Diabetic subjects are of potential interest in this regard because some studies have reported lower serum testosterone concentrations in them than in nondiabetic men,[5,21] and low levels of testosterone predict insulin resistance and the development of type 2 diabetes in older adults.[6] Therefore, andropause in diabetic men may be more prevalent than in nondiabetic subjects and may have deleterious effects on glycemic control, considering also that certain clinical characteristics of andropause may influence diabetic control and therapeutic compliance.

Previous epidemiologic studies[21] have shown that 21% of diabetic patients and 13% of nondiabetic men, aged 40 to 79 years (mean ± SD: 63.9 ± 8.5 years), with a BMI (26.7 ± 3.3 kg/m^2) very similar to that of our own patients, had a testosterone serum level below a categorically defined normal level of 3.5 ng/mL. Here we observed that 20% of type 2 diabetic patients aged 63.6 ± 7.9 years (mean ± SD), with a BMI of 27.6 ± 3.3 kg/m^2, had subnormal levels of total testosterone (≤3.41 ng/mL). Our study expands current knowledge in the sense that, as occurs in nondiabetic men,[2,17] the percentage of type 2 diabetic patients with subnormal levels of free testosterone is much higher than the percentage observed with subnormal levels of total testosterone. Additionally, our data show that the percentage of diabetic men with subnormal levels of testosterone increases with aging, as occurs in healthy, aging men.[2] Although our study includes only a small group of aging controls, we believe that they are representative of healthy aging men in terms of the androgen environment because their mean total testosterone levels (5.2 ng/mL) are very similar to those found in a much larger sample of 875 nondiabetic men (5.3 ng/mL) of similar age in the Rancho Bernardo study.[21] Our diabetic patients had mean values of total testosterone 0.7 ng/mL (13.5%) lower than older, healthy controls, a result that agrees with previous reports indicating that the existence of chronic diseases subtracts 10% to 15% from the values of androgens found in men in good health.[22]

Our study has some advantages with respect to previous observational and intervention studies on the effect of aging on the endogenous androgen environment,[2,9,23,24] because we incorporated not only a hormonal criterion in the definition of partial androgen deficiency, but also a clinical criterion. This is noteworthy because the symptoms and signs of andropause are an important part of the diagnostic complex[20] and because aging patients may exhibit normal serum testosterone values associated with clinical symptoms of andropause.[25] Furthermore, the clinical significance of slightly low serum testosterone in the absence of clinical manifestations consistent with androgen deficiency is not clear.[4] Thus, the diagnosis of andropause requires both the presence of the clinical syndrome and confirmation by testosterone levels.[4,26] Our patients with subnormal total testosterone levels exhibited a positive questionnaire in 87.5% of the cases and those with subnormal free testosterone levels exhibited a positive questionnaire in 93.1% of the cases. It is true that diabetic patients may present erectile dysfunction related, for example, to diabetic neuropathy and not to androgen deficiency, but when the item related to erection was eliminated the sensitivity of the questionnaire remained practically the same (89% using a total testosterone level of ≤3.4 ng/mL and 90% using the criterion of a free testosterone level of ≤11 pg/mL). The sensitivity and specificity of our questionnaire compares favorably with the ADAM questionnaire because sensitivity in our patients with subnormal total testosterone levels was 90% and specificity was 74%, both higher than the 88% and 60% found in the validated ADAM questionnaire,[13] even though we used stricter criteria and the number of items was lower (8 *v* 10).

Currently, the main drawbacks in the design of clinical protocols and the inclusion of patients for the study of andropause lie in establishing the best assay for testosterone measurement and in defining a baseline testosterone cut-off level. Aging increases serum hormone-binding globulin (SHBG) serum levels in men[22] and may therefore increase the values of total testosterone, which is the reason why some investigators do not recommend their use in older men.[4] However, this limitation may be only theoretical in older diabetic men because they exhibit significantly lower SHBG values as compared with nondiabetic controls.[21] Total testosterone assays have been recommended as the first-line method[20,25,27] because it is a good measurement, less expensive, and less complex than free or bioavailable testosterone determinations. Although we recognize that free testosterone analysis by direct analog immunoassay has some limitations when used for diagnostic purposes, its use has not been formally discouraged in andropause.[20] At least in this work, we found that it has some clinical value, because 93.1% of our patients with subnormal free testosterone levels had a positive questionnaire, whereas 79% of patients with free testosterone concentrations above 11 pg/mL had a negative questionnaire. In addition, their concentrations increased under testosterone replacement therapy, as shown in Fig 2. There are no well designed clinical trials indicating that one method is better than any other for the definition of men with androgen deficiency and possible re-

sponders to androgen therapy. Owing to the absence of consensus in the definition of a cut-off level of serum testosterone levels below which a patient can be considered as androgen-deficient and, therefore, suitable for testosterone therapy, we used 3 types of cut-off. One of them (total testosterone $\leq$ 3.4 ng/mL), although arbitrary, was based on our own control population and is very similar to the cut-off values used in epidemiologic studies performed both in diabetic men (total testosterone $\leq$ 3.5 ng/mL),[21] as well as in the general population (total testosterone $\leq$ 3.25 ng/mL).[2] Our choice of the cut-off levels for total and free testosterone is reinforced by the percentage of patients with values below the hormonal criteria for andropause who exhibited a positive questionnaire.

Considering that low levels of testosterone are associated with the development of insulin resistance[6] and that they predict insulin resistance, β-cell dysfunction, and hepatic glucose output in eugonadal subjects,[28] it was of interest to analyze the relationships between testosterone levels and glycemic control in diabetic patients. Our results revealed that testosterone concentrations were unrelated to fasting glucose, fructosamine, insulin, or C-peptide serum values but, surprisingly, they were positively correlated with HbA_{1c} concentrations. Furthermore, type 2 diabetic patients with clinical and hormonal criteria of andropause did not present either lower (or higher) insulin or C-peptide values or higher levels of HbA_{1c} or fructosamine with respect to those without andropause, as would be expected if low levels of testosterone had a deleterious effect on the pathogenic mechanisms involved in type 2 diabetes. Previous studies have reported an inverse relationship between endogenous plasma testosterone and fasting blood glucose in aging patients[6,8] and in diabetic men.[21] However, in other analyses, no correlation between total or free testosterone serum levels and glucose metabolism parameters, such as fasting blood glucose, insulin, and C-peptide values, was found in type 2 diabetic patients.[5,8,23,29] Our results are in agreement with recent work on patients with mild type 2 diabetes, in which no independent associations between the plasma levels of sex steroid hormones and insulin resistance, hepatic glucose output, or insulin secretion were found.[28]

Nevertheless, the cross-sectional design in the analysis of correlations does not establish a causal relationship. Therefore, to study whether testosterone influences glycemic control in type 2 diabetes, a better approach is to study the direct effects of testosterone substitution therapy. Treatment with 150 mg testosterone enanthate increased testosterone levels in our diabetic patients to the midnormal range, as recommended in consensus documents.[20] In agreement with our cross-sectional results, in the patients treated with testosterone for 6 months, a time in which the body fat mass increases and lean mass decreases in elderly nondiabetic men with subnormal values of testosterone treated with testosterone,[9,23,24] we failed to observe a significant decrease in the dose of hypoglycemic agents or in the values of fasting blood glucose, insulin, C-peptide, fructosamine, or HbA_{1c} concentrations. The results of the intervention study should be interpreted cautiously, considering both the design and the relatively small number of patients treated. Nevertheless, given the paucity of information on androgen replacement therapy in aging diabetic men, we believe it is necessary to perform small intervention studies analyzing both efficacy and safety before recommending the initiation of large-scale trials.

In conclusion, our results indicate that low concentrations of endogenous testosterone do not have a deleterious effect on glucose metabolism in aging type 2 diabetic men and show, for the first time, that andropause does not have an adverse effect on glycemic control and that the correction of subnormal levels of testosterone by physiologic substitutive therapy is not associated with any improvement or worsening in overall glycemic control.

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