OBSERVATIONS

Androgen Therapy Improves Insulin Sensitivity and Decreases Leptin Level in Healthy Adult Men With Low Plasma Total Testosterone

A 3-month randomized placebo-controlled trial

In men, an association between lower plasma total testosterone (PTT) and insulin resistance has been found in cross-sectional studies (1,2) and in one nested case-control study (3) without any possible conclusion in terms of causality or direction of the relationship. Indeed, to obtain such information, randomized controlled trials are needed. Until now, only one clinical trial has suggested that testosterone therapy improves insulin sensitivity in obese men (4). Cross-sectional studies concerning leptin regulation by androgens have provided no definitive conclusions as to whether the negative association between androgens and leptin level is independent (5) or dependent (6). This randomized controlled trial was designed to assess the role of androgens on insulin sensitivity and leptin regulation in healthy adult men.

This study was a randomized, double-blind, unicentric, controlled, clinical trial. Three treatments (testosterone, dihydrotestosterone [DHT], and placebo) were compared in parallel groups during a 3-month period. All of the examinations were performed by only two physicians, using a standardized protocol. Blood was drawn between 8:00 A.M. and 9:30 A.M. after an overnight fast to determine fasting plasma glucose, insulin, leptin, sex hormones, lipids, coagulation and fibrinolysis parameters, hepatic enzymes, and prostate-specific antigen (PSA) and blood cell count. Then, a standard 75-g oral glucose tolerance test and a digital rectal examination were performed. In addition, between days 10 and 20, all of the subjects were monitored to measure sex hormones in order to adapt the treatment dose. The study protocol was approved by the Henri Mondor Hospital Ethics Committee. All of the included subjects gave written informed consent.

Men with low levels of PTT (confirmed by two measurements) were selected from a large occupation-based population. The inclusion criteria were as follows: *1*) either PTT ≤3.4 ng/ml [5th percentile value of PTT distribution in the 1,718 men of the TELECOM Study (7)] from 1985 to 1987 and <4.0 ng/ml (13th percentile value) from 1992 to 1993 (3) or PTT <4.0 ng/ml from 1992 to 1993 and <4.0 ng/ml a few days before inclusion; 2) no history of vascular thrombosis or ischemic heart disease; 3) no treatment by androgens, anti-androgens, and antidiabetic or antithrombotic drugs; *4*) normal values of plasma prolactin, estradiol, and thyroxin; 5) no current prostatic disease and a normal PSA value. A total of 18 healthy men with stable low plasma androgens (Table 1) and a range of PTT from 1.4 to 3.7 ng/ml at baseline were included.

The 18 selected men were randomly assigned to one of three treatment groups: testosterone, DHT, or placebo. The randomization code was known only to the study manager. Treatment was a gel administered every morning by percutaneous route. The daily dose during the first weeks was 125 mg for the testosterone and 35 mg for the DHT treatment groups. The adaptation of treatment doses between days 10 and 20 aimed at obtaining a trough level of PTT between 4 and 10 ng/ml for the testosterone group and a trough level of plasma DHT between 4 and 10 ng/ml for the DHT group. To maintain the double blinding, the study manager also sometimes changed the dose of placebo. The subjects were asked not to change their dietary and physical activity. Compliance to treatment was assessed by interview and by measuring sex hormones and gonadotropins at the end of the trial.

Plasma glucose, total cholesterol, HDL cholesterol, triglycerides, apolipoprotein (apo)-A1, apoB, hepatic enzymes, and blood cell count were assayed on the same day of venipuncture. PSA and fibrinolysis markers were measured within 3 days after venipuncture. For hormone measurements at baseline and at the end of the trial, plasma was separated by centrifugation immediately after sampling and frozen at −20°C until the end of the trial, then all of the samples were thawed and the analyses performed in a single batch. All of the analysts were blind to the treatment allocation. Insulin was determined by the immunoradiometric assay method (Medgenix Diagnostics, Fleurus, Belgique), leptin by a commercial radioimmunoassay (RIA) (Linco Research, St. Charles, MO), follicle-stimulating and luteinizing hormone by the Automated Chemiluminescence System 180 (Ciba Corning), and androgens and estradiol by RIA (7). The only missing datum was one 2-h plasma insulin measurement at 3 months in a subject treated by DHT.

The primary end points to assess insulin sensitivity were fasting plasma insulin–to–fasting plasma glucose ratio and homeostasis model assessment (HOMA) index. Plasma leptin, 2-h plasma glucose and insulin, and blood pressure were taken as secondary criteria. Treatment tolerance was assessed by interview, by prostatic examination, and by PSA, as well as by weight, electrocardiogram (ECG), lipid, hemoglobin, hematocrit, fibrinolysis markers, and hepatic enzyme variations.

A sample size of 36 subjects was needed to detect a difference of 5 mg/dl for the decrease of fasting plasma glucose, assuming SD = 5 mg/dl, using a two-tailed Student's *t* test with $\alpha = 0.05$ and $\beta = 0.20$. However, we could not reach that number, and the recruitment was closed after having included 18 subjects. To evaluate the treatment effect, the difference between the values at entry and at the end of the treatment period was calculated for each subject, and then the Kruskal-Wallis nonparametric test was used. When statistical significance ($P \leq 0.05$) was reached for any overall three-group comparison, two-by-two comparisons were performed using the Bonferroni test to correct for multiple comparisons.

At baseline, the three treatment groups were similar with respect to age, BMI, waist-to-hip ratio (WHR), blood pressure, plasma glucose, lipids, insulin, leptin, androgens, and sex hormone–binding globulin, as well as hemoglobin, hematocrit, coagulation, and fibrinolysis parameters (data not shown). At the end of the trial, a significant difference was shown for the variation of fasting plasma insulin ($P < 0.05$), fasting plasma insu-

Table 1—*Baseline characteristics and variations in the three treatment groups (after minus before)*

	Testosterone	DHT	Placebo	*P*
n	6	6	6	
Age (years)	52.8 ± 4.2	51.2 ± 3.9	55.4 ± 3.6	0.80
BMI (kg/m^2)	29.9 ± 0.9	27.8 ± 0.9	28.0 ± 1.1	0.84
WHR	0.95 ± 0.02	0.96 ± 0.02	0.96 ± 0.03	0.99
Systolic blood pressure (mmHg)	152 ± 5	143 ± 7	126 ± 8	0.08
Diastolic blood pressure (mmHg)	93 ± 4	88 ± 2	80 ± 5	0.17
Fasting plasma glucose (mg/dl)	101 ± 5	97 ± 2	99 ± 4	0.97
Total cholesterol (mg/dl)	212 ± 14	228 ± 11	221 ± 14	0.70
HDL cholesterol (mg/dl)	45 ± 4	44 ± 4	42 ± 6	0.81
Triglycerides (mg/dl)	126 ± 20	142 ± 18	123 ± 18	0.64
Fasting plasma insulin (μU/ml)	14 ± 4	18 ± 4	13 ± 3	0.52
Leptin (ng/ml)	10.1 ± 4.5	6.4 ± 1.3	6.2 ± 1.5	0.81
Plasma total testosterone (ng/ml)	2.4 ± 0.1	2.9 ± 0.3	2.7 ± 0.3	0.20
Plasma bioavailable testosterone (ng/ml)	0.6 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.06
Plasma SHBG (nmol/l)	16.5 ± 1.9	16.9 ± 2.6	21.0 ± 3.3	0.32
Δ Fasting plasma glucose (mg/dl)	4 ± 3	−1 ± 3	3 ± 4	0.42
Δ Fasting plasma insulin (μU/ml)	−0.8 ± 2.0	−6.2 ± 2.2	2.7 ± 1.6	0.02*
Δ Fasting plasma insulin/fasting plasma glucose	−0.23 ± 0.32	−1.09 ± 0.29	0.43 ± 0.35	0.003*
Δ HOMA index	−0.09 ± 0.53	−1.54 ± 0.69	0.73 ± 0.39	0.012*
Δ Leptin (ng/ml)	−1.2 ± 1.7	−1.8 ± 0.6	0.4 ± 0.4	0.05†
Δ Total cholesterol (mg/dl)	−7 ± 7	−4 ± 5	−12 ± 5	0.66
Δ HDL cholesterol (mg/dl)	−1 ± 2	−5 ± 1	1 ± 2	0.09
Δ Triglycerides (mg/dl)	6 ± 18	11 ± 12	19 ± 24	0.78
Δ Systolic blood pressure (mmHg)	4 ± 5	−3 ± 5	21 ± 7	0.052†
Δ Diastolic blood pressure (mmHg)	4 ± 5	5 ± 5	14 ± 3	0.22
Δ Weight (kg)	3.3 ± 1.1	1.4 ± 1.0	−0.3 ± 1.0	0.09

Data are means ± SEM. *$P < 0.01$ for DHT vs. placebo; †$P < 0.05$ for DHT vs. placebo.

lin–to–fasting plasma glucose ratio ($P < 0.01$), and HOMA index ($P < 0.05$), which all decreased under androgens. The two-by-two comparisons showed a significant improvement only for DHT compared with placebo ($P < 0.01$ for all of these indexes of insulin sensitivity). No significant differences were observed for 2-h plasma glucose and insulin among the three groups (data not shown), whereas plasma leptin significantly decreased under androgen treatment ($P < 0.05$), mainly with DHT ($P < 0.05$ for DHT vs. placebo). Systolic blood pressure increased in the placebo group ($P = 0.052$) (Table 1).

The only serious event was the discovery of a prostatic nodular hyperplasia, benign at biopsy, in a subject treated by testosterone. A trend for an increase in weight was observed under androgen treatment ($P = 0.09$), mainly with testosterone (Table 1), without any modification of waist circumference and WHR (data not shown). No change was observed on the ECG recordings. No significant difference was shown among the three groups for lipids (Table 1), PSA, hepatic enzymes, coagulation, and fibrinolysis parameters, but hemoglobin and hematocrit increased under androgens ($P < 0.05$ and $P < 0.01$, respectively), mainly with testosterone (data not shown).

This randomized, controlled, double-blind trial provides evidence that in healthy men, androgen treatment, particularly DHT, improves insulin sensitivity and decreases plasma leptin level without notable side effects. The three treatment groups were quite identical at baseline concerning glucose tolerance status. In the placebo group, one subject was diabetic according to 2-h plasma glucose (227 mg/dl, with fasting plasma glucose at 85 mg/dl), and none had impaired glucose tolerance (IGT) or impaired fasting glucose (IFG). In the DHT group, one subject had IGT, and none had diabetes or IFG. In the testosterone group, all of the subjects had normal glucose tolerance. The primary differences at baseline concerned bioavailable testosterone with a trend for a higher level in the DHT group, which should have blunted (not increased) the response to DHT treatment and blood pressure, probably explaining the nearly significant improvement of systolic blood pressure under androgens by a regression to the mean phenomenon in the placebo group. On the contrary, the parallel decrease in fasting plasma insulin and leptin and the improvement in insulin sensitivity under androgens appear very consistent. Our study may appear limited because of the sample size (half of that planned), enjoining the use of conservative nonparametric tests, and causing the final statistical analysis to be equivalent to a planned intermediary analysis. Indeed, to have confirmed the a priori hypotheses in these conditions of weak statistical power emphasizes the effect of androgens, mainly DHT, to improve insulin sensitivity and to decrease leptin concentrations in healthy men with low PTT. Very few side effects were observed, including a tendency for weight increase and an increase in hemoglobin and hematocrit, although these were reversible a few months later (data not shown), thus indicating good patient

compliance to the allocated treatment, also confirmed by hormone measurements.

From the literature, we can speculate that the effect of androgens on insulin sensitivity could be caused by changes in body composition and fat mass distribution. Indeed, androgens are known to increase fat-free mass and muscle size and to decrease visceral fat mass (8) by inhibiting lipoprotein lipase activity, therefore inhibiting triglyceride uptake and accelerating triglyceride release from abdominal adipose tissue (9). In turn, a decrease in abdominal fat mass may induce an improvement in insulin sensitivity via a reduction in circulating free fatty acids (10). In our study, no significant variation of waist circumference was found under androgens, but this measurement was probably too imprecise to detect changes. In the only previous controlled clinical trial having compared testosterone, DHT, and placebo gel treatments, 31 abdominally obese subjects with a moderately low PTT level (mean 4.5 ng/ml) were treated during 9 months (4). In that study, the testosterone group had a significant decrease in visceral fat mass seen by computerized tomography, and a marked augmentation of glucose disposal rate was observed with euglycemic-hyperinsulinemic clamp, whereas plasma triglycerides and total cholesterol had decreased. Leptin was not measured. No significant improvement was shown with DHT treatment. This striking contrast with our study concerning the respective effects of testosterone and DHT could be explained by the higher PTT level at baseline and mostly by an undertreatment in the DHT group in the trial by Marin et al. (4). We used larger doses of DHT and adapted the doses of testosterone and DHT after 2 weeks, when necessary, according to the circulating androgen level, whereas in the trial by Marin et al. (4) no monitoring of sex hormones level was performed.

The decrease in plasma leptin concentration is also probably explained by the supposed reduction in adipose tissue mass (11), but the influence of androgens on leptin could also be mediated by a stimulation of the splanchnic β-adrenoceptors (12) or by a direct suppressive effect on *ob* gene expression (13). Nevertheless, our data clearly demonstrate the role of androgens to decrease leptin levels in healthy men, as previously suggested (5).

In conclusion, this clinical trial demonstrates that androgens improve insulin sensitivity and decrease leptin levels in adult men. We recruited healthy subjects ranked in the lowest 10 percentiles of the PTT distribution from a large occupation-based population by systematically measuring PTT. Therefore, these data can most likely be extrapolated to healthy men in the first decile of the PTT distribution. The pathways through which androgens exert their inhibiting effects on insulin and leptin in humans deserve further fundamental research. In parallel, as low levels of testosterone are predictive of the development of insulin resistance and type 2 diabetes (14), and as type 2 diabetic patients are known to have a lower level of PTT than nondiabetic men (15), larger studies on androgen treatment in insulin-resistant nondiabetic subjects and in type 2 diabetic patients are necessary.

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