Effect of Chronic Cabergoline Treatment and Testosterone Replacement on Metabolism in Male Patients with Prolactinomas

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Key Words

Prolactin · Testosterone deficiency · Metabolic parameters · Cabergoline · Testosterone replacement · Hyperprolactinemia · Metabolic syndrome · Insulin resistance · Pituitary tumors · Pollutants

Abstract

Introduction: Hyperprolactinemia and hypogonadism are reportedly associated with an impaired metabolic profile. The current study aimed at investigating the effects of testosterone replacement and cabergoline (CAB) treatment on the metabolic profile in male hyperprolactinemic patients. **Patients and Methods:** Thirty-two men with prolactinomas, including 22 with total testosterone (TT) <8 nmol/l (HG, 69%) and 10 with TT >8 nmol/l (non-HG, 31%), were entered in the study. In all patients, metabolic parameters were assessed at diagnosis and after 12- and 24-month treatment. **Results:** Compared to non-HG patients, at baseline the HG patients had higher waist circumference (WC). TT significantly correlated with body mass index (BMI). Twelve-month CAB induced PRL normalization in 84%. HG prevalence significantly decreased (28%) and non-HG prevalence significantly increased (72%). Anthropometric and lipid parameters, fasting insulin (FI), insulin sensitivity index (ISI₀), homeostatic model assessment of insulin secretion (HOMA-β) and homeostatic

model assessment of insulin resistance (HOMA-IR) significantly improved compared to baseline. TT was the best predictor for FI. Percent change (Δ) of TT significantly correlated with ΔCholesterol, ΔWeight and ΔBMI. Compared to non-HG patients, the HG patients had a higher weight, BMI, WC and HOMA-β. In HG, testosterone replacement was started. After 24 months, PRL normalized in 97%. HG prevalence significantly decreased (6%) and non-HG prevalence significantly increased (94%). Anthropometric and lipid parameters, FI, ISI₀, HOMA-β and HOMA-IR significantly improved compared to baseline, with FI, ISI₀, HOMA-β and HOMA-IR further ameliorating compared to the 12-month evaluation. Compared to non-HG patients, the HG patients still had a higher weight, BMI and WC. **Conclusions:** In hyperprolactinemic hypogonadal men, proper testosterone replacement induces a significant improvement in the metabolic profile, even though the amelioration in the lipid profile might reflect the direct action of CAB.

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Introduction

The pathogenesis of metabolic syndrome (MetS) is complex; however, visceral obesity and insulin resistance are acknowledged as important causative factors [1–4].

Indeed, testosterone has been demonstrated to regulate body composition and metabolic profile [5–9]. In healthy subjects, testosterone levels have been found to be inversely correlated with waist circumference (WC) [5–9], a surrogate clinical marker of visceral obesity, and with the amount of visceral adipose tissue [10–12]. On the other hand, testosterone clearly influences insulin sensitivity. Indeed, testosterone deficiency is associated with insulin resistance in patients on androgen deprivation therapy [5, 13, 14]. Moreover, testosterone deficiency has been shown to predict the development of MetS [15], and, in turn, MetS has been demonstrated to induce testosterone deficiency, with WC and fasting insulin (FI) being the main determinants of this vicious interrelationship in patients with MetS [16]. In men with prostate cancer, androgen deprivation-induced testosterone deficiency results in insulin resistance, diabetes mellitus, impaired lipid profile and MetS [11–13, 17, 18]. Men with Klinefelter's syndrome and idiopathic hypogonadotropic hypogonadism exhibit significantly higher glucose and insulin levels upon an oral glucose load as compared to healthy controls [19]. In such patients, discontinuation of testosterone replacement (TR) results in an acute increase in FI and homeostatic model assessment of insulin resistance (HOMA-IR) and a decrease in insulin sensitivity within a few weeks, suggesting a direct negative effect of testosterone deficiency on the pathogenesis of peripheral insulin resistance [19]. Whether a correction of testosterone deficiency is associated with an improvement in the metabolic profile is still a matter of debate. To date, discordant results have been reported about the effects of replacement treatment with testosterone on metabolic profile in male patients with testosterone deficiency. Four studies [20–23] have found clinically negligible [20–22] or no [23] effects of TR on lipid fractions in unselected patients with testosterone deficiency despite the significant decrease in body fat, whereas in another meta-analysis [24], a significant reduction in lipid fractions has been found after TR in patients with MetS. Conversely, studies investigating the effects of TR on the glucose profile in men with MetS or diabetes mellitus have consistently reported a significant improvement in fasting glucose (FG), glycated hemoglobin and HOMA index [24, 25]. In two recent meta-analyses [26, 27], a significant improvement of glucose and lipid profile has been documented after androgen therapy in patients with MetS and diabetes mellitus.

Hyperprolactinemia, a common secondary cause of testosterone deficiency, has been also implicated in the pathogenesis of obesity, glucose intolerance and MetS.

PRL excess has been demonstrated to negatively impact lipid metabolism [28–35] and to cause disorders of glucose metabolism, inducing the impairment of glucose tolerance and hyperinsulinemia [36–45]. In patients with hyperprolactinemia, the dopamine agonists bromocriptine and cabergoline (CAB) represent the cornerstone of medical treatment [46, 47]. Short-term treatment with CAB has been shown to significantly decrease body weight in obese men with prolactinomas [48], to reduce body fat percentage [49, 50] and to significantly improve insulin resistance as well as glucose and lipid profile [51, 52]. Long-term CAB treatment [53] has been demonstrated to significantly ameliorate the lipid, glucose and insulin profile in patients with prolactinomas, independently of the degree of reduction in PRL levels, particularly in case of the employment of high doses [53]. Consistently, in patients with prolactinomas long-term therapy with CAB has recently been demonstrated to significantly reduce MetS prevalence, to improve lipid profile and to reduce insulin resistance by decreasing FI, HOMA-IR and homeostatic model assessment of insulin secretion (HOMA- β) and increasing insulin sensitivity index (ISI₀) [54], with the CAB dose being the best predictor of a percent decrease in FI [54]. Noteworthy, in men with prolactinomas the recovery of the gonadal function and testosterone deficiency, even beginning soon after starting CAB treatment, is fully achieved in approximately half of the patients during long-term treatment [55–57]. Therefore, the burden and the differential role of testosterone deficiency and PRL excess and their correction by medical therapy on the modulation of metabolism in such patients are yet to be completely elucidated. To our knowledge, long-term prospective studies evaluating the metabolic outcome of CAB and TR in hyperprolactinemic male patients with testosterone deficiency are still lacking. The current study aimed at investigating the effects of 12- and 24-month continuous CAB treatment and of testosterone normalization either by the use of CAB or TR on metabolic parameters and MetS prevalence in male hyperprolactinemic patients.

Patients and Methods

Inclusion and Exclusion Criteria

This prospective study included male patients with a new established diagnosis of hyperprolactinemia starting treatment with CAB. Inclusion criteria were: (1) age >18 years and (2) baseline PRL >2 $\times$ upper limit of normal. Exclusion criteria were represented by the presence at the study entry of the following conditions: (1) previous pituitary surgery and/or radiotherapy; (2) hypopituitarism without or on replacement treatment with cortico-

Table 1. Patients' profile at study entry

Patients	32 (100)
Age, years	42±5
Microadenoma	7 (22)
Macroadenoma	25 (78)
Tumor volume, ml	4,468.24±6,053.72
PRL levels, µg/l	2,028.1±4,407.7
Testosterone levels, nmol/l	7.5±4.6
CAB starting dose, mg/week	0.25–0.5

Values represent n (%), mean ± SD or range.

steroids, levothyroxine and recombinant human GH; (3) PRL and GH co-secreting pituitary tumors, and (4) suspicion of drug or alcohol abuse. Patients with treatment duration <12 months and those discontinuing CAB for a period >1 month were not considered for the final analysis. Similarly, patients with testosterone deficiency discontinuing sexual steroid replacement treatment for a period >1 month were excluded from the current analysis. The patients included in the study provided written informed consent in line with the confidentiality statement of data collection according to the Italian privacy policy.

Patients

Forty-five newly diagnosed consecutive adult male patients with prolactinomas attended the outpatient clinic of the Neuroendocrine Disease Unit at the 'Federico II' University of Naples between September 1, 2010 and December 31, 2011. Pituitary imaging revealed a microadenoma in 7 and a macroadenoma in 38 patients. All patients started treatment with CAB. Thirteen patients did not enter the study because of previous pituitary surgery and radiotherapy in 3 (6.7%), treatment duration <12 months as a result of resistance to therapy requiring surgery in 7 (15%) and hypopituitarism on chronic replacement treatment with recombinant human GH in 1 (2%), with corticosteroids in 1 (2%) and with corticosteroids plus levothyroxine in 1 (2%). Therefore, 32 patients, aged 42 ± 5 years (range 18–67 years), including 7 with microadenoma and 25 with macroadenoma, were considered for the current analysis. The patients' profile at study entry is shown in table 1.

Study Protocol

This is a prospective study. Clinical and hormonal evaluations were performed as previously described [54]. At diagnosis and thereafter at 3- to 6-month intervals, all patients were admitted to the hospital for a complete physical, biochemical and endocrine examination. At each time point, clinical parameters, including height, weight and WC were recorded. The body mass index (BMI) was calculated as weight-to-squared-height ratio and registered at the study entry; according to the BMI, patients were classified as normal weight (BMI = 18–24.9), overweight (BMI = 25–29.9) and obese (BMI ≥30) [58]. Blood pressure was measured at the right arm, after the subjects had been in a relaxed sitting position for 5 min, using a standard mercury sphygmomanometer; three measurements were taken and averaged to give the blood pressure values used in this analysis. Systemic arterial hypertension was de-

finied as systolic blood pressure >130 mm Hg and diastolic blood pressure >90 mm Hg according to the guidelines of the American Society of Hypertension [59]. Similarly, biochemical parameters, including FG, FI, serum triglycerides (TG), total cholesterol (CHOL), HDL and LDL cholesterol, were evaluated during the study. On the basis of plasma glucose levels, a diagnosis of impaired glucose tolerance and diabetes mellitus was made according to WHO guidelines [60]. MetS was diagnosed when patients met at least three of the following criteria: WC >102 cm, TG >150 mg/dl, HDL cholesterol <40 mg/dl, systolic blood pressure >130 mm Hg, diastolic blood pressure >85 mm Hg and FG >100 mg/dl according to the National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (NCEP-ATP III) criteria [61]. The visceral adiposity index (VAI) was calculated by using the formula reported by Amato et al. [62]: $VAI = [WC/39.68 + (1.88 \times BMI)] \times (TG/1.03) \times (1.31/HDL)$. Insulin resistance was assessed using HOMA index in line with Matthews et al. [63], by calculating the following parameters: $HOMA-IR = [insulin (mU/l) \times FG (mmol/l)]/22.5$ as the surrogate index of insulin resistance and $HOMA-\beta = [20 \times insulin (mU/l)/FG (mmol/l) - 3.5]$ as the surrogate index of insulin secretion [63]. In order to assess baseline insulin sensitivity, ISI_0 was calculated according to the following formula [64]: $ISI_0 = 10,000/insulin (\mu U/ml) \times FG (mg/dl)$. Serum PRL and total testosterone (TT) levels were assessed in all patients at diagnosis and every 3–6 months during the following period. Blood samples were collected at 7.00–8.00 a.m. after overnight fasting. On the basis of TT levels, patients were classified as with (TT <8 nmol/l, i.e. <230 ng/dl, HG) or without (TT >8 nmol/l, non-HG) testosterone deficiency. According to age quartiles, patients were classified as follows: <29 years = quartile 1, 29.1–36.5 years = quartile 2, 36.6–51 years = quartile 3 and >51.1 years = quartile 4. This study considered three points: the baseline, the 12-month (short-term) and the 24-month (long-term) evaluation.

Treatment Protocol

According to the standard protocol of the center [65, 66], in patients with microprolactinomas, CAB was administered orally at a starting dose of 0.25 mg twice weekly for the first 2 weeks and then 0.5 mg twice weekly. Dose adjustment was carried out every 3–6 months on the basis of serum PRL levels. In patients with macroprolactinomas, CAB was administered at a starting dose of 0.25 mg once a week for the first week and then twice weekly. Dose adjustment was performed at 3- to 6-month intervals on the basis of serum PRL levels. In patients whose PRL levels did not normalize, the CAB dose was progressively increased up to 5.5 mg/week. In patients achieving serum PRL levels <5 µg/l (lower limit of normal), the CAB dose was reduced to maintain serum PRL levels in the normal range. Thus, the final CAB dose ranged from 0.25 to 5.5 mg/week. In patients still showing testosterone deficiency after PRL normalization, androgen replacement treatment with the injectable testosterone enanthate (TR) was started. TR was administered at a starting dose of 250 mg every 4 weeks; dose adjustment was performed at 3- to 6-month intervals on the basis of serum TT levels measured 21 days after the last injection. In patients whose TT levels did not normalize, the TR dose was increased to 250 mg every 3 weeks. In patients achieving serum TT levels >31.2 nmol/l (i.e. 900 ng/dl, upper limit of normal), the TR dose was reduced to 250 mg every 6 weeks in order to maintain serum TT levels in the normal range.

Table 2. Effects of chronic treatment with CAB on disease control, MetS prevalence and metabolic complications in the whole patient cohort

	Baseline (A)	12 months (B)	24 months (C)	p (A vs. B)	p (B vs. C)	p (A vs. C)
Tumor volume, ml	4,468.24±6,053.72	1,224.41±2,391.79	689.67±1,361.08	0.000	0.000	0.000
PRL, µg/l	2,028.1±4,407.7	21.8±31.8	8.6±11.3	0.000	0.05	0.000
PRL normalization, %	–	84	97	–	–	–
TT, nmol/l	7.48±4.6	13.1±7.5	16.2±6.3	0.000	0.03	0.000
Median CAB dose (range), mg	–	1 (0.5–3.5)	1 (0.25–5.5)	–	–	–
Weight, kg	96.1±12.2	92.1±11.4	88.8±10.3	0.000	0.22	0.017
BMI	31.7±3.9	30.4±3.6	29.3±3.4	0.000	0.22	0.017
WC, cm	104.6±10.1	101.3±10.3	98.8±10.2	0.000	0.43	0.000
FG, mmol/l	5.3±1.3	5.2±0.7	5.2±0.9	0.51	0.89	0.96
FI, mU/l	17.1±6.8	11.6±5.2	7.8±4.2	0.000	0.004	0.000
CHOL, mmol/l	5.69±0.8	5.02±0.6	4.98±0.9	0.000	0.66	0.007
HDL cholesterol, mmol/l	1.1±0.2	1.2±0.3	1.2±0.25	0.2	0.62	0.13
TG, mmol/l	2.05±1	1.63±0.65	1.41±0.53	0.007	0.16	0.025
LDL cholesterol, mmol/l	3.68±0.8	3.11±0.6	3.2±0.8	0.001	0.71	0.006
MetS, % (n)	50 (16)	18.7 (6)	12.5 (4)	0.018	0.7	0.003
HOMA-IR	4.1±2.2	2.7±1.2	1.8±1.07	0.000	0.009	0.000
HOMA-β	292.6±340.3	161.7±95.8	115.1±86.5	0.000	0.043	0.001
ISI ₀	8.4±6.8	12.3±8.1	19.4±12.9	0.000	0.012	0.000
VAI	1.63±0.94	1.98±0.93	1.73±0.9	0.01	0.25	0.69

Assays

Glucose and lipid levels were measured by standard methods. Insulin, PRL and TT levels were measured by chemiluminescent immunometric assay using commercially available kits (Immulite; DPC, Llanberis, UK). For insulin, the sensitivity was 4 mU/ml; the intra-assay coefficient of variation (CV) values were 5.5–10.6%, and the corresponding interassay CV values were 6.2–10.8%. For PRL, the sensitivity was 0.16 µg/l; the intra-assay CV values for PRL concentrations of 22 and 164 µg/l were 2.3 and 3.8%, respectively; the corresponding interassay CV values were 6%. Normal PRL levels were 5–20 µg/l. Hyperprolactinemia was defined as a serum PRL level >20 µg/l in two different samples taken at a time interval of >1 week. For TT, the sensitivity was 0.347 nmol/l; the intra-assay CV values were 8.0, 4.1 and 3.2% for the levels of 3.4, 15.3 and 31 nmol/l, respectively; the corresponding interassay CV values were 9.3, 9.0 and 8.8% at 3.9, 15.2 and 29.4 nmol/l, respectively. Normal TT levels were 10.4–31.2 nmol/l (i.e. 300–900 ng/dl). Testosterone deficiency was defined as a serum TT level of lower than 8 nmol/l (i.e. 230 ng/dl) on two different samples taken at a time interval of >1 week. Dihydrotestosterone (DHT) and 17β-estradiol concentrations were measured using commercially available ELISA kits (MyBioSource, Inc., San Diego, Calif., USA). For DHT, the sensitivity was 12 pg/ml; intra-assay CV values were ≤8%, and the corresponding interassay CV values were ≤12%. For 17β-estradiol, the sensitivity was 0.06 ng/ml; intra-assay CV values were ≤8%, and the corresponding interassay CV values were ≤12%.

Statistical Analysis

Data were analyzed using SPSS Software for Windows, version 19.0 (SPSS, Inc., Cary, N.C., USA). Data are reported as mean ± SD unless otherwise specified. The comparison between

the numerical data before and after treatment with CAB was made with the nonparametric Wilcoxon test. The nonparametric Mann-Whitney U test was used to compare patients with and without testosterone deficiency. The comparison between prevalence was performed with the χ^2 test corrected by the Fisher exact test when necessary. The correlation study was done by calculating the Pearson correlation coefficients. Regression analysis was done to evaluate the association of MetS with PRL, testosterone normalization and CAB dose and to identify the best-predictive factors of change in parameters related to MetS. Significance was set at 5%.

Results

Clinical, metabolic and hormonal parameters in the whole patient cohort at baseline and after 12- and 24-month treatment are shown in table 2.

Baseline

At baseline, the mean tumor volume was 4,468.24 ± 6,053.72 ml; mean PRL and TT levels of all patients were 2,028.1 ± 4,407.7 µg/l and 7.48 ± 4.6 nmol/l (215.6 ± 132.6 ng/dl), respectively. TT was >8 nmol/l (>230 ng/dl) in 10 patients (non-HG, 31%) and <8 nmol/l (<230 ng/dl) in 22 (HG, 69%) (fig. 1). 71.8, 90.6 and 78% of patients, respectively, complained about asthenia, libido reduction and erectile dysfunction. Normal weight, overweight and

Fig. 1. Treatment protocol and consequent changes in testosterone deficiency prevalence. At baseline, testosterone levels were <8 nmol/l in 22 patients (HG, 69%) and >8 nmol/l in 10 patients (non-HG, 31%). All patients started treatment with CAB. Twelve-month CAB induced a significant decrease in HG prevalence (28%, $p = 0.002$) and a significant increase in non-HG prevalence (72%, $p = 0.002$). At the 12-month follow-up, TR was started in patients still showing testosterone deficiency. After the 24-month follow-up, a significant decrease in HG prevalence (6%, $p = 0.04$) and a significant increase in non-HG prevalence (94%, $p = 0.04$) were seen.

obesity were found in 3, 37.5 and 59.5% of patients, respectively. Impaired glucose tolerance was found in 8 patients (25%), whereas 1 patient (3%) had diabetes mellitus requiring treatment with metformin at the dose of 2 g/day. As shown in figure 2, MetS was diagnosed in 16 patients (50%), including 12 obese patients (75%, 1 with diabetes mellitus and 7 with impaired glucose tolerance) and 4 overweight patients (25%, 1 with impaired glucose tolerance). HOMA-IR was >2.6 in 25 patients (78%). Compared to non-HG, HG patients had significantly lower TT ($p = 0.000$) and higher PRL ($p = 0.001$) and WC ($p = 0.014$), with weight and BMI being slightly but not significantly different between the 2 groups (table 3). MetS prevalence was not significantly different in HG (60%) and non-HG (45.4%) patients ($p = 0.7$, fig. 1). According to the age, 9 patients (28%) were in quartile 1, 7 (22%) in quartile 2, 8 (25%) in quartile 3 and 8 (25%) in quartile 4. MetS was found in 11% of patients in quartile 1, in 57% of those in quartile 2, in 75% of those in quartile 3 and in 62% of those in quartile 4. No significant difference was found in any hormonal and metabolic param-

eters among the different quartiles, whereas MetS prevalence was significantly higher ($p = 0.023$) in patients in quartile 3 as compared to those in quartile 1.

12-Month Evaluation

At a mean CAB dose of 1.39 ± 0.7 mg/week, the tumor volume significantly decreased ($p = 0.000$) by 66% compared to the baseline evaluation, whereas PRL levels significantly decreased by 98% compared to the baseline evaluation and fully normalized in 84% of patients. TT levels significantly increased ($p = 0.000$), inducing a significant decrease in HG prevalence (28%, $p = 0.002$) and a significant increase in non-HG prevalence (72%, $p = 0.002$) (fig. 1). DHT and 17β -estradiol levels were available and measurable in stored sera in a subgroup of 14 patients (8 non-HG and 6 HG). DHT significantly increased after 12 months of CAB ($p = 0.001$) as compared to baseline, both in the HG ($p = 0.028$) and the non-HG patients ($p = 0.017$). Similarly, 17β -estradiol significantly decreased after 12 months of CAB ($p = 0.001$) as compared to baseline, both in the HG ($p = 0.043$) and the non-HG patients ($p = 0.028$). After 12 months of CAB, prevalence of asthenia (34.4%, $p = 0.006$), libido reduction (9.4%, $p = 0.000$) and erectile dysfunction (18.7%, $p = 0.000$) significantly decreased as compared to baseline. Normal weight, overweight and obesity were found in 6% ($p = 0.9$), 37.5% ($p = 1$) and 57% ($p = 0.9$) of patients, respectively. In the whole patient cohort, weight ($p = 0.000$), BMI ($p = 0.000$), WC ($p = 0.000$), CHOL ($p = 0.000$), LDL ($p = 0.001$), TG ($p = 0.007$), FI ($p = 0.000$, fig. 3), ISI_0 ($p = 0.000$, fig. 3), HOMA- β ($p = 0.000$, fig. 3) and HOMA-IR ($p = 0.000$, fig. 3) were all significantly improved, and VAI significantly increased ($p = 0.01$) compared to baseline. No significant change was found in the prevalence of impaired glucose tolerance (15.6%, $p = 0.53$) and diabetes mellitus (3%, $p = 1$), and no change was required in the metformin dosage in the only diabetic patient. The percent decrease in tumor volume was similar in the HG (63%) and the non-HG (74%) patients ($p = 0.502$). Similarly, the percent decrease in PRL was similar between the HG and non-HG patients ($p = 0.41$). PRL levels were still increased in 3 HG (13.6%) and 1 non-HG (10%) patients. As shown in table 3, in both HG and non-HG, a significant improvement was found in weight, BMI, WC, CHOL, LDL, FI, ISI_0 , HOMA- β and HOMA-IR; in non-HG HDL and TG also improved. Compared to non-HG, the HG patients had lower TT ($p = 0.000$) and higher weight ($p = 0.008$), BMI ($p = 0.003$), WC ($p = 0.000$) and HOMA- β ($p = 0.017$), with CHOL being slightly but not significantly different between the 2 groups (table 4). The

Fig. 2. MetS prevalence in the whole patient cohort (a) and in patient subgroups (b). MetS prevalence significantly decreased after 12 (18.7%, $p = 0.018$) and 24 (12.5%, $p = 0.003$) months compared to baseline (28%). In the HG patients, MetS prevalence slightly de-

creased after 12 months and was significantly reduced after 24-month CAB treatment ($p = 0.002$). In the non-HG patients, a slight but not significant difference was found in MetS prevalence throughout the study.

percent decrease in all metabolic parameters was similar in the HG and non-HG patients. Overall, MetS prevalence fell to 18.7% ($p = 0.018$, fig. 1). MetS was recorded in 5 obese patients, including 4 with impaired glucose tolerance and 1 with diabetes mellitus, and in 1 overweight patient with impaired glucose tolerance. HOMA-IR was above 2.6 in 16 patients (50%, $p = 0.038$). MetS prevalence was not significantly different in HG (33%) and non-HG patients (13%, $p = 0.42$, fig. 1). Among patients with MetS, only 1 still had hyperprolactinemia. No significant difference was found in all hormonal and metabolic parameters and in MetS prevalence among different quartiles. At the 12-month follow-up, TR was started in patients who still showed testosterone deficiency.

24-Month Evaluation

At a mean CAB dose of 0.88 ± 0.4 mg/week, the tumor volume further decreased ($p = 0.000$) by 78% as compared to that at the 12-month evaluation. The PRL levels were significantly reduced compared to baseline and further decreased as compared to the 12-month evaluation, with complete normalization in all patients except 1 non-HG patient (97%). TT levels significantly increased ($p = 0.000$) compared to baseline and further improved ($p = 0.03$) compared to short-term evaluation, with complete normalization in all patients except 2, inducing a significant decrease in HG prevalence (6%, $p = 0.04$) and a significant increase in non-HG prevalence (94%, $p = 0.04$) (fig. 1). DHT significantly increased further after 24 months ($p =$

0.008) as compared to the 12-month evaluation; particularly, in the HG patients, DHT significantly improved after 24 months ($p = 0.027$) as compared to baseline, whereas in the non-HG patients DHT significantly increased after 24 months ($p = 0.012$) as compared to baseline and further improved after 24 months of therapy as compared to the 12-month evaluation ($p = 0.012$). After 24 months, DHT was normal in all patients except 2 HG patients. Similarly, 17β -estradiol significantly decreased after 24 months ($p = 0.05$) as compared to baseline, with no further significant improvement after 24 months as compared to the 12-month evaluation. In the HG patients, 17β -estradiol did not show further significant changes after 24-month treatment as compared to baseline and 12 months. In the non-HG patients, 17β -estradiol significantly improved after 24 months ($p = 0.028$) as compared to baseline, with no further change after 24-month therapy as compared to the 12-month evaluation. After 24 months, 17β -estradiol was normal in all patients except one. A slight but not significant further change was observed in the prevalence of asthenia (18.7%, $p = 0.25$), libido reduction (9.4%, $p = 1$) and erectile dysfunction (12.5%, $p = 0.7$) as compared to the 12-month evaluation. Normal weight, overweight and obesity were found in 9% ($p = 0.6$), 47% ($p = 0.6$) and 44% ($p = 0.3$) of patients, respectively. In the whole patient cohort, weight ($p = 0.017$), BMI ($p = 0.017$), WC ($p < 0.000$), CHOL ($p = 0.007$), LDL ($p = 0.006$), TG ($p = 0.025$), FI ($p = 0.000$, fig. 3), ISI_0 ($p = 0.000$, fig. 3), HOMA- β ($p = 0.001$, fig. 3) and HOMA-

Table 3. Effects of short- and long-term treatment on metabolic complications in HG and non-HG patients

	Baseline (A)	12 months (B)	24 months (C)	p (A vs. B)	p (B vs. C)	p (A vs. C)
<i>HG patients (n = 22)</i>						
Tumor volume, ml	3,870.70±6,572.9	851.77±1,277.31	530.09±890.39	0.000	0.000	0.001
PRL levels, µg/l	2,820±5,151	24±35.4	9.7±12	0.000	0.007	0.000
PRL normalization, %	–	81.8	100	–	–	–
TT, nmol/l	5.1±1.8	12.4±6.6	20.8±5.2	0.000	0.01	0.000
Weight, kg	99±12.9	88.5±11.2	84.8±9.3	0.000	0.000	0.000
BMI	32.7±4.1	29.3±3.6	28±3	0.001	0.000	0.000
WC, cm	107.3±10.9	97.5±8.6	95.4±8.6	0.002	0.005	0.002
FG, mmol/l	5.4±1.5	5.2±0.7	5.2±0.9	0.92	0.55	0.79
FI, mU/l	17.4±7.2	10.5±4.6	7.8±3.8	0.000	0.007	0.000
CHOL, mmol/l	5.8±0.9	4.8±0.5	4.9±1	0.002	0.78	0.005
HDL cholesterol, mmol/l	1.07±0.24	1.13±0.24	1.16±0.26	0.25	0.38	0.26
TG, mmol/l	2±1.1	1.5±0.7	1.3±0.5	0.06	0.10	0.003
LDL cholesterol, mmol/l	3.8±0.9	3±0.5	3.1±0.9	0.003	0.78	0.011
MetS, % (n)	60 (13)	33 (7)	11 (2)	0.13	0.16	0.002
HOMA-IR	4.2±2.4	2.5±1.2	1.8±1.1	0.001	0.013	0.001
HOMA-β	324.4±404.2	136.8±81.2	116.6±86.9	0.007	0.09	0.002
ISI ₀	8.6±7.7	13.8±9.2	19±12.4	0.028	0.008	0.001
VAI	1.6±0.9	1.9±1	1.7±0.9	0.08	0.29	0.81
<i>Non-HG patients (n = 10)</i>						
Tumor volume, ml	5,595.28±4,421.50	2,176.74±4,032.53	1,097.52±2,176.69	0.012	0.018	0.008
PRL levels, µg/l	285.3±276.5	17.1±23.6	4.6±8.7	0.008	0.015	0.008
PRL normalization, %	–	70	90	–	–	–
TT, nmol/l	12.7±4.7	16.3±6.5	21.7±6.2	0.005	0.028	0.008
Weight, kg	89.5±6.9	84.7±11	82.5±9.7	0.007	0.007	0.008
BMI	29.6±1.9	28.3±3.5	27.2±3.1	0.008	0.008	0.008
WC, cm	98.8±4.1	94.6±8.5	93.2±8.5	0.007	0.007	0.007
FG, mmol/l	5.2±0.8	5.2±0.5	5.2±0.4	0.70	0.66	0.68
FI, mU/l	16.4±6.1	10.5±4.5	7.6±3.9	0.006	0.008	0.008
CHOL, mmol/l	5.5±0.7	4.9±0.6	4.8±0.9	0.007	0.11	0.012
HDL cholesterol, mmol/l	1.1±0.3	1.1±0.2	1.2±0.8	0.024	0.012	0.008
TG, mmol/l	2±0.9	1.6±0.6	1.3±0.5	0.007	0.2	0.051
LDL cholesterol, mmol/l	3.5±0.6	2.9±0.5	3±0.9	0.008	0.011	0.008
MetS, % (n)	45.4 (4)	13 (1)	13 (1)	0.27	1	0.27
HOMA-IR	3.9±1.8	2.3±1.1	1.7±1.1	0.008	0.028	0.008
HOMA-β	222.6±98.3	137.2±78.4	112.7±86.8	0.008	0.011	0.008
ISI ₀	7.9±4.4	13.6±9	20.7±14.4	0.008	0.028	0.021
VAI	1.7±1	1.9±0.9	1.6±0.9	0.78	0.82	0.84

IR ($p = 0.000$, fig. 3) were all significantly improved compared to baseline, with FI ($p = 0.004$), ISI₀ ($p = 0.012$), HOMA-β ($p = 0.043$) and HOMA-IR ($p = 0.009$) also significantly improving compared to the 12-month evaluation. No significant difference was found in VAI as compared to the study entry. Impaired glucose tolerance was still found in 3 patients (9%, $p = 0.6$), whereas 2 patients acquired normal glucose tolerance; diabetes mellitus was confirmed in 1 patient with no change in the insulin sensitizer dosage. The percent decrease in tumor volume was similar in the HG (74%) and non-HG (88%)

patients ($p = 0.444$). In the same way, the percent decrease in PRL was similar between the HG and non-HG patients ($p = 0.78$). As shown in table 3, in both HG and non-HG patients, a significant improvement was found in weight, BMI, WC, CHOL, LDL, TG, FI, ISI₀, HOMA-β and HOMA-IR as compared to the baseline evaluation, with weight, BMI, WC, FI, ISI₀ and HOMA-IR being further improved compared to the 12-month evaluation. Moreover, in the non-HG patients HDL, LDL and HOMA-β improved as compared to the 12-month follow-up. Compared to the non-HG patients, the HG patients still had a

Fig. 3. Effects of treatment with CAB on insulin resistance and sensitivity. **a** CAB induced a significant decrease ($p = 0.000$) in FI after 12-month therapy and a further significant reduction ($p = 0.000$) after long-term treatment. Correction of testosterone deficiency further improved FI ($p = 0.004$) as compared to the 12-month evaluation. **b** Compared to baseline, HOMA-IR significantly decreased after both short- ($p = 0.000$) and long-term therapy ($p = 0.000$). TT normalization induced a further significant

reduction as compared to the 12-month evaluation ($p = 0.009$). **c** Compared to baseline, HOMA- β significantly decreased after both 12- ($p = 0.000$) and 24-month CAB treatment ($p = 0.001$), with a further significant improvement after TR as compared to the 12-month evaluation ($p = 0.043$). **d** ISI₀ significantly increased after 12- ($p = 0.000$) and 24-month CAB therapy ($p = 0.000$). Correction of hypogonadism resulted in a further significant improvement of insulin sensitivity ($p = 0.012$).

higher weight ($p = 0.004$), BMI ($p = 0.004$) and WC ($p = 0.001$) (table 4). The percent decrease in all metabolic parameters was similar in the HG and non-HG patients. Overall, MetS prevalence significantly fell to 12.5% ($p = 0.003$, fig. 1). MetS was recorded in 1 obese diabetic patient and in 3 overweight patients with impaired glucose tolerance. HOMA-IR was above 2.6 in 5 patients (16%, $p = 0.000$). MetS prevalence was not significantly different in the HG (11%) and non-HG (13%, $p = 0.6$) patients and significantly decreased in the HG patients ($p = 0.002$) compared to the baseline evaluation (fig. 1). Among the patients with MetS, all achieved PRL and TT normalization. No significant difference was found in all hormonal

and metabolic parameters and in MetS prevalence among the different quartiles. However, MetS prevalence significantly decreased ($p = 0.044$) in patients in quartile 3 after 24-month treatment (12.5%) as compared to baseline evaluation.

Correlation Study

At baseline, TT significantly correlated with BMI ($r = -0.344$, $p < 0.05$), whereas no significant correlation was found between PRL levels and metabolic parameters. At the 12-month evaluation, the CAB dose significantly correlated with HOMA- β ($r = 0.367$, $p = 0.039$) and TT with weight ($r = -0.461$, $p = 0.008$), BMI ($r = -0.366$, $p = 0.04$)

Fig. 4. Correlation study in the current patient cohort. At the 24-month evaluation, TT (**a**) significantly correlated with ISI_0 ($r = 0.356$, $p = 0.045$) and the CAB dose (**b**) with FI ($r = 0.439$, $p = 0.012$).

Table 4. Comparison of short- and long-term treatment on metabolic complications in HG and non-HG patients

	Baseline			12 months			24 months		
	HG	non-HG	p	HG	non-HG	p	HG	non-HG	p
PRL, $\mu\text{g/l}$	2,820.3 $\pm$ 5,151.9	285.3 $\pm$ 276.5	0.001	24 $\pm$ 35.4	17.1 $\pm$ 23.6	0.53	9.7 $\pm$ 12	4.6 $\pm$ 8.7	0.12
TT, nmol/l	5.1 $\pm$ 1.8	12.7 $\pm$ 4.7	0.000	12.4 $\pm$ 6.6	16.3 $\pm$ 6.5	0.000	20.8 $\pm$ 5.2	21.7 $\pm$ 6.2	0.61
Weight, kg	99 $\pm$ 12.9	89.5 $\pm$ 6.9	0.06	88.5 $\pm$ 11.2	84.7 $\pm$ 11	0.008	84.8 $\pm$ 9.3	82.5 $\pm$ 9.7	0.004
BMI	32.7 $\pm$ 4.1	29.6 $\pm$ 1.9	0.06	29.3 $\pm$ 3.5	28.3 $\pm$ 3.5	0.003	28 $\pm$ 3	27.2 $\pm$ 3.1	0.004
WC, cm	107.3 $\pm$ 10.9	98.8 $\pm$ 4.1	0.014	97.5 $\pm$ 8.6	94.6 $\pm$ 8.5	0.000	95.3 $\pm$ 8.3	93.2 $\pm$ 8.5	0.001
FG, mmol/l	5.4 $\pm$ 1.5	5.2 $\pm$ 0.8	0.92	5.2 $\pm$ 0.7	5.2 $\pm$ 0.5	0.64	5.2 $\pm$ 0.9	5.2 $\pm$ 0.4	0.65
FI, mU/l	17.4 $\pm$ 7.2	16.4 $\pm$ 6.1	0.65	13.8 $\pm$ 9.2	10.5 $\pm$ 4.5	0.09	7.8 $\pm$ 3.8	7.6 $\pm$ 3.9	0.77
CHOL, mmol/l	5.8 $\pm$ 0.9	5.5 $\pm$ 0.7	0.39	4.8 $\pm$ 0.5	4.9 $\pm$ 0.6	0.053	4.9 $\pm$ 1	4.8 $\pm$ 0.9	0.70
HDL cholesterol, mmol/l	1.1 $\pm$ 0.2	1.1 $\pm$ 0.3	0.81	1.1 $\pm$ 0.2	1.1 $\pm$ 0.2	0.48	1.2 $\pm$ 0.3	1.2 $\pm$ 0.8	0.93
TG, mmol/l	2 $\pm$ 1.1	2 $\pm$ 0.9	0.40	1.5 $\pm$ 0.7	1.6 $\pm$ 0.6	0.46	1.3 $\pm$ 0.5	1.3 $\pm$ 0.5	1
LDL cholesterol, mmol/l	3.8 $\pm$ 0.9	3.5 $\pm$ 0.6	0.14	3 $\pm$ 0.5	2.9 $\pm$ 0.5	0.46	3.1 $\pm$ 0.9	3 $\pm$ 0.9	0.82
MetS, % (n)	60 (13)	45.4 (4)	0.7	33 (7)	13 (1)	0.42	11 (2)	13 (1)	0.60
HOMA-IR	4.2 $\pm$ 2.4	3.9 $\pm$ 1.8	0.74	2.5 $\pm$ 1.2	2.3 $\pm$ 1.1	0.17	1.8 $\pm$ 1.1	1.7 $\pm$ 1.1	0.80
HOMA- β	324.4 $\pm$ 404.2	222.6 $\pm$ 98.3	0.97	136.8 $\pm$ 81.2	137.2 $\pm$ 78.4	0.017	116.6 $\pm$ 86.9	112.7 $\pm$ 86.8	0.98
ISI_0	8.6 $\pm$ 7.7	7.9 $\pm$ 4.4	0.74	13.8 $\pm$ 9.2	13.6 $\pm$ 9	0.17	19 $\pm$ 12.4	20.7 $\pm$ 14.4	0.80
VAI	1.6 $\pm$ 0.9	1.7 $\pm$ 1	0.96	1.9 $\pm$ 1	1.9 $\pm$ 0.9	0.86	1.7 $\pm$ 0.9	1.6 $\pm$ 0.9	0.41

and WC ($r = -0.512$, $p = 0.003$). Percent change (Δ) in TT significantly correlated with Δ Weight ($r = -0.381$, $p = 0.031$), Δ BMI ($r = -0.366$, $p = 0.039$) and Δ CHOL ($r = 0.434$, $p = 0.013$). No significant correlation was found between the PRL nadir and the percent change in any clinical or metabolic parameter. At the 24-month evaluation, TT significantly correlated with ISI_0 ($r = 0.356$, $p = 0.045$, fig. 4) and the CAB dose with FI ($r = 0.439$, $p = 0.012$, fig. 4). Δ PRL significantly correlated with Δ Weight

($r = 0.432$, $p = 0.014$) and Δ BMI ($r = 0.443$, $p = 0.011$), and the CAB dose with Δ CHOL ($r = -0.64$, $p = 0.000$) and Δ LDL ($r = -0.72$, $p = 0.000$). No significant correlation was found between the PRL nadir and the percent change in any clinical or metabolic parameter. At multiple regression analysis, TT was the best predictive factor for FI ($t = -2.076$, $p = 0.047$) at the 12-month evaluation, whereas the CAB dose was the best-predictive factor of FI ($t = 2.2$, $p = 0.037$) at the 24-month follow-up.

Discussion

The results of the current study demonstrated that treatment with CAB improves the metabolic profile and reduces the MetS prevalence and that proper androgen replacement therapy in such patients strongly contributes to the significant amelioration of visceral obesity and insulin resistance in male hyperprolactinemic patients with concomitant testosterone deficiency. The underlying mechanisms responsible for this metabolic amelioration may be related to the action of either PRL and/or testosterone normalization or to the direct action of CAB and/or testosterone administration.

The association of sexual steroids with the impairment of metabolism and the consequent cardiovascular deterioration in men is controversial. The impact of testosterone on metabolism has been extensively elucidated, whereas published data are limited for DHT and inconsistent for estrogens. A meta-analysis of 49 cross-sectional studies [67] found that patients with any cardiovascular disease (CVD), including obesity and MetS, showed significantly lower TT but also DHT levels and higher serum estradiol levels as compared with individuals without CVD. This association of low testosterone and high estradiol with CVD was confirmed in the logistic regression model after adjusting for age, BMI, diabetes and hypertension [67]. Estradiol levels have also been positively associated with the progression of carotid intima-media thickness and incident stroke [68, 69] but have been negatively associated with mortality in a different study [70].

On the other hand, PRL excess has been demonstrated to negatively impact lipid, glucose and insulin metabolism. The close relationship among chronic hyperprolactinemia, weight gain and obesity has been extensively investigated over the past years [29–32, 71, 72]. In hyperprolactinemic patients receiving treatment with CAB, the decrease in body weight and body fat following PRL normalization has been ascribed mainly to the direct activation of dopamine type 2 receptor (D₂R) by CAB [50]. The reduction in body weight after PRL normalization during treatment with dopamine agonists has been consistently documented in several investigations [54, 71, 72]. In the present study, short- and long-term treatment with CAB induced a significant reduction in weight, BMI and WC, suggesting a clinically relevant improvement of visceral obesity, as confirmed by the significant decrease in the MetS prevalence both after short- and long-term treatment. However, in the whole patient cohort the percent change in weight and BMI has been found to be correlated to the increase in TT levels at the 12-month evalua-

tion and to the decrease in PRL levels after 24 months. Both were induced solely by the treatment with CAB, therefore suggesting an effect of the decrease in PRL and the increase in testosterone levels on the metabolic improvement. On the other hand, the negative impact of testosterone deficiency on the body composition and visceral obesity [5–9, 15] is confirmed in the current series, since testosterone has been found to be inversely correlated to weight, BMI and WC, and particularly so in the HG patients, whose weight, BMI and WC were significantly higher than those in the non-HG subjects at both evaluations. Men with testosterone and DHT levels in the lower quartiles have been found to have more than 2-fold higher odds of exhibiting MetS [73], and testosterone and DHT have been proposed as potential biomarkers of MetS which are not causally related to the MetS onset over time [73]. It is noteworthy that changes in body weight have been shown to influence testosterone levels, at least in ageing men [74], and losing 5% of weight has been found to be associated with a significant increase in testosterone, which further increased with additional weight loss. This seems to suggest that the rise in testosterone levels might represent the consequence rather than the cause of losing weight [74]. In the present study, the HG patients still showed a higher weight, BMI and WC as compared to the non-HG patients after long-term treatment despite the increase in TT levels. In this light, the current results seem to support the hypothesis that visceral obesity might be mainly influenced by testosterone deficiency and that weight loss might reflect a direct beneficial effect of both CAB treatment and adequate androgen replacement. Interestingly, VAI was found to be significantly increased after short-term CAB in both the HG and non-HG patients and decreased after 24-month treatment, with similar values as when compared to baseline, thus confirming the previous speculation that the significant improvement of adipose tissue dysfunction may occur only after long-term CAB therapy [54] and suggesting a potential direct beneficial effect of TR on adipose dysfunction. The significant reduction in body weight, BMI and WC recorded in the current series could explain per se the clinically relevant improvement seen also in the lipid and glucose profile as well as the significant decrease in MetS prevalence.

The main evidence in the literature supports the role of the normalization of PRL and testosterone as an important determinant of the metabolic outcome in hyperprolactinemic and hypogonadal patients, respectively. Indeed, decreased HDL and increased CHOL and TG have been reported in patients with prolactinomas as com-

pared to controls [75–78], and a direct correlation between lipid metabolism and PRL levels has been proposed [48]. PRL can directly act on adipose tissue and may be involved in the lipid metabolism of mature adipocytes [79–82]. Moreover, D₂R is also expressed on human adipocytes, suggesting a regulatory role for peripheral dopamine in adipose functions [83]. Previous studies have demonstrated that dopamine agonist-induced D₂R activation ameliorates various features of MetS including dyslipidemia, even apart from its impact on food intake and body weight [84–87]. Several investigations have reported the improvement in lipid profile after treatment with either bromocriptine or CAB in patients with prolactinomas, regardless of changes in body weight and BMI [50, 53, 88]. In line with these findings, the significant improvement in CHOL, LDL and HDL cholesterol as well as in TG and VAI after 5-year CAB treatment has recently been demonstrated [54], independently of baseline weight, BMI and WC and of the degree of their reduction during treatment, supporting the hypothesis of a beneficial direct action of dopaminergic activation on adipose dysfunction in patients with hyperprolactinemia [54]. On the other hand, an impaired lipid profile has also been described in patients with testosterone deficiency. A negative correlation between testosterone and/or DHT levels and lipid fractions has also been demonstrated [18, 89]. Testosterone deficiency has been found to be associated with dyslipidemia [90, 91] and so can predict the development of an adverse lipid profile [92]. In mice, testosterone and DHT have been found to inhibit pluripotent stem cell differentiation into the adipogenic lineage [93] and preadipocyte differentiation into adipocytes *in vitro* [94]. Additionally, testosterone has been shown to increase lipolysis adipocytes of male rats [95] and to promote leptin resistance in humans [96]. Replacement treatment for testosterone deficiency has been reported to impact lipid profile, albeit with discordant results. TR has been found to induce clinically negligible [20–22] or no effects [23] on lipid fractions in unselected patients despite the significant decrease in body fat, reporting a small but significant decrease in HDL [20] and CHOL levels [21]. In other studies [24, 97–100], a significant reduction in lipid fractions has been found after androgen treatment in obese and diabetic patients. In the current study, 12 and 24 months of continuous CAB treatment induced a significant decrease in TG, CHOL and LDL, with a slight but not significant impact on HDL. However, after 12 months of TR no further change of lipid fractions was observed either in the whole patient cohort or in the HG group, which suggests that in patients with

hyperprolactinemia androgen treatment might not exert a direct beneficial effect on the lipid profile. Conversely, in non-HG patients a significant improvement was found in TG after short-term CAB and in HDL after both evaluations. Altogether, these results confirm the previous hypothesis of a beneficial direct action of dopaminergic activation on lipid metabolism [54]. The strong correlation between CAB dose and percent change in CHOL and LDL found in the current series strengthens the hypothesis that the improvement in lipid profile may reflect the direct effect of CAB treatment on lipids rather than the sole association between PRL normalization and BMI [53, 54].

Similarly, the CAB-induced correction of PRL excess reportedly plays a role in improving the glucose profile and insulin resistance in hyperprolactinemic patients. A link between elevated PRL levels and glucose abnormalities or hyperinsulinemia has been documented in several studies, showing that exposure to PRL results in enhanced insulin secretion and β -cell proliferation [38, 41] and that overexpression of PRL in β -cells leads to inappropriately elevated serum insulin concentrations, increased islet insulin content and sustained β -cell replication [42]. A significant improvement in glucose metabolism and insulin resistance has been described in patients with Cushing's disease [101] and prolactinomas [50, 51, 53] following treatment with CAB. Recently, the significant improvement in FI, HOMA-IR, HOMA- β and ISI₀ after CAB therapy has been demonstrated in normal weight, overweight and obese patients [54], regardless of changes in body weight and BMI, confirming the hypothesis of a beneficial direct effect of CAB on insulin secretion and sensitivity [54]. Noteworthy, the CAB dose turned out to be the best predictor of the percent decrease in FI [54], strengthening the hypothesis of a direct effect of CAB on pancreatic β -cell insulin secretion [54]. On the other hand, the literature provides strong evidence of a link between testosterone deficiency and abnormalities in the glucose and insulin profile. An association between testosterone and/or DHT deficiency and impaired FG, insulin resistance and diabetes mellitus has emerged in several studies [18, 89–91, 102], and testosterone deficiency has been shown to predict the development of diabetes mellitus [19, 26, 103]. Men with chronic androgen deficiency due to Klinefelter's syndrome and idiopathic hypogonadotropic hypogonadism exhibit significantly higher steady-state plasma glucose concentrations upon an oral glucose load as compared to healthy control subjects [19], with glucose and insulin levels being almost doubled in comparison with controls [19]. According to

in vitro evidence, testosterone may facilitate insulin sensitivity in fat and muscle cells by upregulating the expression of insulin-induced downstream proteins [104]. Dose-dependent effects of testosterone on the expression of insulin receptor substrate 1 and glucose transporter 4 have been observed in vitro [105]. TR has been reported to ameliorate glucose and insulin metabolism. The improvement in FG, glycated hemoglobin and HOMA index has been demonstrated in men with obesity and diabetes mellitus after androgen treatment [23–25, 97–100]. Two recent meta-analyses [26, 27] have described a significant improvement in FG, glycated hemoglobin and HOMA index after testosterone therapy in patients with MetS and diabetes mellitus. In the whole patient cohort of the current study, FI, HOMA-IR and HOMA- β were found significantly decreased, and ISI_0 significantly increased after both the 12- and 24-month CAB treatment. Complete TT normalization further improved the parameters of insulin resistance and insulin sensitivity, particularly in the HG patients whose FI and ISI_0 further ameliorated after the addition of TR. Interestingly, the CAB dose was found to be significantly correlated with HOMA- β after the 12-month and with FI after the 24-month treatment, confirming the hypothesis of a direct dopaminergic regulatory role in insulin secretion [54]. Conversely, TT significantly correlated with ISI_0 at the 24-month evaluation, which suggests a potential direct beneficial action of androgen therapy on the regulation of peripheral insulin sensitivity. Moreover, the testosterone and CAB doses were the main predictive factors of FI after the 12- and 24-month follow-up, respectively. Considering these results together, it might be speculated that testosterone deficiency strongly and directly influences FI, promoting insulin resistance and that the improvement of the insulin profile seen after testosterone normalization can be directly ascribed to treatment with CAB, thus confirming the hypothesis of a direct beneficial effect of CAB itself on insulin secretion [54]. Androgen replacement further contributes to significantly improve peripheral insulin sensitivity.

However, a potential limitation of the current results is related to the impact of environmental pollutants on androgen levels in patients living in urban and industrialized areas, as in the present study. In fact, an increasing number of studies suggest that chemicals and pollutants in the environment, such as those arising from the traffic which are caused and spread by human activities, can affect the male reproductive system [106–116] and act as an endocrine disruptor because of their adverse effects on reproduction [117, 118] and disruption of steroidogene-

sis and spermatogenesis [119, 120]. Nickel pollution, taken as water-soluble, sulfidic, oxidic or metallic form, is known to modify the function of proteins in mammalian testes in terms of fertility and/or hormone production [119]. In a cohort of males from Eastern Slovakia heavily exposed to persistent organochlorinated pollutants, such as hexachlorobenzene, testosterone levels have been found to be decreased [121]. BMI has been shown to be significantly correlated to blood hexachlorobenzene levels and has been proposed as an imaginary compartment closely related to the total body fat mass, representing a depot of persistent organochlorinated pollutants able to negatively impact testosterone levels [121]. Therefore, on the basis of this evidence, the potential impact of environmental pollutants on testosterone deficiency seen in the current patient cohort cannot be excluded.

In conclusion, in male patients with prolactinoma, hyperprolactinemia and concomitant testosterone deficiency are associated with MetS in approximately 50% of cases. Visceral obesity appears to be mainly influenced by testosterone deficiency, and weight loss seems to reflect a direct beneficial effect of both CAB treatment and adequate androgen replacement. Thus, simultaneous correction of both PRL excess and testosterone deficiency is mandatory in order to improve insulin resistance and metabolic abnormalities in such patients. CAB might act as direct modulator of adipose tissue dysfunction and insulin secretion, whereas androgen replacement might directly influence peripheral insulin sensitivity regulation. Further studies are still needed to confirm and extend these data, to better elucidate the burden and the differential role of PRL, dopamine agonists, testosterone and androgen replacement in the modulation of metabolism in patients with prolactinomas.

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