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The effect of testosterone on cardiovascular risk factors in men with type 2 diabetes and late-onset hypogonadism treated with metformin or glimepiride

Robert Krysiak [a,*], Wojciech Gilowski [a,b], Bogusław Okopień [a]

[a] Department of Internal Medicine and Clinical Pharmacology, Medical University of Silesia, Katowice, Poland
[b] Cardiology Department, Chrzanow District Hospital, Chorzów, Poland

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ABSTRACT

Background: Men with type 2 diabetes are often characterized by abnormal plasma testosterone levels. This study was aimed at investigating whether testosterone treatment has an impact on cardiovascular risk factors in patients with type 2 diabetes and late-onset hypogonadism (LOH), chronically treated with hypoglycemic agents.
Methods: This study included 51 men with type 2 diabetes, 26 of whom had already been treated with metformin and 25 with glimepiride for at least 6 months. On the basis of patient preference, 15 men receiving metformin and 12 receiving glimepiride were treated with intramuscular testosterone enanthate (100 mg weekly) for 12 weeks. Plasma lipids, glucose homeostasis markers, as well as plasma levels of androgens, uric acid, high-sensitivity C-reactive protein (hsCRP), homocysteine and fibrinogen were determined before and at the end of the study.
Results: With the exception of insulin sensitivity, plasma hsCRP and homocysteine, there were no differences between patients treated with metformin and glimepiride. Testosterone enanthate administered to both groups of patients increased plasma testosterone, reduced plasma hsCRP and improved insulin sensitivity. Testosterone-metformin combination therapy reduced also circulating levels of uric acid, homocysteine and fibrinogen. These effects, stronger in patients treated with metformin than glimepiride, correlated with the impact of testosterone on insulin sensitivity.
Conclusions: Our results suggest that testosterone may bring more clinical benefits to metformin- than sulfonylurea-treated men with diabetes and LOH.

Introduction

The prevalence of diabetes mellitus has increased exponentially throughout the world and it is estimated that the total number of people with this disorder will increase from 328 million to 592 million by the year 2035 [1]. Patients with diabetes have a two- to four-fold increased risk of cardiovascular disease over non-diabetic patients, and cardiovascular disease is the major cause of death in patients with type 2 diabetes. More than 60% of patients with type 2 diabetes die of myocardial infarction or stroke, and an even greater proportion of patients have serious burdensome complications [2]. Diabetic patients without a history of cardiovascular disease have a 5-year cardiovascular mortality that is similar to that of non-diabetic patients who have a history of myocardial infarction [3]. Because of an increase in longevity and increasingly sedentary lifestyle, type 2 diabetes is one of the most common chronic conditions among older adults and is often present with co-morbidities and geriatric syndromes [4].

Interestingly, observational studies revealed the association between glucose homeostasis abnormalities and circulation levels of testosterone [5,6]. Plasma glucose concentrations inversely correlated with total testosterone. Men with type 2 diabetes had lower plasma testosterone levels than non-diabetics [7,8]. Decreased testosterone levels have been also associated with reduced insulin sensitivity and vascular disease in men with type 2 diabetes [9], as well as with subsequent development of insulin resistance

Abbreviations: CRP, C-reactive protein; DHEA-S, dehydroepiandrosterone sulphate; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; HOMA1-IR, the homeostatic model assessment 1 of insulin resistance ratio; hsCRP, high-sensitivity C-reactive protein; IGT, impaired glucose tolerance; LDL, low-density lipoprotein; LOH, late-onset hypogonadism; SD, standard deviation.

* Corresponding author.
E-mail address: r.krysiak@interia.pl (R. Krysiak).

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and type 2 diabetes [10,11]. Moreover, low circulating levels of total testosterone in men have been associated with an increased prevalence of dyslipidemia [12], atherosclerosis [13], and an overall mortality [14]. Patients with low plasma testosterone levels are characterized by increased cardiovascular morbidity and mortality [15,16].

Unlike the dramatic fall in estrogen levels at the time of menopause in women, testosterone concentrations decline gradually with age [17]. In a large population of men between 40 and 70 years of age, total testosterone level declined by about 1.6% per year, while free and albumin-bound testosterone levels declined by 2–3% per year [18]. As a result of this decline, some middle-aged and elderly men may develop late-onset hypogonadism (LOH), defined as a clinical and biochemical syndrome associated with advancing age, which is characterized by typical symptoms and deficiency in serum testosterone levels [19]. The risk of its development is higher in the case of coexisting type 2 diabetes [20,21].

A high prevalence of diabetes in a geriatric population and its association with LOH cause that some patients may benefit from testosterone therapy. Unfortunately, no previous study has investigated the impact of exogenous testosterone on cardiovascular morbidity or mortality. The aim of this study was to investigate whether the addition of testosterone to oral hypoglycemic therapy affects plasma levels of cardiovascular risk markers in men with type 2 diabetes. C-reactive protein (CRP), uric acid, homocysteine and fibrinogen were chosen for investigation because their increased values are strongly associated with an enhanced risk of atherosclerosis and its complications [22–26].

Materials and methods

The participants of the study ($n = 51$) were recruited among men (55–79 years old) with type 2 diabetes treated for at least 6 months with a constant dose of metformin (2.55–3 g daily) or glimepiride (2–4 mg daily) and complying with lifestyle intervention. To be admitted to the study, they had to meet the inclusion criteria of LOH: total testosterone level below 3.0 ng/mL on two different occasions and the presence of the following symptoms: decreased frequency of morning erection, erectile dysfunction and decreased frequency of sexual thoughts. The exclusion criteria were as follows: prostate cancer, severe lower urinary tract symptoms (the American Urological Association International Prostate Symptom Score exceeding 19), baseline prostate-specific antigen greater than 4 ng/mL (greater than 3 ng/mL in men at high risk of prostate cancer), breast cancer, myocardial infarction, acute coronary event, unstable angina, stroke or coronary revascularization procedure within 6 months preceding the study, severe heart failure (classes II–IV according to the New York Heart Association Functional Classification), uncontrolled arterial hypertension, hematocrit exceeding 50%, untreated obstructive sleep aspnea, concomitant treatment with other hypoglycemic agents or drugs to interact with metformin, sulfonylureas or testosterone, and poor patient compliance. The local ethics committee approved the study protocol. All subjects included in the study signed informed consent after careful explanation of the study procedures. After appropriate discussion of the uncertainties of the risks and benefits of testosterone therapy in older men, the included patients were divided into four groups: patients treated with metformin and testosterone ($n = 15$), patients receiving only metformin ($n = 11$), patients treated with glimepiride and testosterone ($n = 12$), as well as patients receiving only glimepiride ($n = 13$). Testosterone enanthate was administered intramuscularly at the dose of 100 mg once weekly. Throughout the study, lasting 12 weeks, patients received metformin or glimepiride at the same dose as before the beginning of the study and complied with dietary recommendations.

Venous blood samples were drawn from the antecubital vein in a quiet, temperature-controlled room (24–25 °C) after the patients had been in a recumbent position for at least 15 min before and at the end of the study period. Samples, taken 12 h after the last meal, which occurred between 8:00 and 9:00 a.m. and assessed in duplicate by a person blinded to subject identity and study sequence. Pre- and post-treatment samples from each patient were assayed in the same assay run. Plasma lipids (total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides), fasting glucose and plasma uric acid were assayed by routine laboratory techniques (Roche Diagnostics, Basel, Switzerland). LDL-cholesterol levels were measured directly. Glycated hemoglobin (HbA_{1c}) was determined using DCA 2000 analyzer (Bayer Ames Technicon, Tarrytown, NY, USA). Plasma insulin, total testosterone and dehydroepiandrosterone sulphate (DHEA-S) were measured using commercially available enzyme immunoassay kits (DRG Instruments GmbH, Marburg, Germany). The homeostatic model for insulin resistance (HOMA1-IR) was calculated as follows: fasting glucose (mg/dL) × fasting insulin (mIU/L)/405. Circulating levels of CRP evaluated using a high-sensitivity monoclonal antibody assay (hsCRP) (MP Biomedicals, Orangeburg, NY, USA). Plasma levels of homocysteine were determined by enzyme-linked immunosorbent assay (Diazyme, San Diego, CA, USA). Plasma fibrinogen concentration was measured according to the Clauss method using a commercial enzyme-linked immunosorbent assay kit (Biomerieux, Marcy l'Etoile, France). The intra- and interassay coefficients of variation for the investigated parameters were below 6.1% and 8.8%, respectively.

The normality of the quantitative variables was verified using the Shapiro–Wilk test. Outcomes for the non-normal variables (triglycerides, HOMA1-IR, hsCRP, homocysteine, fibrinogen and hormones) were natural-log transformed to fit a normal distribution curve. Comparisons between the groups were performed using analysis of covariance followed by Bonferroni *post hoc* tests after consideration of age, smoking, body mass index, blood pressure, duration of hypoglycemic treatment and metformin and glimepiride dose as potential confounders. Student's paired t test was applied to compare pre- and post-treatment data within the same group. Categorical variables were analyzed by χ^2 test. Correlations between the study parameters were calculated using Kendall's tau test. Values of $p < 0.05$ were considered statistically significant.

Results

Demographic data and baseline blood test values in patients who completed the study are presented in Tables 1 and 2. At the beginning of the study, metformin- and glimepiride-treated patients were comparable with respect to age, body weight, medical background and clinical characteristics. Patients treated with metformin exhibited higher levels of homocysteine and lower levels of plasma hsCRP and HOMA1-IR than subjects receiving glimepiride. In both metformin- and glimepiride-treated patients, baseline values did not differ between patients receiving and not receiving later testosterone.

One patient who was treated with glimepiride reported dizziness and declined to participate further in the study. Another subject (receiving metformin and testosterone) was withdrawn from the study because of elevated hematocrit. No other serious adverse events occurred during the study.

In the patients treated with metformin or glimepiride who did not decide on testosterone treatment, the lipid profile, glucose homeostasis and plasma levels of the investigated cardiovascular risk factors remained at the similar level throughout the study.