Endokrynologia Polska
DOI: 10.5603/EP.a2017.0009
Tom/Volume 68; Numer/Number 3/2017
ISSN 0423–104X

Effect of testosterone replacement therapy on vitamin D and FGF-23 levels in congenital hypogonadism

Wpływ testosteronowej terapii zastępczej na stężenia witaminy D i FGF-23 w hipogonadyzmie wrodzonym

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Abstract

Introduction: Patients with hypogonadism are at increased risk of cardiac and metabolic diseases and osteoporosis. Vitamin D and fibroblast growth factor-23 (FGF-23) play a role in the regulation of bone mineral metabolism and endothelial functions. Low vitamin D levels are reported in hypogonadism, while there is no data about the effect of testosterone replacement therapy (TRT). We investigated the effect of TRT on vitamin D and FGF-23 levels along with endothelial functions and insulin resistance in hypogonadal patients.

Material and methods: Patients with congenital hypogonadotrophic hypogonadism (CHH) (n = 32, age 20.6 ± 1.58 years) were enrolled. TRT was implemented in transdermal form. The demographic parameters, FGF-23, 25(OH)D3, asymmetric dimethylarginine (ADMA), and homeostatic model assessment of insulin resistance (HOMA-IR) levels, were measured both before and after TRT.

Results: After a follow-up period of 3.63 ± 1.33 months, ADMA and FGF-23 levels were significantly increased ($p = 0.03$ and $p = 0.005$ respectively), while 25(OH)D3 and HOMA-IR index were not significantly changed. The body mass index and waist circumference levels of the patients were also increased ($p < 0.001$ and $p = 0.02$) along with a significant decrease in the HDL cholesterol levels ($p = 0.006$).

Conclusions: The results show that short-term TRT increases plasma FGF-23 and ADMA levels but does not alter the vitamin D levels in young, treatment naive patients with CHH. Whether this is an early implication of TRT-related adverse effects in this very young and treatment naïve population of CHH is not clear. Future prospective studies are required to find out the long-term effects of TRT on cardiometabolic morbidity and mortality in this specific population. **(Endokrynol Pol 2017; 68 (3): 311–316)**

Key words: *hypogonadism; vitamin D; fibroblast growth factor-23; asymmetric dimethyl arginine*

Streszczenie

Wstęp: U chorych z hipogonadyzmem występuje zwiększone ryzyko chorób sercowych I metabolicznych oraz osteoporozy. Witamina D i czynnik wzrostu fibroblastów-23 (FGF-23) uczestniczą w regulacji metabolizmu kostnego i czynności śródbłonka. Istnieją doniesienia na temat niskiego stężenia witaminy D w hipogonadyzmie, natomiast brakuje danych dotyczących wpływu testosteronowej terapii zastępczej (TRT) na to stężenie. Autorzy zbadali wpływ TRT na stężenia witaminy D i FGF-23 oraz na czynność śródbłonka i poziom insulinooporności u chorych z hipogonadyzmem.

Materiał i metody: Do badania włączono chorych z wrodzonym hipogonadyzmem hipogonadotropowym (CHH) (n = 32, wiek 20,6 ± 1,58 roku). Chorzy otrzymywali TRT w postaci przezskórnej. Przez rozpoczęciem leczenia i po jego zakończeniu u chorych zebrano dane demograficzne, zmierzono stężenia FGF-23, 25(OH)D3 i asymetryczej dimetyloargininy (ADMA) oraz określono wskaźnik insulinooporności HOMA-IR.

Wyniki: Po okresie obserwacji trwającym 3,63 ± 1,33 miesiąca stwierdzono istotne zwiększenie stężeń ADMA i FGF-23 (odpowiednio $p = 0,03$ i $p = 0,005$), natomiast stężenie 25(OH)D3 i wskaźnik HOMA-IR nie zmieniły się istotnie. Ponadto zaobserwowano u chorych zwiększenie wskaźnika masy ciała i obwodu pasa ($p < 0,001$ I $p = 0,02$) oraz istotne zmniejszenie stężenia cholesterol frakcji HDL ($p = 0,006$).

Wnioski: Wyniki badania pokazują, że krótkotrwałe stosowanie TRT u młodych chorych z CHH, uprzednio nieleczonych, powoduje zwiększenie osoczowego stężenia FGF-23 i ADMA, lecz nie wpływa na stężenie witaminy D. Nie jest jasne, czy jest to wczesny efekt działań niepożądanych TRT w tej grupie bardzo młodych pacjentów z CHH. Konieczne są dalsze prospektywne badania w celu ustalenia długookresowego wpływu TRT na chorobowość i śmiertelność w związku z chorobami sercowymi i metabolicznymi w tej szczególnej populacji. **(Endokrynol Pol 2017; 68 (3): 311–316)**

Słowa kluczowe: *hipogonadyzm; witamina D; czynnik wzrostu fibroblastów-23;* asymetryczna *dimetyloarginina*

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Introduction

The clinical features of hypogonadism are far beyond the lack of fertility. The risk of cardiac and metabolic disorders such as obesity, type 2 diabetes, and atherosclerotic cardiovascular diseases [1, 2] are significantly increased. Also, bone mineral metabolism disorders, such as osteoporosis, are prevalent in patients with hypogonadism [3–5]. Positive correlations between testosterone and vitamin D levels were reported in several cross-sectional studies in hypogonadal subjects [6-8]. However, very few studies have been performed so far to investigate the effect of testosterone replacement therapy (TRT) on vitamin D levels [9].

Fibroblast growth factor-23 (FGF-23) is a novel osteocyte-derived hormone, which regulates phosphate, vitamin D, and bone mineral metabolism [10–12]. FGF-23 also plays role in the pathogenesis of endothelial dysfunction and atherosclerosis [13-15]. There is a competitive interaction between the levels of FGF 23 and asymmetric dimethylarginine (ADMA), a well-documented surrogate for endothelial dysfunction [15]. Despite the significant impairment of endothelial functions and bone mineral metabolism in hypogonadism, little is known about the levels of FGF-23 and the role it plays in these patients [16, 17]. Also, there are no data about the effect of TRT on FGF-23 levels in patients with hypogonadism.

We designed the following study to search for any effect of TRT on the vitamin D and FGF-23 levels in patients with hypogonadism. Also, we searched for any relationship between FGF-23 and Vitamin D levels or the surrogate markers of endothelial dysfunction and insulin resistance.

Material and methods

Military service is compulsory for every young man in Turkey. Gulhane Military Medical Academy School of Medicine is the tertiary medical centre for all the military recruits. Patients with hypogonadism are referred to the Department of Endocrinology and Metabolism for treatment and follow-up. Some of these hypogonadal patients, generally the ones living in the rural regions, have never been treated before. Information about the enrolment criteria and the testosterone replacement procedures of our hypogonadal cohort were given in detail in previous articles [18–20]. Briefly, young patients diagnosed as CHH, who were not treated with testosterone replacement and who did not have any other chronic diseases, were enrolled.

Thirty-two treatment-naive patients (age 20.6 ± 1.58 years) with CHH were enrolled in the study. The diagnosis of CHH was based on a failure to undergo spontaneous puberty, specific physical findings of hypogonadism, such as infantile genitalia (Tanner stages 1 or 2), eunuchoid proportions, high-pitched voice, and sparse male-pattern body hair; and laboratory results showing hypogonadotrophic hypogonadism (low testosterone, normal or low gonadotrophins, and normal pituitary functions). None of the patients had any chronic disorders or were previously given testosterone or human chorionic gonadotropin (hCG) therapy. All patients gave informed consent, and the Local Ethical Committee of Gulhane School of Medicine approved the study. This study has been registered to Clinicaltrials.gov (NCT02111473).

The height, weight, and waist circumference (WC) of the patients were measured with their underwear. Body mass index (BMI) was computed as the ratio of weight to the square of height (kg/m^2). WC was measured, after the patients exhaled, from the line on the iliac crest, which is parallel to the ground. Pubertal developments of the patients were assessed according to the Tanner stages. Pituitary hormones were evaluated in all patients to exclude panhypopituitarism. Pituitary or hypothalamic mass lesions were excluded by magnetic resonance imaging. The 25(OH)D3 and FGF-23 levels of some patients presented here were involved in a previous case control study [17]. The present study is designed to evaluate the effect of TRT during the follow-up period.

Testosterone replacement therapy

The patients enrolled in this study were treated with transdermal testosterone gel (Testogel 50 mg gel) applied every night. The blood samples for the evaluation of the baseline metabolic parameters were taken before the first testosterone dosage. The patients were then reevaluated in the third and/or sixth months of treatment.

Sample collection and laboratory measurements

For biochemical analyses, all blood samples were collected from the antecubital vein, between 08:00 and 09:00 after an overnight fasting. The samples were centrifuged for 15 minutes at 4000 rpm, aliquoted, and immediately frozen at –80°C for analyses. Fasting blood glucose (FBG), total cholesterol, triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) levels were measured by the enzymatic colorimetric method with an Olympus AU2700 auto analyser using reagents from Olympus Diagnostics (GmbH, Hamburg, Germany). Low-density lipoprotein cholesterol (LDL-C) was calculated by Friedewald's formula [21]. The serum basal insulin, testosterone, FSH, and LH were measured by the electrochemiluminescence method with the *UnicelDXI 800 Access Immunoassay System* (Miami, FL, ABD). Insulin sensitivity was calculated

Table I. *Clinical and laboratory characteristics of patients before and after testosterone replacement therapy*
Tabela I. *Parametry kliniczne i laboratoryjne u chorych przed leczeniem i po zastosowaniu testosteronowej terapii zastępczej*

	Before treatment (n = 32)	After treatment (n = 32)	p*
Age (years)	20.6 ± 1.58		
SBP [mmHg]	123.1 ± 17.3	123.1 ± 13.0	0.98
DBP [mmHg]	73.5 ± 8.2	69.2 ± 9.9	0.11
BMI [kg/m^2]	21.8 ± 2.7	23.4 ± 3.1	**< 0.001**
WC [cm]	80.8 ± 10.4	84.6 ± 10.6	**0.02**
FSH [mIU/mL] **	0.64 (0.33-0.96)	0.43 (0.29-0.93)	0.79
LH [mIU/mL] **	0.29 (0.20-0.94)	0.38 (0.20-0.89)	0.31
Total Testosterone [ng/dl]	32.6 ± 22.1	194.8 ± 205.8	**< 0.001**
HDL-C [mg/dl]	48.1 ± 9.6	43.1 ± 9.0	**0.006**
TG [mg/dl]**	120.0 (70.0-225.0)	108 (90.0-174.0)	0.63
FBG [mg/dl]	88.8 ± 6.4	93.0 ± 5.7	**0.007**
INSULIN**	11.5 (6.3-16.5)	10.4 (8.2-15.5)	0.50
HOMA-IR**	2.36 (1.43-3.26)	2.34 (1.77-3.60)	0.12
FGF-23 [pg/mL]	66.3 ± 26.7	88.8 ± 37.6	**0.005**
ADMA [μmol/L]	0.75 ± 0.15	0.87 ± 0.21	**0.03**
25[OH]D3 [nmol/L]	17.3 ± 8.2	20.0 ± 8.3	0.23

***** Paired samples t test.
** Mann-Whitney *U* test. Results are given as mean (25–75%)
SBP — Systolic blood pressure, DBP — Diastolic blood pressure, BMI — Body mass index, WC — Waist circumference, FSH — Follicle stimulating hormone, LH — Luteinising hormone, HDL-C — High-density lipoprotein cholesterol, TG — Triglyceride, FBG — Fasting blood glucose, HOMA-IR — Homeostatic model assessment-insulin resistance, FGF-23 — Fibroblast growth factor-23, ADMA — Asymmetric dimethylarginine

by using the homeostatic model assessment of insulin resistance (HOMA-IR) index by the formula: HOMA-IR = (insulin × glucose)/405 [22]. Plasma asymmetric dimethylarginine (ADMA) levels were determined by ELISA (Immundiagnostik, Bensheim, Germany). The minimum detectable concentration for ADMA was 0.05 μmol/L. Plasma FGF-23 levels were determined by ELISA, (Human Intact FGF-23 ELISA Kit, Inc., San Clemente, CA, USA). The minimum detectable concentration for FGF-23 was 1.0 pg/mL. Intra-assay coefficient of variation (CV) ranged from 2.6% to 4.4%, while inter-assay CV ranged from 6.1% to 6.5% for FGF-23. Measurements were carried out using ELISA plate reader Bio-Tek Synergy HT [Biotek Instruments Inc., Winooski, VT, USA]. Plasma 25(OH)D3 levels were measured by Immuchrom kits (Immuchrom, Hessen, Germany) using isocratic HPLC method with UV detector in the Shimadzu Prominence HPLC system.

Statistical analysis

All data were recorded on a computer database and analysed using SPSS 18.0 package program (SPSS, Inc.,Chicago, IL, USA). Results are expressed as mean ± S.D. The correlations were performed by using the Pearson's or Spearman's Correlations tests. The variables were assessed for normality using Kolmogorov-Smirnov test. Intra-group changes at two time points were analysed by paired samples t-test. Differences were considered significant at $p < 0.05$.

Results

The clinical and laboratory characteristics of the patients, both before and after the TRT, are given in Table I. The follow-up period of the patients under TRT is 3.63 ± 1.33 months. According to the results, the secondary sexual characteristics of the patients were significantly improved from Tanner stages 1–2 to stages 3–4. Also, the BMI, WC measures ($p < 0.001$ and $p = 0.02$), and FBG levels ($p = 0.007$) were significantly increased and HDL-C levels were significantly decreased ($p = 0.006$) during the follow-up period. On the other hand, serum FGF-23 and ADMA levels significantly increased ($p = 0.005$ and $p = 0.03$, respectively), while the 25(OH)D3 and HOMA-IR index were not significantly changed (Fig. 1). No significant correlation was found between the serum levels of total testosterone, FGF-23, 25(OH)D3, ADMA, or the HOMA-IR indexes. No significant side effects were reported during the study period.

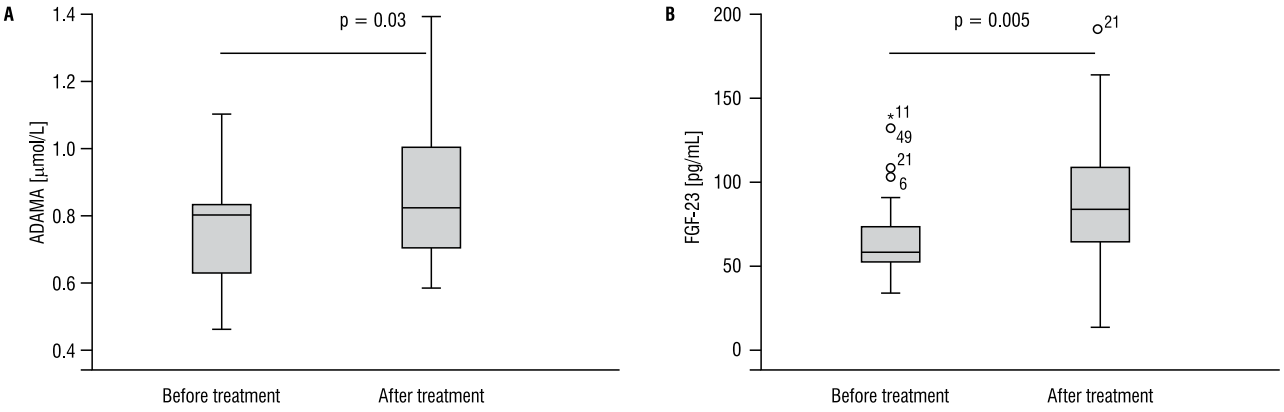

Figure 1. *Comparison of plasma ADMA* **(A)** *and FGF-23* **(B)** *levels before and after testosterone replacement therapy*
Rycina 1. *Porównanie stężeń w osoczu ADMA* **(A)** *I FGF-23* **(B)** *przed i po testosteronowej terapii zastępczej*

Discussion

The results of the present study show that TRT for about four months has no significant effect on Vitamin D levels but increases plasma FGF-23 and ADMA levels in treatment naïve, young patients with congenital hypogonadism. The BMI and WC of these patients also increase along with a decrease in HDL-C levels. Whether these findings are the early implications of TRT-related adverse cardio-metabolic outcomes will be discussed below.

Osteoporosis and increased fracture risk is a significant feature of hypogonadism [23–26]. The exact mechanism of impaired skeletal health in hypogonadal subjects is not clearly explained [27]. Recent data has shown that vitamin D levels are lower and significantly correlated with low testosterone levels in hypogonadal males [6–8]. The expression of vitamin D receptor and vitamin D metabolising enzymes in the human testis [28] or the impairment of the renal 1α-hydroxylase activity due to low testosterone levels [29] show a close functional relationship between testosterone and vitamin D. Another key regulator of vitamin D and phosphate metabolism is FGF-23, a bone-derived hormone improving renal phosphate excretion and preventing the activation of vitamin D levels [11, 12]. The role of FGF-23 in hypogonadal patients has not been clearly defined. In an interventional study of healthy male volunteers who were experimentally exposed to gonadotropin releasing hormone (GnRH) analogue, no alteration was observed in FGF-23 levels [16]. Recently, we have reported that FGF-23 levels in congenital hypogonadism are not significantly different from the healthy controls, while the vitamin D levels tend to be lower [17].

Both metabolic and cardiovascular diseases are prevalent in patients with hypogonadism [30–33]. Several studies show that TRT improves cardiovascular risk factors such as fasting glucose levels, insulin sensitivity, arterial blood pressure, or total cholesterol levels [34–36]. However, the metabolic benefits of TRT may have been exaggerated at least in some of the previous studies [37]. Contrary to the data on the metabolic and cardiovascular benefits, increased cardiovascular events were reported in hypogonadal patients under TRT [38–41]. Most of these studies showing adverse effects of TRT were by and large performed on frail elderly populations with several confounders. However, we also reported endothelial dysfunction, inflammation and insulin resistance after TRT in unconfounded, young hypogonadal patients [18-20]. To our knowledge, the present study is the first to search for the effect of TRT on 25(OH)D3 and FGF-23 levels . FGF 23 is not only secreted from osteocytes but also from the vascular endothelium [42]. FGF-23 elevation plays a role in the pathogenesis of endothelial dysfunction [14, 43], atherosclerosis [13], and left ventricular hypertrophy [44]. On the other hand, ADMA is an endogenous inhibitor of nitric oxide production and a promising biomarker for cardiovascular diseases [45]. To our knowledge, very few studies have reported the effect of TRT on ADMA levels in the literature. Two of these studies, which have significant limitations, report decreased ADMA levels after TRT. ADMA levels were measured only a few days after a single injection in one study [46], and only 10 middle aged or elderly patients with different aetiologies and several comorbidities were enrolled in the other [47]. However, plasma ADMA levels did not improve but worsened after TRT in our homogenous study population. This is in accordance with our previous report of a similar follow-up study in a CHH population [20]. The mechanism of this increase in ADMA levels after TRT is not clear. There is a significant interaction between the elevated FGF-23

and ADMA levels in terms of the renal and cardiovascular outcomes [15]. The increase in both markers after TRT may be an early indicator of adverse cardiovascular events in CHH.

There are several limitations of this study. The relatively short follow-up period and the small study population preclude further mechanistic comments. In addition, the effect of the season and sun exposure on the measurement of 25(OH)D3 levels were ignored in the study. Most importantly, this specific population of treatment naïve young patients with CHH may not represent the elderly hypogonadal patients. In our CHH group, the secondary sexual characteristics significantly improved, but the BMI and WC were increased after the TRT. A general response that we observed in this study population is a significant improvement in the secondary sexual characteristics along with a significant increase in BMI and WC [17–20]. It is likely that TRT ignites pubertal growth in these young patients, which is not seen in elderly hypogonadal patients of other aetiologies This may partly explain the rapid increase in BMI and waist circumference of these patients. Despite these limitations, this study has significant advantages regarding the homogeneous study population and lack of confounding factors, such as chronic metabolic disorders and concomitant medications.

Conclusions

The results of the present study show that TRT in young, treatment naive patients with CHH does not have a significant effect on 25(OH)D3 levels, but it increases plasma FGF-23 and ADMA levels. However, whether these metabolic effects will result in adverse cardiovascular outcomes in the long term is not clear. Future prospective studies are required to find out the long-term effects of testosterone replacement on vitamin D levels and cardiovascular morbidity and mortality in this specific population.

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