

The assessment of testosterone and radioisotopic index of bone metabolism and bone mineral density in men with testosterone deficiency after one year of testosterone therapy

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Abstract

BACKGROUND: Testosterone deficiency in men is characterized by typical symptoms of hypogonadism and negative influence on the preservation of bone mass. In this study, we analysed the relationship between testosterone concentration and bone metabolism. Moreover, we assessed the impact of one-year compensation of testosterone deficiency in elderly men on bone metabolism and bone mineral density. Radioisotopic methods of bone metabolism assessment provide new research opportunities.

MATERIALS AND METHODS: Men with total testosterone concentration (TT) ≤ 3 ng/ml were included into this study. Patients with disorders or injuries of bone system, elevated prostate-specific antigen (PSA), enlarged prostate, disorders of thyroid and liver, diabetes mellitus or a history of chemotherapy as well as those treated for a long time with antibiotics were excluded from this study. The results of 50 men aged 57.52 ± 6.71 years obtained before the treatment (I test) and after one year of oral testosterone supplementation (test II) were analysed in this study. The following examinations and analyses were performed: interview and physical examination, orthopaedic, neurological and urological consultations, blood biochemistry, determination of hormones levels, assessment of Testosterone Deficiency Syndrome (TDS), densitometric and radioisotope assessment of bone metabolism. Moreover, radioisotopic index of bone metabolism was calculated.

Testosterone therapy with oral preparation Undestor Testo Caps (Organon) containing 40 mg of testosterone lasted for 12 months. Statistical analysis was performed using Statistica 12 and Excel 2010 programs. Correlations between results before and after treatment were analysed.

RESULTS: After 12 months of treatment, testosterone concentration increased by mean 78% and the level of luteinizing hormone (LH) decreased by 62%. TDS index increased from 0.53 ± 0.21 (in test I) to 1.91 ± 0.60 (in test II). After the therapy this index was significantly higher in all men ($p < 0.0001$). Moreover, BMD was also improved following therapy, however, the difference between test I and II was statistically insignificant. The greatest change was found in case of IBM (Index of Bone Metabolism). We observed a positive correlation between IBM and BMD before treatment ($r = 0.7991$), however, its strength decreased after one-year therapy ($r = 0.6757$).

CONCLUSIONS: In our opinion, IBM is more sensitive than other methods of the assessment of changes occurring in bone system under the influence of testosterone therapy. The observed changes in IBM were proportional to changes in testosterone concentration. Testosterone level, TDS and radioisotopic assessment of bone metabolism may be used as prognostic and therapeutic factors of osteoporosis and bone fractures in elderly men.

KEY words: testosterone, bone metabolism, densitometry

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Introduction

Testosterone deficiency is characterized by typical symptoms of hypogonadism, it may be associated with the deterioration of quality of life and may negatively affect the function of many organs and systems. Over the age of 40, total testosterone (TT) concentration is reduced by mean 1% per year [1, 2].

In men, the correct concentration of sex hormones exerts beneficial effects on the preservation of bone mass [3]. These findings are well-known and have been presented in the literature [4–7]. However, there is still a need for further studies concerning the relationship between testosterone levels and bone metabolism which is the main factor in the process of bone remodelling. Unravelling these associations can help to determine procedures of osteoporosis treatment in men. It is interesting how changes in the concentration of testosterone correlate with alterations in bone metabolism and with the progression of osteoporosis. Radioisotopic methods of bone metabolism assessment provide new research possibilities. Along with the densitometry, they may contribute to the widening of the spectrum of analysis of bone turnover occurring in older men with testosterone deficiency. Therefore, we decided to take up this issue using wide spectrum of diagnostic tools.

Main objectives: the assessment of the impact of testosterone deficiency compensation in elderly men on metabolism and bone mineral density; the determination of correlation between bone mineral density (BMD) and radioisotopic index of bone metabolism (IBM) and the concentration of testosterone (T) in elderly men with low testosterone levels treated with oral preparation of testosterone for a year; the inclusion of radioisotopic evaluation of bone metabolism using own methods and programs into imaging diagnostics of osteoporosis in men with osteoporosis.

Materials and methods

In this study, the results of biochemical and hormonal tests of patients subjected to isotopic examination of bone system or densitometry were analysed. Also the records of patients with signs of hypogonadism referred to endocrinologist were evaluated. A total of 317 patients' records were examined. Men aged 50–65 years with total testosterone concentration ≤ 3 ng/ml (normal range: $3 \leq TT \leq 10.6$ ng/ml) were selected from this group. In order to confirm the reproducibility of results, TT level was tested two times (the second analysis was performed after 2 weeks from the first measurement). Blood samples were drawn in the morning (7–8 a.m.) after a night fasting. Patients with increased level of prostate-specific antigen (PSA) were excluded from further analysis. Study group contained only patients with PSA ≤ 3 ng/ml. All eligible participants underwent ultrasound examination of prostate and those with enlarged organ were also excluded from study. Also patients with skeletal disorders that may affect the metabolism, bone inflammation, arthritis, trauma, fractures and surgery within bone system, disorders of thyroid and liver, diabetes, history of chemotherapy or chronic intake of glucocorticosteroids and antibiotics were not enrolled into study. Control ultrasound examination of the prostate was performed additionally after 6 and 12 months of therapy onset. Finally, the results of 50 male subjects aged 57.52 ± 6.71 years obtained before treatment (test I) and after one year of oral testosterone supplementation (test II) were

analysed. All eligible patients were informed about the purpose and methods of the study. Each of them provided written consent to participate in the study and to publish its anonymous results. The study was approved by Bioethics Committee of Medical University (protocol no. RNN/123/09/KB).

Basic research and consultations:

- interview and physical examination (age, height, weight, BMI)
- orthopaedic, neurological and urological consultations

Parameters determined in the serum

- **biochemical analysis included:** ionized calcium (Ca^{2+}), vitamin D_3 , phosphates (P), gamma-glutamyl transpeptidase (GGTP), aspartate aminotransferase (AspAT) (glutamic oxaloacetic transaminase), alanine aminotransferase (ALAT) (glutamic pyruvic transferase), amylase, glucose and cholesterol (chol). The determination of the concentration of aforementioned parameters was performed with the use of reagents from Bor-Pol Gliwice, and the measurement was made on A15 analyzer (BioSystems).
- **analysis of hormones included:** total testosterone (TT), prostate-specific antigen (PSA), luteinizing hormone (LH), thyroid stimulating hormone (TSH), dehydroepiandrosterone sulphate (DHEA-S). The determination of hormones levels was performed using ELFA (Enzyme Linked Fluorescent Assay) technique and bioMérieux reagents.

Testosterone Deficiency Syndrome (TDS) was calculated using the following formula:

testosterone concentration (ng/ml)/LH concentration (mIU/ml). TDS value < 1 is considered as hormonal indicator of age-related hypogonadism [2].

Densitometric examination

Densitometry was performed with DEXA (Dual Energy X-ray Absorptiometry) technique on NORLAND X46 densitometer. Bone mineral density (BMD) and the T-score of femoral bone neck were measured.

Radioisotopic analysis of bone metabolism

Radioisotope analysis of bone system was performed with Philips Brightview XCT gamma camera (Philips, Guildford, UK) using own method and a developed program — BONS. The study involved dynamic acquisition of femoral bone performed in rear projection after the injection of radiopharmaceutical ($\text{Tc}^{99\text{m}}\text{MDP}$) with an activity of 11 MBq/kg of body mass as well as static acquisitions performed after 180 min. from radiopharmaceutical injection.

Following the analysis of data obtained using the developed BONS program and static and dynamic bone scintigrams, radioisotopic index of bone metabolism (IBM) was calculated from the formula:

$$\text{IBM} = \frac{(G_{\text{tot}} - G_{\text{roi}}) \times 361.2 \times \ln(2)}{G_{\text{roi}} \times \text{EXP}(T_0 - T_1)}$$

where:

IBM — radioisotopic index of bone metabolism; G_{tot} — number of counts per one matrix pixel in the study of the whole body; G_{roi} — number of counts per one matrix pixel in the selected area of interest (ROI); T_0 — time of dynamic phase beginning; T_1 — time of static phase beginning.

Table 1. The results of anthropometric examination

N = 50	Age [years]	Weight [kg]	Height [cm]	BMI
Test I	57.52 ± 6.71	85.40 ± 9.69	175.18 ± 6.32	27.85 ± 3.01
Test II	57.52 ± 6.71	84.20 ± 8.80	175.18 ± 6.32	27.45 ± 2.65
p	ns	ns	ns	ns

IBM is dimensionless and its normal range for healthy men is $87.31 \leq \text{IBM} \leq 104.79$. The method of the determination of radioisotopic index of bone metabolism (IBM) and results obtained in studies of healthy subjects and patients with various diseases have been published in the literature [8–11].

Testosterone therapy

Study participants received oral preparation Undestor Testo Caps (Organon) containing 40 mg of testosterone for 12 months during morning and evening meals.

Statistical analysis

Statistical analysis of data was performed using Excel 2010 and Statistica 12 (pl). Mean value, median and standard deviation were calculated. The Shapiro-Wilk test was used to verify normal distribution of variables. ANOVA test was used for the comparison of data showing no departures from normality. Mean value, median, maximum and minimum values and standard deviation were calculated for measurable variables. Correlations between results obtained before and after treatment were analysed. All calculations were performed using 95% confidence area.

Results

The results of preliminary study are presented in Table 1.

The distribution of BMI values in tests I and II was normal. In test I, 10 patients had normal body mass, 26 were overweight and 14 were obese. In test II (post-treatment), 14 men had normal body mass, 28 were overweight and 8 were obese. Slightly reduced BMI was observed in 19 patients in test II in comparison to test I. However, this difference in BMI was not statistically significant ($p < 0.05$).

The results of densitometry are presented in Table 2.

The distribution of BMD values in tests I and II was normal. In test I, normal bone mineral density was found in 11 patients, in 24 patients osteopenia was observed, while osteoporosis was found

Table 2. The results of densitometric examination

N = 50	BMD [g/cm ²]
Test I	0.8913 ± 0.0748
Test II	0.8995 ± 0.0799

in 15 men. In test II, normal bone mineral density was found in 14 patients, osteopenia — in 25 patients, while osteoporosis — in 11 men. There were no statistically significant differences in BMD between test I and II ($p < 0.05$).

In this study, a positive correlation between IBM and BMD was observed ($r = 0.7991$). After one year of testosterone supplementation the strength of this correlation decreased ($r = 0.6757$), which can be due to a lower accuracy of densitometric assessment of BMD in comparison to radioisotopic assessment of IBM.

The results of biochemical and hormonal parameters analyses are presented in Table 3.

All results of biochemical and hormonal parameters analyses performed before and after the treatment were within normal range. Differences between the results of test I and II were statistically insignificant, except for P in case of which the difference was significant ($p = 0.0087$). IBM values and their scattering in test I vs. test II are shown in Figure 1. In both tests, the distribution of IBM values was normal. In test I, IBM below the lower limit of the normal range was found in 18 patients (36%). In test II, the increase in radioisotopic index of bone metabolism value was observed in all patients. However, the results of 7 men (14%) were still below the normal range. The rest of the results were within the normal range. The difference in IBM before treatment (test I) and after therapy (test II) was statistically significant ($p < 0.0001$). No results exceeding the upper level of norm were observed. A strong correlation between IBM in tests I and II as well as the ascending regression line (Fig. 1) indicate the increase in bone metabolism index in all subjects. LH and TT values and their

Table 3. The results of biochemical and hormonal parameters analyses

n = 50	Normal range	Test I	Test II	p
PSA	0 ÷ 4 [ng/ml]	1.75 ± 0.83	1.83 ± 0.89	ns
TSH	0.3 ÷ 4 [mU/ml]	2.31 ± 1.17	2.61 ± 1.65	ns
DHEA-S	880 ÷ 3180 [ng/ml]	1756 ± 426	1956 ± 826	ns
Ca ²⁺	0.98 ÷ 1.13 [mmol/l]	1.03 ± 0.03	1.01 ± 0.04	ns
P	2.5 ÷ 4.8 [mg/dl]	3.25 ± 0.47	3.01 ± 0.52	0.0087
GGTP	11 ÷ 50 [U/l]	29.89 ± 9.07	32.11 ± 6.42	ns
AspAT	12 ÷ 40 [U/l]	29.11 ± 5.22	30.56 ± 5.81	ns
cholesterol	150 ÷ 250 [mg/dl]	209 ± 33.18	201 ± 36.22	ns
glucose	55 ÷ 100 [mg/dl]	82.44 ± 9.65	80.18 ± 6.19	ns
ALAT	9 ÷ 40 [U/l]	31.01 ± 5.82	28.83 ± 7.51	ns

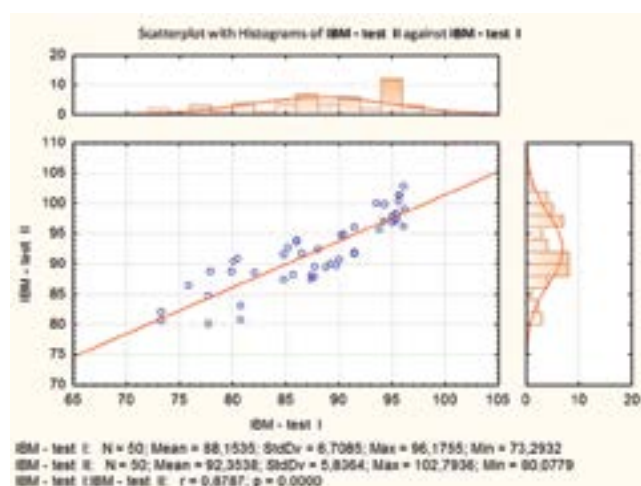


Figure 1. The scattering of IBM values in test II vs. test I

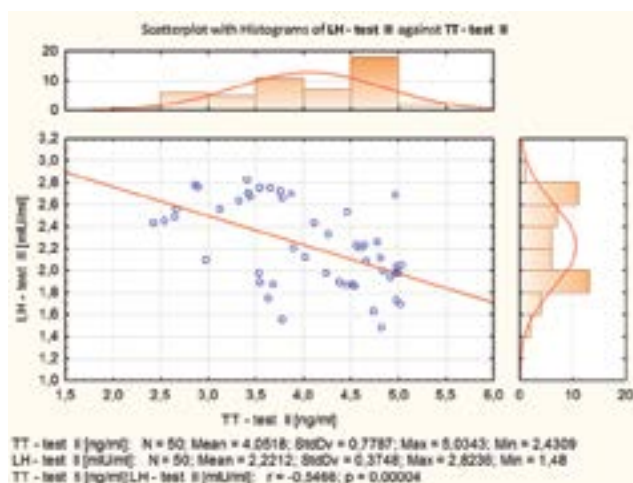


Figure 3. The scatter of LH vs. TT values in test II

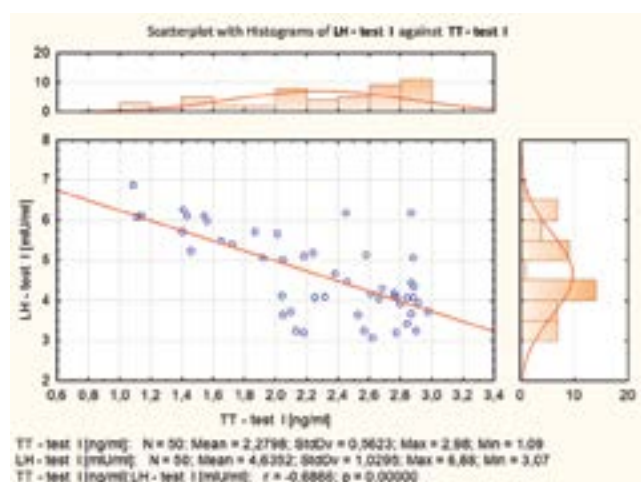


Figure 2. The scatter of LH vs. TT values in test I

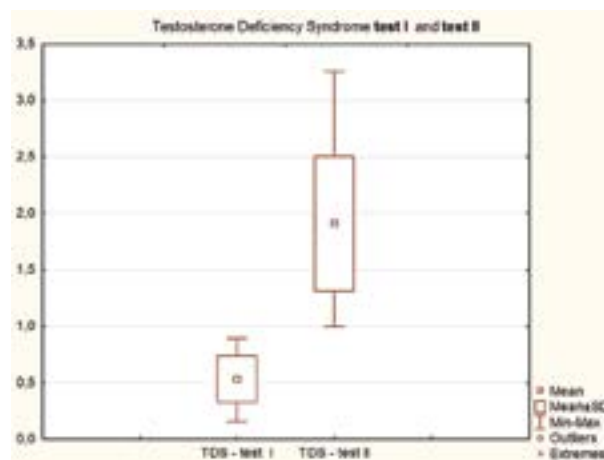


Figure 4. Testosterone deficiency syndrome in test I and II

scatter are presented in Figure 2. In test I, mean testosterone level in the study group was 2.27 ± 0.56 ng/ml, while LH value was 4.64 ± 1.03 mIU/ml. A negative correlation with medium strength was observed between LH and TT values. TT and LH values after one year testosterone therapy (test II) are presented in Figure 3. In test II, mean testosterone value in study group was 4.06 ± 0.78 ng/ml, while LH value was 2.22 ± 0.37 mIU/ml. After 12 months of testosterone supplementation (test II), the increase in testosterone was observed in comparison to test I (before treatment). Mean increase in TT value after the therapy was 78%, and the difference in levels between test I and test II was statistically significant ($p = 0.0001$). At the same time, the decrease in LH by mean 62% was seen, and the difference between test I and test II was statistically significant ($p = 0.0001$). Similarly to test I, the correlation between LH and TT values in test II was negative, however, it was of lower strength. The decrease in the strength of the correlation may indicate different individual-related influence of one-year testosterone treatment on the increase of testosterone concentration and the decrease in LH levels. Changes in the calculated index of testosterone deficiency

— TDS — before and after the therapy are presented in Figure 4. Before treatment, TDS < 1 was observed in all study participants. Mean value of TDS was 0.53 ± 0.21 . After therapy, this index was higher than 1 in all men, and its mean value was 1.91 ± 0.60 . Vitamin D₃ values and their scatter in relation to IBM values in test I are presented in Figure 5. Before treatment, we observed a strong positive correlation between D₃ and IBM values. We also found a positive correlation between D₃ and BMD values ($r = 0.6451$). In patients with osteoporosis, levels of vitamin D₃ were below the lower limit of norm. The increase in vitamin D₃ concentration was accompanied by the increase in bone mineral density. Vitamin D₃ values and their scatter in relation to IBM in test II are presented in Figure 6. After treatment, we observed a strong positive correlation between D₃ and IBM values. Its strength slightly decreased in comparison with test I ($r = 0.8452$). Correlation between D₃ and BMD values was positive and it had similar strength to the correlation before therapy ($r = 0.6528$). In patients with osteoporosis, vitamin D₃ values were below the lower limit of norm. Again, the increase in vitamin D₃ concentration was accompanied by the increase in bone mineral density.

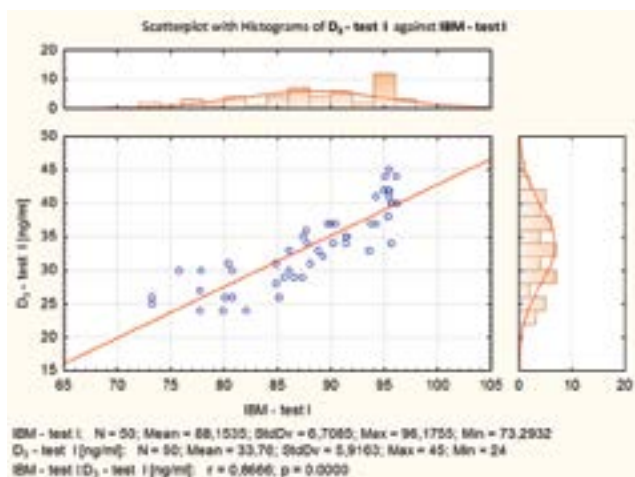


Figure 5. The scatter of vitamin D₃ values in relation to IBM values in test I

Discussion

In men, androgens exert a great impact on bone system throughout the life. The highest concentration of testosterone is reached between 25 and 30 years and then the slow reduction by 0.4–0.8% per year in total testosterone and by about 1.3–2% in free testosterone is observed. Late hypogonadism occurs over the age of 60 yrs. The reduction in testosterone levels with age exacerbates muscle weakness and muscle mass loss and leads to osteopenia and osteoporosis [12–17]. There are a number of methods assessing changes in bone system [18, 19]. Densitometry is the most common and generally available one. However, its sensitivity is low. In this study, T-score index was used only in the differentiation between normal bone density, osteopenia and osteoporosis. In turn, the method of radioisotopic assessment of bone metabolism (developed by authors) is a sensitive indicator of changes in bone metabolic processes. The results of our study show that densitometry is less sensitive than radioisotopic assessment of bone metabolism.

TDS index, which is included in endocrinology guidelines and provides additional diagnostic value, was used during the design of testosterone therapy. When TDS is below 1, testosterone replacement therapy should last 6–12 months. In this study, testosterone supplementation lasted for 12 months. Before final qualification to study group, a number of tests were performed in order to exclude men with disorders that may result in the lowering of testosterone levels. Study group comprised 50 men with reduced testosterone levels and no other comorbidities. Clinical features of testosterone deficiency and hypogonadism were confirmed using TDS index (Fig. 4), which value did not exceed 1. After 12 months of oral testosterone therapy, a significant increase in its value was observed. Before treatment, TDS was 0.53 ± 0.21 , and after therapy it was 1.91 ± 0.60 . This change was associated with the rise in testosterone concentration by mean 78% and simultaneous decrease in LH level by mean 62%. The greatest increase in TT following therapy was observed in men with lowest baseline concentration of testosterone. Change in bone mineral density in densitometry was small and changes between test I and II were not statistically

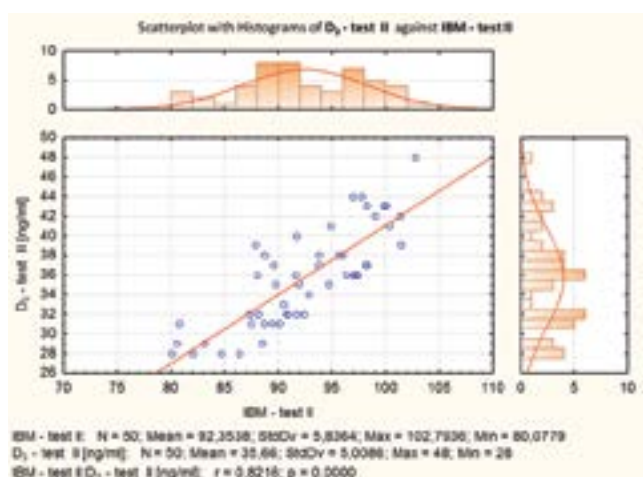


Figure 6. The scatter of vitamin D₃ values in relation to IBM in test II

significant. The analysis of radioisotope index of bone metabolism was included in the assessment of bone turnover. In this analysis, the greatest change was found in case of IBM values (Fig. 1). After the therapy, we observed a statistically significant increase in its level in all participants in comparison with test I ($p < 0.0001$). In our opinion, IBM is more sensitive than other methods assessing changes occurring in bone system after one year of testosterone treatment.

In studies in which other treatment methods were used, mean increase in TT concentration was in the range of $88 \div 128\%$ [2].

Conclusions

1. In men with hypogonadism, one year of oral testosterone therapy in the dose 40 mg/day significantly increased its total plasma level. Changes observed in IBM were proportional to the increase in TT concentration.
2. Radioisotopic method of bone metabolism (IBM) assessment is a sensitive and useful method in the diagnosis of bone turnover in patients treated with testosterone.
3. TT, TDS and IBM values may be prognostic and therapeutic factors of osteoporosis and bone fractures in elderly men.

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