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ORIGINAL ARTICLE

The effect of testosterone on thyroid autoimmunity in euthyroid men with Hashimoto's thyroiditis and low testosterone levels

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Abstract

What is known and objective: Thyroid autoimmune diseases occur much more frequently in women than men. Unfortunately, no previous study has determined whether sex hormones produce any effect on thyroid antibody titres. The primary study aim was to assess whether exogenous testosterone affects thyroid autoimmunity in men with Hashimoto's thyroiditis and low testosterone levels.

Methods: The study population consisted of 34 euthyroid men with autoimmune thyroiditis and testosterone deficiency. On the basis of patient preference, these patients were either treated with oral testosterone undecanoate (120 mg daily; n = 16) or remained untreated (n = 18). Circulating levels of thyrotropin, free thyroxine, free triiodothyronine, prolactin and total testosterone, as well as serum titres of thyroid peroxidase and thyroglobulin antibodies, were measured at the beginning of the study and 6 months later. The structure parameters of thyroid homeostasis (Jostel's thyrotropin index, SPINA-GT and SPINA-GD) were also calculated. Moreover, semen analyses were performed in eight patients in each group.

Results and discussion: In testosterone-naïve men, serum hormone levels and antibody titres remained at the similar levels throughout the study. Apart from increasing serum testosterone levels, testosterone undecanoate reduced titres of thyroid peroxidase and thyroglobulin antibodies and increased SPINA-GT. The drug produced a neutral effect on circulating levels of thyrotropin, free thyroid hormones, prolactin and testosterone, Jostel's thyrotropin index, SPINA-GD and semen parameters. Testosterone-induced changes in antibody titres correlated with the effect of treatment on SPINA-GT and with serum testosterone levels.

What is new and conclusion: This study is the first one to have shown that exogenous testosterone may have a protective effect on thyroid autoimmunity in men with Hashimoto's thyroiditis and testosterone deficiency.

KEYWORDS

hypothalamic-pituitary-thyroid axis, testosterone, thyroid autoimmunity, thyroid function tests

1 | WHAT IS KNOWN AND OBJECTIVE

Hashimoto's or autoimmune thyroiditis is one of the most frequent human disorders, as well as by far the most common cause of thyroid hypofunction in developed countries.[1,2] The disease is characterized by replacement of follicular cells by lymphocytes and, at a later stage, also by fibrous tissue.[3,4] The biological hallmark of Hashimoto's thyroiditis is the presence of antibodies directed against various antigens of the thyroid gland, particularly thyroid peroxidase antibodies (TPOAb) and thyroglobulin antibodies (TgAb).[1,2]

Thyroid autoimmune disorders develop much more frequently in women than men,[5] and this dimorphism may be at least in part attributed to differences in the production of sex hormones. Surprisingly, very little is known about the role of testosterone in the development of these disorders. Men with primary hypothyroidism, 62.5% of whom were diagnosed with autoimmune thyroiditis, were characterized by low levels of serum testosterone.[6] Hashimoto's thyroiditis was found to develop more frequently in men with high than in men with low values of the estradiol:testosterone ratio.[7] Autoimmune thyroiditis occurs much more frequently in subjects with Klinefelter syndrome, the most common form of male hypogonadism.[8] The (CAG)n repeat polymorphism of the androgen receptor gene influenced the age of onset of Hashimoto's thyroiditis.[9] In the obese strain of chickens, testosterone prevented the development of spontaneous autoimmune thyroiditis.[10,11] Moreover, its administration to rats with autoimmune thyroiditis, by either parenteral injection or implantation, resulted in disappearance of mononuclear cellular infiltration and reappearance of normal gland architecture.[12]

Despite the female preponderance, an increasing number of men are being diagnosed with Hashimoto's thyroiditis.[5] Unfortunately, to the best of our knowledge, no interventional study with the use of exogenous testosterone has been conducted to date in humans. Therefore, the primary aim of the current research was to investigate whether testosterone undecanoate affects thyroid autoimmunity in men with autoimmune thyroid disease and low testosterone levels.

2 | METHODS

2.1 | Patients

The participants of the study were selected among adult men (40-70 years old) fulfilling the criteria of testosterone deficiency: serum levels of total testosterone less than 3.0 ng/mL on two different occasions combined with reduced frequency of morning erection, erectile dysfunction and/or decreased frequency of sexual thoughts.[13] We included 34 drug-naïve men with recently diagnosed autoimmune thyroiditis (defined as serum TPOAb titres above 100 U/mL and the reduced echogenicity of the thyroid parenchyma on thyroid ultrasonography), in whom serum levels of thyrotropin and free thyroid hormone were within the reference range (thyrotropin between 0.4 and 4.5 mU/L, free thyroxine between 10.2 and 21.2 pmol/L and free triiodothyronine between 2.3 and 6.7 pmol/L). Assuming a power of 80% and a significance level of 0.05, 15 or more men had to be included in each arm to detect a 20% difference in thyroid antibody titres.

Individuals were excluded if they met at least one of the following criteria: positive serum antibodies against thyrotropin receptor, other autoimmune disorders, body mass index above 40 kg/m^2, prostate cancer, severe lower urinary tract symptoms (the American Urological Association International Prostate Symptom Score exceeding 19), prostate-specific antigen greater than 4 ng/mL (or prostate-specific antigen above 3 ng/mL in men at high risk of prostate cancer), breast cancer, myocardial infarction, stroke or coronary revascularization procedure preceding the study, congestive heart failure, haematocrit exceeding 50%, untreated obstructive sleep apnoea, diabetes mellitus, impaired renal or liver function, any treatment within 6 months before the beginning of the study and poor patient compliance.

The study protocol was approved by the institutional review board, and all participants signed a written informed consent. The principles of the Declaration of Helsinki were applied throughout the study.

2.2 | Study design

At the beginning of the study, all participants were informed about the benefits and possible adverse effects of testosterone therapy. On the basis of patient preference, 16 men were then treated for 6 months with exogenous testosterone, while the remaining patients (n = 18) did not receive any treatment and served as a control group. The total daily dose of testosterone undecanoate (120 mg orally) was divided into three equal doses and administered for 6 months.

2.3 | Laboratory assays

Venous blood samples were obtained between 8.00 and 9.00 AM, 12 hours after the last meal, in a quiet, temperature-controlled room (24-25°C) at the beginning and at the end of the study (6 months later). Serum titres of TPOAb and TgAb, as well as serum levels of thyrotropin, free thyroid hormones (free thyroxine and free triiodothyronine), prolactin and testosterone, were assayed by direct chemiluminescence using acridinium ester technology (ADVIA Centaur XP Immunoassay System, Siemens Healthcare Diagnostics). All measurements were carried out in duplicate. The structure parameters of thyroid homeostasis were calculated using SPINA-Thyr 4.0.1 for Windows software. Jostel's thyrotropin index was calculated as follows: ln [thyrotropin] + 0.1345 × free thyroxine.[14] SPINA-GT was calculated as follows: $\beta_T \times (D_T + \text{thyrotropin}) \times (1 + K_{41} \times \text{standard concentration of thyroxine-binding globulin} + K_{42} \times \text{standard concentration of transthyretin} \times \text{free thyroxine})/(\alpha_T \times \text{thyrotropin})$.[15] SPINA-GD was calculated using the following formula: $\beta_{31} \times (K_{M1} + \text{free}$

thyroxine) (1 + K_{30} × standard concentration of thyroxine-binding globulin) × free triiodothyronine/(α_{31} × free thyroxine).[16] Constants in the equations were as follows: β_T = 1.1 × 10^{-6}/s, D_T = 2.75 mU/L, K_{41} = 2 × 10^{10} L/mol, standard concentration of thyroxine-binding globulin = 300 nmol/L, K_{42} = 2 × 10^{8} L/mol, standard concentration of transthyretin = 4.5 mmol/L, α_T = 0.1/L, β_{31} = 8 × 10^{-6}/s, K_{M1} = 5 × 10^{-7} mol/L, K_{30} = 2×10^{9} L/mol and α_{31} = 0.026/L.[15,16]

2.4 | Semen analyses

Semen analyses were performed after 3-5 days of sexual abstinence in eight patients from each group at the beginning and at the end of the study (the remaining men did not agree on semen analyses). Each semen sample, collected in a sterile container and analysed within the first hour after collection, was evaluated for semen volume, sperm concentration, total sperm count, total sperm motility, progressive sperm motility and sperm normal morphology.

2.5 | Statistical analysis

All values were log-transformed to meet the assumption of normally distributed data. Between- and within-group comparisons were performed by Student's *t* tests for independent samples and Student's paired *t* tests, respectively. The significance of the results was determined using a 95% confidence interval. A *t* statistic and two sample means were used to generate an interval estimate of the difference between two population means. Correlations were assessed using Pearson's correlation coefficient (*r*). Differences were described as statistically significant if 95% confidence intervals did not include the null value and/or two-tailed *P* values were below 0.05.

3 | RESULTS

There were no significant differences between the study groups in age, body mass index, smoking habits, thyroid antibody titres, serum levels of thyrotropin, free thyroxine, free triiodothyronine, prolactin and testosterone, as well in values of the calculated parameters of thyroid homeostasis (Table 1). In testosterone-naïve men, serum hormone levels, antibody titres and values of all indices remained at the similar levels throughout the study. In turn, testosterone undecanoate reduced titres of thyroid peroxidase and thyroglobulin antibodies, increased SPINA-GT and increased serum testosterone levels. The drug produced a neutral effect on circulating levels of thyrotropin, free thyroid hormones, prolactin and testosterone, as well as on Jostel's thyrotropin index and SPINA-GD. At the end of the study, titres of TPOAb and TgAb were lower, while values of SPINA-GT and levels of testosterone were higher in testosterone-treated than testosterone-naïve men (Table 2).

Semen parameters did not differ between men belonging to both subgroups and did not change throughout the study (Table 3).

Baseline titres of TPOAb correlated with baseline titres of TgAb titres (r = 0.59, P < 0.001). There were also inverse correlations between baseline thyroid antibody titres and baseline SPINA-GT (TPOAb: r = −0.25, P < 0.05; TgAb: r = −0.28, P < 0.05). Sperm concentration, total sperm count, total sperm motility, progressive sperm motility and sperm normal morphology inversely correlated with TPOAb titres (r values in the range between −0.24 [P < 0.05] and −0.35 [P < 0.01]), while sperm concentration, total sperm count, progressive sperm motility and sperm normal morphology positively correlated with SPINA-GT (r values in the range between 0.23 [P < 0.05] and 0.37 [P < 0.01]). Treatment-induced changes in

TABLE 1 Baseline characteristics of patients

Variable	Testosterone-treated men	Testosterone-naive men	Difference (95% CI)
Number of patients	16	18	
Age (years; mean [SD])	58 (8)	57 (6)	−1 [−6, 4]
Smokers (%)	31	28	
Body mass index (kg/m^2; mean [SD])	28.8 (4.7)	28.4 (4.3)	−0.4 [−3.5, 2.7]
TPOAb (IU/mL; mean [SD])	882 (354)	902 (380)	20 [−238, 278]
TgAb (IU/mL; mean [SD])	784 (305)	811 (364)	27 [−209, 263]
Thyrotropin (mIU/L; mean [SD])	2.6 (1.2)	2.4 (1.1)	−0.2 [−1.0, 0.6]
Free thyroxine (pmol/L; mean [SD])	14.2 (2.0)	14.0 (1.9)	−0.2 [−1.6, 1.2]
Free triiodothyronine (pmol/L; mean [SD])	3.4 (0.7)	3.5 (0.6)	0.1 [−0.4, 0.6]
Jostel's thyrotropin index (mean [SD])	2.9 (0.3)	2.8 (0.2)	−0.1 [−0.3, 0.1]
SPINA-GT index (pmol/s; mean [SD])	2.22 (0.37)	2.28 (0.29)	0.06 [−0.17, 0.29]
SPINA-GD index (nmol/s; mean [SD])	22.14 (2.84)	23.12 (2.63)	0.98 [−0.93, 2.89]
Testosterone (ng/mL; mean [SD])	2.2 (0.5)	2.3 (0.4)	0.1 [−0.2, 0.4]
Prolactin (ng/mL; mean [SD])	11 (5)	10 (4)	−1 [−4, 2]

Abbreviations: CI, confidence interval; IU, international unit; SD, standard deviation; SPINA, structure parameter inference approach; TgAb, thyroglobulin antibodies; TPOAb, thyroid peroxidase antibodies.

TABLE 2 The effect of testosterone undecanoate on thyroid antibody titres, hormones and thyroid function in euthyroid men with Hashimoto's thyroiditis and low testosterone levels

Variable	Testosterone-treated men	Testosterone-naive men	Difference (95% CI)
TPOAb (IU/mL; mean [SD])			
Baseline	882 (354)	902 (380)	20 [−238, 278]
After 6 mo	610 (283)[b]	878 (342)	268 [47, 489][a]
Change	−272 (134)	−24 (103)	248 [165, 331][c]
TgAb (IU/mL; mean [SD])			
Baseline	784 (305)	811 (364)	27 [−209, 263]
After 6 mo	602 (211)[b]	779 (281)	177 [2, 352][a]
Change	−182 (134)	−32 (116)	150 [63, 237][c]
Thyrotropin (mIU/L; mean [SD])			
Baseline	2.6 (1.2)	2.4 (1.1)	−0.2 [−1.0, 0.6]
After 6 mo	2.2 (1.2)	2.3 (1.0)	0.1 [−0.7, 0.9]
Change	−0.4 (0.8)	−0.1 (0.6)	0.3 [−0.2, 0.8]
Free thyroxine (pmol/L; mean [SD])			
Baseline	14.2 (2.0)	14.0 (1.9)	−0.2 [−1.6, 1.2]
After 6 mo	15.3 (2.4)	14.4 (2.2)	−0.9 [−2.5, 0.7]
Change	1.1 (1.1)	0.4 (1.0)	−0.7 [−1.5, 0.1]
Free triiodothyronine (pmol/L; mean [SD])			
Baseline	3.4 (0.7)	3.5 (0.6)	0.1 [−0.4, 0.6]
After 6 mo	3.6 (0.8)	3.5 (0.6)	−0.1 [−0.6, 0.4]
Change	0.2 (0.7)	0.0 (0.4)	−0.2 [−0.6, 0.2]
Jostel's thyrotropin index (mean [SD])			
Baseline	2.9 (0.3)	2.8 (0.2)	−0.1 [−0.3, 0.1]
After 6 mo	2.8 (0.3)	2.8 (0.2)	0.0 [−0.2, 0.2]
Change	−0.1 (0.2)	0.0 (0.2)	0.1 [−0.1, 0.3]
SPINA-GT index (pmol/s; mean [SD])			
Baseline	2.22 (0.37)	2.28 (0.29)	0.06 [−0.17, 0.29]
After 6 mo	2.61 (0.30)[b]	2.40 (0.25)	−0.21 [−0.40, −0.02][a]
Change	0.39 (0.36)	0.12 (0.20)	0.27 [−0.48, −0.06][c]
SPINA-GD index (nmol/s; mean [SD])			
Baseline	22.14 (2.84)	23.12 (2.63)	0.98 [−0.93, 2.89]
After 6 mo	21.76 (3.11)	22.47 (2.18)	0.71 [−1.15, 2.57]
Change	−0.38 (0.40)	−0.65 (0.52)	−0.27 [−0.60, 0.06]
Testosterone (ng/mL; mean [SD])			
Baseline	2.2 (0.5)		0.1 [−0.2, 0.4]
After 6 mo	5.9 (1.5)[b]	2.5 (0.4)	−3.4 [−4.1, −2.7][a]
Change	3.7 (1.6)	0.2 (0.2)	−3.5 [−4.3, −2.7][c]
Prolactin (ng/mL; mean [SD])			
Baseline	11 (5)	10 (4)	−1 [−4, 2]
After 6 mo	12 (6)	12 (5)	0 [−4, 4]
Change	1 (4)	2 (3)	1 [−1, 3]

Abbreviations: CI, confidence interval; IU, international unit; SD, standard deviation; SPINA, structure parameter inference approach; TgAb, thyroglobulin antibodies; TPOAb, thyroid peroxidase antibodies.

[a]Statistically significant difference between both groups.

[b]Statistically significant difference between post-treatment and baseline values in the same group.

[c]Statistically significant difference between the changes in both groups.

TABLE 3 Seminal characteristics of euthyroid men with Hashimoto's thyroiditis and low testosterone levels[a]

	Testosterone-treated men	Testosterone-naive men	Difference (95% CI)
Semen volume (mL; mean [SD])			
Baseline	1.7 (0.7)	1.6 (0.6)	−0.1 [−0.8, 0.6]
After 6 mo	1.6 (0.6)	1.5 (0.5)	−0.1 [−0.7, 0.5]
Change	−0.1 (0.2)	−0.1 (0.1)	0.0 [−0.1, 0.1]
Sperm concentration ($\times 10^6$/mL; mean [SD])			
Baseline	10.2 (4.0)	10.0 (3.6)	−0.2 [−4.3, 3.9]
After 6 mo	9.6 (3.2)	9.2 (3.4)	−0.4 [−4.0, 3.2]
Change	−0.6 (0.3)	−0.8 (0.4)	−0.2 [−0.6, 0.2]
Total sperm count ($\times 10^6$; mean [SD])			
Baseline	17.4 (7.6)	16.1 (7.5)	−1.3 [−9.4, 6.8]
After 6 mo	15.4 (8.0)	13.9 (7.1)	−1.5 [−9.6, 6.6]
Change	−2.0 (1.2)	−2.2 (1.4)	−0.2 [−1.6, 1.2]
Total sperm motility (%; mean [SD])			
Baseline	25.2 (10.2)	24.0 (12.8)	−1.2 [−13.6, 11.2]
After 6 mo	23.4 (12.0)	21.5 (11.3)	−1.9 [−13.4, 9.6]
Change	−1.8 (1.0)	−2.5 (1.5)	−0.7 [−2.1, 0.7]
Progressive sperm motility (%; mean [SD])			
Baseline	18.3 (7.4)	17.8 (6.9)	−0.5 [−8.2, 7.2]
After 6 mo	17.7 (6.7)	16.8 (7.5)	−0.9 [−8.5, 6.7]
Change	−0.6 (0.5)	−1.0 (0.8)	−0.4 [−1.1, 0.3]
Sperm normal morphology (%; mean [SD])			
Baseline	2.8 (1.2)	2.5 (1.5)	−0.3 [−1.8, 1.2]
After 6 mo	3.0 (1.0)	2.6 (1.7)	−0.4 [−1.9, 1.1]
Change	0.2 (0.4)	0.1 (0.3)	−0.1 [−0.5, 0.3]

[a]Data obtained from 8 men from each study group.

TPOAb correlated with the changes in TgAb (r = 0.56, P < 0.001). Testosterone-induced changes in antibody titres inversely correlated with baseline testosterone levels (TPOAb: r = −0.28, P < 0.05; TgAb: r = −0.31, P < 0.05) and positively correlated with baseline antibody titres (TPOAb: r = 0.57, P < 0.001; TgAb: r = 0.52, P < 0.001), baseline thyrotropin levels (TPOAb: r = 0.29, P < 0.05; TgAb: r = 0.25, P < 0.05) and with the effect of treatment on SPINA-GT (TPOAb: r = 0.30, P < 0.05; TgAb: r = 0.27, P < 0.05). Treatment-induced changes in SPINA-GT correlated with baseline thyrotropin levels (r = 0.38, P < 0.001). The effect of treatment on TPOAb titres correlated with the impact of therapy on testosterone levels (r = 0.38, P < 0.001). No other correlations were found.

4 | DISCUSSION

This study is the first to have shown that testosterone replacement therapy with oral testosterone preparations reduces thyroid antibody titres in men with autoimmune thyroiditis. The lack of changes in TPOAb and TgAb in the control group indicates that testosterone action cannot be attributed to a spontaneous resolution of thyroid autoimmunity or to seasonal fluctuations in antibody titres. The study purposely included only men with thyrotropin and free thyroid hormone levels within the reference range. Taking into account that patients with autoimmune hypothyroidism should receive thyroid hormone replacement,[17] as well as that exogenous levothyroxine reduces thyroid antibody titres,[18] excluding men with thyroid hypofunction minimized the possibility that the alleviation of thyroid autoimmunity is a consequence of the effect of levothyroxine (not testosterone) or is secondary to pharmacokinetic or pharmacodynamic interactions between levothyroxine and testosterone. Because the study population consisted of only drug-naïve subjects, the obtained results cannot be also explained by a modulatory impact of testosterone on other medications found to reduce thyroid antibody titres (vitamin D, selenium, myo-inositol or statins).[19-22]

Interestingly, treatment-induced improvement in thyroid autoimmunity inversely correlated with baseline testosterone levels, while treatment-induced changes in TPOAb correlated with an increase in testosterone levels. This finding suggests that, from a thyroid point of view, men with autoimmune thyroiditis co-existing with severe forms of hypogonadism may gain more benefits from testosterone treatment

than individuals with milder forms of testicular failure and individuals with the intact hypothalamic-pituitary-testicular axis. There are two possible explanations for the lack of correlations between the changes in TgAb titres and serum testosterone levels. Firstly, measurements of TgAb are characterized by lesser sensitivity and lesser specificity in the diagnosis of Hashimoto's thyroiditis than measurements of TPOAb.[23] Secondly, serum levels of testosterone do not seem to reflect well its tissue content in men receiving testosterone preparations.[13]

Because of the inclusion criteria baseline thyrotrope and thyroid cell function was only slightly impaired by the presence of autoimmune process and lymphocyte infiltration. This finding explains why, despite reducing antibody titres, testosterone did not change thyrotropin, free thyroxine and free triiodothyronine levels. However, the effect of oral testosterone on antibody titres was paralleled by a stimulatory effect on SPINA-GT, estimating the maximum thyroidal capacity and being a more sensitive marker of thyroid cell function than circulating levels of thyrotropin and free thyroid hormones.[15,24] Hoermann et al[24] observed lower values of this marker in euthyroid subjects with autoimmune thyroid disease than in healthy controls, while Dietrich et al[15,16] observed lower intra-individual variation of SPINA-GT than of thyrotropin, free thyroxine and free triiodothyronine. This may suggest that testosterone therapy improves secretory function of the thyroid gland even in euthyroid patients. It is likely that this effect is stronger in subjects with impaired activity of the hypothalamic-pituitary-thyroid axis, because treatment-induced changes in antibody titres and SPINA-GT correlated with baseline thyrotropin levels. Unlike SPINA-GT, neither Jostel's index, quantitatively assessing the thyrotropic function of the anterior lobe of the pituitary gland,[14] nor SPINA-GD, determining thyroid hormone conversion efficiency, changed during testosterone treatment.[15,16] Therefore, it seems that testosterone undecanoate does not affect hypothalamic-pituitary-thyroid axis activity at the level of thyrotropes and does not modulate global deiodinase activity.

The observational nature of our study allows us only to speculate about the molecular mechanisms underlying the obtained results. The reduction in antibody titres may be secondary either to alleviation of immune processes or to a direct effect on thyrocytes. An argument in favour of the first explanation is the above-mentioned presence of correlations between baseline testosterone levels and antibody titres, as well as between treatment-induced changes in titres of TPOAb and the effect of testosterone on its serum levels. Hashimoto's thyroiditis develops as a consequence of a stimulatory effect of T and B cells infiltrating the thyroid gland on cell cytotoxicity, apoptosis and thyroid antibody production.[25] The proinflammatory state in Hashimoto's thyroiditis is partially mediated by proinflammatory cytokines, which seem to contribute to low-grade systemic inflammation and gland destruction.[26] In turn, low testosterone levels were found to be associated with low-grade systemic inflammation, while administration of exogenous testosterone inhibited the production of proinflammatory cytokines (interleukin-1β, interleukin-6, tumour necrosis factor-α).[27,28] It is possible that the increase in tissue testosterone content secondary to its oral administration suppresses thyroid autoimmunity *via* androgen receptors expressed in primary lymphoid organs and peripheral immune cells.[29] However, this effect does not seem to be mediated by prolactin, stimulating immune cells and many immunological responses, as well as inducing the progression of numerous autoimmune disorders.[30] We think so because testosterone did not affect circulating levels of prolactin, as well as because, both at baseline and during treatment, prolactin levels did not correlate with testosterone levels and thyroid antibody titres. According to the alternative explanation, the inhibitory effect on thyroid immunity is a consequence of the improvement in thyroid secretory function. In line with this explanation, values of SPINA-GT correlated with antibody titres. Moreover, androgens receptors were found in the thyroid gland,[31] testosterone up-regulated androgen receptors in rats[32] and induced thyrocyte proliferation.[33] Finally, exogenous levothyroxine reduced titres of TPOAb and TgAb in men with autoimmune thyroiditis.[18] Therefore, it is also possible that increased endogenous thyroxine production induced by testosterone administration behaves similarly to exogenous levothyroxine.

According to the protocol, the participants of the study were allocated to one of two groups based on their preference. Because the subjects under study were aware of their hormonal status and of group allocation, questionnaire analyses assessing sexual functioning would be highly questionable and therefore were not performed. However, in the previous study of our research team,[18] the beneficial effect of levothyroxine on thyroid antibody titres in men with autoimmune hypothyroidism was accompanied by the improvement in erectile function and, to a lesser extent also, by the improvement in intercourse satisfaction, orgasmic function, sexual desire and overall satisfaction. This may suggest that exogenous testosterone, apart from its well-known direct effect on sexual functioning,[34] may reverse sexual dysfunction in euthyroid men with autoimmune thyroiditis and low testosterone levels also indirectly by improving thyroid autoimmunity and thyroid function. In line with this explanation, thyroid hormone receptors were identified in Sertoli, Leydig, sperm and peritubular cells, while thyroid hormones were found to stimulate proliferation and function of Leydig and Sertoli cells.[35]

Unlike positive effects on sexual functioning, exogenous testosterone therapy negatively affects fertility and therefore is used in male contraception.[36,37] By suppressing the pulsatile release of gonadotropin-releasing hormone, luteinizing hormone and follicle-stimulating hormone, testosterone preparations reduce testicular testosterone production and deprive developing sperm of the signals required for normal maturation.[36,37] Interestingly, testosterone produced a neutral effect on all semen parameters assessed in the subpopulation of patients participating in the current study. It is possible that the unfavourable direct effect of testosterone on semen quality was counterbalanced by an indirect beneficial effect associated with the reduction in thyroid autoimmunity. This explanation is supported by the finding that semen quality was impaired in men with autoimmune hypothyroidism.[38] Moreover, in our study, sperm concentration, total sperm count, total sperm motility, progressive sperm motility and sperm normal morphology inversely correlated with TPOAb titres and, with the exception of total sperm motility, positively correlated with SPINA-GT.

Some limitations of the study should be pointed out. The main limitation is a small number of participants and its non-randomized

design. Moreover, the Upper Silesia, where the study was carried out, is an area with low-selenium status[39] and sufficient iodine supply.[40] It cannot be totally ruled out that the effect of testosterone is different in iodine-deficient and/or selenium-adequate areas. Furthermore, the study protocol does not allow us to verify whether other forms of testosterone therapy: intramuscular preparations, topical gels, transdermal patches, buccal tablets, topical solutions or pellet implants, have the same impact on thyroid autoimmunity as oral testosterone undecanoate. Finally, the question whether exogenous testosterone reduces thyroid antibody titres in eugonadal men requires further research.

5 | WHAT IS NEW AND CONCLUSION

In conclusion, oral testosterone administered for 6 months reduced thyroid antibody titres, which was paralleled by an increase in SPINA-GT in euthyroid men with autoimmune thyroiditis and low testosterone levels, and these effects depended on both baseline antibody titres and baseline testosterone levels. Our findings indicate that testosterone therapy may reduce thyroid autoimmunity, at least in men with impaired activity of the hypothalamic-pituitary-testicular axis. Because of study limitations, the obtained results should be verified in large clinical trials.

6 | INSTITUTIONAL APPROVAL

The study was approved by the Bioethical Committee of the Medical University of Silesia.

CONFLICT OF INTEREST

No conflicts of interest have been declared.

ETHICAL APPROVAL

The study protocol was approved by the institutional review board by (the Bioethical Committee of the Medical University of Silesia), and all participants signed a written informed consent. The principles of the Declaration of Helsinki were applied throughout the study.

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