

# The Impact of Testosterone on Metformin Action on Hypothalamic-Pituitary-Thyroid Axis Activity in Men: A Pilot Study

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## Abstract

The effect of metformin on thyrotrope function seems to be sex dependent. The aim of this study was to determine the role of endogenous testosterone in the impact of metformin on hypothalamic-pituitary-thyroid axis activity. The study population consisted of 2 groups of men with nonautoimmune hypothyroidism matched for age, weight, insulin sensitivity, and thyrotropin levels. The first group ( $n = 11$ ) included subjects with low serum testosterone levels, while the second ( $n = 12$ ) men with testosterone levels within the reference range. Because of concomitant type 2 diabetes, all men were treated with metformin (2550–3000 mg daily). Circulating levels of glucose, prolactin, testosterone, gonadotropins, thyrotropin, and free thyroid hormones were measured, while the structure parameters of thyroid homeostasis and the degree of insulin sensitivity were calculated at baseline and 16 weeks later. In both study groups, metformin decreased plasma glucose levels and improved insulin sensitivity. However, only in men with low testosterone levels, the drug decreased thyrotropin levels, reduced Jostel's thyrotropin index, and increased SPINA-GT. Metformin-induced changes in thyrotropin and Jostel's index correlated with their baseline values, baseline levels of testosterone, and with the effect of treatment on insulin sensitivity. In men with neither low or normal testosterone levels, metformin affected free thyroid hormones, prolactin, testosterone, gonadotropins, and SPINA-GD. The obtained results suggest that the impact of metformin on thyrotrope function depends on the androgen status of a patient.

## Keywords

hypothalamic-pituitary-thyroid axis, hypothyroidism, metformin, testosterone, thyroid function tests

Metformin, being the first-line drug in the treatment of type 2 diabetes mellitus and other insulin-resistant states,<sup>1</sup> is commonly used in clinical practice. Because of the lack of the blood-brain barrier, the pituitary accumulates substantial amounts of metformin, which suggests that the gland may be an important target for metformin action.<sup>2</sup> In numerous studies, metformin reduced circulating levels of thyrotropin,<sup>3–6</sup> prolactin<sup>7,8</sup> and gonadotropins,<sup>9,10</sup> but these effects were observed only if baseline hormone levels were elevated. The impact of metformin on thyrotropin levels was reported both in hypothyroidism<sup>3–5</sup> and resistance to thyroid hormone.<sup>6</sup> In patients with thyroid hypofunction, the effect on thyrotrope function was not accompanied by the changes in free thyroxine and free triiodothyronine levels. Treatment-induced decrease in thyrotropin concentration was present in both autoimmune and nonautoimmune hypothyroidism, and the impact of metformin on serum thyrotropin levels did not correlate with thyroid antibody titers.<sup>3</sup>

Recently, Krysiak et al<sup>11</sup> have observed the effect of metformin on serum thyrotropin levels is characterized by sexual dimorphism. Despite similar baseline characteristics, metformin affected thyrotropin levels only in women but not in men. This finding and the fact that most thyroid disorders develop several times more

frequently in women than men<sup>12</sup> allow us to suspect that the effect of metformin on thyrotrope function is partially determined by the extent of production and/or metabolism of endogenous sex steroids. In line with this hypothesis, men with hypergonadotropic hypogonadism were characterized by an increased prevalence of hypothyroidism.<sup>13</sup> Moreover, testosterone and fluoxymesterone administered to hypogonadal men resulted in a blunted response of thyrotropin to administration of thyrotropin-releasing hormone,<sup>14,15</sup> suggesting the inhibitory effect of testosterone on thyrotropin secretion.

To the best of our knowledge, no previous study has assessed the role of endogenous sex hormones in affecting metformin action at the level of the pituitary. Therefore, the aim of the present study was to compare the impact of metformin on

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hypothalamic-pituitary-thyroid axis activity and thyroid function tests in men with impaired testosterone production resulting from late-onset hypogonadism and in patients with testosterone levels within the reference range.

## Methods

The study was approved by the local institutional review board (the Bioethics Committee of the Medical University of Silesia) and the study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to participation.

### Study Population

The participants of this case-control study were men (35-65 years old) with new-onset type 2 diabetes mellitus (fasting plasma glucose at least 126 mg/dL and/or plasma glucose concentration 2 hours after a glucose load at least 200 mg/dL), who had been previously diagnosed with late-onset hypogonadism. We included only subjects ( $n = 11$ ) with nonautoimmune subclinical hypothyroidism, defined as thyrotropin between 4.5 and 10 mIU/L, free thyroxine between 10.1 and 21.1 pmol/L, free triiodothyronine between 2.2 and 6.5 pmol/L and thyroid peroxidase and thyroglobulin antibody titers in the reference range, in whom testosterone levels were  $<3.0$  ng/mL. The control group consisted of 12 men with asymptomatic nonautoimmune subclinical hypothyroidism, in whom testosterone levels were in the range between 4 and 12 ng/mL. The control subjects were selected among 56 men with recently diagnosed and previously untreated type 2 diabetes, based on a computer algorithm aimed at obtaining 2 study groups matched for age, body mass index, plasma glucose levels, insulin sensitivity, and thyrotropin levels.

The study protocol excluded subjects with type 1 diabetes, glycosylated hemoglobin levels above 9.5%, autoimmune thyroid disorders, pituitary or adrenal disorders, impaired renal or hepatic function, empty sella syndrome, men receiving any drugs (with the exception of metformin during the study period), as well as subjects with poor compliance.

### Study Design

Throughout the study period, all participants were treated with metformin and complied with dietary recommendations. The patients were started on 850 mg of metformin once daily, and this dose was gradually (over 2-4 weeks) titrated. The final dose of this drug (2550-3000 mg daily in 3 divided doses) was administered for 16 weeks. Compliance was assessed by tablet counting

and interviews with the patients during each visit, taking place every 2 to 4 weeks.

### Laboratory Assays

Venous blood samples were taken from the antecubital vein between 8 and 9 AM at least 12 hours after the last meal before and at the end of the study and assessed in duplicate by a person blinded to all clinical data. To limit the impact of external conditions on the measured parameters, before blood collection, the participants had been resting in a quiet, temperature-controlled (24-25°C) room for at least 30 minutes in the seated position. Plasma levels of glucose were assessed by a routine laboratory technique (Roche Diagnostics, Basel, Switzerland). Serum levels of thyrotropin, free thyroxine, free triiodothyronine, insulin, prolactin, testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were assayed by direct chemiluminescence using acridinium ester technology (ADVIA Centaur XP Immunoassay System, Siemens Healthcare Diagnostics, Munich, Germany). The homeostasis model assessment 1 of insulin resistance index (HOMA1-IR) was calculated based on mean values of glucose and insulin using the following equation: serum insulin [mIU/L]  $\times$  plasma glucose [mg/dL]/405. The structure parameters of thyroid homeostasis were calculated using SPINA-Thyr 4.0.1 for Mac Universal software as described previously.<sup>16-18</sup>

### Statistical Analysis

Prior to statistical analysis, all quantitative data were log-transformed to meet the assumption of homogeneity of variance. Group differences were tested for significance using Student's *t*-tests for independent samples with Bonferroni's correction for multiple comparisons. Baseline and posttreatment values were compared using Student's paired *t*-test. To verify the correctness of statistical analysis, apart from parametric statistics, median values of the measured variables were compared using nonparametric tests (the Mann-Whitney *U* test and the Wilcoxon matched-pairs test). Because the results of nonparametric statistics did not differ from the ones obtained after using parametric tests, they are not shown. Qualitative variables were compared using chi-squared tests. Pearson's *r*-tests were used to test correlations between the measured variables. The clinical importance of the result was assessed on the basis of the 95% confidence interval. Differences were regarded as statistically significant if 95% confidence intervals did not include the null value and/or 2-tailed *P* values were  $<.05$ . All statistical analyses were performed using the Statistica 12.0 PL software package (number: JPZP507D199115ARC-N-E, StatSoft Polska, Kraków, Poland).

**Table 1.** Baseline Characteristics of Participants

	Men With Low Testosterone Levels	Control Men	Difference (95% Confidence Interval)
Number of patients	11	12	...
Age, y (mean [SD])	52 (8)	51 (8)	−1 (−8 to 6)
Smoking, %	27	25	...
Body mass index, kg/m <sup>2</sup> (mean [SD])	29.4 (3.4)	29.0 (3.0)	−0.4 (−3.2 to 2.4)
Fasting glucose, mg/dL (mean [SD])	146 (16)	144 (15)	−2 (−15 to 11)
HOMA1-IR (mean [SD])	5.1 (1.2)	4.9 (1.0)	−0.2 (−1.2 to 0.8)
Thyrotropin, mIU/L (mean [SD])	7.4 (1.4)	7.3 (1.3)	−0.1 (−1.3 to 1.1)
Free thyroxine, pmol/L (mean [SD])	13.4 (1.8)	13.6 (1.4)	0.2 (−1.2 to 1.6)
Free triiodothyronine, pmol/L (mean [SD])	3.2 (0.7)	3.2 (0.6)	0.0 (−0.6 to 0.6)
Prolactin, ng/mL (mean [SD])	15 (5)	14 (4)	−1 (−5 to 3)
FSH, IU/L (mean [SD])	6.2 (2.4)	5.2 (1.9)	−1.0 (−2.9 to 0.9)
LH, IU/L (mean [SD])	6.0 (2.8)	5.5 (2.2)	−0.5 (−2.7 to 1.7)
Testosterone, ng/mL (mean [SD])	2.3 (0.4)	5.8 (1.0)	3.5 (2.8 to 4.2) <sup>a</sup>
Jostel's thyrotropin index, mean [SD])	3.8 (0.2)	3.8 (0.1)	0.0 (−0.1 to 0.1)
SPINA-GT index, pmol/s (mean [SD])	1.40 (0.12)	1.42 (0.14)	0.02 (−0.09 to 0.13)
SPINA-GD index, nmol/s (mean [SD])	22.08 (3.12)	21.76 (2.95)	−0.32 (−2.95 to 2.31)

FSH, follicle-stimulating hormone; HOMA1-IR, homeostasis model assessment 1 of insulin resistance index; LH, luteinizing hormone; SD, standard deviation.

<sup>a</sup>Statistically significant difference between the groups.

## Results

At study entry, both groups were comparable with respect to age, smoking, body mass index, glucose homeostasis markers, serum levels of thyrotropin, free thyroxine, free triiodothyronine, prolactin, FSH, and LH, as well as Jostel's index, SPINA-GT, and SPINA-GD. Expectedly, serum testosterone levels were lower in men with late-onset hypogonadism than in the control subjects (Table 1).

Metformin treatment was well tolerated, and all men completed the study protocol. The final daily dose of this drug did not differ between the study groups (late-onset hypogonadism,  $2.78 \pm 0.21$  mg/daily; control subjects,  $2.81 \pm 0.19$  mg daily; difference, 0.03 [−0.14–0.20]).

In both study groups, metformin decreased plasma glucose levels and reduced HOMA1-IR. Only in men with late-onset hypogonadism, the drug decreased thyrotropin levels, reduced Jostel's index, and increased SPINA-GT. Neither in men with low testosterone levels nor in men with testosterone concentrations within the reference range, metformin affected free thyroid hormones, prolactin, FSH, LH, testosterone, and SPINA-GD. The impact of metformin on HOMA1-IR, thyrotropin, Jostel's index, and SPINA-GT was stronger, while posttreatment values were lower in men with late-onset hypogonadism than in the control subjects (Table 2).

In patients with low testosterone levels, metformin-induced changes in thyrotropin, Jostel's thyrotropin index, and SPINA-GT correlated with baseline thyrotropin levels (thyrotropin,  $r = 0.42$ ,  $P < .001$ ; Jostel's index,  $r = 0.40$ ,  $P < .001$ ; SPINA-GT,  $r = 0.30$ ,  $P < .05$ ), and with the mean baseline value of Jostel's

thyrotropin index (thyrotropin,  $r = 0.38$ ,  $P < .001$ ; Jostel's index,  $r = 0.34$ ,  $P < .001$ ; SPINA-GT,  $r = 0.32$ ,  $P < .05$ ). The impact of metformin on thyrotropin and Jostel's thyrotropin index also correlated with baseline testosterone concentration (thyrotropin,  $r = -0.38$ ,  $P < .001$ ; Jostel's index,  $r = -0.37$ ,  $P < .01$ ), as well as with treatment-induced changes in HOMA1-IR (thyrotropin,  $r = 0.32$ ,  $P < .05$ ; Jostel's index,  $r = 0.34$ ,  $P < .01$ ). No other correlations were found. The most important correlations between the impact of metformin on thyrotropin levels and on other measured variables in men with type 2 diabetes with nonautoimmune hypothyroidism and low testosterone levels are shown in Figures 1 and 2.

## Discussion

In the present study, we have found for the first time that the effect of metformin on the hypothalamic-pituitary-thyroid axis in men depends on endogenous testosterone secretion. Metformin affected thyrotropin levels only in subjects with low testosterone levels but not in patients with testosterone levels within the reference range. Moreover, in subjects with late-onset hypogonadism, the extent of metformin-induced reduction in serum thyrotropin inversely correlated with baseline testosterone levels. The obtained results may, at least in part, explain sexual dimorphism in the impact of metformin on circulating thyrotropin levels.<sup>11</sup> According to the study protocol, supported by the baseline characteristics of the participants, both treatment arms were well matched for age, body mass index, insulin sensitivity, and thyrotropin levels. This minimized a possible impact of differences in anthropometric measurements and glucose

**Table 2.** Impact of Metformin on Glucose Homeostasis Markers, Serum Hormone Levels, and Calculated Parameters of Thyroid Homeostasis in Men With Type 2 Diabetes With Nonautoimmune Hypothyroidism and Late-Onset Hypogonadism or Testosterone Levels Within the Reference Range

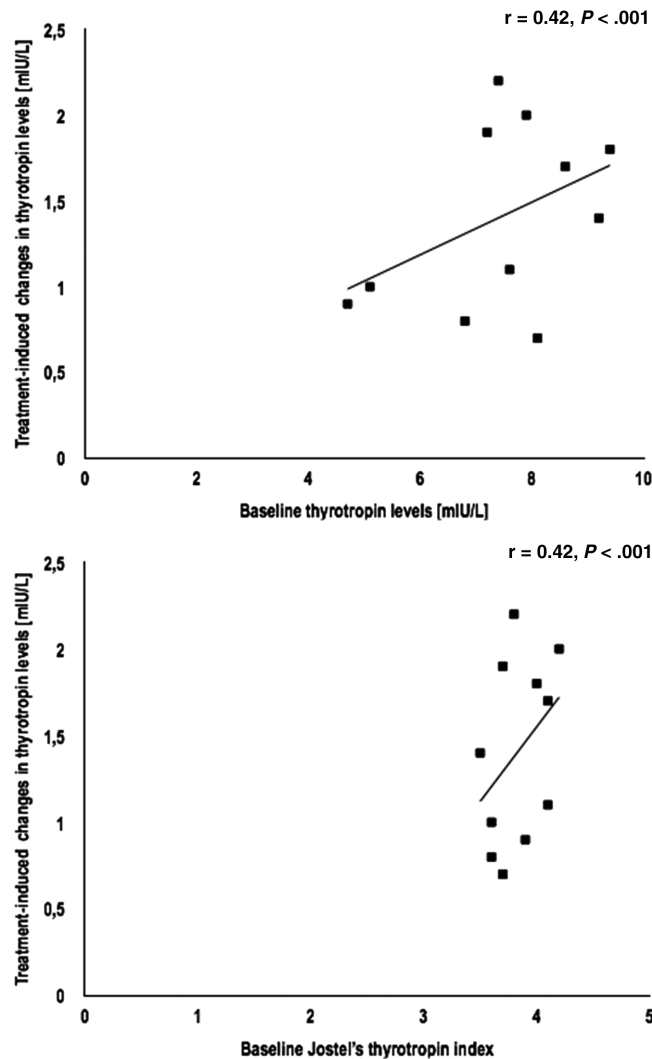
	Men With Low Testosterone Levels	Control Men	Difference (95% Confidence Interval)
Fasting glucose, mg/dL (mean [SD])			
At the beginning of the study	146 (16)	144 (15)	−2 (−15 to 11)
At the end of the study	117 (14) <sup>b</sup>	118 (12) <sup>b</sup>	1 (−11 to 13)
Change	−29 (11)	−26 (9)	3 (−6 to 12)
HOMA1-IR (mean [SD])			
At the beginning of the study	5.1 (1.2)	4.9 (1.0)	−0.2 (−1.2 to 0.8)
At the end of the study	3.4 (0.7) <sup>b</sup>	4.1 (0.8) <sup>b</sup>	0.7 (0.1 to 1.3) <sup>a</sup>
Change	−1.7 (0.8)	−0.8 (0.4)	0.9 (0.4 to 1.4) <sup>c</sup>
Thyrotropin, mIU/L (mean [SD])			
At the beginning of the study	7.4 (1.4)	7.3 (1.3)	−0.1 (−1.3 to 1.1)
At the end of the study	6.0 (1.3) <sup>b</sup>	7.2 (1.2)	1.2 (0.1 to 2.3) <sup>a</sup>
Change	−1.4 (0.5)	−0.2 (0.1)	1.2 (0.9 to 1.5) <sup>c</sup>
Free thyroxine, pmol/L (mean [SD])			
At the beginning of the study	13.4 (1.8)	13.6 (1.4)	0.2 (−1.2 to 1.6)
At the end of the study	13.8 (2.1)	13.4 (1.7)	−0.4 (−2.1 to 1.3)
Change	0.4 (0.9)	−0.2 (0.7)	−0.6 (−1.3 to 0.1)
Free triiodothyronine, pmol/L (mean [SD])			
At the beginning of the study	3.2 (0.7)	3.2 (0.6)	0.0 (−0.6 to 0.6)
At the end of the study	3.5 (0.7)	3.4 (0.5)	−0.1 (−0.6 to 0.4)
Change	0.3 (0.4)	0.2 (0.2)	−0.1 (−0.4 to 0.2)
Prolactin, ng/mL (mean [SD])			
At the beginning of the study	15 (5)	14 (4)	−1 (−5 to 3)
At the end of the study	13 (4)	12 (3)	−1 (−4 to 2)
Change	−2 (2)	−2 (1)	0 (−1, 1)
FSH, IU/L (mean [SD])			
At the beginning of the study	6.2 (2.4)	5.2 (1.9)	−1.0 (−2.9 to 0.9)
At the end of the study	5.8 (2.2)	4.9 (2.0)	−0.9 (−2.7 to 0.9)
Change	−0.4 (0.4)	−0.3 (0.4)	0.1 (−0.2 to 0.4)
LH, IU/L (mean [SD])			
At the beginning of the study	6.0 (2.8)	5.5 (2.2)	−0.5 (−2.7 to 1.7)
At the end of the study	5.5 (2.0)	5.2 (1.8)	−0.3 (−2.1 to 1.5)
Change	−0.5 (0.4)	−0.3 (0.2)	−0.2 (−0.5 to 0.1)
Testosterone, ng/mL (mean [SD])			
At the beginning of the study	2.3 (0.4)	5.8 (1.0)	3.5 (2.8 to 4.2) <sup>a</sup>
At the end of the study	2.5 (0.5)	5.9 (1.1)	3.4 (2.6 to 4.2) <sup>a</sup>
Change	0.2 (0.2)	0.1 (0.2)	−0.1 (−0.3 to 0.1)
Jostel's thyrotropin index (mean [SD])			
At the beginning of the study	3.8 (0.2)	3.8 (0.1)	0.0 (−0.1 to 0.1)
At the end of the study	3.6 (0.1) <sup>b</sup>	3.8 (0.1)	0.2 (0.1 to 0.3) <sup>a</sup>
Change	−0.2 (0.1)	0.0 (0.1)	0.2 (0.1 to 0.3) <sup>c</sup>
SPINA-GT index, pmol/s (mean [SD])			
At the beginning of the study	1.40 (0.12)	1.42 (0.14)	0.02 (−0.09 to 0.13)
At the end of the study	1.53 (0.16) <sup>b</sup>	1.41 (0.11)	−0.12 (−0.23 to −0.01) <sup>a</sup>
Change	0.13 (0.04)	−0.01 (0.02)	−0.14 (−0.17 to −0.11) <sup>c</sup>
SPINA-GD index, nmol/s (mean [SD])			
At the beginning of the study	22.08 (3.12)	21.76 (2.95)	−0.32 (−2.95 to 2.31)
At the end of the study	23.45 (3.25)	23.47 (2.87)	0.02 (−2.63 to 2.67)
Change	1.37 (0.52)	1.71 (0.46)	0.34 (−0.10 to 0.78)

FSH, follicle-stimulating hormone; HOMA1-IR, homeostasis model assessment 1 of insulin resistance index; LH, luteinizing hormone; SD, standard deviation.

<sup>a</sup>Statistically significant difference between the groups.<sup>b</sup>Statistically significant difference between posttreatment and baseline values in the same group.<sup>c</sup>Statistically significant difference between the changes in both groups.

homeostasis markers, resulting from the presence of late-onset hypogonadism,<sup>19</sup> as well as excluded differences in the baseline activity of the hypothalamic-pituitary-thyroid axis. The obtained results suggest that endogenous testosterone may modulate the impact of

metformin on thyrotrope function. However, taking into account a neutral effect of metformin on plasma levels of free thyroid hormones, even in subjects with late-onset hypogonadism, this hypothesis needs verification in further studies.

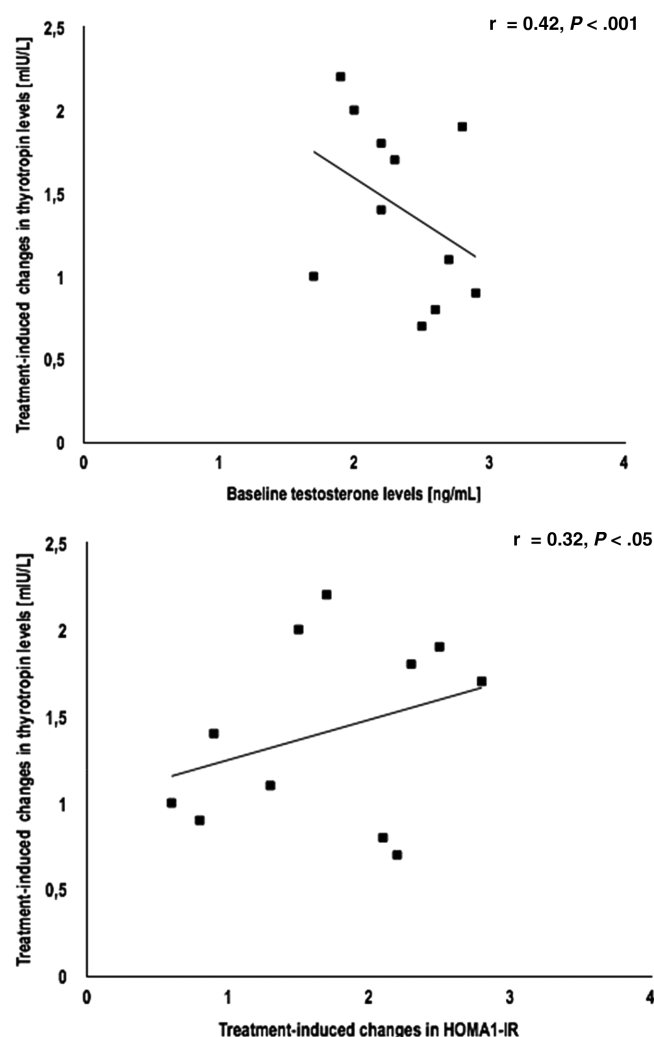


**Figure 1.** Correlations between the impact of metformin on serum thyrotropin levels and baseline values of thyrotropin and Jostel's thyrotropin index in men with type 2 diabetes with nonautoimmune hypothyroidism and low testosterone levels.

Similarly to women,<sup>3,11</sup> metformin-induced changes in thyrotropin levels in testosterone-deficient men were more pronounced in subjects with markedly elevated thyrotropin levels. These changes were paralleled by a decrease in Jostel's thyrotropin index, which is a parameter estimating the degree of pituitary dysfunction based on thyrotropin and free thyroxine levels. Interestingly, the mean value of this index in the study population (3.8) was close to the upper limit of the reference range (1.3-4.1). Taking into account that low Jostel's thyrotropin index is a marker of thyrotropic insufficiency,<sup>16</sup> its relatively high mean value indirectly suggests increased thyrotrope function in the enrolled patients. Thus, the obtained results are in line with previous findings indicating that a metformin-induced decrease in serum thyrotropin correlates with the degree of thyrotropin hypersecretion.<sup>3-5</sup> Moreover, the presence of correlations between treatment-induced

changes in thyrotropin levels and Jostel's thyrotropin index may imply that the effect of metformin on serum thyrotropin results from its action at the level of the pituitary and/or hypothalamus, rather than from the impact on thyrotropin metabolism and excretion.

Another interesting finding of the current study was that despite a neutral effect on serum levels of free thyroxine and free triiodothyronine, metformin administered to patients with late-onset hypogonadism led to a small increase in SPINA-GT, estimating the maximum secretory capacity of the thyroid gland,<sup>17,18</sup> and this effect correlated with the changes in Jostel's thyrotropin index. Although no previous study has assessed the effect of metformin on the calculated parameters of thyroid homeostasis in subjects with thyroid dysfunction, metformin increased SPINA-GT in young women with hyperprolactinemia and intact hypothalamic-pituitary-thyroid axis activity.<sup>20</sup> Although this finding suggests



**Figure 2.** Correlations between the impact of metformin on serum thyrotropin levels and baseline values of testosterone and treatment-induced changes in HOMA1-IR in men with type 2 diabetes with nonautoimmune hypothyroidism and low testosterone levels. HOMA1-IR, homeostasis model assessment 1 of insulin resistance index.

that metformin slightly improves thyroid function, its clinical relevance remains to be established. The effect of therapeutic doses of metformin at the level of the thyroid gland is probably negligible. However, very high doses of this drug may, at least in male rats, result in symptoms of hyperthyroidism.<sup>21</sup> Taking into account the correlation between treatment-induced changes in Jostel's thyrotropin index and in SPINA-GT, it is possible that metformin administered to subjects with low testosterone levels increases thyrotropin sensitivity in the thyroid gland, with a subsequent decrease in thyrotropin production. In line with this explanation, follicular cells express the androgen receptor,<sup>22</sup> while dihydrotestosterone was found to exert an inhibitory effect on human thyroid cell growth.<sup>23</sup> Unlike thyrotropes and thyroid cells, irrespective of the androgen status of patients, metformin does not seem to affect the conversion rate of thyroxine to triiodothyronine, which

is supported by no changes in SPINA-GD, being a marker of total deiodinase activity.<sup>17,18</sup>

We can only hypothesize about the molecular mechanisms explaining our findings. In our previous observations, testosterone reduced thyroid antibody titers in men with autoimmune thyroiditis.<sup>24</sup> However, this effect does not explain the obtained results because the study purposely included only subjects with nonautoimmune hypothyroidism. A likely explanation is the association of pituitary/hypothalamic action of metformin with the improvement in insulin sensitivity. The impact of metformin on HOMA1-IR was more pronounced in men with low testosterone than in subjects with testosterone levels within the reference range, and only in men with late-onset hypogonadism correlated with treatment-induced changes in serum thyrotropin and Jostel's thyrotropin index. Taking into account that the central dopaminergic system plays an important



role in the regulation of glucose homeostasis,<sup>25</sup> differences in metformin action on insulin sensitivity between patients with low and normal testosterone levels may be, at least in part, explained by baseline differences in dopaminergic transmission in brain dopaminergic circuits. One of them, the tuberoinfundibular pathway, originating in the hypothalamus and projecting to the pituitary gland, is also a well-known regulator of thyrotrope function.<sup>26</sup> In line with this explanation, castrated rats were characterized by lower dopamine levels than intact male rats,<sup>27</sup> while testosterone increased dopamine content in hypothalamic cell cultures.<sup>28</sup> This explanation is also supported by the findings that metformin stimulated dopaminergic transmission in the hypothalamus of women with insulin resistance<sup>29</sup> and that the effect of metformin on thyrotropin secretion was inversely proportional to baseline central dopaminergic tone.<sup>30</sup> Alternatively, the association between the androgen status of the patient and the impact of metformin on thyrotrope function may be explained by testosterone action on adenosine 5'-monophosphate-activated protein kinase. This master sensor of whole-body energy homeostasis and an important mediator of metabolic effects of metformin<sup>31</sup> is expressed strongly in thyrotropes<sup>32</sup> and plays a role in mediating the impact of metformin on gonadotropin secretion,<sup>32</sup> while its activity is characterized by sexual dimorphism.<sup>33</sup> Although no previous study assessed the effect of orchidectomy or androgen therapy on adenosine 5'-monophosphate-activated protein kinase in men, its activity in females is modulated by estradiol.<sup>33</sup>

All observed correlations, while statistically significant, have not been strong. Even lower values of the coefficients of determination indicate that only small amounts of the variance can be explained by the measured variables. Hence, it seems that the impact of metformin on the hypothalamic-pituitary-thyroid axis is partially independent of endogenous testosterone secretion and the degree of improvement in insulin sensitivity. This means that other variables, not investigated in the current study, also contribute to the different effect of metformin on thyrotropin levels and Jostel's thyrotropin index between men with late-onset hypogonadism and men with testosterone levels within the reference range.

Some study limitations merit comment. The most important limitation is the small sample size, resulting from difficulties in including in one research center a larger group of untreated patients with new-onset type 2 diabetes mellitus, nonautoimmune subclinical hypothyroidism, and late-onset hypogonadism. Second, because the real meaning of changes in Jostel's thyrotropin index and SPINA-GT is still unclear, the obtained results should be interpreted with caution. Moreover, taking into account that the participants

were characterized by low selenium status<sup>34</sup> and adequate iodine intake,<sup>35</sup> it is not certain whether the impact of metformin is the same in areas with adequate selenium and inadequate iodine consumption. Furthermore, it remains to be elucidated whether metformin affects hypothalamic-pituitary-thyroid axis activity in men with thyroid hypothyroidism of autoimmune origin. Finally, the question of whether endogenous testosterone determines metformin action on thyrotrope function in subjects with normal or only slightly impaired insulin sensitivity requires further research.

To sum up, despite improving glucose homeostasis in both study groups, metformin decreased thyrotropin levels, lowered Jostel's thyrotropin index, and increased SPINA-GT only in men with low testosterone levels. The effect of treatment on thyrotropin and Jostel's thyrotropin index depended on baseline levels of thyrotropin and testosterone, as well as correlated with the impact of metformin on insulin sensitivity. These findings suggest that metformin action on the hypothalamic-pituitary-thyroid axis activity depends on the androgen status of a patient. Because of the small sample size, the current study should be regarded as a pilot study, and larger multicenter trials are required to confirm the obtained results.

## Conflicts of Interest

The authors declare no conflicts of interest.

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This work was not supported by any external source of funding. The experiments comply with the current law of Poland.

## Data Sharing

Data presented in this manuscript cannot be shared. For any questions, please contact r.krysiak@interia.pl.

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