

Effect of metformin on thyroid stimulating hormone and thyroid volume in patients with prediabetes: A randomized placebo-controlled clinical trial

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Background: The people with prediabetes have insulin resistance (IR). IR may affect thyroid function, size and nodules. We investigated the effects of metformin on the thyroid gland in prediabetic people. **Materials and Methods:** In a randomized, double-blind placebo-control clinical trial, 89 people with prediabetes, aged 18-65 years were studied for 3 months. They were divided into two, metformin ($n = 43$) and placebo ($n = 46$) treated groups. Serum thyroid stimulating hormone (TSH) was measured and thyroid nodules and volume was studied by ultrasonography. The data were compared between and within groups, before and after the study. **Results:** Mean of the baseline characteristics in metformin and placebo-treated groups had no statistically significant difference. At the end of the study, serum TSH was not significantly different between the two groups. However, if the TSH range was divided into two low normal (0.3-2.5 $\mu\text{U/ml}$) and high-normal (2.6-5.5 $\mu\text{U/ml}$) ranges, significant decrease was observed in metformin-treated group with a high-normal basal serum TSH ($P = 0.01$). Thyroid volume did not change in metformin-treated group. However, in placebo-treated group, the thyroid was enlarged ($P = 0.03$). In 53.9% of participants, thyroid nodule was observed. There was just a decrease in the volume of small solid (not mixed) nodules from median of 0.07 ml to 0.04 ml in metformin-treated group ($P = 0.01$). **Conclusion:** In prediabetic people, metformin decreases serum TSH, only, in those people with TSH $>2.5 \mu\text{U/ml}$ and reduces the size of small solid thyroid nodules. It also prevents an increase in the thyroid volume.

Key words: Insulin resistance, metformin, prediabetes, thyroid, thyroid nodule, thyroid volume

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INTRODUCTION

Prediabetes is defined as impaired fasting plasma glucose (FPG) ($100 \text{ mg/dL} \leq \text{FPG} \leq 125 \text{ mg/dL}$) and/or impaired glucose tolerance (IGT), 2-h postglucose values of 140-199 mg/dl during oral glucose tolerance test (OGTT) with 75 g glucose.^[1] Prediabetes is a kind of insulin resistance (IR), a condition in which people have plasma glucose concentration higher than normal, but not high enough to be classified as diabetes. Persons with prediabetes have an increased risk of developing type 2 diabetes, heart and cerebrovascular diseases. IR is characterized by an inadequate physiological response of peripheral tissues to circulating insulin and results in metabolic and hemodynamic disturbances.^[2] While prediabetic people could not manage to maintain, the appropriate degree of hyperinsulinemia, they may develop type 2 diabetes.^[3]

It is supposed that functional changes in the thyroid gland might have cooperation with metabolic syndrome and its related components including, obesity, IR, lipid and glucose metabolism abnormalities, and high blood pressure.^[4] In patients with IR, the prevalence of goiter and thyroid nodule is likely more common.^[5] Insulin-like growth factor-1 (IGF-1) is an important hypertrophic and cell cycle progression factor for a number of cell types. It was previously pointed out that thyroid stimulating hormone (TSH) in association with insulin or IGF-1 stimulates cell cycle progression and proliferation in various thyroid cell culture systems. The IGF system includes a network of ligands (IGF-1 and IGF-2), which are very similar to insulin; IGF-1 receptor, has structural similarity with the insulin receptor. Insulin/IGF-1 signaling pathway has long been known to modulate regulation of thyroid gene expression and might be considered as additional important factors in thyroid cell proliferation and differentiation. Very recently, an elegant cell culture work by Malaguarnera

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et al. showed that insulin receptor isoforms, IGF-1 receptor, IGF-1 and IGF-2, were expressed at high concentrations in thyroid follicular cell precursors and highly decreased in differentiating cells. Insulin and IGFs promoted the growth of thyroid cancer precursors.^[6]

Patients with IR are candidates to receive metformin that decreases IR.^[7]

As the prevalence of diabetes mellitus and thyroid disease is relatively high in Isfahan^[8-10] and as far as we know, the effect of metformin on thyroid function of patients with prediabetes has not been studied yet, we decided to investigate, prospectively, the effect of metformin on the thyroid gland (volume and function) in prediabetic patients.

MATERIALS AND METHODS

Subjects

Our study was a randomized double-blind placebo-controlled clinical trial. People with prediabetes (impaired FPG [100-125 mg/dl] and/or IGT [2-h postglucose values of 140-199 mg/dl during OGTT) and being 18-65 years-old were, initially, included in the study. A total of 124 participants during April 2013 to March 2014 were, randomly, enrolled (62 people in each group); however, only 89 people completed the 3-month follow-up period. The randomization method was computer-based generated. Patients and study personnel were blinded to treatment assignment.

Why some participants did not finish, the study is being mentioned later in the text. The participants were selected among the first-degree relatives of type-2 diabetes mellitus who are being screened for a diabetes primary preventive program in Isfahan Endocrine and Metabolism Research Center.

Those patients with thyroid malignancy or any other malignancies, previous use of levothyroxine for suppression therapy at any time, obesity due to endocrine disorders, pregnancy and lactation, liver failure, neurological or psychological disorders (depression, epilepsy, schizophrenia), heart failure, hypothyroidism or hyperthyroidism, smoking and exposure to iodinated contrast material in the previous 6 months were excluded from the study. Serum creatinine ≥ 1.4 mg/dl in women and ≥ 1.5 mg/dl in men was among the exclusion criteria.

The Ethics Committee of Isfahan University of Medical Sciences approved this study, and written informed consent was obtained from all study participants.

Thirty-five people dropped out of the study. The subjects excluded were 19 in metformin-treated group (Group I). Two persons failed to take the medication, and 4 did not come for follow-up visits, 1 developed subclinical hyperthyroidism, 2 got new onset diabetes, 3 people were prescribed levothyroxine by another physician, 1 participant had a retro-sternal goiter with pressure effect on the trachea and had been referred to a surgeon, 6 persons had drug intolerance (gastrointestinal upset and malaise).

Sixteen people were excluded in the placebo-treated group (Group II). Three persons failed to take the medication, 4 participants were not willing to continue the study, 2 of them developed the new onset diabetes, 1 person had been prescribed levothyroxine by another physician, 2 people had normal glucose tolerance test (GTT), 1 person had drug intolerance (gastrointestinal upset), 1 participant had subclinical hypothyroidism and 2 did not come for follow-up visits.

Methods

Anthropometric measurements

Measurements of height and body weight of participants were recorded by a physician using Secca scale. Body weights (kg) and heights (m) were measured without shoes and/or cap. Body mass index (BMI) was obtained by dividing the body weight (kg) to the square of height (m).

Interventions

Pretreatment baseline evaluation and a 12-week treatment period with visits at 4, 8, and 12 weeks after random assignment were done in our center by the same doctor (MK). Drug adherence was assessed by pills count. In both groups, we prescribed 500 mg of either metformin or placebo, with weekly increment of 1 tablet (500 mg) consequently to achieve the ultimate dose of 1500 mg in participants whom tolerate this dose-escalation protocol.

In people whom could not tolerate the final dose of metformin, they continued with the lower tolerable dose. If even 500 mg of medication was not tolerable, they were excluded (6 people in metformin-treated and 1 person in placebo-treated group).

Biochemical evaluations

After taking history and a physical examination, blood samples were taken between 8:00 and 9:00 a.m., after 8-h of overnight fasting, and stored in -20°C at Isfahan Endocrine and Metabolism Research Center laboratory. For each patient laboratory tests were performed within a week (Creatinine, FPG, T3, T4, TSH, T3RU, antithyroperoxidase antibodies [TPO-Ab], thyroglobulin antibody [TgAb], insulin, HbA1C, and GTT). TPO-Ab and TgAb positive

were defined as the concentration higher than 60 IU/ml according to the used lab kit.

All the laboratory tests, but GTT were repeated 3 months later. Thyroid function tests were performed using immunoassay system by an automated analyzer, Siemens 2010 (ADVIA Centaur CP Immunoassay System). Plasma glucose was measured by the glucose oxidase technique (by Pars azmun kit, Iran). IR was estimated based on the calculation of the homeostasis model assessment (HOMA) index for each participant. HOMA-IR calculated by using the formula: (Fasting plasma insulin [mU/L] × FPG [mmol/L]) ÷ 22.5.^[11]

Evaluation of the thyroid gland morphology

Thyroid ultrasonography was performed by one radiologist, who was blind to all clinical conditions of the participants, using a 7-9 MHz linear probe (Logiq 500 Pro, GE Medical Systems, WI, USA). Volume of thyroid gland and nodules were calculated according to the ellipsoid formula: Volume (mL) = depth (cm) × width (cm) × length (cm) × π/6 for each lobe. Thyroid volume was expressed as ml. All patients with thyroid nodules ≥1 cm or having suspicious criteria of malignancy on sonography report were suggested to undergo fine needle aspiration biopsy.

Statistical analysis

All continuous data were expressed as the mean ± standard error of the mean, and/or median and range. Qualitative variables were reported as percentage and number. Data were analyzed with SPSS software (Statistical Package for the Social Sciences, version 13, SSPS Inc., Chicago, IL, USA). Statistical comparisons were performed by means of independent samples' *t*-test for quantitative data with a normal distribution and Chi-square, or Fisher exact tests were used to compare qualitative variables between the two groups. Paired *t*-test was used to do within group comparisons in terms of numerical variables with the normal distribution. To compare the numerical variables, not normally distributed, between and within groups, the Mann-Whitney U and the Wilcoxon signed-rank tests were used, respectively. To calculate the correlation coefficient, Pearson's correlation coefficient was used. *P* < 0.05 were considered as statistically, significant.

RESULTS

The baseline characteristics of participants in the metformin and placebo-treated groups are shown in Table 1. There was no statistically significant difference between baseline age, weight, BMI, TSH level, thyroid volume, nodule diameter, FPG and HOMA-IR between the two groups (NS). At basal the mean of HbA1c was 5.6% ± 0.09% in metformin-treated group versus 5.4% ± 0.08% in placebo-treated group

(*P* = 0.2). It was 5.44% ± 0.06% in metformin-treated group after intervention and 5.38% ± 0.09% after receiving placebo (*P* = 0.06).

After 3 months of follow-up, the metformin group did not show any significant decrement on TSH level over all.

Table 1: Baseline characteristics of prediabetic people treated by metformin or placebo

Characteristics Groups (people)	Treatment group		<i>P</i>
	Metformin (<i>n</i> = 43)	Placebo (<i>n</i> = 46)	
Women/men (<i>n</i> [%])	39 (91)/4 (9)	38 (83)/8 (17)	0.3
Age (years)			
Mean±SEM	51.1±1.22	47.7±1.36	0.07
Median	51	47.5	
Range	36-65	24-64	
Weight (kg)			
Mean±SEM	73.4±2.1	74.5±2	0.7
Median	72.5	74	
Range	50-111.1	49-117	
BMI (kg/m ²)			
Mean±SEM	30.5±0.7	29.84±0.7	0.53
Median	29.6	29.9	
Range	21.5-43	18.7-41	
TSH (μU/mL)			
Mean±SEM	2.02±0.16	2.14±0.19	0.63
Median	1.8	1.7	
Range	0.35-5.5	0.39-5.5	
Thyroid volume at onset (ml)			
Mean±SEM	8.46±0.67	8.8±0.56	0.7
Median	7.35	8.1	
Range	2.2-23.5	3.3-21.5	
TPO-Ab			
Mean±SEM	167.23±56.34	182.82±53.28	0.84
Median	37	37	
Range	37-1300	37-1300	
TgAb			
Mean±SEM	72.38±16.72	57.84±12.09	0.75
Median	24	20.5	
Range	7.5-500	4-301	
FPG			
Mean±SEM	105.63±1.98	102.09±2.27	0.2
Median	108	104	
Range	87-125	83-125	
BS-2 h OGTT			
Mean±SEM	134.56±5.16	125.71±4.82	0.2
Median	129	122.5	
Range	77-199	73-193	
HOMA-IR			
Mean±SEM	3.26±0.25	2.71±0.17	0.07
Median	3.15	2.43	
Range	0.64-6.91	0.62-6.23	

FPG = Fasting plasma glucose; SEM = Standard error of the mean; BMI = Body mass index; TSH = Thyroid-stimulating hormone; TPO-Ab = Thyroperoxidase antibodies; TgAb = Thyroglobulin antibody; BS-2 h OGTT = The 2-h plasma glucose value after a 75-g oral glucose tolerance test; HOMA-IR = Homeostasis model assessment-insulin resistance

However, after dividing them according to their baseline serum TSH to two subgroups of 0.3-2.5 $\mu\text{U/ml}$ and 2.6-5.5 $\mu\text{U/ml}$, TSH level revealed statistically, a significant reduction in those with higher baseline serum TSH level ($P = 0.01$) [Table 2 and Figure 1].

At the end of 3 months, there were no significant differences in the posttreatment thyroid volume in metformin-treated group ($P = 0.3$). However, there were a significant increase in the posttreatment thyroid volume in placebo-treated group ($P = 0.03$) [Table 3 and Figure 2].

The median baseline nodules size in metformin-treated group and placebo-treated group was 0.16 ml (range, 0.002-7.46 ml) and 0.13 ml (range, 0.01-1 ml), respectively. After 3 months of treatment, the median size were 0.1 ml (range, 0-6.3 ml) and 0.05 ml (range, 0-1 ml), respectively. However, there were no significant differences in the posttreatment volume of nodules in both groups [Table 4]. Among the 89 patients with prediabetes, 53.9% had thyroid nodules (29.21% in metformin-treated group and 24.72% in placebo-treated group). Seventeen persons (19.1%) had one thyroid nodule and 31 (34.8%) had multinodules. In the metformin-treated group, 17 people (39.5%) had no nodules on the thyroid

gland, 9 (21%) persons had one thyroid nodule and 17 (39.5%) people had multinodules. In the placebo-treated group, 24 patients (52.2. %) had no nodules on the thyroid gland, 8 (17.4%) persons had one thyroid nodule and 14 (30.4%) persons had multinodules. The numbers of selected nodules were 52. At the end of the study, no significant differences in nodule size was observed between the two groups, but when the solid nodules were separated from mixed nodules (solid and cystic), there was statistically, significant reduction in the volume of solid nodules in metformin-treated group ($P = 0.01$) [Table 5 and Figure 3].

In metformin-treated group, the mean of HOMA-IR, before and after-treatment was 3.26 ± 0.25 and 2.56 ± 1.3 , respectively ($P = 0.001$). However, in placebo-treated group, there was no difference before and after the treatment (2.7 ± 0.17 vs. 2.27 ± 0.19 , $P = 0.073$) [Table 6].

Fine-needle aspiration biopsy had been performed for 9 cases (10.1%) (7 cases [7.09%] in metformin-treated group and 2 cases [3.01%] in placebo-treated group) due to nodule size (≥ 1 cm) and other ultra-sonic criteria of malignant nodule, which all of them were benign. Some of the participants were not keen for doing procedure. Pearson's

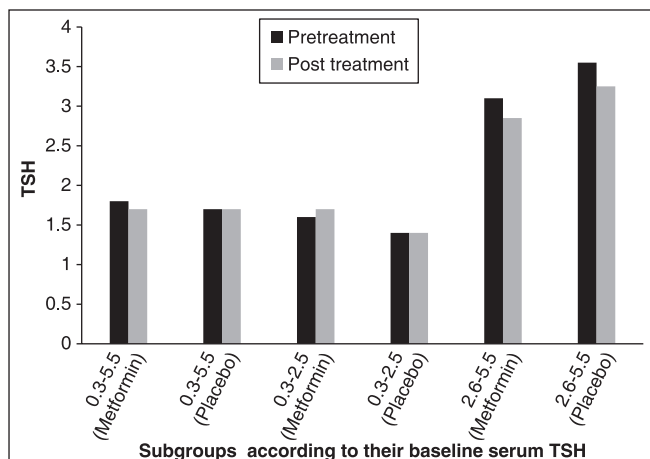


Figure 1: Changes in thyroid stimulating hormone levels after 3 months of follow-up between prediabetic participants treated with metformin and placebo

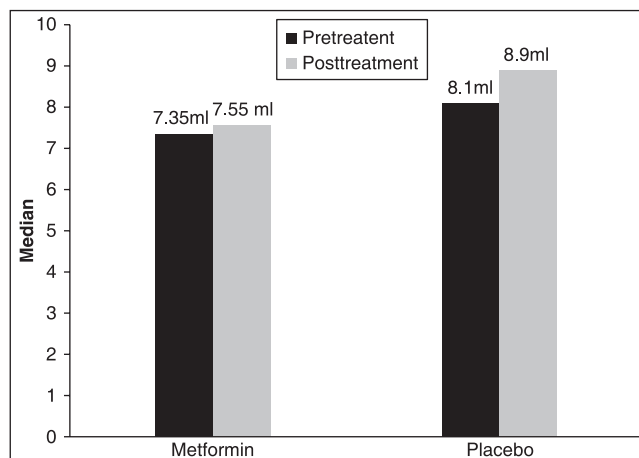


Figure 2: Median thyroid volume (ml) before and after 3 months of treatment with metformin or placebo in people with prediabetes

Table 2: Changes in TSH levels after 3 months of follow-up between prediabetic participants treated with metformin and placebo

TSH range	TSH = 0.3–5.5		TSH = 0.3–2.5		TSH = 2.6–5.5	
	Group I ^c (n = 43)	Group II ^c (n = 46)	Group I (n = 32)	Group II (n = 32)	Group I (n = 11)	Group II (n = 14)
TSH ($\mu\text{U/ml}$) pretreatment						
Median	1.8	1.7	1.6	1.4	3.1	3.55
Range	0.35-5.5	0.39-5.5	0.35-2.5	0.39-2.34	2.6-5.5	2.6-5.5
TSH ($\mu\text{U/ml}$) posttreatment						
Median	1.7	1.7	1.7	1.4	2.85	3.25
Range	0.6-5.5	0.8-4.7	0.6-4.35	0.8-3.75	1.5-4.5	1.5-4.7
P*	0.4	0.6	0.06	0.18	S 0.01	0.06

^cGroup I = Patients with prediabetes who received metformin (M); ^cGroup II = Patients with prediabetes who received placebo; TSH = Thyroid-stimulating hormone; S = Significant means $P < 0.05$; *Using Wilcoxon signed ranks test

Table 3: Changes on thyroid gland volume after 3 months of follow-up between prediabetic participants treated by metformin and placebo

Groups (participants) (n = 89)	Mean ± SEM (ml)		P
	Pretreatment	Posttreatment	
Metformin (n = 43)	8.46±0.72	8.8±0.7	0.3*
Placebo (n = 46)	8.7±0.72	9.23±0.7	S 0.03**

SEM = Standard error of the mean; S = Significant means $P < 0.05$; *From Wilcoxon signed ranks test; **From independent samples test

Table 4: Changes on volume of nodules after 3 months of follow-up between prediabetic people treated by metformin and placebo

Treatment groups nodules (n = 52)	Metformin (n = 35)	Placebo (n = 17)
Pretreatment		
Nodule volume (mean ± SEM)	0.82±0.29	0.26±0.07
Median	0.16	0.13
Range	0.002-7.46	0.01-1
Posttreatment (ml)		
Nodule volume (mean ± SEM)	0.67±0.23	0.17±0.06
Median	0.1	0.05
Range	0-6.3	0-1
P*	0.2	0.08

SEM = Standard error of the mean; *From Wilcoxon signed ranks test

Table 5: Changes on volume of thyroid solid nodules after 3 months of follow-up between prediabetic people treated by metformin and placebo

Groups nodules (n = 27)	Metformin-treated (n = 16)	Placebo-treated (n = 11)
Nodule volume pretreatment (ml)		
Median	0.07	0.05
Range	0.005-0.6	0.01-0.5
Nodule volume posttreatment (ml)		
Median	0.04	0.07
Range	0.005-0.44	0.01-0.6
P*	S 0.01	0.75

S = Significant means $P < 0.05$; *From Wilcoxon signed ranks test

Table 6: Changes of, HOMA-IR, before and after 3 months of follow-up between prediabetic people treated by metformin or placebo

Groups (patients)	Metformin (n = 43)	Placebo (n = 46)
HOMA-IR pre		
Mean±SEM	3.26±0.25	2.7±0.17
Median	3.15	2.43
Range	0.64-6.91	0.62-6.23
HOMA-IR post		
Mean±SEM	2.56±1.3	2.27±0.19
Median	2.35	2.05
Range	0.66-6.27	0.35-5.96
P*	S 0.001	0.07

SEM = Standard error of the mean; HOMA-IR = Homeostasis model assessment-insulin resistance; *S = Significant means $P < 0.05$; *Paired t-test

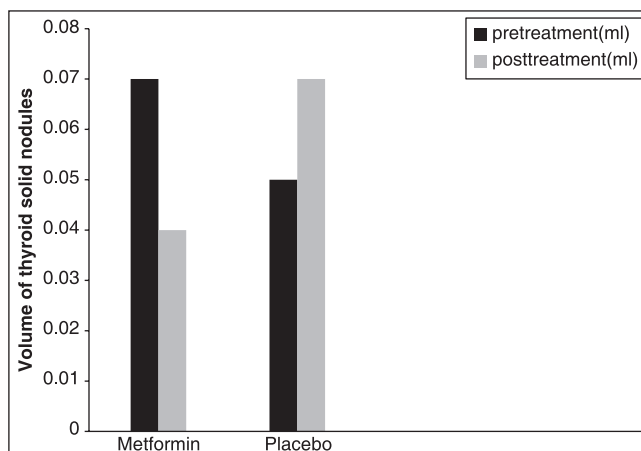


Figure 3: Changes on volume of thyroid solid nodules after 3 months of follow-up between prediabetic people treated by metformin and placebo

correlation between Δ HOMA-IR and Δ TSH in metformin and placebo group were ($r = 0.13$, $P = 0.4$) and ($r = -0.02$, $P = 0.9$), respectively.

At basal TPO-Ab was positive in 9 people (49.3%) in metformin-treated group and 13 persons (50.7%) in placebo-treated group ($P = 0.84$). After intervention, it was 56.5% and 43.5%, respectively. However, percentage of the changes in TPO-Ab positivity was not different statistically between metformin and placebo-treated groups ($P = 0.9$).

At basal TgAb was positive in 13 people (30.2%) in metformin-treated group and 10 persons (45.5%) in placebo-treated group ($P = 0.75$). After prescribing metformin and placebo, it was 54.1% and 49.5%, respectively. The percentage of changes in TgAb positivity was not different statistically between metformin and placebo-treated groups ($P = 0.09$).

There were no statistically, significant changes between FPG, HbA1c and BMI before and after treatment in both metformin and placebo-treated group (data not shown). However, HOMA-IR decreased in metformin-treated group [Table 6].

DISCUSSION

The people who recruited to this study are from Isfahan city. They are using iodized salt since 1989 and are iodine sufficient according to median urinary iodine concentration in different surveys since that time.^[9,10] It is supposed that the effect of iodine insufficiency on thyroid volume must have been eliminated during such long period. Although, goiter prevalence is still high in this city and investigating the other causes of thyroid enlargement rather than iodine deficiency should be sought.^[10] One of these factors might be IR, that has to be studied here.

Obesity and obesity-associated IR are increasing in Iran including Isfahan in recent years.^[12]

In some case-control studies which have been conducted in Isfahan city, the role of thyroid autoimmunity as the cause of goiter has been shown.^[9,10,13,14] The concentrations of TPO-Ab and TgAb in prediabetic participants were shown in Table 1.

There was no difference between two groups from view point of autoimmunity. Metformin did not affect on thyroid autoimmunity at least in this short period (3 months). It seems the effect of metformin on TSH >2.5 μ U/ml and thyroid volume must be independent of autoimmunity.

However, TSH has been recognized as the main growth factor for thyroid cells, although, this interpretation does not explain the role of TSH-dependent and independent mechanisms and positive, negative signaling interaction.^[11] TSH is not only involved in the control of differentiated functions, but also regulates the expression of growth factors and their receptors.^[15,16] This has been demonstrated, for example, for the expression of epidermal growth factor receptors,^[17] and for IGF-I-dependent signaling.^[18] Actually, TSH promotes the insulin, IGF-I signaling.^[19-22] Moreover, exposure to TSH-or cyclic adenosine monophosphate-elevating agents increase the responsiveness of thyroid cells to stimulation with insulin, IGF-I, and IGF-II.^[20] Accumulated evidence indicates that IGF-I-dependent, TSH-independent signaling may be of major importance for growth regulation of the human thyroid gland. This assumption is supported by the findings in conditions not accompanied by increased TSH secretion, such as in acromegaly, in which high intrathyroidal IGF-I levels may contribute to the enlargement of the thyroid.^[23] Thus, TSH induces the expression of growth factors and their receptors and may contribute to an increased responsiveness to growth factor-stimulated tyrosine kinase signaling, which consequently will stimulate tissue proliferation via the other pathways.

Duntas *et al.*^[24] have proposed, an emerging hypothesis to explain the effect of metformin on TSH involves the action of metformin on 5'-adenosine monophosphate activated protein kinase (AMPK). AMPK regulates cellular metabolism and integrates nutritional and hormonal signals in the hypothalamus, being a central target for both modulation of insulin sensitivity and feedback of thyroid hormones on appetite and energy expenditure. Peripherally, AMPK is dose and time dependently stimulated by triiodothyronine (T3).

In our study, metformin has decreased HOMA-IR, an index of IR. However, it has no statistically significant change on FPG, HbA1c and BMI. It suggests that the effect of

metformin on TSH >2.5 μ U/ml and thyroid volume and small thyroid nodule volume must probably be through insulin, IGF-I and their receptors. Our study supports that the metformin has a more strong regulatory effect on hormonal signal in the hypothalamus in comparison with cellular metabolism and nutritional signals.^[24]

In the liver, metformin suppresses hepatic gluconeogenesis by activating AMPK. The opposite effect is observed in the central nervous system where metformin inhibits hypothalamic AMPK.^[25] Although, few studies on the regulation of the hypothalamic isoforms (a1 and a2) of AMPK are available, the results provided by López *et al.*^[26] support the concept that the effects of metformin on hypothalamic AMPK activity can counteract T3 effects at the hypothalamic level. As an alternative hypothesis, the central effect of metformin could be mediated by a reduction of circulating fatty acids.^[27]

Some studies on the TSH effect of metformin in diabetic patients had been conducted.^[7,24,28]

With our best knowledge, the study on the effect of metformin on TSH and thyroid volume of prediabetic people had not been conducted, previously.

In our study, in which the effect of metformin compared with placebo on TSH and thyroid volume of prediabetic people was investigated. After 3 months of treatment, a significant decrease in TSH levels did not observe. However, when the TSH range was divided into two low and high-normal ranges, a decrease in TSH was significantly observed only in those patients with a basal high-normal TSH concentration [Table 2 and Figure 1]. There was also slightly increased thyroid volume in both groups that were significant in placebo-treated group [Table 3 and Figure 2]. In addition, a significant reduction was seen in the size of small solid nodules in metformin-treated group, compared with placebo-treated group [Tables 4, 5 and Figure 3].

In a retrospective study, conducted in Italy by Cappelli *et al.*, TSH levels in diabetic patients treated with metformin were studied, and they concluded that a significant reduction of TSH was seen in patients who had high-normal serum TSH (2.5-4.5 [μ U/ml]).^[28]

The result of our study is similar to Cappelli *et al.* study; however, it has some differences. First of all, our patients had prediabetes, and the study was prospective. In our study, 11 participants had TSH levels in the range of 2.5-5.5 (μ U/ml). There was a significant decrease in TSH level after treatment with metformin (3.1 μ U/ml vs. 2.85 μ U/ml, $P = 0.01$). However, such a reduction in placebo-treated group was not observed.

In a study by Rezzónico *et al.*, in 14 insulin resistant women that treated with metformin for 6 months compared with 15 female patients who were not treated, they found that TSH levels were not significantly different. However, the volume of small solid nodules in the metformin group, significantly decreased.^[29]

In our study, from 89 patients, 48 patients had thyroid nodules, 26 patients in metformin-treated group and 22 patients were in placebo-treated group. In some patients, due to multiple nodules, comparison of volume of nodules before and after treatment was not possible. Therefore only nodules in 33 of the patients were compared. The numbers of selected nodules were 52.

At the end of the treatment, there were no significant differences in the posttreatment thyroid nodule size between two groups, but when the solid nodules were separated from mixed nodules (solid and cystic), there were statistically significant reduction in the volume of solid nodules in metformin-treated group. This means that a total of 27 nodules were studied, 16 in metformin-treated group and 11 were in placebo-treated group and a significant reduction in volume of solid nodule was observed ($P = 0.017$) [Table 5].

In a study by Imani *et al.*, on thyroid of 263 people in the normal population of Isfahan, Iran, who 54% and 46% of the population were male and female, respectively, the prevalence of thyroid nodules by ultrasound was 22.4%. Among this normal population, 11.4% ($n = 30$) had one thyroid nodule and 8.7% ($n = 23$) had multinodules. The prevalence of thyroid nodules in males and females were 16.3% and 30%, respectively.^[30] In our study, 13.5% and 86.5% of patient were male and female, respectively. The prevalence of thyroid nodules was 53.9%.

In our study, 19.1% ($n = 17$) and 34.8% ($n = 31$) of patients had one thyroid nodule and multinodules, respectively. The prevalence of thyroid nodules in males and females were 58.4% ($n = 7$) and 53.2% ($n = 41$) respectively. Our prediabetic participants are recruited from the same population (Isfahan city). However, the prevalence of thyroid nodule by ultrasonography is 53.9% versus 22.4%. It is much higher than the normal population. This difference suggests the role of some factors like IR in the pathogenesis of thyroid nodule formation which is more common in prediabetic people in comparison with normal population.

Since, in prediabetic participants, the prevalence of thyroid nodule in male and female is almost equal (58.4% in men vs. 53.2% in women), it seems that the presence of IR blunts the effect of gender on thyroid nodule formation. However, in the normal population of Isfahan, its prevalence in women is twice as men.

In a Turkish study on 124 patients with prediabetes by Anil *et al.*, the percentage of patients with thyroid nodules in the prediabetes and control group was 51.3% and 23.7%, respectively ($P < 0.001$).^[31] In the present study, the prevalence of thyroid nodules in patients with prediabetes was 53.9%. The prevalence of thyroid nodules in prediabetic people in Iran and Turkey is similar to each other.

In a meta-analysis study by Lupoli *et al.*, they concluded that the metformin induces a reduction in TSH levels both in overt and in subclinical hypothyroidism. In contrast, no change in TSH levels was found in euthyroid people.^[32] In the present study, the effect of metformin compared with placebo on TSH and thyroid volume of prediabetic people who were euthyroid. We did not include hypothyroid patients. After 3 months of treatment, a significant decrease in TSH levels did not observe. In future study, hypothyroid prediabetic people should also be included in order to let us compare the findings with other similar researches.

Our study has some limitations. 3 months of study period, might be too short to observe the changes in thyroid volume and nodule size. The numbers of male participants in this study was low. However, as the thyroid disease is more prevalent in the female people and the tendency of women to participate in the research projects, it is expected to have such gender differences in future studies. However, bigger sample size may help solve the problem.

CONCLUSION

In prediabetic people, metformin decreases serum TSH in those with TSH > 2.5 $\mu\text{U/ml}$ and reduces the size of small solid thyroid nodules. It also prevents an increase in the thyroid volume. This effect might be through the decrease in IR and hormonal not nutritional and metabolic signals regulation.

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AUTHOR'S CONTRIBUTION

MK contributed in the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. AA contributed in the conception of the work, conducting the study, revising the

draft, approval of the final version of the manuscript, and agreed for all aspects of the work. MA contributed in the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. TM has done all ultrasound, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. BI conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. AF conducting the statical analysis of this study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. AN conducting the hormonal study, approval of the final version of the manuscript, and agreed for all aspects of the work.

REFERENCES

- American Diabetes Association. Standards of medical care in diabetes - 2014. *Diabetes Care* 2014;37:s14-64.
- Ascaso JF, Pardo S, Real JT, Lorente RI, Priego A, Carmena R. Diagnosing insulin resistance by simple quantitative methods in subjects with normal glucose metabolism. *Diabetes Care* 2003;26:3320-5.
- Reaven G. The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. *Endocrinol Metab Clin North Am* 2004;33:283-303.
- Kokkoris P, Pi-Sunyer FX. Obesity and endocrine disease. *Endocrinol Metab Clin North Am* 2003;32:895-914.
- Rezzonico J, Rezzonico M, Pusiol E, Pitoia F, Niepomniscz H. Introducing the thyroid gland as another victim of the insulin resistance syndrome. *Thyroid* 2008;18:461-4.
- Kimura T, Van Keymeulen A, Golstein J, Fusco A, Dumont JE, Roger PP. Regulation of thyroid cell proliferation by TSH and other factors: A critical evaluation of *in vitro* models. *Endocr Rev* 2001;22:631-56.
- Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, *et al.* Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002;346:393-403.
- Amini M, Afshin-Nia F, Bashardoost N, Aminorroaya A, Shahparian M, Kazemi M. Prevalence and risk factors of diabetes mellitus in the Isfahan city population (aged 40 or over) in 1993. *Diabetes Res Clin Pract* 1997;38:185-90.
- Aminorroaya A, Janghorbani M, Amini M, Hovsepian S, Tabatabaei A, Fallah Z. The prevalence of thyroid dysfunction in an iodine-sufficient area in Iran. *Arch Iran Med* 2009;12:262-70.
- Aminorroaya A, Amini M, Hovsepian S. Prevalence of goitre in Isfahan, Iran, fifteen years after initiation of universal salt iodization. *J Health Popul Nutr* 2010;28:351-8.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-9.
- Janghorbani M, Amini M, Willett WC, Mehdi Gouya M, Delavari A, Alikhani S, *et al.* First nationwide survey of prevalence of overweight, underweight, and abdominal obesity in Iranian adults. *Obesity (Silver Spring)* 2007;15:2797-808.
- Aminorroaya A, Momenzadeh M, Hovsepian S, Haghghi S, Amini M. Thyroid autoantibodies in women with and without thyroid disorders in an iodine-replete area. *East Mediterr Health J* 2008;14:325-32.
- Hashemipour M, Amini M, Aminorroaya A, Dastjerdi MI, Rezvani H, Kachoei A, *et al.* High prevalence of goiter in an iodine replete area: Do thyroid auto-antibodies play a role? *Asia Pac J Clin Nutr* 2007;16:403-10.
- Derwahl M, Studer H. Pathogenesis and treatment of multinodular goiter. In: Fagin JA, editor. *Thyroid Cancer*. Boston/Dordrecht/London: Kluwer; 1998. p. 155-86.
- Studer H, Derwahl M. Mechanisms of nonneoplastic endocrine hyperplasia - a changing concept: A review focused on the thyroid gland. *Endocr Rev* 1995;16:411-26.
- Westermarck K, Karlsson FA, Westermarck B. Thyrotropin modulates EGF receptor function in porcine thyroid follicle cells. *Mol Cell Endocrinol* 1985;40:17-23.
- Eggo MC, Bachrach LK, Burrow GN. Interaction of TSH, insulin and insulin-like growth factors in regulating thyroid growth and function. *Growth Factors* 1990;2:99-109.
- Hofbauer LC, Rafferteder M, Janssen OE, Gärtner R. Insulin-like growth factor I messenger ribonucleic acid expression in porcine thyroid follicles is regulated by thyrotropin and iodine. *Eur J Endocrinol* 1995;132:605-10.
- Burikhanov R, Coulonval K, Pirson I, Lamy F, Dumont JE, Roger PP. Thyrotropin via cyclic AMP induces insulin receptor expression and insulin Co-stimulation of growth and amplifies insulin and insulin-like growth factor signaling pathways in dog thyroid epithelial cells. *J Biol Chem* 1996;271:29400-6.
- Eggo MC, King WJ, Black EG, Sheppard MC. Functional human thyroid cells and their insulin-like growth factor binding proteins: Regulation by thyrotropin cyclic 39.59 adenosine monophosphate, and growth factors. *J Clin Endocrinol Metab* 1996;81:3056-62.
- Clark R. The somatogenic hormones and insulin-like growth factor-1: Stimulators of lymphopoiesis and immune function. *Endocr Rev* 1997;18:157-79.
- Cheung NW, Boyages SC. The thyroid gland in acromegaly: An ultrasonographic study. *Clin Endocrinol (Oxf)* 1997;46:545-9.
- Duntas LH, Orgiazzi J, Brabant G. The interface between thyroid and diabetes mellitus. *Clin Endocrinol (Oxf)* 2011;75:1-9.
- Lim CT, Kola B, Korbonits M. AMPK as a mediator of hormonal signalling. *J Mol Endocrinol* 2010;44:87-97.
- López M, Varela L, Vázquez MJ, Rodríguez-Cuenca S, González CR, Velagapudi VR, *et al.* Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. *Nat Med* 2010;16:1001-8.
- Wiersinga WM, Chopra IJ, Teco GN. Inhibition of nuclear T3 binding by fatty acids. *Metabolism* 1988;37:996-1002.
- Cappelli C, Rotondi M, Pirola I, Agosti B, Formenti A, Zarra E, *et al.* Thyrotropin levels in diabetic patients on metformin treatment. *Eur J Endocrinol* 2012;167:261-5.
- Rezzónico J, Rezzónico M, Pusiol E, Pitoia F, Niepomniscz H. Metformin treatment for small benign thyroid nodules in patients with insulin resistance. *Metab Syndr Relat Disord* 2011;9:69-75.
- Imani EF, Aminorroaya A, Soheilipour F, Adibi A, Sirous M, Roohi E, *et al.* Sonographic and functional characteristics of thyroid nodules in a population of adult people in Isfahan. *Endokrynol Pol* 2010;61:188-91.
- Anil C, Akkurt A, Ayturk S, Kut A, Gursoy A. Impaired glucose metabolism is a risk factor for increased thyroid volume and nodule prevalence in a mild-to-moderate iodine deficient area. *Metabolism* 2013;62:970-5.
- Lupoli R, Di Minno A, Tortora A, Ambrosino P, Lupoli GA, Di Minno MN. Effects of treatment with metformin on TSH levels: A meta-analysis of literature studies. *J Clin Endocrinol Metab* 2014;99:E143-8.

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