Letter to the Editor

Association of Thyroid Nodules with Various Elements in Urine and Blood Serum: A Case-Control Study*

LI Xiang Zhi[1,2,&], WU Cai Lian[1,2,&], CEN Li Ting[1,2], HUANG Ming Le[1,2], HUANG Min Min[1,2], QIU Mei Ting[1,3,#], and LU Xiao Ling[1,2,#]

Thyroid nodules are a common clinical problem caused by various factors, including environmental and nutritional factors and genetic background. The environment can modulate and influence not only the risk of thyroid nodules but also the severity of disease in humans[1]. The environment is awash with elements, and all mineral elements, including heavy metals and trace elements, are considered potentially toxic. A significantly higher incidence of thyroid cancer has been reported in populations exposed to low doses of metallic elements in volcanic areas over a long period[2]. As research on thyroid disorders continues, it is becoming clear that abnormalities in trace elements in the body can affect the development of thyroid-related disorders.

Thyroid-stimulating hormone (TSH) is a known thyroid growth factor, and serum TSH levels can be used to predict the stages of differentiated thyroid cancer[3]. Thyroxine (T4) and triiodothyronine (T3) are key enzymes involved in the synthesis of thyroid hormones. Trace elements are elements that make up just under 0.01% of the total body weight and include Iron (Fe), Zinc (Zn), Copper (Cu), Cadmium (Cd), Selenium (Se), Cobalt (Co), and Fluorine (F). These elements are not abundant but are closely related to the health of the human body. Trace elements can act as endocrine disruptors that interfere with the thyroid gland and can be used as blood markers for thyroid disease[4].

Appropriate iodine supplementation promotes healthy development in pregnant women[5]. In areas with or without iodine deficiency, adequate intakes of Se and Zn may prevent the formation of thyroid nodules[6]. Simultaneously, excess or deficiency of trace minerals can cause diseases, resulting in thyroid dysfunction and even thyroid tumors. In this study, we attempted to determine the correlation between the surrounding environment and onset of thyroid nodules by determining the levels of thyroid-related hormones and human elements in the serum and urine of a particular group of people.

The serum levels of T3, T4, and TSH in patients with thyroid nodules better reflect the differences in hormone levels between patients and the normal population, whereas the differences in urine trace elements better reflect the effects of environmental factors on both populations. Therefore, to study the effects of elemental exposure to environmental factors on thyroid disease, we randomly selected 120 participants as study subjects in the Second Affiliated Hospital of Guangxi University of Science and Technology and tested urine and blood samples from patients in the two groups; the inclusion and exclusion criteria of the study population are shown in Supplementary Figure S1, available in www.besjournal.com.

Blood analyses included an HDL cholesterol kit (direct method-peroxidase scavenging), triglyceride kit (GPO-PAP), and total cholesterol kit (CHOD-PAP), all of which were performed using a fully automated biochemistry analyzer (Hitachi 008AS) from Mecon Biotech Ltd. FT3 and FT4 levels were detected by chemiluminescence using free triiodothyronine and free thyroid kits on a MAGLUMI X8 Automated Chemiluminescent Immunoassay System (Snibe Diagnostic, China).

Urine was analyzed using flame atomic absorption spectrometry and inductively coupled

doi: *10.3967/bes2024.035*

*The College Students' Innovative Entrepreneurial Training Plan Program [202110594024] and Science and Technology Plan Projects of Liuzhou [2022CAC0299].

1. Guangxi Key Laboratory on Precise Prevention and Treatment for Thyroid Tumor, The Second Affiliated Hospital of Guangxi University of Science and Technology, Guangxi University of Science and Technology, Liuzhou 545006, Guangxi, China; 2. Department of Public Health, School of Medicine, Guangxi University of Science and Technology, Liuzhou 545005, Guangxi, China; 3. Academic and Research Department, The Second Affiliated Hospital of Guangxi University of Science and Technology, Guangxi University of Science and Technology, Liuzhou 545006, Guangxi, China

plasma mass spectrometry. Urine samples were first diluted with 1% nitric acid solution, and then the trace elements were determined by inductively coupled plasma spectrometry (ICP-MS). The mass-to-charge ratios of the ionized elements were separated and characterized, and their charge ratios were determined and quantified using internal standard curves. More than 80% of the measured values for Vanadium (V), Fe, Ni, Cu, Zn, As, Se, Sr, Cd, and Cesiu (Cs) exceeded the instrumental detection limits for inclusion in the statistical analyses (Supplementary Table S1, available in www.besjournal.com).

Continuous variables were presented as means and standard deviations, and categorical variables were presented as medians and interquartile ratios. Associations between sociodemographic and clinical characteristics and urinary characteristic levels of thyroid trace elements (T3, T4, and TSH) were analyzed using chi-square tests, Pearson's correlation analyses, independent sample t-tests, and Kruskal-Wallis tests (K-W tests), respectively. All analyses were performed using SPSS version 26 (IBM SPSS Statistics ver. 26.0).

In demographic analysis, the mean age of the participants in the control group was 55.76 ± 3.99 years, and the mean age of the participants in the thyroid nodule group was 51.45 ± 10.61 years. Chi-square tests were performed for gender, type of disease, and age group, and the results indicated no differences in age groups (Supplementary Tables S2–S3, available in www.besjournal.com).

We tested serum elements [Potassium (K), Sodium (Na), Chloride (Cl), Calcium (Ca)] as well as lipids [cholesterol (TC), triglycerides (TG), high density protein (HDL) and low-density protein (LDL)] and indicators of thyroid function (TSH, T3, T4 and FT3, FT4) in both groups, using the mean and standard deviation to express the overall picture of the data in both groups.

The TSH level in the thyroid nodule group 2.44 ± 4.18 μIU/mL was lower than that in the control group (2.79 ± 3.04 μIU/mL) and the T3 level in the thyroid nodule group 1.52 ± 1.31 nmol/L was significantly lower than that in the control group 1.77 ± 2.48 nmol/L, while the T4 level in the thyroid nodule group (126.93 ± 29.79 nmol/L) was significantly higher than that in the control group (121.97 ± 31.96 nmol/L). Using an independent sample T-test of serum test results from both groups, we found significant differences between the nodule and control groups for Na (P < 0.001). (Supplementary Table S4, available in www.besjournal.com)

To investigate the relationship between thyroid hormones and urinary elements in the nodal group, we used Pearson correlation analysis and found a positive correlation between T3 and Zn (r = 0.338, P = 0.0154), significant positive correlation between T4 and Cd (r = 0.453, P = 0.0008). Meanwhile, Fe (r = 0.380, P = 0.0060), Cu (r = 0.319, P = 0.0225), Ni (r = 0.312, P = 0.0258), Se (r = 0.287, P = 0.0408) with T4 had a weaker positive correlation (Table 1).

The final measurements obtained for the various elements were described using the median and interquartile spacing (Supplementary Table S5, available in www.besjournal.com); the Kruskal-Wallis non-parametric test was performed on the datasets.

Table 1. Correlation analysis of thyroid hormones and in thyroid nodule group

Elements	T3	T4	TSH	FT3	FT4
V	−0.0746 (0.6031)	0.0549 (0.7019)	−0.2469 (0.0807)	−0.1298 (0.3639)	−0.0110 (0.9391)
Fe	−0.0811 (0.5715)	0.3800 (0.0060)[b]	−0.1601 (0.2619)	−0.1260 (0.3782)	0.0270 (0.8510)
Ni	0.0332 (0.8172)	0.3120 (0.0258)[b]	−0.0463 (0.7470)	−0.0049 (0.9730)	−0.0827 (0.5611)
Cu	0.0737 (0.6071)	0.3190 (0.0225)[b]	−0.1273 (0.3732)	0.0288 (0.8412)	−0.1179 (0.4101)
Zn	0.3380 (0.0154)[b]	0.0545 (0.7043)	0.1055 (0.4614)	0.3590 (0.0098)[b]	−0.0600 (0.6757)
As	−0.0443 (0.7578)	0.1568 (0.2720)	−0.0354 (0.8050)	−0.0732 (0.6098)	−0.0720 (0.6157)
Se	−0.0789 (0.5820)	0.2870 (0.0408)	−0.1271 (0.3739)	−0.0847 (0.5547)	−0.0062 (0.9655)
Sr	−0.0310 (0.8290)	0.1387 (0.3316)	−0.2262 (0.1105)	−0.0743 (0.6043)	−0.0728 (0.6115)
Cd	−0.0362 (0.8009)	0.4530 (0.0008)[b]	−0.0818 (0.5681)	−0.0589 (0.6815)	−0.0719 (0.6163)
Cs	−0.1235 (0.3879)	0.1682 (0.2380)	−0.1832 (0.1983)	0.1514 (0.2889)	−0.0318 (0.8246)

Note. [b]0.3 ≤ |r| ≤ 0.5, low correlation. V, Vanadium; Fe, Iron; Ni, Nickel; Cu, Copper; Zn, Zinc; As, Arsenium; Se, Selenium; Sr, Strontium; Cd, Cadmium; Cs, Cesiu. The data were represented as r (P).

Significant differences were found for Fe (P = 0.010) and V (P = 0.003) in the thyroid nodule group, with a significant positive effect on the thyroid nodules (Table 2).

To investigate whether an association exists between the elements, Pearson correlation analysis was performed for each element (Figure 1). Significant correlations existed between Fe and Ni (r = 0.7450) and Se (r = 0.5000) and weaker correlations with Cu (r = 0.4850) and Cs (r = 0.4080). High correlation was noted between Ni and Se (r = 0.5810) and Cu (r = 0.5520), while weak correlation was observed with Cs (r = 0.4830) and Zn (r = 0.3950). A strong correlation was observed between Cu and Cs (r = 0.7030), whereas Se (r = 0.6830), Cd (r = 0.6410), Zn (r = 0.6190), Sr (r = 0.4380), and As (r = 0.3320) were weakly correlated. There was a significant correlation between Zn and Se (r = 0.5730), and a weaker correlation between As and Cd (r = 0.3490). A significant correlation was observed between Se and Cs (r = 0.7200). There was a weak correlation between Sr and Cd (r = 0.4640), whereas Cd was weakly correlated with Cs (r = 0.4070).

I, Se, Fe, Zn, and Cu are also involved in the synthesis of thyroid hormones, particularly Se and Fe, which are factors that limit epidemiological and interference studies on benign thyroid diseases[7]. A study by Hanif et al.[8] showed that Fe levels were significantly higher in the hypothyroid group than in the control group, suggesting a correlation between Fe and hypothyroidism. In the present study, a significant correlation was observed between elemental Fe levels in the thyroid nodule group and control population.

Winther et al.[9] noted that Se concentrations in the functioning thyroid gland are generally higher than those in other tissues and organs; therefore, Se supplementation is often used to treat thyroid disorders. In the present study, we found that

Table 2. Kruskal-Wallis test for urinary elements between thyroid nodule and control groups

Variables	V	Fe	Ni	Cu	Zn	As	Se	Sr	Cd	Cs
K-W test	8.813	6.638	32.080	0.880	1.286	0.038	0.907	0.730	1.224	0.791
P value	0.003*	0.010*	0.149	0.348	0.257	0.845	0.341	0.393	0.269	0.374

Note. *Significant at P < 0.05 in contrast to the control. V, Vanadium; Fe, Iron; Ni, Nickel; Cu, Copper; Zn, Zinc; As, Arsenium; Se, Selenium; Sr, Strontium; Cd, Cadmium; Cs, Cesiu.

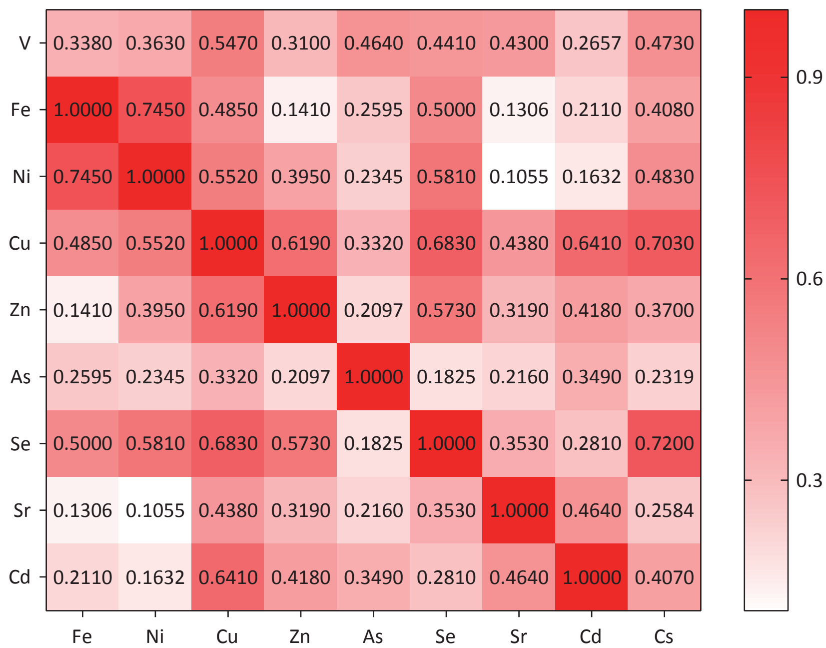


Figure 1. Correlation between the elements examined in the urine of thyroid nodule group. Fe, Iron; Ni, Nickel; Cu, Copper; Zn, Zinc; As, Arsenium; Se, Selenium; Sr, Strontium; Cd, Cadmium; Cs, Cesiu.

urinary Se levels were significantly lower in patients with thyroid disease than in controls, and by Pearson's correlation analysis, we found a positive correlation between Se and T4.

In a study by Ferrari et al.[10], Cd was found to accumulate in the thyroid, pancreas, liver, and kidneys, and diseases, such as multinodular goiter, occurred in the thyroid in response to chronic Cd toxicity. In this study, we observed significantly higher cadmium levels in the nodule group of patients than in the control group, and a positive correlation between cadmium levels and T4 in the thyroid nodule group of patients, which is in agreement with the findings of Ferraris' study.

Furthermore, we observed lower values for the metal elements Fe, Zn, and Cu relative to the control group, while Cu was mainly captured in the blood by copper cyanide (CP). Some studies have shown that urinary Cu decreases in patients with hyperthyroidism and increases in patients with hypothyroidism. In the present study, we found a significant correlation between Cu and T4 levels in the thyroid nodule group, suggesting that the elemental Cu levels in patients with thyroid nodules were consistent with those in patients with hyperthyroidism. In addition, Zn is required for the receptor activity of thyroid hormone (T3) and for the conversion of thyroxine to triiodothyronine and affects T4 levels by increasing the production of thyroxine-binding protein. In the present study, mean urinary Zn levels were lower in the nodule group than in the control group, yet T4 was higher in the nodule group than in the control group, and correlation study analysis found a correlation between Zn and the nodule group hormone as well, consistent with this finding.

This study had some limitations. First, the sample size was small and there was still a large gap compared with the overall population; second, the amount of information collected was not sufficiently and comprehensively collected, and in addition to analyzing the data of serum and urine samples of the population, the participants did not measure the effects of estrogen and androgen on thyroid hormones and failed to measure the patients' Body Mass Index (BMI) and Fasting Blood Glucose (FBG). Moreover, kits and techniques for studying trace elements in serum are not widely available and trace elements are not mandatory for serum testing, making it difficult to study trace elements in whole blood and serum. In the future studies, we plan to improve this aspect.

In our current study of the thyroid nodule population, we found links between elements, such as Fe, Zn, Cu, Cd, Se, and thyroid-related hormones, and between elements and elements; and that the relationship between hormones and elements, and between elements and the organism, is relatively complex, suggesting that environmental factors and elemental content between different regions have a certain influence on the organism. We believe that continued in-depth research in this direction will further reveal the influence of environment and elements on diseases, thus providing a basis for the prevention of thyroid diseases.

Acknowledgements We thank all participants in this study and the staff working on the indicator examinations at the Liuzhou CDC.

Author Contribution LI Xiang Zhi and WU Cai Lian: Project development, data collection, data analysis, and manuscript writing; HUANG Ming Le and HUANG Min Min: Data collection and manuscript writing. CEN Li Ting contributed to data acquisition. QIU Mei Ting and LU Xiao Ling contributed to the sample testing. All the authors have read and approved the final version of the manuscript.

Ethics Approval This study was approved by the Ethics Committee of the Second Affiliated Hospital of Guangxi University of Science and Technology.

Conflict of Interest All authors declare that they have no relevant conflicts of interest.

[&]These authors contributed equally to this work.

[#]Correspondence should be addressed to LU Xiao Ling, Tel: 86-772-2056080, E-mail: 824090772@qq.com; QIU Mei Ting, Tel: 86-772-3198003, E-mail: 530165677@qq.com

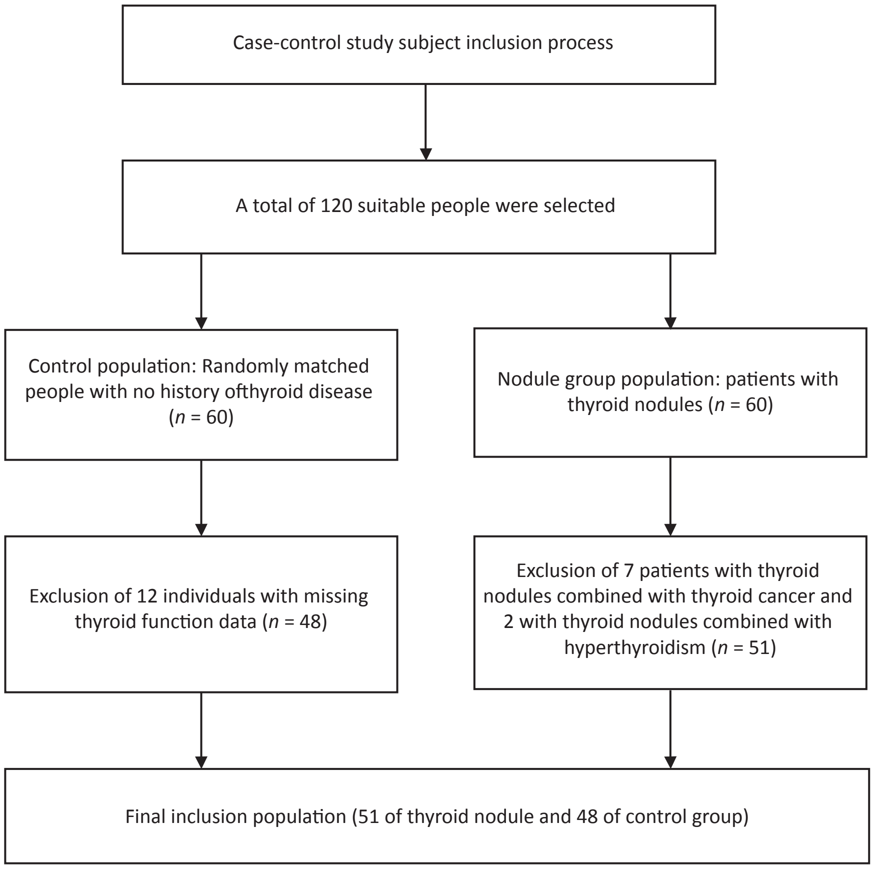
Biographical notes of the first authors: LI Xiang Zhi, male, born in 1989, MD, majoring in environmental pollutants and tumor research; WU Cai Lian, female, born in 2001, majoring in preventive medicine.

Received: September 16, 2023;
Accepted: December 11, 2023

REFERENCES

1. Nettore IC, Colao A, Macchia PE. Nutritional and environmental factors in thyroid carcinogenesis. Int J Environ Res Public Health, 2018; 15, 1735.
2. Markou K, Georgopoulos N, Kyriazopoulou V, et al. Iodine-induced hypothyroidism. Thyroid, 2001; 11, 501−10.
3. Soleimanisardoo L, Rouhani M, Sardoo FS. The effect of thyroid stimulating hormone on stage of differentiated thyroid carcinoma. Endocrinol Diabetes Metab, 2021; 4, e00266.
4. Stojsavljević A, Rovčanin B, Jagodić J, et al. Alteration of trace elements in multinodular goiter, thyroid adenoma, and thyroid cancer. Biol Trace Elem Res, 2021; 199, 4055−65.
5. Sun R, Qian TT, Liu LC, et al. Effect of iodine supplementation on iodine nutrition and thyroid function in pregnant women: a cross-sectional study. Biomed Environ Sci, 2022; 35, 641−7.

6. Turan E, Turksoy VA. Selenium, zinc, and copper status in euthyroid nodular goiter: a cross-sectional study. Int J Prev Med, 2021; 12, 46.
7. Köhrle J. Selenium and the thyroid. Curr Opin Endocrinol Diabetes Obes, 2015; 22, 392−401.
8. Hanif S, Ilyas A, Shah MH. Statistical evaluation of trace metals, TSH and T_4 in blood serum of thyroid disease patients in comparison with controls. Biol Trace Elem Res, 2018; 183, 58−70.
9. Winther KH, Rayman MP, Bonnema SJ, et al. Selenium in thyroid disorders - essential knowledge for clinicians. Nat Rev Endocrinol, 2020; 16, 165−76.
10. Ferrari SM, Fallahi P, Antonelli A, et al. Environmental issues in thyroid diseases. Front Endocrinol (Lausanne), 2017; 8, 50.



Supplementary Figure S1. Flowchart of study population inclusion exclusion.

Supplementary Table S1. Detection limit and recovery rate of urine testing instrument

Element	IDLs (μg/L)	Thyroid (*n* = 51)	Detection rate (%)	Contral (*n* = 48)	Detection (%)	Spiked recovery (%)
Be	0.009	9	17.65	9	18.37	75.2–104.2
Al	5.04	20	39.22	17	34.69	
V	0.0032	51	100.00	46	93.88	84.8–110.0
Cr	0.14	17	33.33	17	34.69	86.2–105.1
Mn	0.06	7	13.73	7	14.29	82.2–105.4
Fe	1.07	42	82.35	40	81.63	85.2–116.0
Co	0.053	4	7.84	19	38.78	80.0–101.9
Ni	0.063	45	88.24	46	93.88	82.8–103.4
Cu	0.11	49	96.08	48	100.00	81.1–105.9
Zn	1.33	51	100.00	48	100.00	83.5–107.1
Ga	0.0028	1	1.96	19	38.78	
As	0.03	51	100.00	48	100.00	91.2–109.6
Se	0.4	44	86.27	47	95.92	81.7–102.9
Sr	0.41	51	100.00	47	95.92	90.5–104.8
Ag	0.13	0	0.00	0	0.00	
Cd	0.0013	51	100.00	48	100.00	80.1–105.5
Cs	0.055	51	100.00	48	100.00	
Ba	0.16	10	19.61	31	63.27	
Tl	0.017	31	60.78	31	63.27	
Pb	0.078	9	17.65	16	32.65	
U	0.0006	2	3.92	7	14.29	

Note. IDLs: Instrumental detection limit.

Supplementary Table S2. Gender and disease distribution in thyroid nodule and control groups

Variables	Thyroid nodule *n* = 51 (%)	Controls *n* = 48 (%)	Chi-square test *P*-value
Gender			
Male	4 (7.84)	25 (53.06)	< 0.001
Female	47 (92.16)	23 (46.94)	
Thyroid nodule			
No	0 (0.00)	48 (100.00)	< 0.001
Yes	51 (100.00)	0 (0.00)	

Supplementary Table S3. Age distribution in thyroid nodule and control groups

Age (years)	Thyroid nodule *n* = 51 (%)	Controls *n*= 48 (%)	Chi-square test *P*-value
< 20	0 (0.00)	1 (2.08)	0.161
21–30	2 (3.92)	1 (2.08)	
31–40	7 (13.73)	6 (12.50)	
41–50	10 (25.49)	5 (12.50)	
51–60	20 (39.22)	15 (31.25)	
> 60	9 (17.65)	20 (39.58)	

Supplementary Table S4. Various elements and thyroid hormone levels in thyroid nodule and control groups

Serological assays	Thyroid nodule *n* = 51 (%)	Controls *n* = 48 (%)	Independent *t* test *P*
K (mmol/L)	3.97 ± 0.29	3.99 ± 0.34	0.817
Na (mmol/L)	143.47 ± 1.70**	140.67 ± 4.47	< 0.001
Cl (mmol/L)	106.11 ± 2.27**	103.65 ± 5.71	0.007
Ca (mmol/L)	2.34 ± 0.09	2.30 ± 0.12	0.118
TC (mmol/L)	4.96 ± 1.06	4.85 ± 1.11	0.611
TG (mmol/L)	1.27 ± 0.49	1.73 ± 1.62	0.065
HDL (mmol/L)	1.36 ± 0.25	1.29 ± 0.41	0.289
LDL (mmol/L)	2.99 ± 0.89	2.78 ± 0.88	0.248
TSH (μIU/mL)	2.44 ± 4.18	2.79 ± 3.04	0.636
T3 (nmol/L)	1.52 ± 1.31	1.77 ± 2.48	0.509
T4 (nmol/L)	124.50 ± 34.62	121.97 ± 31.96	0.707

Note. **Significant at the 0.01 level (two-tailed).