ARE THE ELEMENTS ZINC, COPPER, MAGNESIUM, AND RUBIDIUM RELATED TO NUTRITION AND IODINE DEFICIENCY IN PREGNANT BULGARIAN WOMEN FROM IODINE DEFICIENT REGION?

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SUMMARY

Objective: Trace elements are essential for the biochemistry of the cell. Their reference values have been found to differ considerably in pregnant women stratified by age, place of residence, anthropometric status, and length of pregnancy. In optimal amounts, these elements reduce the risk of pregnancy complications. Subclinical hypothyroidism in pregnancy is associated with adverse maternal and neonatal outcomes. The aim of the study was to determine the effects of zinc (Zn), copper (Cu), magnesium (Mg), and rubidium (Rb) on pregnant women in an iodine deficiency region and find the relationship with the thyroid status and nutrition.

Methods: We evaluated the iodine status of 61 healthy pregnant women from an iodine deficient region in Bulgaria. Thyroid stimulating hormone (TSH) and thyroxin free (FT4) levels were measured using ELISA.

Results: We found elevated levels of copper that differed the most between the first and second trimesters; Cu and TSH were found to be positively correlated ($p < 0.05$). Lower Cu levels were found in pregnant women consuming pulses more than 2–3 times a week ($p = 0.033$). The women consuming fish more than 2–3 times a week had higher levels of Rb. We found a pronounced iodine deficiency in more than half of the examined women in the first to third trimesters, without any effect of pregnancy on the ioduria ($p=0.834$). All second and third trimester cases were associated with severe ioduria (< 150 μg/L).

Conclusion: The high Cu levels were associated with subclinical hypothyroidism (SCH) and less pulse consumption during pregnancy in an iodine deficiency endemic area. SCH was found in 24% of the pregnant women in such an area while in 13% of them SCH had progressed to overt hypothyroidism.

Key words: trace elements, subclinical hypothyroidism, iodine-endemic area, pregnant women

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INTRODUCTION

The qualitative and quantitative composition of trace elements (TE) in human biological fluids and tissues has been actively studied for several decades (1). These substances enter into the composition of enzymes, participate in the antioxidant protection and in oxidation-reduction processes; they are involved in the exchange of hormones and proteins and in some immune processes, etc. TEs are acquired through the diet (mainly from plant products) with their levels in the body varying by geographical location, environmental pollution, sex, and age of individuals. The need for trace elements in humans fluctuates widely, and for most of them, it is not precisely established. Significant differences in the reference values of serum trace elements have been observed between different groups matched by age, place of residence, anthropometric status, and length of pregnancy. The diet during pregnancy should adequately provide sufficient amounts of these elements to meet the needs of the baby and the mother, with their pre-pregnancy levels being also of importance (2). Supplying the optimal amounts of the trace elements reduces the risk of pregnancy complications such as miscarriage, premature birth, foetal hypotrophy, preeclampsia, and foetal death (3). In the United States, subclinical hypothyroidism (SCH) during pregnancy affects approximately 2% to 2.5% of women, while overt hypothyroidism affects only 0.2% to 0.5% of all pregnant women (4). The prevalence of SCH depends on several factors such as supplemental iodine, age, and race. Deficiencies of iodine, magnesium, selenium, molybdenum, zinc and other TEs have been observed in pregnant women with euthyroid goitre and iron deficiency anaemia in endemic areas (5). Subclinical hypothyroidism during pregnancy is associated with numerous adverse outcomes for the mother and newborn such as pregnancy loss, gestational diabetes and hypertension, preterm delivery, premature rupture of membranes, preeclampsia, intrauterine growth restriction, placental abruption, low birth weight, low Apgar score, small for gestational age, and neonatal death (4).

Trace Elements and Thyroid Status

Deficiencies of iodine, magnesium, selenium, molybdenum, zinc, and some other trace elements have been found in pregnant women with euthyroid goitre and iron deficiency anaemia living in endemic areas (5). All the evidence suggests that most women in Europe are iodine deficient during pregnancy, but less than 50% of them receive iodine supplementation (6). Only nine out of 40 European countries meet the requirement that iodized salt should be used in no less than 90% of households (7). Bulgaria is recognized as an iodine-deficient country in the Balkans, as a substantial portion of its territory is known to have a low concentration of iodine (8).

MATERIALS AND METHODS

This cross-sectional study included sixty-one healthy pregnant women aged 27.57 ± 4.56 years from Asenovgrad, Bulgaria, and nearby villages. They did not get any trace element and mineral-containing supplements during gestation. We assessed the iodine status of these women during their regular gynaecological consultations at Asenovgrad hospital in 2020. This is a random selection of pregnant women. Group selection criteria were as follows. Age – participants should fall in the specified age group to ensure that pregnancy is comparable in terms of risks and micronutrient needs. This is usually between the ages of 18 and 35. Health – participants must be in good health and free of serious illnesses that could affect the results of the study. Thyroid function level – inclusion of participants with normal thyroid hormone levels is important for the effects of thyroid dysfunction on micronutrients and pregnancy to be ruled out. Absence of chronic diseases – participants must have no chronic diseases that could affect trace element analyses or thyroid function. The tests are conducted once in the corresponding trimester in which the woman is. Morning urine samples from the participants were taken during their prophylactic examinations or, from some of the pregnant women, during their stay in a healthcare facility. The iodine concentrations were measured using the Sandell-Kolthoff reaction (9). The working protocol was based on the recommendations of the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) (10, 11). Blood samples were taken from each patient. The sera were centrifuged at 3,000 g/min for 10 min at 4° C and stored at −80° until analysis. Thyroid stimulating hormone (TSH) and thyroxin free (FT4) were determined using the ELISA method (Globe Diagnostics, Italy). Before measuring the concentrations of magnesium (Mg), copper (Cu), zinc (Zn), and rubidium (Rb), we performed a microwave assisted acid digestion of the serum samples using a microwave digestion system with closed vessels (Multiwave GO, Anton Paar, Austria). We used an optimized pseudomatrix-matched calibration with external standards prepared in 15% HNO_3 with 130 mg/L Na content using rhodium as an internal standard. Multi-element determination was carried out on Thermo Scientific iCAP Qc ICP–MS (Thermo Scientific, Germany), equipped with a collision cell, working in a kinetic energy discrimination (KED) mode, with helium as collision gas. The accuracy was checked with two levels of standard reference materials (Seronorm Trace Elements, SERO AS, Norway). The digestion procedure, the method for the pseudomatrix-matched calibration, as well as the ICP-MS operating conditions, have been described in detail previously (12).

For the purposes of the study, we compiled questionnaires about the diet of the women. Statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistical tests including mean, standard deviation, and median were calculated according to the standard methods. Continuous variables were analysed for normality by Kolmogorov-Smirnov and Shapiro-Wilk tests. The data distribution was normal. The student's t test and ANOVA test were used to compare the continuous variables with normal distribution in two or more independent groups. Differences with $p < 0.05$ were considered statistically significant. The study conformed to the tenets of the declaration of Helsinki and was approved by the Ethics Committee of the Medical University of Plovdiv (No. P-172/21 01 2020). All pregnant women signed informed consent for participation and were informed about the type, risks, and expected benefits of the study.

RESULTS

Table 1 presents the mean trace elements reference values in pregnancy.

The distributions of Mg, Cu, Zn, and Rb by trimester are shown in Figures 1a, 1b, 1c, and 1d, respectively. A statistically significant difference between trimesters was found only for the copper levels, with the largest differences being between the first and second trimesters ($p < 0.01$). Our results suggest that TSH and FT4 had normal mean values during pregnancy. There was no statistically significant difference between the TSH and FT4 levels of women in the three trimesters ($p > 0.05$). Figures 2a and 2b show that Cu and TSH levels were positively correlated ($p = 0.029$) while Cu levels were negatively correlated with the FT4 levels ($p = 0.05$). Women with FT4 levels below 9 pmol/L had Rb concentration of 4.03 ± 2 µmol/L compared to the concentration of the other group (5.07 ± 2.5 µmol/L), a dependence trend was seen for the Rb levels compared with the FT4 levels ($p = 0.071$). Pregnant women with a low Zn level (below 10.7 µmol/L) had a statistically significantly higher TSH (1.9 ± 1.2 mIU/L) than the group with higher zinc (0.8 ± 0.5 mIU/L) ($p = 0.042$). Only magnesium level did not show statistically significant correlations with these hormones ($p > 0.05$). We found that women consuming fish more than 2–3 times a week had high levels of Rb ($p < 0.05$) (Fig. 3a), while their Zn levels showed some dependency trends (Fig. 3b). The Mg (Fig. 3c) and Cu (Fig. 3d) concentrations in pregnant women consuming fish failed to reach significance. Pregnant women who consumed pulses more than 2–3 times per week were found to have lower copper concentrations (26.9 ± 5.6) than those of women that consumed less than

Table 1. Trace elements in pregnant women (N = 61)

	Mean (SD)	Median	95% CI	Min	Max	SEM
Mg (mmol/L)	0.76 (0.06)	0.77	0.74–0.78	0.61	0.87	0.008
Mg (ppm)[a]	18.64 (1.57)	18.80	18.21–19.07	14.74	21.10	0.216
Cu (μmol/L)	29.79 (4.78)	31.45	28.47–31.12	17.07	36.89	0.65
Cu (ppb)[b]	1,893.3 (304.04)	1,998	1,809.49–1,977.1	1,084	2,344	41.76
Zn (μmol/L)	9.05 (1.39)	8.90	8.67–9.44	6.77	13.06	0.19
Zn (ppb)b	591.86 (91.22)	582	566.72–617.01	442	854	12.53
Rb (μmol/L)	4.64 (2.38)	3.95	3.98–5.30	1.55	12.92	0.32
Rb (ppb)[b]	396.92 (203.85)	338	340.73–453.11	133	1,104	28

[a]ppm = 1×10^{-6} g/g = 1 μg/g; [b]ppb = 1×10^{-9} g/g = 1 ng/g

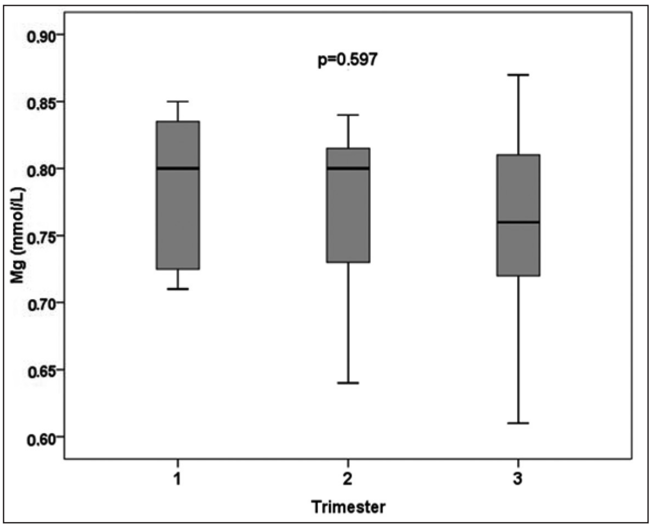

Fig. 1a. *Distribution of serum Mg by trimesters in pregnant women.*

One-way ANOVA test

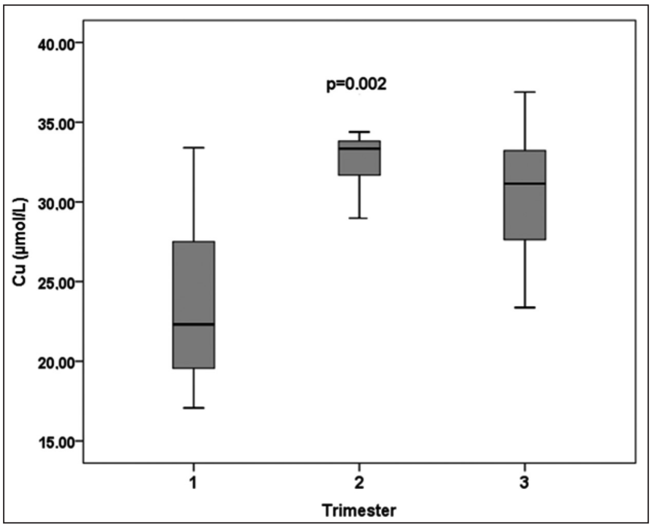

Fig. 1b. *Distribution of serum Cu by trimesters in pregnant women.*

*Statistically significant difference (p < 0.05), one-way ANOVA test

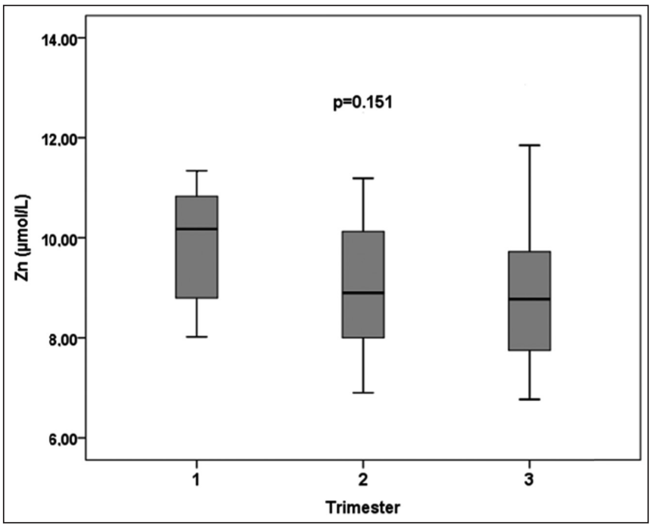

Fig. 1c. *Distribution of serum Zn by trimesters in pregnant women.*

One-way ANOVA test

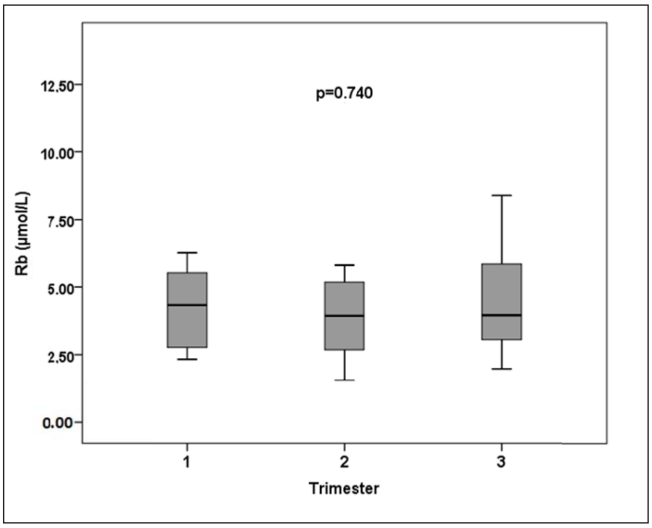

Fig. 1d. *Distribution of serum Rb by trimesters in pregnant women.*

One-way ANOVA test

2–3 times (30.4 ± 4.3, p = 0.033), lower TSH levels (1.9 ± 1.5) in comparison with women who consumed pulses less than 2–3 times (2.2 ± 2.4, p = 0.089), and higher FT4 levels (11.9 ± 3.2) than those of the women who rarely consumed pulses (11.4 ± 2.9, p = 0.067), albeit as a dependency trend. Rare consumption of legumes less than 2–3 times (OR = 5.06, 95% CI: 1.05–24.39, p = 0.043) and

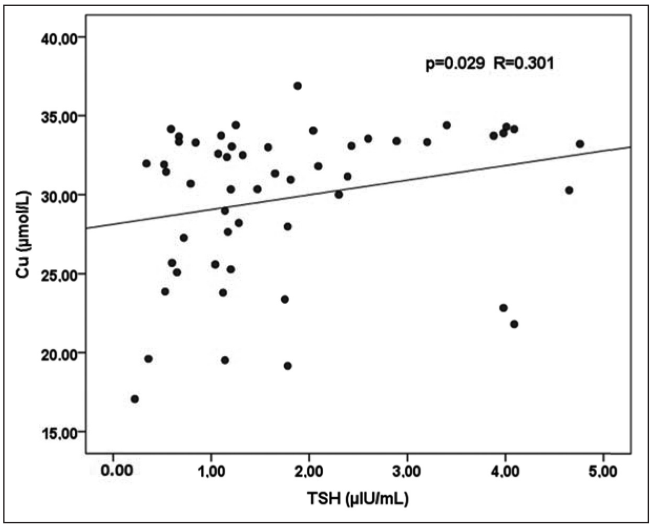

Fig. 2a. *Correlation between amount of copper and levels of TSH.*

Pearson's correlation coefficient

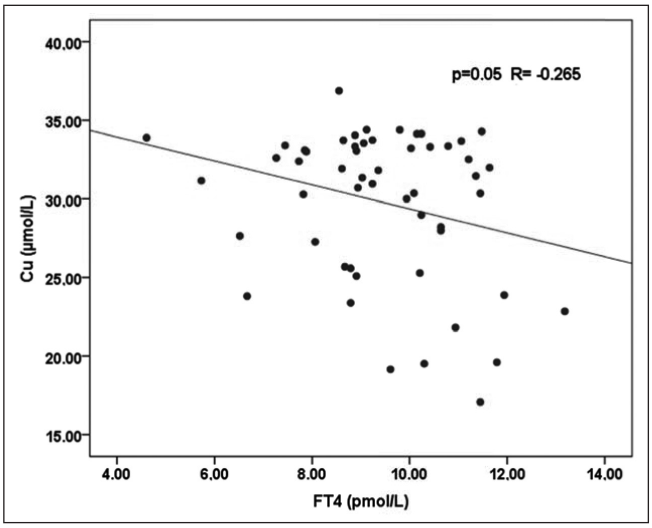

Fig. 2b. *Correlation between amount of copper and levels of FT4.*

Pearson's correlation coefficient

fish (OR = 30.33, 95% CI: 2.69–341.36, p = 0.006) is a risk factor for high copper levels. Rare consumption of fish appears to be a protective factor against high levels of rubidium (OR = 0.024, 95% CI: 0.001–0.674, p = 0.028). Regarding other food sources (milk, eggs, coffee, meat, nuts, fruits), no significant differences were found (p > 0.05).

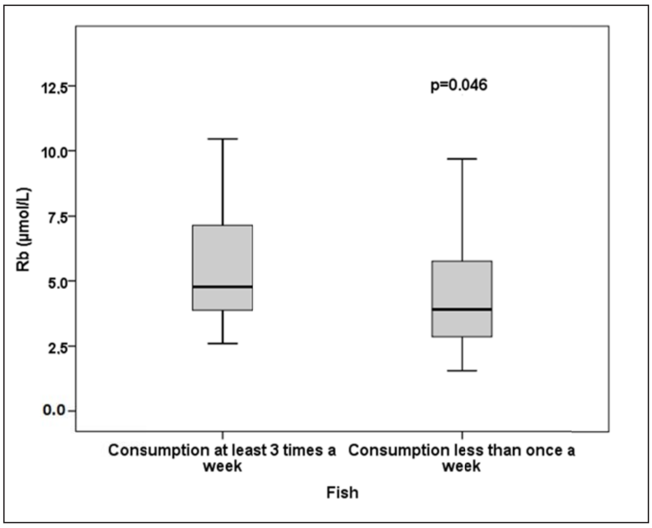

Fig. 3a. *Concentration of Rb in pregnant women consuming fish.*

*Statistically significant difference (p < 0.05), independent samples t-test

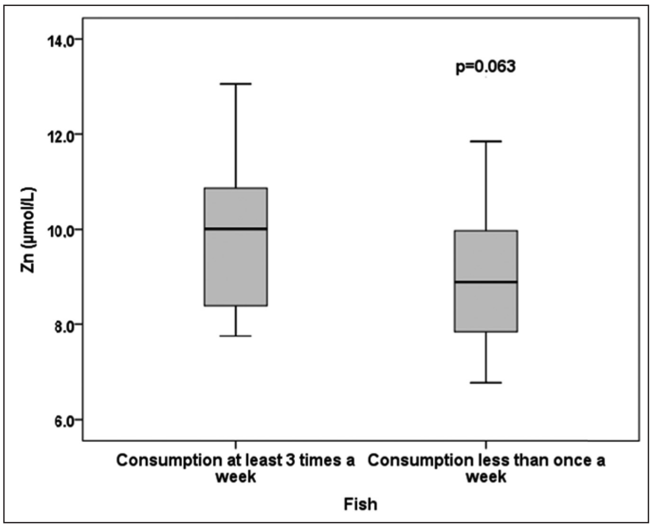

Fig. 3b. *Concentration of Zn in pregnant women consuming fish.*

Independent samples t-test

Table 2 shows the indicators for iodine status assessment of pregnant women from Asenovgrad, Bulgaria.

In assessing the iodine status of the examined women, we noticed that the proportion of iodine deficient women was the highest when assessed on the basis of their level of ioduria, which was found to be below 150 µg/L in more than half of all pregnant

Table 2. *Indicators for iodine status assessment of pregnant women from Asenovgrad, Bulgaria*

Indicators	Iodine concentration in urine (µg/L)	FT4 (pmol/L)	TSH (mIU/L)
Number	61	61	61
Mean (SD)	131.08 (64.07)	9.27 (1.79)	1.92 (1.29)
SEM	8.20	0.23	0.17
Median	124.0	9.24	1.47
95% CI	114.67–147.49	8.81–9.73	1.59–2.25
Min	32.92	4.61	0.22
Max	323.0	13.18	4.76

FT4 – free thyroxin; TSH – thyroid-stimulating hormone

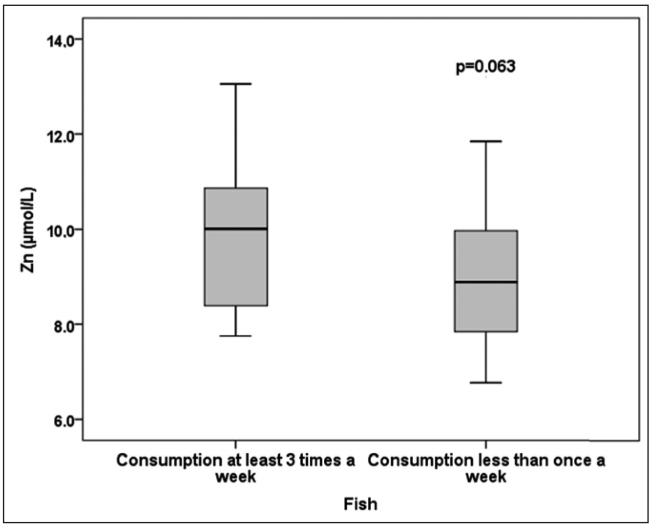

Fig. 3c. *Concentration of Mg in pregnant women consuming fish.*

Independent samples t-test

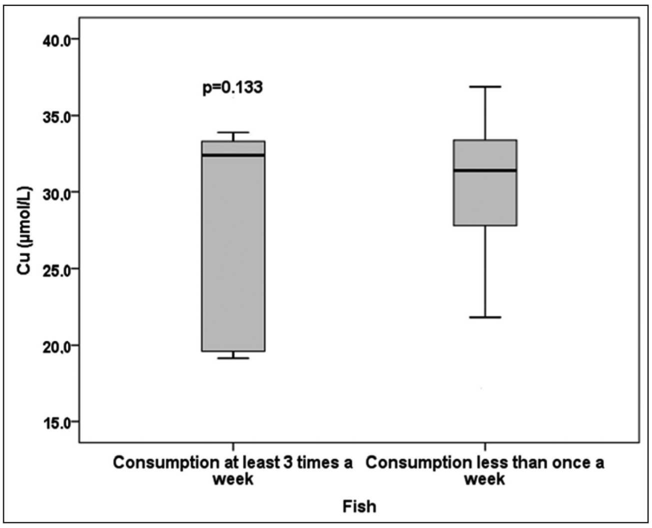

Fig. 3d. *Concentration of Cu in pregnant women consuming fish.*

Independent samples t-test

women in the study – 39 (63.9%) out of 61 women. About a third of the pregnant women had an optimal iodine intake, with 4.9% of them having an iodine intake exceeding the optimal intake. Table 3 and Figure 4 show the dynamics of iodine status of the examined women in the different periods of pregnancy. There is a pronounced iodine deficiency in more than half of the pregnant women from the first to the third trimesters. Their proportion is the highest in the third trimester, with the duration of pregnancy not affecting ioduria ($p = 0.834$). FT4 concentrations below the lower reference limit of 9 pmol/L (as measured by ELISA) were found in 27 (44%) women, all of them in the third trimester. The mean FT4 concentration was 9.89 ± 2.7 pmol/L in the first trimester women, 8.88 ± 1.7 pmol/L in women in the second trimester, and 9.33 ± 1.4 pmol/L in women in the third trimester. The level of this hormone was not affected by the term of pregnancy ($p = 0.313$). Twenty-one (78%) of the women had FT4 concentrations below the lower reference limit, with their ioduria below 150 µg/L. As of 2016, new guidelines have redefined the normal reference range of TSH during pregnancy, with the upper reference range of 2.5 mIU/L in the first trimester and 3.0 mIU/L in the second and third trimesters (13, 14). Fifteen (24%) of the examined women had their TSH levels above the respective range limits according to trimester, with 4 of these women in the first trimester, 5 in the second, and 6 in the third trimester. All women in the second and third trimesters had severe ioduria below 150 µg/L (median 138, 95% CI: 101.65–165.51 and median 118, 95% CI: 106.63–142.4). As irregularities were also found in the FT4 levels, we can assume that there was a clinically manifest iodine deficiency disorder. The term of pregnancy did not affect statistically significantly the level of TSH in these women ($p = 0.833$). The level of TSH was 2.1 ± 1.6 mIU/L in the women in the first trimester, 1.8 ± 1.2 mIU/L in the women in the second trimester, and 1.9 ± 1.2 mIU/L in those in the third trimester.

DISCUSSION

Providing the body with sufficient amounts of trace elements can only be achieved through food and water, making them crucial in providing the body with adequate amounts of these elements. Their currently available reference values in pregnant women, however, vary considerably in different populations stratified by age, place of residence, anthropometric status, and length of pregnancy (15). There is strong evidence that serum selenium (Se), Zn, and Cu influence thyroid hormone levels. Evidence also suggests that Zn, Cu and cadmium (Cd) may interact with each other, micronutrient abnormalities may impair iodine uptake by the thyroid gland (16). The plasma concentrations of selenium, zinc, and copper are correlated with the concentrations of FT4 and TSH in the thyroid gland. Supplementation with selenium, zinc, and copper is known to affect the thyroid peroxidase antibody (TPO-Ab) status (17). The effects of some other trace elements

Table 3. *Iodine status in urine (µg/L) of pregnant women by trimesters*

Indicators	1st trimester	2nd trimester	3rd trimester
Number	9	19	33
Mean (SD)	150.11 (100.5)	133.58 (66.25)	124.52 (50.45)
SEM	33.51	15.2	8.78
Median	133	138	118
95% CI	72.83–227.4	101.65–165.51	106.63–142.4
Min	33	46	35
Max	323.0	289	226

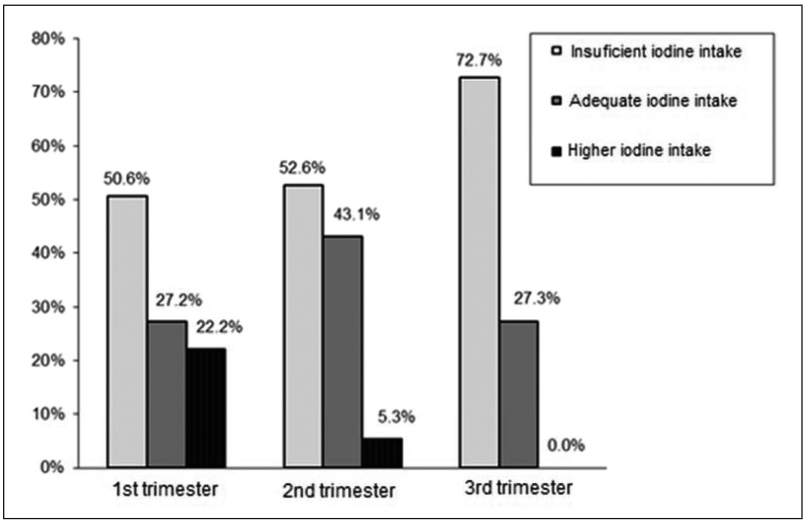

Fig. 4. *Iodine status of pregnant women by trimesters.*

such as copper and zinc on thyroid hormones, on the other hand, are still inconclusive. Overall, trace elements can affect the thyroid hormones, which are essential for the proper function of the body's antioxidant defence (18).

The normal range for plasma zinc concentrations in healthy non-pregnant women is 10.7–18.4 µmol/L (15). The plasma zinc concentration in 88% of the pregnant women in our study was <10.7 µmol/L, which makes them zinc deficient. Because of the haemodilution and low albumin levels, its serum concentration decreases during pregnancy, and the additional zinc requirement for the foetal and placental tissues must be compensated by increased maternal intake (19).

Magnesium deficiency (<0.66 mmol/L) was found in 28.3% of the examined pregnant women (reference range for magnesium 0.6 to 1.07 mmol/L) (15). Our results for zinc and magnesium concentrations in pregnant women are not consistent with those reported in the literature, which are elevated for the respective period of pregnancy (99.77 ± 13.48 for Zn, and 1.55 ± 0.15 for Mg) (20). Magnesium deficiency is more likely to occur in pregnant women with major consequences for the mother, the foetus, and the child, including impaired foetal growth and premature birth due to induced hyperexcitability, particularly in stressful situations for the mother (21).

Very little is known about the normal concentration of rubidium in pregnancy and the effects it has particularly on pregnant women. In non-pregnant women, this trace element should be in the range of 0.96–6.5 µmol/L (15), however, its concentration rises as high as 2.1–9.4 µmol/L (22) during pregnancy according to literature data. In our study, 7.5% of the women had Rb concentration above 9.4 µmol/L. There is very little information about rubidium (Rb) in the literature, especially about the concentration of this trace element in pregnant women (23). Although Rb is recognized to be crucial for the embryonic development (24, 25), excessive amounts of the metal in the body can potentially be quite toxic.

The normal range of Cu reference values for non-pregnant women is 12.6–24.3 µmol/L (15), while these values for pregnant women are elevated, according to literature data (20). We found 83% of the pregnant women in our study to have copper concentrations above 24.3 µmol/L. Acquisition of copper by the human body is achieved mainly through food (75%) and water (25%). In pregnant women, plasma copper concentration increases with the gestational age (20). In our study, we found the copper concentrations in the second trimester (32.1 ± 2.6 µmol/L) to be statistically significantly higher than those in the first trimester ($p<0.001$); Cu levels were high also in the third trimester (30.2 ± 4 µmol/L). This rise in the concentration of copper as pregnancy proceeds can be partially accounted for by the synthesis of ceruloplasmin, a key copper-binding protein, as a result of the rising levels of maternal oestrogen. A reduced biliary excretion of copper due to hormonal changes during pregnancy might also explain the increase in copper levels (26). The amount of copper taken in with food and water is relatively small, while the excess amounts of copper in the body are dealt with by reducing the absorption or increasing the excretion. Copper concentrations may be elevated in pregnant women, due to the higher levels of copper-containing proteins (27), which may cause premature births and low foetal weight compared to pregnancies in which copper concentration is within normal range (22). The relationship between thyroid hormones metabolism and trace element levels is of biological significance. Yet, in the literature, the comparison between the levels of trace elements in hypothyroid patients and healthy individuals has yielded conflicting results. Copper levels are correlated with elevated TT3 and TT4 levels (28). Our research has demonstrated that high copper levels are associated with low FT4 levels and high TSH levels (Fig. 2a and 2b). The 2014 Health and Nutrition Examination Survey showed that there was a positive correlation between the serum copper levels and the FT4 and TT4 levels in women (28). In addition, a study on children with congenital hypothyroidism demonstrated that copper concentration and TT4 levels were positively correlated (29). A case-control study from 2017 reported that blood concentrations of trace elements, including Cu, Zn, and Mn, were significantly higher in patients with hypothyroidism (30), which is consistent with our results.

Zinc is involved in the regulation of thyroid function, acting in the synthesis of thyrotropin-releasing hormone, the peripheral deiodination of T4 (tetraiodothyronine), and the binding of thyroid hormones to nuclear receptors. A 2019 meta-analysis reported that patients with hypothyroidism showed lower plasma concentrations of selenium and zinc compared to healthy controls, and plasma concentrations of copper and magnesium did not differ between patients with hypothyroidism and healthy controls (31). With the exception of selenium, research on the effects of trace elements on the thyroid gland is very limited at this point.

Limitations of the Study

Our study had several limitations. This cross-sectional study could not establish a causal relationship between maternal thyroid disorders and iodine status. Finally, we were unable to monitor iodine nutrition at different periods during pregnancy and postpartum. Using single samples for iodine determination is acceptable for large populations. Urinary iodine varies between individuals both during the day and between days, but these fluctuations can be levelled out with a sufficiently large sample size, and this is one of the limitations of our study. However, ioduria is a practical and convenient method for assessing the iodine status of risk groups of the population and is used in the periodic monitoring conducted according to the National Strategy for Overcoming Iodine Deficiency Diseases in Bulgaria.

CONCLUSIONS

Twenty-four percent of the pregnant women in an iodine deficient region had subclinical hypothyroidism (elevated TSH levels above the upper reference limit with normal FT4 levels), and 8 (13%) had overt hypothyroidism (elevated thyroid-stimulating hormone levels above the upper reference limit at low FT4 levels). Elevated serum copper levels are associated with subclinical hypothyroidism and less consumption of pulses during pregnancy in an iodine-endemic area. Pregnant women with low zinc levels below 10.7 µmol/L had statistically significantly higher levels of thyroid-stimulating hormone compared to the higher zinc group. Fish consumption more often than 2–3 times per week during pregnancy is associated with higher Rb levels. High Rb levels tend to be positively correlated with FT4. More micro- and macronutrient studies are needed to show the relationship with nutrition, especially in at-risk populations such as pregnant women and especially in iodine deficient regions.

Conflicts of Interest

None declared

Authors' Contributions

MGB, PDG, TID, DMD and AVB – material preparation, data collection, and analysis; MGB – first draft of the manuscript. All authors contributed equally to the study conception and design and read and approved the final manuscript.

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