Amelioration of iodine supply is notably associated with thyroid function in healthy subjects and in patients with euthyroid Hashimoto's thyroiditis

Izboljšanje preskrbe z jodom je pomembno povezano z delovanjem ščitnice pri zdravih posameznikih in pri bolnikih z evtirotičnim Hashimotovim tiroiditisom

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Izvleček

Izhodišča: Podatkov o povezavi med delovanjem ščitnice in preskrbo z jodom je malo. Zato je bil naš namen ugotoviti, kako so se zdravi posamezniki (ZP) in bolniki z evtirotičnim Hashimotovim tiroiditisom (EvHT) soočili z zvečanim jodiranjem kuhinjske soli v letu 1999, ko se je le-to povečalo s prejšnjih 10 mg kalijevega jodida na kg soli na 25 mg na kg.

Metode: V retrospektivni raziskavi smo pregledali medicinsko dokumentacijo 24.899 bolnikov, med letoma 1995 in 2002 prvič napotenih v naš terciarni center, ki ima že več kot 20 let stabilno področje zajemanja bolnikov. ZP so bili negativni in bolniki z EvHT pozitivni za protitelesa proti ščitnični peroksidazi in/ali tiroglobulinu. Pridobili smo podatke o koncentraciji tirotropina (TSH), prostega tiroksina (pT_4) in prostega trijodtironina (pT_3). Izračunali smo razmerje pT_4/pT_3.

Rezultati: V obdobju 1995–1998 je imelo 917 ZP značilno nižje razmerje pT_4/pT_3 (2,6±0,6) kot 644 ZP v obdobju 1999–2002 (2,9±0,9, p<0,001) na račun značilno višjega pT_3 (mediana 5,7 in 5,2 pmol/L, p<0,001). V obdobju 1995–1998 je imelo 482 bolnikov z EvHT nižje razmerje pT_4/pT_3 (2,4±0,6) kot 846 bolnikov z EvHT v obdobju 1999–2002 (2,8±0,7, p<0,001) na račun značilno višjega pT_3 (mediana 5,8 in 4,9, p=0,018). ZP so imeli višje razmerje pT_4/pT_3 kot bolniki z EvHT v 1995–1998 (p<0,001), ne pa tudi v 1999–2002 (p=0,206).

Zaključki: Izboljšanje preskrbe z jodom je povezano z zvečanjem razmerja pT_4/pT_3 na račun zniževanja pT_3 in s primerljivo ščitnično funkcijo pri ZP in pri bolnikih z EvHT.

Abstract

Background: Our aim was to establish how healthy subjects (HS) and patients with euthyroid Hashimoto's thyroiditis (EuHT) faced the increase in mandatory salt iodization in Slovenia in 1999 from previous 10 mg of potassium iodide to 25 mg per kg since data about thyroid function with respect to iodine supply are scant.

Methods: In this retrospective study we reviewed records of 24,899 patients referred to our tertiary referral centre for the first time between 1995 and 2002; the centre has had a stable catchment area for more than 20 years. HS were negative and patients with EuHT positive for thyroid peroxidase antibodies and/or thyroglobulin antibodies. Thyrotropin (TSH), free thyroxine (fT_4), and free triiodothyronine (fT_3) levels were obtained. The fT_4/fT_3 ratio was calculated.

Results: In the period 1995–1998, 917 HS had significantly lower fT_4/fT_3 ratio than 644 HS in the period 1999–2002 (2.6±0.6 and 2.9±0.9, respectively, p<0.001) on account of significantly higher fT_3 (median 5.7 and 5.2 pmol/L, p<0.001). Similarly, in 1995–1998, 482 patients with EuHT had a lower fT_4/fT_3 ratio than 846 EuHT in 1999–2002 (2.4±0.6 and 2.8±0.7, respectively, p<0.001) on account of significantly higher fT_3 (median 5.8 and 4.9, respectively, p=0.018). HS had a higher fT_4/fT_3 ratio than patients with EuHT in 1995–1998 but not in 1999–2002 (p<0.001 and p=0.206, respectively).

Conclusions: Amelioration of iodine supply is associated with an increase in the fT_4/fT_3 ratio on account of lower fT_3, and with a similar thyroid function in HS and in patients with EuHT.

Introduction

Thyroid function and size depend primarily on adequate iodine supply. Optimal intake of iodine lies within a very narrow interval between 120 and 220 µg per day (1). Lower or higher iodine intake represents an increased risk of thyroid disorders, and already a little change in iodine supply significantly influences the epidemiology of thyroid diseases (2,3).

In 1953, Slovenia introduced mandatory iodization of table salt, but the country was still among those with mild iodine deficiency (4). In 1999, Slovenia increased the amount of potassium iodide from previous 10 mg to 25 mg per kilogram of table salt. After the increase, the median urinary iodine concentration was 148 µg/L compared to only 82.9 µg/g creatinine before 1999 (4). According to the WHO criteria, after 1999, Slovenia therefore became an area with adequate iodine supply (5). The increase in iodine content in salt has brought changes in the incidence of thyroid diseases. An important decrease in the incidence of diffuse goiter (below 1 %) and thyroid autonomy was established (4,6). The incidence of euthyroid and hypothyroid Hashimoto's thyroiditis (HT) grew from 1999 to 2006, reaching twice the value from 1999, and then stabilized (4). Until now, no data on the incidence of HT in Slovenia before 1999 has been available. Similar studies that evaluated the epidemiology of thyroid diseases after the amelioration of iodine supply have also described an increased incidence of thyroid antibodies and HT (7,8). Other studies disproved the link between the increased intake of iodine and appearance of HT (9,10). Most studies only followed the levels of thyroid peroxidase antibodies (TPOAb), and a few also the levels of thyroglobulin antibodies (TgAb). In Greece, several years after the increased salt iodization TgAb were observed more frequently (11). It turned out that this might only be a transitory phenomenon, because in almost half of the cases TgAb disappeared in the following few years. The remaining patients developed thyroid dysfunction (11).

Nevertheless, little data on the incidence of HT, and even less data on the thyroid function before and after the increase in mandatory iodization of salt is available. Therefore, our aim was to evaluate the incidence of euthyroid HT and, first of all, to establish the thyroid function in healthy subjects (HS) and in patients with euthyroid HT (EuHT) before and after the increase in mandatory salt iodization in Slovenia.

Subjects and methods

Subjects

This retrospective clinical study was carried out by manually reviewing medical records of 24,899 patients who

were referred to the Outpatient Thyroid Department of the Department of Nuclear Medicine at the University Medical Centre Ljubljana for the first time between 1995 and 2002. For more than 20 years, this department has had a stable catchment area of 1 million inhabitants. Consequently, the number of new cases in a certain year offers a good estimate of the incidence of different thyroid diseases as shown previously (4). Each patient was clinically examined by one of the ten skilled thyroid specialists. In accordance with clinical practice guidelines, thyroid ultrasound was regularly performed and the concentration of thyrotropin (TSH) was measured in all patients at the first examination. In majority of patients, the concentration of free thyroxine (fT_4), free triiodothyronine (fT_3), TPOAb and TgAb was also determined. In this study we included only HS and patients with EuHT. We did not include patients with hypothyroid or hyperthyroid HT. HS had a normal serum concentration of TSH, fT_4 and fT_3, a normal thyroid ultrasound pattern, and did not have increased levels of TPOAb and TgAb. Patients with EuHT had a normal serum concentration of TSH, fT_4 and fT_3, a hypoechoic thyroid ultrasound pattern and increased levels of TPOAb and/or TgAb. From the medical records we obtained clinical data including age, gender, thyroid diagnosis, thyroid ultrasound pattern, and laboratory data on TSH, fT_4, fT_3, TPOAb and TgAb concentrations. We calculated the fT_4/fT_3 ratio.

This study was approved by the Republic of Slovenia National Medical Ethics Committee – NMEC (number 73/04/13 from 20.05.2013).

Laboratory tests

All laboratory tests were performed at the Department of Nuclear Medicine. Between 1995 and 1997, TSH, fT_4 and fT_3 concentrations were measured using "Amerlite TSH-30 Ultrasensitive", "Amerlite fT4", and "Amerlite fT3" assays (Kodak Clinical Diagnostics), in 1998 and 1999, these parameters were determined by ECi automated instrument

Table 1: Healthy subjects and patients with euthyroid Hashimoto's thyroiditis in the periods 1995–1998 and 1999–2002.

Parameter	Period 1995–1998		Period 1999–2002	
Diagnosis	HS	EuHT	HS	EuHT
Number of subjects	917	482	644	846
% of subjects	[a] 8.1	[a] 4.3	4.7	6.2
Number of women (%)	779 (85)	466 (96.7)	492 (76.4)	793 (93.7)
Number of men (%)	138 (15)	16 (3.3)	152 (23.6)	53 (6.3)
Age (years) Mean SD	[a,c] 41.5 15.5	[b] 44.2 14.5	45.1 18.2	46.3 6.2

Legend: HS, healthy subjects; EuHT, patients with euthyroid Hashimoto's thyroiditis; % of subjects, % of healthy subjects or patients with euthyroid Hashimoto's thyroiditis out of all firstly examined subjects.
a p <0.001 compared to the period 1999–2002
b p <0.05 compared to the period 1999–2002
c p <0.001 compared to patients with EuHT in the same period

(Ortho Clinical Diagnostics), whereas between 2000 and 2002, TSH, fT_4 and fT_3 were measured by automated methods on the ADVIA Centaur instrument (Bayer Diagnostics, now Siemens Healthcare Diagnostics). In all three periods, chemiluminescence tests were performed. Sensitive third generation tests were used for the measurement of TSH. For TSH, reference values were between 0.17 and 2.87 mU/L in the years 1995, 1996 and 1997, between 0.465 and 4.68 mU/L in 1998 and 1999, and between 0.35 and 5.5 mU/L in 2000, 2001 and 2002. For fT_4, reference values were between 11.7 and 28.0 pmol/L in 1995, 1996 and 1997, between 10.0 and 28.2 pmol/L in 1998 and 1999, and between 11.5 and 22.7 pmol/L in the years 2000, 2001 and 2002. For fT_3, reference values were between 4.3 and 7.6 pmol/L in 1995, 1996 and 1997, between 4.26 and 8.1 pmol/L in 1998 and 1999, and between 3.5 and 6.5 pmol/L in the years 2000, 2001 and 2002.

In the year 1995, concentrations of TPOAb and TgAb were measured by chemiluminescence immunoassays "LUMI-test anti-TPO" and "LUMI-test anti--TG" (Brahms Diagnostica), in the years 1996 to 1999 by the enzyme immunoassays "AB-TG" and "AB-TPO" performed on an automated BOSS instrument (Sorin Biomedica), and in the years 2000 to 2002 on the ADVIA Centaur instrument (Bayer Diagnostics, now Siemens Healthcare Diagnostics). For TPOAb, the sample was considered negative if the concentration was below 200 kU/L in 1995, below 150 kU/L in 1996 and 1997, and below 15 kU/L between 1998 and 2002. For TgAb, the sample was considered negative if the concentration was

Table 2: The concentration of TSH, free thyroid hormones and the ratio between the free thyroid hormones in healthy subjects and in patients with euthyroid Hashimoto's thyroiditis in the periods 1995–1998 and 1999–2002.

Characteristic	Period 1995–1998		Period 1999–2002	
Diagnosis	HS	EuHT	HS	EuHT
TSH (mU/L) Median Range N	[a,c] 0.93 0.17–4.67 917	[a] 1.95 0.17–4.67 482	[c] 1.5 0.35–5.46 644	2.8 0.4–5.5 846
fT_4 (pmol/L) Median Range N	[b,c] 14.40 7.00–33.40 797	12.90 7.40–25.00 260	[c] 14.10 8.10–28.60 641	13.60 7.80–27.50 352
fT_3 (pmol/L) Median Range N	[a,c] 5.70 2.00–15.30 797	[b] 5.80 2.90–10.10 260	5.20 1.60–11.10 641	4.90 2.90–8.10 352
fT_4/fT_3 Mean SD N	[a,c] 2.60 0.64 797	[a] 2.35 0.55 260	2.86 0.86 641	2.79 0.68 352

Legend: HS, healthy subjects; EuHT, patients with euthyroid Hashimoto's thyroiditis; N, number of subjects; TSH, thyrotropin; fT4, free thyroxine, fT3, free triiodothyronine; SD, standard deviation.
a p<0.001 compared to the period 1999–2002
b p<0.05 compared to the period 1999–2002
c p<0.001 compared to patients with EuHT in the same period

below 200 kU/L in 1995, 1996 and 1997, below 100 kU/L in 1998 and 1999, and below 60 kU/L in the years 2000, 2001 and 2002.

Thyroid ultrasound

In each patient, thyroid ultrasound was performed by one out of ten skilled thyroid specialists using ALOKA ultrasound machines with a 7.5 MHz probe. Both thyroid lobes were examined with respect to echogenicity.

Statistical methods

The measured values in both groups of subjects and in both observed periods (before and after the increase in iodine supply) were compared with the Student's two-tailed t test. When the distribution was not normal, we compared the values with the Wilcoxon's test. Frequencies of different variables in both observed periods were compared with the χ^2 test. Statistical analysis was performed using Statistica software (StatSoft) programme. Statistically significant P values were taken into account, when P was below 0.05.

Results

Characteristics of subjects

Between 1995 and 1998, in the period before the increase in iodine supply, 11,199 subjects were examined for the first time, whereas between 1999 and 2002, in the period after the increase in iodine supply, the corresponding number was 13,700. Between 1995 and 1998, we detected 917 HS and 482 patients with EuHT, while between 1999 and 2002, 644 HS and 846 patients with EuHT were detected (Table 1). In the first period, the ratio between the number of women and men was significantly higher than in the second period (8.1 and 6.3, respectively, p=0.029).

Before the increase in iodine supply, the incidence of EuHT was significantly lower than after the increase (Table 1). In both investigated periods, EuHT was more frequent in women than in men (p<0.001). When we calculated the ratio between the number of new cases of EuHT in both sexes from the data in Table 1, we established a significant decrease from 29.1 in 1995–1998 to 15.0 in 1999–2002 (p=0.02). As shown in Table 1, patients with EuHT were younger in the period 1995–1998 than in the period 1999–2002 (p=0.016). In the period 1995–1998, patients with EuHT were significantly older than HS (Table 1) whereas in the period 1998–2002, their age was not significantly different (p=0.161).

Laboratory results

The thyroid function tests before and after the increase in iodine supply in HS and in patients with EuHT are presented in Table 2.

In the period 1995–1998, HS had significantly lower concentration of TSH, a slightly higher concentration of fT_4 (p=0.010), significantly higher concentration of fT_3, and significantly lower fT_4/fT_3 ratio than in the period 1999–2002 (Table 2).

In 1995–1998, patients with EuHT had significantly lower concentration of TSH, a similar concentration of fT_4 (p=0.691), significantly higher concentration of fT_3 (p=0.018), and significantly lower fT_4/fT_3 ratio than in 1999–2002. In the period 1995–1998, patients with EuHT had significantly higher concentration of TSH, a lower concentration of fT_4, significantly higher concentration of fT_3, and significantly lower fT_4/fT_3 ratio as HS. In the period 1999–2002, pati-

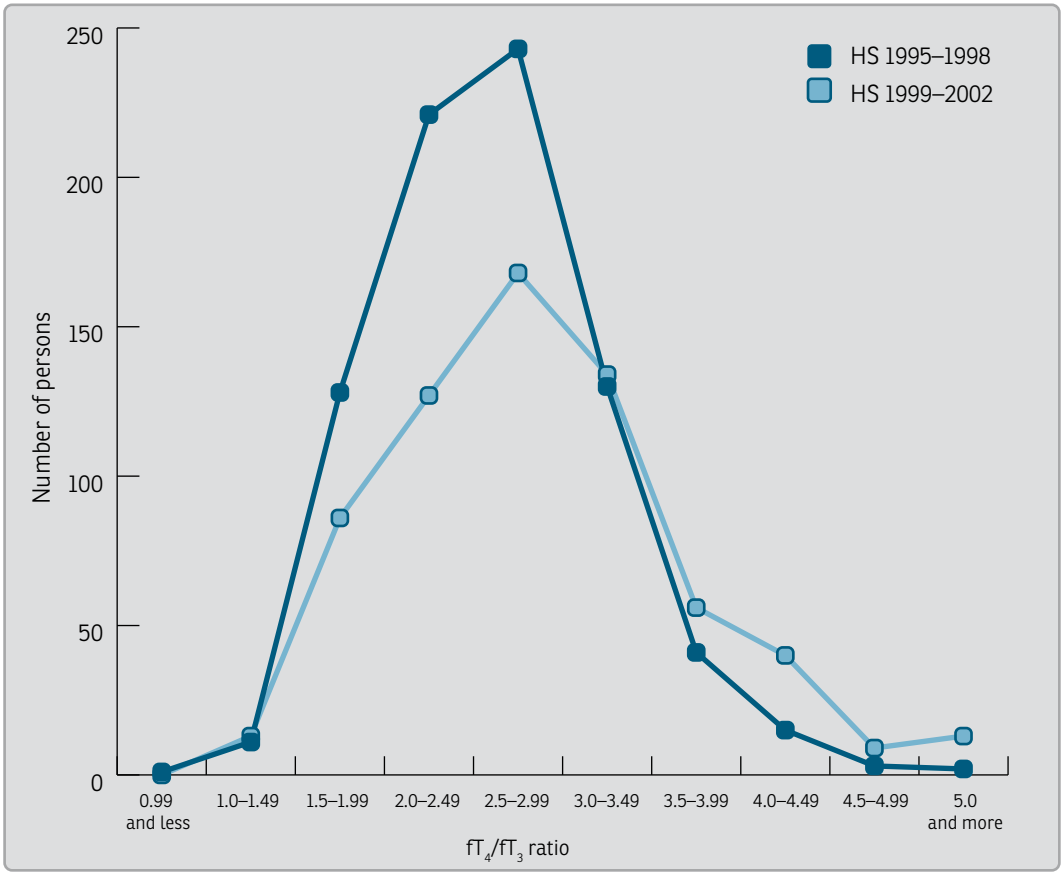

Figure 1: The number of healthy subjects with a certain fT4/fT3 ratio in the period before and after the increase in iodine supply.
Legend: fT4, free thyroxine; fT3, free triiodothyronine; HS, healthy subjects.

ents with EuHT had significantly higher concentration of TSH, a lower concentration of fT_4, a similar concentration of fT_3 (p=0.301), and a similar fT_4/fT_3 ratio (p=0.206) as HS (Table 2).

Figures 1 and 2 show the number of HS and patients with EuHT, respectively, with a certain fT_4/fT_3 ratio in the periods 1995–1998 and 1999–2002. As depicted in Figures 1 and 2, after the increase in iodine supply, there was an increase in the number of subjects with higher values of fT_4/fT_3 ratio. Therefore, the curve is shifted to the right. The change was more pronounced in patients with EuHT than in HS.

Discussion

After the increase in mandatory salt iodization in Slovenia, HS and patients with EuHT have higher fT_4/fT_3 ratio than before the increase on account of lower fT_3 value. Furthermore, after the increase in iodine supply, both groups are similar with respect to fT_4/fT_3 ratio, though before the increase, this ratio was significantly lower in patients with EuHT than in HS. Amelioration of iodine supply is associated with the higher incidence of EuHT.

It is known that iodine supply has a significant impact on the incidence of various thyroid diseases, also on the incidence of EuHT (2,4). After the increase in iodine supply, we found a higher incidence of HT than before in spite of a lower ratio between the number of women and men after the increase and a higher incidence of HT in women than in men in both periods. Our results are in accordance with several reports on the increased incidence of HT in populations where iodine deficiency was followed by a sufficient iodine intake (7,8), and are not consistent with other studies,

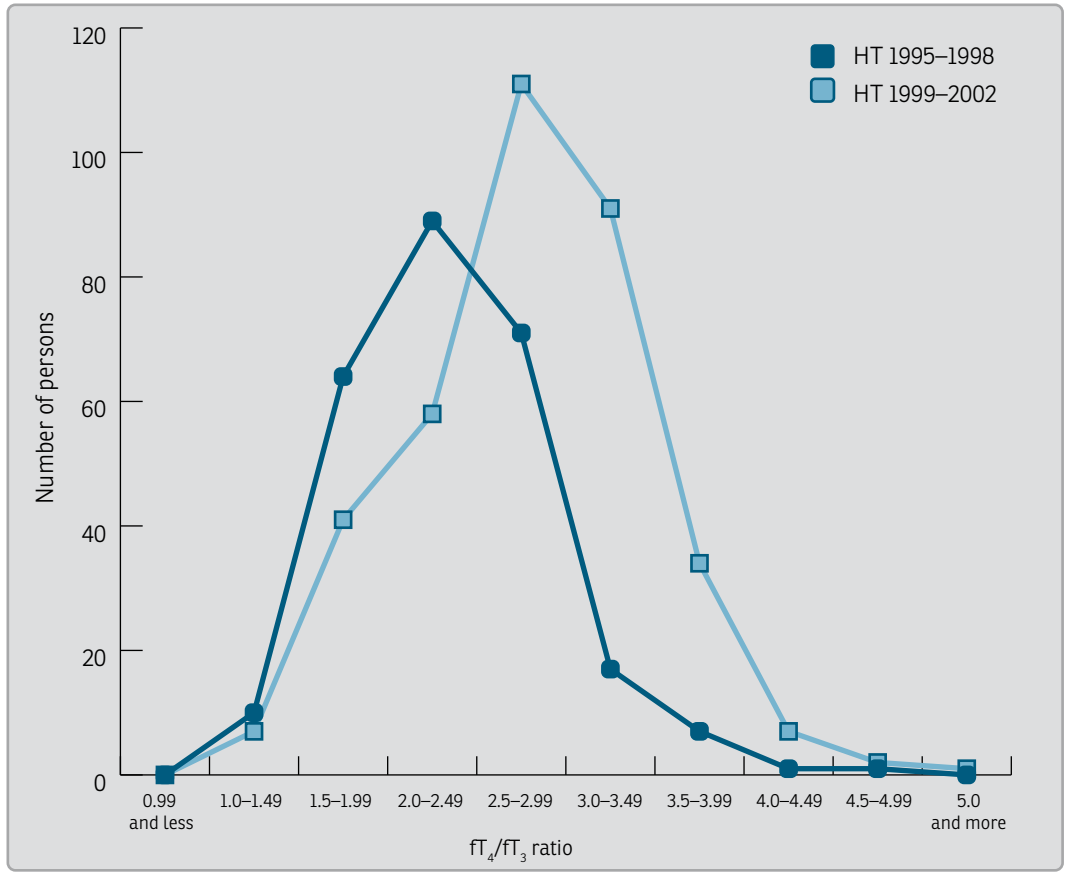

Figure 2: The number of euthyroid patients with Hashimoto's thyroiditis with a certain fT4/fT3 ratio in the period before and after the increase in iodine supply.
Legend: fT4, free thyroxine; fT3, free triiodothyronine; HT, Hashimoto's thyroiditis.

where an increased incidence of HT has not been observed (9). We must be cautious in the interpretation of the results and bear in mind that after the change in iodine supply, several years must elapse before the epidemiological situation with respect to thyroid diseases stabilizes, which was also established in Italy, Denmark and Germany (12-14). With the present data, a previously described increase in the incidence of HT in Slovenia ten years after the increase in iodine supply (6) has been completed with the incidence of HT before the increase. For this reason, this study represents an important contribution to the understanding of the effects of increased iodine supply on the occurrence of EuHT. Possible disadvantage of our retrospective study regarding the incidence of EuHT are different methods applied for the measurement of TPOAb and TgAb between 1995 and 2002. However, we believe that this did not affect the estimated incidence of EuHT significantly, since we included a large number of subjects, and also did not consider absolute concentrations of TPOAb and TgAb but only whether their concentrations were above or below the cut-off value. Although slightly increased level of TgAb may be found in healthy subjects, studies show that these subjects more frequently develop HT (10). Further, beside TPOAb and TgAb levels, a hypoechoic thyroid ultrasound pattern played an important role in the diagnosis of EuHT in our subjects. This way, the diagnosis of EuHT was more reliable than it would be without this information.

We confirmed a higher incidence of EuHT in women than in men which is in agreement with the largest epidemiological study performed in an area with adequate iodine intake (15). However, the ratio between the number of new cases

of EuHT in women and in men in both periods is higher than described (15). A possible speculative explanation for this finding may be that women are more aware than men of thyroid diseases, whereas general practitioners associate thyroid diseases more often with women.

The fT_4/fT_3 ratio is a result of the synthesis and secretion of thyroid hormones and of conversion of fT_4 to fT_3 under the influence of deiodinases (16). Body weight, gender, race or physical activity have no significant effect on this ratio (17). In HS, the relation between thyroid hormones is precisely regulated. The fT_4/fT_3 ratio well reflects the function of the thyroid gland and is a useful tool in the diagnostics and treatment-monitoring of different thyroid diseases (18,19). In the epidemiological studies considering the influence of iodine supply on thyroid disorders, both free thyroid hormones are rarely measured (20). Most likely, this is a consequence of different clinical practice in the diagnostics of thyroid disorders. In some areas, total thyroid hormones are still measured, while in others, beside TSH, only fT_4 is used in the diagnostics. In the available literature, we have not found any information about the change in the fT_4/fT_3 ratio in association with iodine supply. Therefore, according to our knowledge, our results regarding fT_4/fT_3 ratio represent a novelty in this field.

In HS as well as in patients with EuHT, a significantly higher fT_4/fT_3 ratio after the increase in iodine supply than before was ascertained, and was most likely a consequence of established significantly lower fT_3 concentration in that period. Accordingly, slightly higher concentration of TSH in HS and in patients with EuHT after the increase in iodine supply can be explained by the influence of the lower concentration of fT_3 via negative feedback (21). A lower fT_3 concentration after the increase in iodine supply than before the increase was not an unexpected finding. It is known that the thyroid gland adapts to the decreased iodine intake by increasing the synthesis of T_3 (20,22). In rats, an increased expression of deiodinase D1 in the thyroid gland was established in iodine deficiency, which helps to maintain a constant concentration of T_3 in the blood at the expense of T_4 (22). Depending on the status of thyroid function, deiodinases in the thyroid and in the peripheral tissues are constantly changing the balance between the active hormones fT_3, fT_4, and their inactive metabolites (16,23). After the increase in iodine supply, the synthesis of T_3 in the thyroid most likely decreases in comparison with the period before the increase which slightly lowers serum fT_3 concentration and has an inhibitory effect on the deiodinases (16), which further reduce serum concentration of fT_3. This explanation is consistent with our results.

In the period 1995–1998, we found significantly higher fT_4/fT_3 ratio in HS than in patients with EuHT, predominantly on account of lower fT_4 and, to a smaller degree, on account of higher fT_3 concentration in EuHT. In our opinion, in patients with EuHT thyroid function gradually declines (24), which is reflected in lower fT_4/fT_3 ratio. However, in the period 1999–2002, we found no significant difference in the fT_4/fT_3 ratio in HS and in patients with EuHT. To our knowledge, there is no data in the literature whether patients with EuHT adapt to an increase in iodine supply similarly as HS. As shown in our study, the comparable fT_4/fT_3 ratio in HS and in patients with EuHT is a result of similar concentration of fT_3 and somewhat, but not sufficiently higher concentration of fT_4 in HS. It seems that adequate iodine supply has a beneficial effect on thyroid

function in patients with EuHT. This is a completely new finding, since until now it was a general belief that the increase in iodine supply does not affect the course and the incidence of HT favorably.

A limitation of our retrospective study is the occasional change of the methods for the measurement of TSH and free thyroid hormones between 1995 and 2002. However, it should be noted, that the third generation assays were always used for the measurement of TSH. Besides, a factor that increases the reliability of results is a large number of subjects included. In addition, it is known that the concentration of TSH and free thyroid hormones in an individual varies within a narrow range, and is probably largely genetically dependent, and therefore stable (25,26).

In conclusion, amelioration of iodine supply is associated with an increase in the fT_4/fT_3 ratio on account of lower fT_3, and with a similar thyroid function in HS and in patients with EuHT.

Our results emphasize the importance of adequate iodine supply on thyroid function and therefore represent a valuable contribution to knowledge in the field of prevention of iodine deficiency disorders (27).

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