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Assessment of the Effect of Two Distinct Restricted Iodine Diet Durations on Urinary Iodine Levels (Collected over 24 h or as a Single-Spot Urinary Sample) and Na^+/I^- Symporter Expression

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Key Words
Restricted iodine diet · Urinary iodine · Na^+/I^- symporter · Thyroid cancer · Radioiodine therapy

Abstract
Introduction: A restricted iodine diet (RID) may be recommended for depletion of the whole-body iodine pool in patients with differentiated thyroid cancer referred for radioiodine treatment or a whole-body scan. Evaluation of the iodine pool is possible through urinary iodide (UI) measurements, which can be collected in 24-hour (24U) or spot urinary (sU) samples. However, the minimum period required for an RID to lower the iodine pool, the measurement of iodine in sU samples as a iodine pool marker, and the influence of the iodine pool on Na^+/I^- symporter (NIS) expression are debatable in the literature. ***Objectives:*** To compare the effects of 15- and 30-day RID on UI measurements in 24U and sU samples and the impact of RID on NIS expression. ***Methods:*** Thyroidectomized patients went on a 15- or 30-day RID and collected 24U and sU samples before and after the RID. Twenty healthy individuals were evaluated for mRNA NIS expression before and after the RID. ***Results:*** Of 306 patients, only 125 properly complied with both the RID and 24U collection. We observed a correlation between sU and 24U UI before the RID ($n = 306$, $\rho = 0.47$, $p < 0.001$), after a 15-day RID ($n = 79$, $\rho = 0.49$, $p < 0.001$), and after a 30-day RID ($n = 46$, $\rho = 0.73$, $p < 0.001$). There was a significant decrease in UI after the RID. The median UI measurement was 275 µg/l at baseline and 99 and 80 µg/l after a 15- and 30-day RID, respectively. There was a significant increase in NIS expression after a 15-day RID. ***Conclusions:*** A 15-day RID is sufficient to deplete the iodine pool. sU can replace 24U UI as a marker for assessing the iodine pool. NIS expression was increased after a 15-day RID.

Introduction

Total thyroidectomy followed by radioiodine (RAI) therapy is generally the initial treatment of choice for many patients with differentiated thyroid carcinoma

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(DTC) [1–3]. The goals of RAI therapy are: remnant ablation (to facilitate the detection of recurrent disease and initial staging), adjuvant therapy (to decrease the risk of recurrence and disease-specific mortality by destroying suspected but unproven metastatic disease), and RAI treatment (to treat known persistent disease). RAI therapy has additional benefits, such as postdose RAI scanning in postsurgical staging and improving the sensitivity of serum thyroglobulin [1–3].

Several known biological phenomena limit the efficacy of RAI therapy. The most challenging factor is the iodine uptake capacity of thyroid cancer tissue, which is poorer than that of normal thyroid cells [4]. In addition, to maximize the RAI uptake by residual thyroid remnants, persistent disease, or metastases, TSH levels must be increased by either thyroid hormone withdrawal or recombinant TSH administration [1–3]. Moreover, it is valuable to deplete the whole-body iodine pool through a restricted iodine diet (RID) before RAI treatment [5]. Studies have demonstrated a positive relationship between RID and RAI uptake [6–8], and some authors have documented a 2- to 3-fold increase in ^{131}I uptake by the thyroid after an RID [9, 10].

To be sure of iodine pool depletion, it is recommended to use the measurement of urinary iodine (UI), which is a valid index for iodine intake, since 90% of iodine excretion from the body occurs through urine and only a small amount is lost through the skin and intestines [11–19]. Usually UI is collected over a period of 24 h (24U), which is considered the best method for the measurement of iodine status in an individual; however, as collection of 24U samples is problematic, a single-spot urinary sample (sU), which is more feasible, is validated in epidemiological studies [13, 16, 17].

Additionally, the diet is quite unpleasant, resulting in low compliance in many patients. Consequently, it is important, firstly, to establish the minimal effective diet period and, secondly, whether sU could replace 24U as an adequate method of urine collection for iodine determination. Thus, the initial objectives of our study were to evaluate UI results before and after 2 different periods of RID (15 and 30 days) and to compare the results of UI measurements in 24U and sU samples.

In addition, this study had a third objective. It is known that in the dog thyroid, iodine depletion increases the expression of Na^+/I^- symporter (NIS) [20]. Accordingly, considering that low iodine pool concentrations caused by RID may increase the expression of NIS also in humans, we evaluated NIS expression before and after a 15-day RID in healthy individuals and thyroidectomized patients referred for RAI.

Materials and Methods

Subjects

We selected 306 thyroidectomized patients with DTC (208 females and 98 males, age range 20–65 years, median age 37 years) who collected UI samples (24U and sU) before an RID. There were 235 patients with papillary thyroid carcinoma (PTC); 148 (63%) had classic PTC, 74 (31%) had the follicular variant of PTC, 4 (2%) had the tall-cell variant of PTC, 9 (4%) had the Hürthle cell variant, and 71 had follicular thyroid carcinoma (minimally invasive in 60 and widely invasive in 11). After the first UI collection (both 24U and sU), all patients were instructed to follow an RID (table 1) and undergo thyroid hormone withdrawal before having ^{131}I treatment or a whole-body scan. For the RID, the 306 patients were divided into 2 groups of 153 patients; the first group was instructed to follow an RID for 30 days and the second group was to follow an RID for 15 days. Both groups were very similar in terms of age, type of cancer, risk assessment of recurrence, management, and follow-up.

UI collections comprised sU and 24U samples collected before and after the RID. The sU samples were collected in the morning between 8.00 and 11.00 h, at least 2 h after the end of 24U collection, as recommended [13, 17, 19, 21, 22]. Patients were asked how they had collected the samples and whether they had adhered to an RID. Samples that had been improperly collected were discarded. Patients who failed to adhere to an RID were excluded from the analysis.

To analyze NIS expression, we studied 20 healthy individuals with normal thyroid glands (18 females and 2 males, age range 40–65 years, median age 52 years) and 20 DTC patients (12 females and 8 males, age range 25–65 years, median age 39 years) scheduled to receive RAI treatment after total thyroidectomy. Blood samples for NIS mRNA expression were obtained at baseline and 7 and 15 days after the RID. These individuals also collected sU samples for UI measurement.

Signed informed consent forms were obtained from all patients and healthy individuals, and this study was approved by the University Ethics Committee.

Methods

UI Measurement

Samples were assayed for UI using a semiautomated method [13] based on indirect detection of iodine through monitoring of the reduction of ceric ammonium sulfate, as recommended by the International Council for Control of Iodine Deficiency Disorders (ICCIDD) [17, 22]. All urine samples were assayed for iodine content and values were not corrected per gram of creatinine excretion because, as endorsed by the ICCIDD, creatinine levels vary depending on the general nutritional status of the population, which contributes an independent source of variation that invalidates the ratio [22].

The method has a sensitivity of 10 μg of iodine/l. The intra-assay variation coefficient is 12.6% for a sample of 90 μg/l (n = 11) and 2.3% for a sample of 277 μg/l (n = 11). The inter-assay coefficient of variation is 15.5% for a sample of 100 μg/l (n = 47) and 10% for a sample of 262 μg/l (n = 47) [13].

Extraction and Amplification of NIS mRNA

Blood samples (4 ml) were collected in EDTA tubes and immediately stored in trizol. DNA extraction, purification, and quantification were performed following standard protocols [23, 24].

Table 1. Model of an RID that provides less than 50 μg of iodine daily, proposed for patients and healthy individuals in this study [20]

Allowed	Restricted
Noniodized salt, snacks, and potato chips	Iodized salt
Fish, seafood, shrimp, oysters, and algae	Fresh-water fish
Milk, ice cream, cottage cheese, yogurt, cheese, tofu, soy milk	Skimmed milk powder, unsalted butter, margarine
Smoked meat, broth, ham, bacon, sausage, sauerkraut	Fresh meat
Egg yolk, mayonnaise, soy sauce	Egg whites, spices, oil, olive oil, vinegar
Canned fruit, fruit in syrup, salted nuts	Fresh fruits and juices, fruit with salt, nuts and peanuts
Watercress, celery, Brussels sprouts, cabbage, olives, pickles, mushrooms	Lettuce, beets, broccoli, onion, carrots, cabbage flower, peas, spinach, turnips, cucumbers, tomatoes, green beans
Industrialized breads, pizza	French bread, cream crackers, spaghetti, rice, oats, barley, flour, beans, corn, wheat
Chocolate, milk, red candies	Sugar, honey, jam, candy
Tea	Juice and soda

Total RNA (2.5 μg) was used to perform cDNA synthesis. Real-time polymerase chain reaction (RT qPCR) was employed to analyze the relative expression (RE) of NIS mRNA, and the RPS8 gene was used as an internal control. The primer oligonucleotides for the NIS gene were as follows: 5′ primer: 5′-TCTCTCAGTCAACGCCTCT-3′; 3′ primer: 5′-ATCCAGGATGGCCACTT CTT-3′. The amplification yielded a 299-bp DNA product corresponding to fragment 1,801–2,099 according to the published sequence of the NIS gene [25].

The RE of NIS mRNA was calculated using the formula: 2(Cs – Rs)/2(Cn – Rn), where Cs is the cycle threshold number for NIS in the samples, Rs is the cycle threshold value for RPS8 for each sample, Cn is the mean value of the NIS cycle threshold value of individuals without evidence of disease, and Rn is the RPS8 cycle threshold value in the same individuals [26].

Statistics

All data are reported as absolute UI concentration median values (μg/l) and percentages. The tests used were Spearman's correlation (ρ), the Mann-Whitney test for analysis 2 groups, and the Kruskal-Wallis test for more than 2 groups. $p < 0.05$ was considered statistically significant. A Bland-Altman plot (difference plot) was used to analyze the agreement between sU and 24U UI measurements. We used GraphPad Prism for Windows, version 5.03, for graphic construction and statistical analysis.

Results

Comparative Analysis of 15- and 30-Day RID Periods and UI Measurements in 24U and sU Samples

For the baseline comparison between 24U and sU UI measurements, all 306 patients collected samples. However, of the 153 patients instructed to follow an RID for 15 days, only 79 (52%) were able to conclude the RID period. Of the 153 patients selected to follow an RID for 30 days, only 46 (30%) concluded it. Therefore, only 125 patients of the original 306 were able to conclude the RID period; consequently, we analyzed 125 patients for determination of the UI after the RID period. The major reason for discontinuation of the diet was difficulty adhering to an RID for a long period of time due to the taste of noniodized salt, as well as difficulty adhering to an RID outside the home. Additionally, it is not easy for patients who work outside the home to collect, store, and transport 24U samples.

At baseline, median UI values were 210 μg/l for 24U and 226 μg/l for sU (fig. 1a). It is important to note that 184 patients (60%) presented normal UI excretion according to ICCIDD guidelines (between 100 and 300 μg/l) at baseline, while 113 patients (37%) presented values higher than 300 μg/l (considered excessive by the ICCIDD), and 9 patients presented deficient UI excretion (below 100 μg/l).

After a 15-day RID, patients presented a reduction of 64% in UI values (for both 24U and sU; fig. 1d). After a 30-day RID, patients presented a reduction of 70% in UI values (for both 24U and sU; fig. 1g). The difference between UI values in patients who adhered to an RID for 15 and 30 days was not statistically significant ($p = 0.06$). Therefore, we demonstrated that a 15-day RID is efficient at reducing the iodine pool, even in patients with a high iodine intake; accordingly, our study indicates that it is not necessary to maintain an RID for 30 days.

In relation to the method of UI collection, we observed a correlation between sU and 24U UI values at baseline ($n = 306$, $\rho = 0.47$, $p < 0.001$; fig. 1b), which persisted after 15 days of an RID ($n = 79$, $\rho = 0.49$, $p < 0.001$; fig. 1e) and after 30 days of an RID ($n = 46$, $\rho = 0.73$, $p < 0.001$; fig. 1h). The Bland-Altman plots for the 3 periods (baseline, 15-day RID, and 30-day RID; fig. 1c, f, i) showed agreement between UI values, which was better when UI values were

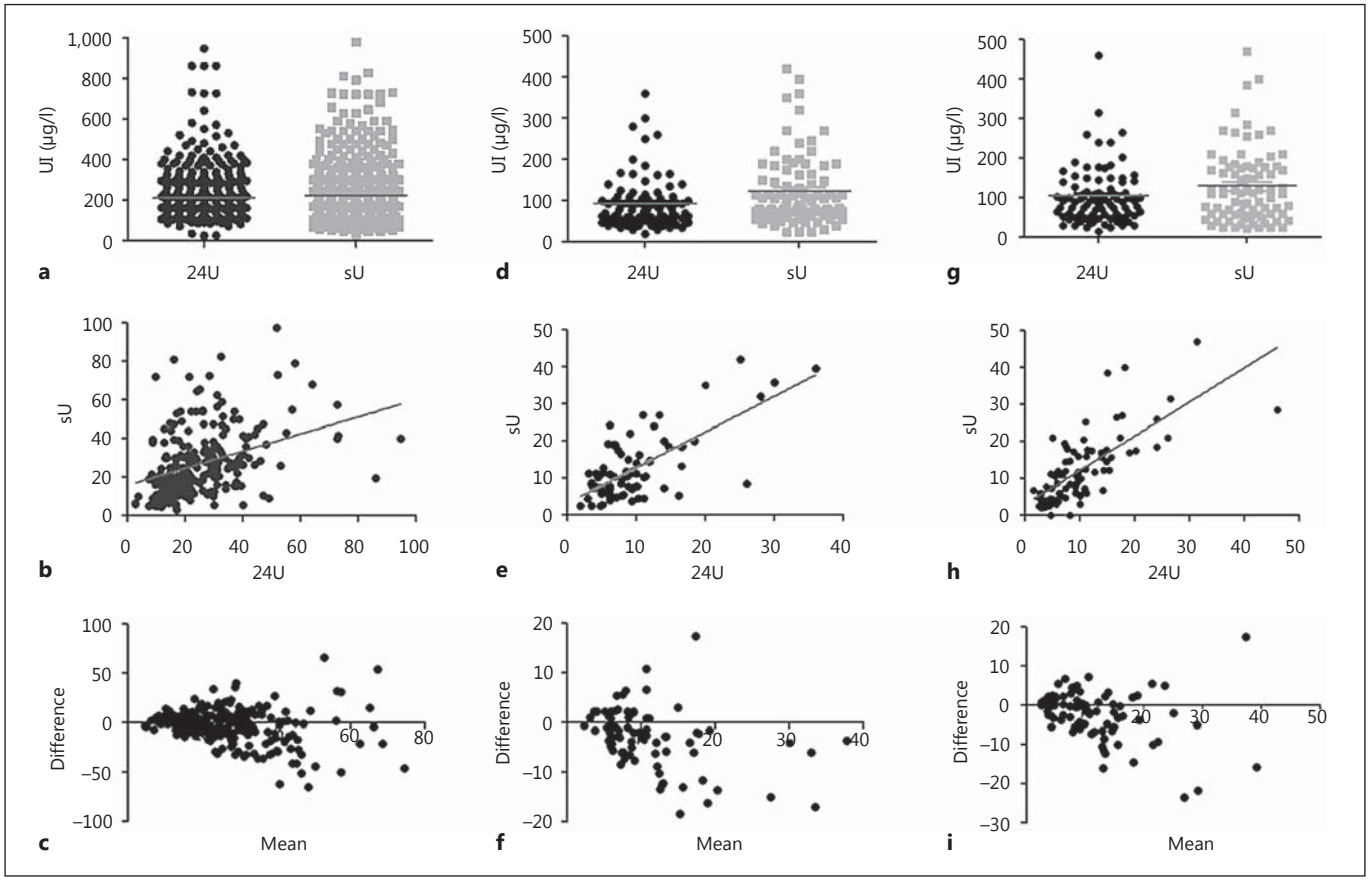

Fig. 1. UI measurements in 24U and sU samples at baseline (n = 306) (**a**), after 15 days of RID (n = 79) (**d**), and after 30 days of RID (n = 46) (**g**). Correlation between 24U and sU UI samples at baseline (**b**), after 15 days of RID (**e**), and after 30 days of RID (**h**). Bland-Altman plots demonstrating the concordance between 24U and sU UI results at baseline (**c**), after 15 days of RID (**f**), and after 30 days of RID (**i**).

lower than 400 µg/l. Therefore, to exclude iodine contamination in patients referred for RAI therapy, we concluded that the whole-body iodine pool can be evaluated through UI measurements collected via sU samples rather than the more complex 24U collections.

NIS Expression Analysis

The 20 healthy individuals who adhered to an RID for the study of NIS expression presented a median UI in sU samples of 222 µg/l at baseline, 146 µg/l after a 7-day RID, and 95 µg/l after a 15-day RID (fig. 2a). Shorter periods of RID (7 and 15 days) were adopted in this case because it is challenging for volunteer healthy individuals to adhere to an RID for a longer period. In this group of patients, the median UI values decreased after 15 days of RID ($p < 0.05$); however, we did not observe a difference between the median UI values at baseline and after 7 days of RID.

As shown in figure 2b, we observed a significant increase in NIS expression in those 20 healthy individuals after 15 days of RID ($p < 0.05$), which was accompanied by a decrease in UI values ($p < 0.05$). The median values of NIS RE (arbitrary units; a.u.) was 0.83 at baseline, 1.58 after 7 days of RID, and 4.57 after 15 days of RID (5.5×, $p < 0.05$), respectively.

In the group of 20 patients with DTC subjected to total thyroidectomy, there was no NIS expression detectable in the blood.

Discussion

The ability to transport, concentrate, and promote the organification of iodide is a property of normal thyroid tissue. The use of RAI for the diagnosis and treatment of

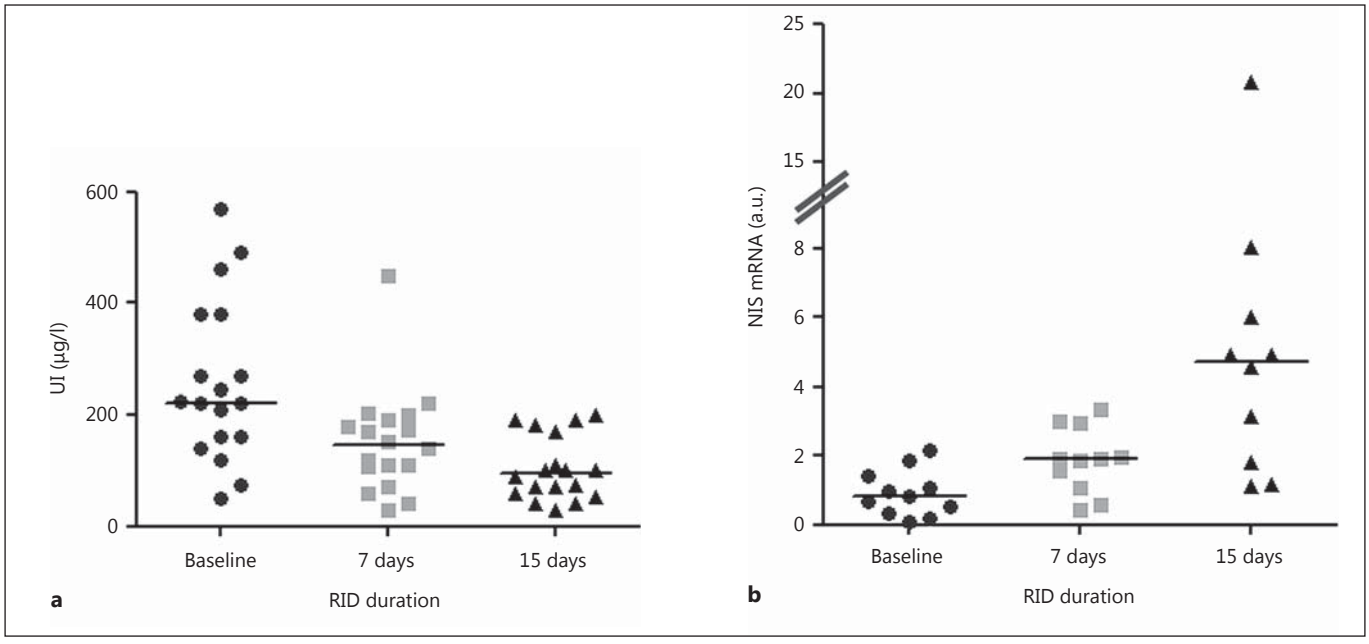

Fig. 2. a UI measurements at baseline (median 222 µg/l), after 7 days of RID (median 146 µg/l), and after 15 days of RID (median 95 µg/l, $p < 0.05$) in 20 healthy individuals. **b** mRNA NIS expression in peripheral blood at baseline (0.83 a.u.), after 7 days of RID (1.58 a.u.), and 15 after days of RID (4.57 a.u., 5.5 times the basal value, $p < 0.05$) in 20 healthy individuals.

patients with DTC is feasible because these features are maintained in thyroid cancer cells [1–3]. Because iodine transport is performed by NIS, several strategies can be used to increase its expression [27]. One of these strategies is iodine depletion in the whole body, which may be achieved through an RID and can be evaluated by a decrease in UI measurements.

Therefore, one of the objectives of our study was to investigate the possibility of using the results of sU UI measurement as an individual marker for the iodine pool in DTC patients referred for RAI therapy. To date, 24U measurement is considered the best measure of iodine status, but it is our observation that 24U collection poses a challenge, especially for patients who work outside their home (our patients complained of difficulty storing their urine collection in the workplace and transporting the samples). Because our results showed a correlation and agreement between UI levels collected through 24U and sU samples, we conclude that the whole-body iodine pool can be evaluated to exclude iodine contamination through sU samples rather than 24U samples in patients referred for RAI therapy. Collection of sU samples allows obtainment of the urinary material in a simple and effective way, avoiding the discomfort of 24-hour collection. In addition, besides the difficulty of collecting 24U samples, many patients were excluded because they were not able to complete the RID, which is a limitation of this study. This limitation, however, shows that adhering to an RID is a very demanding task for patients, particularly for those who work outside the home. The most frequent complaints were the different taste of non-iodized salt and trouble adhering to the diet outside the home.

These results confirm our previous work in a smaller number of patients; we observed a good correlation between UI in 24U and sU samples in patients who received high iodine doses of contrast agents for performance of a tomography [28].

Furthermore, our study corroborates previous results which demonstrated that iodine consumption in Brazil is high in some regions [29, 30]. We observed that 37.4% of the patients had baseline UI levels higher than 300 µg/l, which is considered an excessive iodine intake; on the other hand, 3% of the group had UI levels under 100 µg/l, which is considered deficient according the ICCIDD/World Health Organization [17, 22]. These data are particularly important for thyroid cancer patients, who should have a reduced iodine pool to receive RAI therapy.

They suggest that an RID may be a good strategy for thyroid cancer patients referred for RAI therapy who live in these areas.

According to our results, an RID proved to be a good strategy to reduce the iodine pool. Considering that some researchers have shown a positive relationship between an RID (30–50 μg/day dietary iodine) and RAI uptake [5, 8], we recommend that patients referred for RAI treatment or a whole-body scan, mainly in areas with a high iodine intake, adhere to an RID beforehand [3]. Maybe the RID can be waived in areas with a low iodine intake, as a study has demonstrated [18].

The time required to obtain a reduction of the iodine pool through an RID is another undefined question. It varies from 7 to 30 days according to the recommendations of the American Thyroid Association, the American Association of Clinical Endocrinologists, the European Society for Medical Oncology, and the British Thyroid Association [2, 31–33]. We demonstrated that a 15-day RID is efficient at reducing the iodine pool, even in patients with a high iodine intake, and that it is not necessary to maintain the RID for 30 days. Our results showed that both diets (15 and 30 days) led to a significant decrease in UI values (64% with the 15-day RID and 70% with the 30-day RID) and that the UI median values were not statistically different ($p = 0.06$) after 15 and 30 days of an RID. We hypothesize that one of the reasons for these equivalent data between 15- and 30-day RID is the lack of compliance of patients who went on an RID for 30 days, which made the UI results in this group similar to those of the patients who went on the diet for 15 days. Our results confirm the findings of Morsch et al. [34], who compared RID for 2 and 3 weeks and observed a significant UI decrease in both groups, with no difference between them.

Although several centers recommend an RID to increase the RAI uptake in thyroid tissues remnants and metastasis foci, the efficacy of RID has never been demonstrated convincingly [35]. Previous studies have attempted to demonstrate the benefits of RID by comparing the results of thyroid remnant ablation after RAI treatment in groups of patients who do and do not adhere to an RID [12, 14]. However, considering that the efficacy of ablation depends on multiple factors, such as the administered dose, the TSH level at the moment of ablation, the amount of RAI that is absorbed by remnant tissue, and the volume of the remnant tissue, the contribution of an RID remains undefined. Our study demonstrated the usefulness of a 15-day RID in reducing the UI pool.

Another contribution of this study was the analysis of NIS expression in healthy individuals after a 15-day RID. We demonstrated that the decrease in the iodine pool was followed by a significant increase in NIS mRNA expression in peripheral blood. We showed for the first time that the decrease in the iodine pool in vivo increases the expression of NIS mRNA in peripheral blood. Uyttersprot et al. [20] demonstrated that moderate doses of iodine in vivo inhibited the expression of NIS mRNA in the dog thyroid, but no subsequent study has correlated NIS expression with a decreased UI in humans. Nevertheless, we appreciate that NIS mRNA is also present in other tissues capable of concentrating iodine; the fact that NIS mRNA did not increase after an RID in thyroidectomized patients but did increase in normal controls suggests that it comes from the thyroid.

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Disclosure Statement

The authors have nothing to disclose.

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