

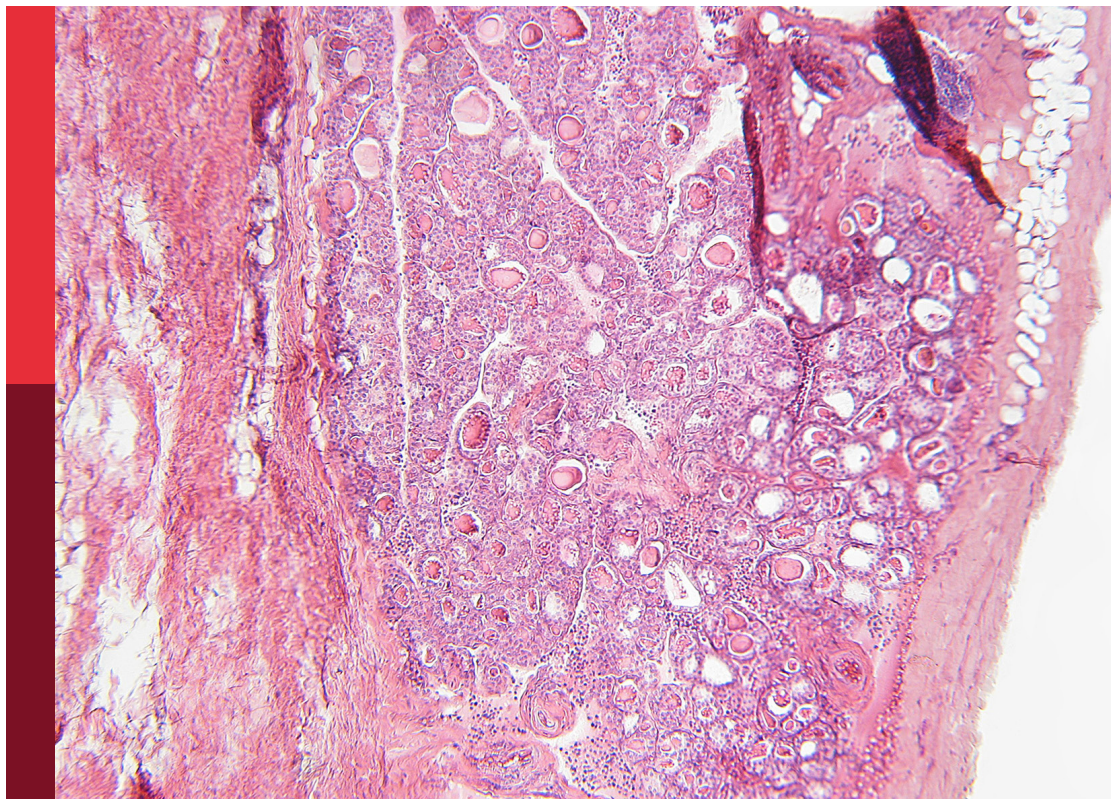
Thyroid hormones and diet

Edited by

Joao D. T. S. Anselmo, Elisa Keating, Joseph V. Martin
and Lutz Schomburg

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Thyroid hormones and diet

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Editorial: Thyroid hormones and diet

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Editorial on the Research Topic

Thyroid hormones and diet

Thyroid hormones—primarily T3 (triiodothyronine) and T4 (thyroxine)—are essential for regulating numerous physiological processes, including development, metabolism and energy production. Diet plays a crucial role in maintaining healthy thyroid function and managing thyroid disorders, especially those resulting from deficiencies or excesses in thyroid hormone levels. The purpose of this Research Topic was to collect recent updates on the role of diet on thyroid function and thyroid health.

The main reason for the diet's importance in thyroid function is the requirement for several micronutrients—also called trace elements—that are essential for thyroid hormone synthesis but cannot be synthesized by the human body. These nutrients have to be furnished by external sources. Iodine and selenium are particularly critical. Iodine is a structural component of thyroid hormones, and its deficiency is a major cause of hypothyroidism. Selenium, in synergy with iodine, supports the activity of enzymes involved in thyroid hormone synthesis and metabolism. Other trace elements such as zinc, copper, and iron also contribute significantly to thyroid hormone production and function (1, 2). Vitamins, in particular vitamin C may also influence thyroid hormone production, as it shown by Wu et al. in a large epidemiological study.

Iodine deficiency remains a global health problem, affecting over two-thirds of the world population (3). It is especially concerning during pregnancy and early childhood due to its negative impact on brain development (4). Goiter—thyroid gland enlargement—is the most common manifestation of iodine deficiency and remains endemic in several regions, particularly in Sub-Saharan Africa. Tuke et al. reported a goiter prevalence of 37.2% among school-aged children in the Guraferda District, Southwest Ethiopia. Their findings highlight the urgent need for comprehensive iodine supplementation programs in such regions, since goiter is the alarming visible sign of iodine deficiency, whereas neuro-developmental impairments and hypothyroidism are less obvious but also of high relevance for the affected population.

Severe iodine deficiency during pregnancy can lead to profound cognitive and physical impairments in the offspring, a condition known as congenital hypothyroidism. Iodine deficiency is largely preventable through dietary supplementation, primarily via iodized salt

(5). Importantly, iodine supplementation may be recommended even in coastal areas, which are believed to be abundant in natural iodine given the geographical proximity to the sea, as demonstrated by [Wu et al.](#) in a study conducted in Hainan Province, China. In fact, many coastal areas are subjected to high rainfall which can leach iodine from the soil into the sea, reducing the iodine content in local food products, including vegetables and grains, but also milk, eggs, and meat. This is notably the case in the Azores islands, where continuous iodized salt supplementation has proven effective (6).

Since iodine is not essential for plant growth, plant-derived foods typically contain low iodine levels. Consequently, individuals following plant-based diets, including vegans and vegetarians, may be at increased risk of iodine deficiency due to the absence of animal-based food sources. [Croce et al.](#) discuss these challenges in their review on iodine nutrition in vegan and vegetarian diets, emphasizing the need for education, better diagnostics and targeted supplementation strategies in these populations. This issue is even more important given the putative increased consumption of goitrogenic foods in these dietary patterns, which certainly deserves further investigation (7).

While the risks of iodine deficiency are well known, the consequences of iodine excess are less thoroughly studied (8). Diets rich in iodine, particularly those that include seaweed or excessive use of iodine-containing supplements and contrast media, can lead to systemic health issues due to the overproduction of thyroid hormones. Excessive iodine intake has also been linked to autoimmune thyroid diseases, such as Graves' disease and Hashimoto's thyroiditis, particularly in genetically susceptible individuals. For example, Japan, a country with a traditionally high iodine intake from seaweed, also has a higher prevalence of autoimmune thyroid conditions (9). Herein, in two different works [Khudair et al.](#) and [Khudair et al.](#) discuss several complications of iodine excess including implications in reproductive health.

[Nie et al.](#) explored the biological mechanism underlying iodine-induced goiter and identified the overactivity of the LNC89/LNC60-COL11A2 axis as a potential contributor. This pathway involves the long non-coding RNAs LNC89 and LNC60 and the collagen gene COL11A2, which showed elevated expression in thyroid tissues of mice exposed to iodine-rich diets.

There is also growing interest in how iodine nutritional status might affect the therapeutic response to radioactive iodine in patients undergoing ablative treatment for hyperthyroidism. [Hu et al.](#) demonstrated that factors such as metabolism and organ function may influence the efficacy of this therapy, with iodine levels playing a potentially modulating role.

Thyroid hormones also play a central role in modulating metabolic rate and energy expenditure (10). During caloric deprivation, such as prolonged fasting, the body adapts by converting T4 into the less active metabolite reverse T3 (rT3), and away from the biologically active T3, thereby reducing the activity of T3-dependent processes. This shift serves to reduce metabolic rate and conserve energy, an evolutionary survival mechanism that gains time by suppressing energy loss. Beyond metabolic adaptation, fasting has been associated with a range of health benefits, including enhanced autophagy, improved antioxidant capacity, and increased cellular stress resistance (11). [Sui et al.](#) review

these mechanisms, emphasizing the importance of tissue-specific regulation of thyroid hormones during extended fasting periods.

It is known that thyroid hormone production follows a circadian rate (12), but the relationship between the frequency of night eating and thyroid function remains unclear. This interesting aspect was investigated by [Zhang et al.](#) showing that the frequency of food ingesting during night may alter thyroid hormone levels. This is particularly relevant in person working night shifts.

Finally, in a retrospective study conducted by [Lu et al.](#), a significant positive association was identified between *Helicobacter pylori* (HP) positivity and elevated T4 levels. Evidences suggest that dietary patterns significantly influence host vulnerability to HP infection and its subsequent clinical manifestations (13), thereby potentially affecting thyroid hormone levels.

In conclusion, Iodine, selenium, and other trace elements are essential for thyroid hormone synthesis and regulation. Daily nutrition is the key aspect to avoid deficiencies in these essential micronutrients for thyroid function and health, and often combined deficiencies prevail when the food quality is poor and the choices are few. While global efforts such as salt iodization have significantly reduced iodine deficiency disorders, challenges persist—particularly among vulnerable populations like children, pregnant women, and individuals following plant-based diets. Moreover, the health risks associated with excessive iodine intake are becoming more apparent and warrant careful monitoring. Future dietary and public health strategies must balance iodine intake to optimize thyroid health while minimizing risks associated with both deficiency and excess, and require also attention to the supply with the other essential trace elements, to provide the thyroid axis with the required substrates for full functionality and long-lasting health.

Author contributions

JA: Writing – original draft, Writing – review & editing. EK: Writing – review & editing. JM: Writing – review & editing. LS: Writing – review & editing.

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Commentary: Effect of gluten-free diet on autoimmune thyroiditis progression in patients with no symptoms or histology of celiac disease: a meta-analysis

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A Commentary on

Effect of gluten-free diet on autoimmune thyroiditis progression in patients with no symptoms or histology of celiac disease: a meta-analysis

by Piticchio T, Frasca F, Malandrino P, Trimboli P, Carrubba N, Tumminia A, Vinciguerra F and Frittitta L (2023) *Front. Endocrinol.* 14:1200372. doi: 10.3389/fendo.2023.1200372

We carefully assessed the meta-analysis by Piticchio et al., 2023, which aimed to “examine all available data in the literature on the effect of a gluten-free diet (GFD) on TgAb, TPOAb, TSH, FT4 and FT3 levels in patients with TH and without symptoms or histology of celiac disease (CD)”. However, we noted some concerns about the quality of this systematic review, which we hope merit consideration.

A systematic literature review is a secondary study that brings together similar studies, published or not, and critically evaluates their methodology. It can gather quantitative results in a statistical analysis or meta-analysis, when possible. It is considered the best level of evidence for making decisions on therapeutic issues, as it synthesizes similar primary studies of good quality (1, 2). To avoid analysis bias in a systematic review, the methods for selecting and analyzing the data are established before the review is carried out, in a rigorous and well-defined process.

Firstly, the authors did not define the methods in a rigorous and well-defined process, at least in terms of protocol registration, as they did not present a previous registration number on any platform. Drawing up a protocol allows the systematic review to be organized, more transparent, and with a lower risk of bias (1). Another issue observed was that Piticchio et al., 2023 reported that the publication was only a meta-analysis as explained in the title, but in the first line of 2.1 Construction of the review under

Materials and Methods, the authors stated that it was a systematic review (SR). There is a difference between carrying out just a meta-analysis and an SR with meta-analysis. A meta-analysis is not carried out in all cases; it should only be carried out if the studies included are similar, i.e., if the sample, the types of studies, the intervention, and the clinical results are homogeneous (1). In this case, as will be seen below, the studies were not similar, which would require subgrouping.

Secondly, the authors of the publication stated that they carried out the meta-analysis according to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) and through the National Heart, Lung, and Blood Institute Quality Assessment Tool for Observational Studies; the analyses can be seen in Supplementary Figure 1. If the included articles carried out an intervention, gluten-free diet (GFD), they could not be classified as observational articles. So, why did the authors not use more appropriate assessment tools, such as 'Risk of Bias in Non-Randomized Studies of Interventions' (Rob 1), 'risk-of-bias tool for randomized trials' (Rob 2) (1), the Jadad Scale (2), and/or A Measurement Tool to Assess Systematic Reviews (3)? It is possible to see that important issues to be analyzed in clinical trials were not addressed in the MOOSE tool used, as shown in Supplementary Figure 1.

Moreover, there was also a lack of tools to help with the writing of the systematic review, such as The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (4). The PRISMA guidelines are designed to ensure transparency and rigor in the reporting of systematic reviews. In the topic of the Introduction section, for example, there was no mention of the subject of gluten and the Gluten-free diet (GFD). The authors also failed to mention whether they followed an acronym, such as PICO, when drawing up the eligibility criteria, objectives, research question, and research strategy (1, 4). The lack of this acronym may have led to the absence of some points: clear objectives in the article, since in the conclusions the authors talked about inflammation, which was not mentioned previously, and did not state how they assessed this outcome. Regarding the eligibility criteria, there was no information on the diet, whether the diet would be totally gluten-free or whether it could be gluten-reduced, and there was no information on which diets would be compared to the gluten-free diet, i.e., specifying the comparator group. In one of the articles included in this meta-analysis, there were multiple interventions, GFD (gluten-free diet) and lifestyle (5). As such, this article needed to be carefully assessed or excluded; it should also be added that there was no mention of which types of studies would be included and excluded in this meta-analysis.

As for the choice of database, only two were searched, PubMed and Scopus, which may have restricted the number of articles found and the number of people in the sample. Furthermore, the search language was restricted to English only. Given the availability of resources for translating articles into other languages, excluding non-English articles from the search does not seem justified. Finally, the authors described that some stages were carried out by two reviewers (item 2.5), sometimes independently (item 2.4), but at no point did they state that the procedure was carried out blindly, as

indicated in the main methodological guidelines for preparing systematic reviews (2).

At the end of the meta-analysis, the authors concluded that the evidence was not sufficient to recommend GFD for all patients diagnosed with Hashimoto's thyroiditis (HT). However, it is not up to the systematic review to "recommend", but rather to gather scientific evidence to support the specialist's decision. In addition the authors also did not assess the certainty of the evidence using the GRADE methodology approach by two independent reviewers. This is an extremely important stage that takes place after the meta-analysis has been carried out. Finally, our research group considered that the research lacks robustness and that there are methodological flaws that need to be readjusted, in addition to revising the eligibility criteria, so that we can answer and fully address the question of whether or not GFD is effective in treating HT in non-celiac disease.

Lastly, we would like to cordially thank the editors for allowing us to clarify these points.

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The influence of extended fasting on thyroid hormone: local and differentiated regulatory mechanisms

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The hypometabolism induced by fasting has great potential in maintaining health and improving survival in extreme environments, among which thyroid hormone (TH) plays an important role in the adaptation and the formation of new energy metabolism homeostasis during long-term fasting. In the present review, we emphasize the potential of long-term fasting to improve physical health and emergency rescue in extreme environments, introduce the concept and pattern of fasting and its impact on the body's energy metabolism consumption. Prolonged fasting has more application potential in emergency rescue in special environments. The changes of THs caused by fasting, including serum biochemical characteristics, responsiveness of the peripheral and central hypothalamus-pituitary-thyroid (HPT) axis, and differential changes of TH metabolism, are emphasized in particular. It was proposed that the variability between brain and liver tissues in THs uptake, deiodination activation and inactivation is the key regulatory mechanism for the cause of peripheral THs decline and central homeostasis. While hypothalamic tanycytes play a pivotal role in the fine regulation of the HPT negative feedback regulation during long-term fasting. The study progress of tanycytes on thyrotropin-releasing hormone (TRH) release and deiodination is described in detail. In conclusion, the combination of the decrease of TH metabolism in peripheral tissues and stability in the central HPT axis maintains the basal physiological requirement and new energy metabolism homeostasis to adapt to long-term food scarcity. The molecular mechanisms of this localized and differential regulation will be a key research direction for developing measures for hypometabolic applications in extreme environment.

KEYWORDS

long-term fasting, hypometabolism, thyroid hormone, extreme environment, deiodinase, tanycyte

1 Introduction

Fasting, with a history spanning nearly thousands of years, has become an ideal lifestyle choice for purportedly maintaining physical and mental well-being, improving metabolic syndrome (1) and combating cancer (2), protecting from chemotherapy toxicity (3) and promoting longevity. Medical fasting for 2 days or more has been applied for preventive and therapeutic purposes in specialized centers (4). Additionally, long-term fasting (LTF) has long been known to induce hypometabolism and hypothermia in humans and animals (5, 6), and has significant potential in the physiological maintenance during emergency rescue, particularly in extreme environments such as mine disasters, cave explorations, and manned deep space explorations. Food deprivation will be last for a long time in such conditions. Reducing energy expenditure is the best approach to enhance survival and rescue efforts. However, the practical application of fasting interventions has become increasingly challenging due to the distinct and individual consequences of short-term complete fasting for up to two days in currently prevalent forms (7, 8) and the limited evidence-based clinical data of LTF (9). More information is necessary for its broader implementations as a therapeutic prescription for diseases or valuable survival strategies for extreme environments of prolonged fasting (10).

Fasting confers healthy benefits to a greater extent than just being attributed to a reduction of caloric intake and may involve a metabolic switch, enhanced autophagy, and antioxidant capacity, as well as cellular stress resistance (11, 12). Metabolic switching from liver-derived glucose to adipose-derived ketones as a fuel source has been observed and contributes to improvements in energy metabolic regulation. Recent studies show that individuals can well tolerate a 10-day complete fasting (CF), experience an energy metabolic substrate shift, and achieve new metabolic homeostasis between days 3 to 6 of CF, with a declining resting metabolic rate (13). Additionally, fasting significantly impacts the regulation of thyroid hormone (TH) levels, which play a crucial role in the regulation of energy metabolism. However, the influence of fasting on energy metabolism and TH metabolism varies depending on the duration and frequency of fasting (14). The physiological mechanisms involved in these effects are complex and required further exploration. The principles of fasting, modifications in TH metabolism, and possible regulatory mechanisms are outlined in the present review. Here we discuss the mechanisms of potential metabolic regulatory and biological effects, and ultimately provide effective practical methods for food conservation, as well as physiological and behavioral adaptations to endure long-term food limitations.

2 Fasting definition and patterns

In humans, fasting typically refers to the dietary pattern of intentionally abstaining from food and caloric beverages for periods ranging from 12 hours to 3 weeks (15). Generally, the body enters a fasting state 8–12 hours after the last meal. Many religious groups incorporate fasting into their rituals for spiritual development and health promotion, including Daoist Bigu, Buddhist fasting, and

Muslim Ramadan fasting (RF) (16). Fasting therapy is an ancient folk health method that has been established as a defined therapeutic approach in modern medicine, particularly in specialized hospitals or clinical departments of integrative medicine. Following expert consensus in Germany (17) and China (18), fasting therapy has become an evidence-based preventive treatment for modern diseases. Essentially, fasting is a physiologically adaptive process that restricts energy intake. Under different fasting modes, the body constantly makes physiological metabolic adjustments, reduces energy requirements, and systematically utilizes its own energy reserves and repairs aging components through processes such as fat breakdown, and autophagy. This leads to an improvement in physiological state and a delay in senescence, helps to prevent and treat conditions like obesity, diabetes, and cardiovascular diseases (19). Different fasting patterns produce different physiological impacts, and the health-improving effects vary accordingly.

In general, fasting can be classified into four main modalities based on fasting duration and interval. 1) Caloric Restriction (CR): This involves typically decreasing daily caloric intake by 15% to 40%. 2) Intermittent Fasting (IF): Refers to a cycle of eating patterns that can range from 12 hours to several days, with recurring periods of little to no caloric intake. Time restricted feeding (TRF) and alternate day fasting (ADF) are forms of IF. TRF limits daily food intake to a 4–8 hours window, while ADF alternates normal food intake with days of restricted food intake. The religious fasting practices of RF fall into this category. 3) Long-term Fasting or prolonged fasting (LTF): This involves completely abstaining from food for more than 2 consecutive days, typically ranging from 3 to 21 days (20, 21). Certain forms of Daoist Bigu belong to this category. 4) Periodic Fasting (PF): Lasts 2 to 7 days periodically on a weekly, monthly, or yearly scale (22). The “5:2” diet, a popular fasting regimen, involves 5 days of ad libitum feeding and two fasting days per week, during which food intake is markedly decreased to approximately 500–600 kcal. These PF/IF protocols reduce energy intake for a period of time, repeatedly mobilizing and storing energy substrates. This results in more frequent but less pronounced alterations compared to continuous fasting (15). During LTF, physiological mechanisms ensure the survival of glucose-dependent tissues and employ alternative energy source to fuel other tissues. LTF is capable of deeply remodeling energy metabolism and causes stronger effects than CR and IF (13, 23). These characteristics highlight the therapeutic potential of LTF. Moreover, extended fasting can effectively reduce resting energy expenditure (EE) and has application potential in emergency rescue under special environment with food shortage, although the inducing efficiency to hypometabolism and the health maintenance mechanism need to be further explored.

3 Influences of fasting on energy metabolic expenditure

LTF fasting reduces BMR and EE, but the effects of short-term fasting and IF have not been concluded. Fasting is a popular

controlled nutritional intervention for its potential benefits in weight loss and over health improvement. Understanding its effects on EE can help tailor fasting regimens and guide to practical applications. The effects of fasting on EE vary depending on the type of fasting utilized, its duration, frequency and refeeding method (24, 25). Under normal conditions, there is an equilibrium between energy intake and expenditure. EE occurs primarily through the resting metabolic rate (RMR, accounting for approximately 60-70% of total EE), followed by physical activity (contributing about 20-30%) and the thermic effect of food (responsible for roughly 10% of total EE) (26). First of all, the effects of short-term fasting or IF on EE are still being determined. It has been observed that resting EE briefly increased during the first few days of fasting, likely due to increased sympathetic nervous system activity and catecholamine release. Other contributions to increased EE may include the energy costs of fatty acid recycling and gluconeogenesis (31, 32). The resting EE was not different after 12 hours and 60 hours of fasting, but the proportional contribution of carbohydrate, fat and protein oxidation was significantly shifted (33). Total EE showed a decrease of -1.9 MJ/day, but resting EE remained unchanged in obese individuals after 6 days of fasting for weight loss (34). Heilbronn et al. observed no significant changes in RMR and RQ from baseline to day 21 of ADF, but there was an increase in fat oxidation (35). Although RF is associated with decreased activity and sleeping time, no significant alteration was observed in RMR or total EE (36). The daily total EE and resting EE decreased about 12.4% and 6.5% respectively, after a month of RF, but there was no statistical difference in the EE of physical activity (37). Some reports indicated that the change in RMR varies at different times of RF duration. RMR was higher in the first week of RF and showed a significant downward trend in subsequent weeks, potentially due to metabolic adaptation mediated both centrally and locally (36, 38). Therefore, while IF regimens and short fasting may initially boost EE, extended fasting and continuous CR prompt a proportional metabolic slowdown. However, LTF and severe CR have been demonstrated to lower RMR and result in metabolic adaptation (13, 27). As early as 1915, Benedict reported a 20-30% decrease in energy metabolism induced by prolonged starvation (28). LTF significantly decreases 24-hour EE and the respiratory exchange ratio, as detected by a respiration chamber (29, 30). The respiratory quotient (RQ) approaches 0.7 with the extension of fasting time in a 10-day CF experiment, indicating fat as the predominant fuel source (13). An important phenomenon is that fasting leads to a significant decrease in serum thyroid hormone T₃, which may contribute to the decline in RMR (39). From an evolutionary perspective, animals instinctively consume more energy to find food in the initial stage of food deprivation (13). By the way, the increased EE in the early stage of food deficiency suggest that we should strengthen psychological guidance to reduce this consumption. This metabolic adaptation likely contributes to conserve energy and enhance survival during prolonged food scarcity such as under some extreme environments.

4 Impacts of fasting on thyroid hormone metabolism

4.1 Effects of fasting on TH levels

As a crucial endocrine regulatory system for basal metabolic rate and EE, hypothalamus- pituitary-thyroid (HPT) axis is profoundly affected by various fasting protocols. To save energy and conserve protein during food shortages, one of the major adaptations is the down-regulation of TH-dependent metabolism. Firstly, the decrease in TH levels during fasting is usually temporary and reversible. Once the individual resumes normal diet habits, the TH levels return to normal. In some cases, LTF or severe CR can lead to a more significant decrease in TH and may even result in low T₃ syndrome. Secondary, it is well known that fasting induces alterations of circulating THs, characterized by a marked decrease in serum triiodothyronine (T₃), free T₃ (FT₃), an increase of reverse T₃ (rT₃) (40, 41), and inconsistent changes in thyroxine (T₄), free T₄ (FT₄) and thyroid stimulating hormone (TSH), which may show a slight decrease or remain unchanged during fasting as depicted in Figure 1 by a meta-analysis during LTF. A 24-hour short-time fasting decreases FT₃ by 6%, but increases rT₃ by 16% in healthy humans (42). Animal experiments with 12- to 48-hour fasting also confirmed the decreases of serum T₃ and T₄ as summarized in a recent review (43). Fasting did not alter plasma thyrotropin-releasing hormone (TRH) levels after a 6-day fasting (44). The concentration of TRH in both the median eminence (ME) and the hypothalamic portal blood decreases during fasting (45). As a typical negative feedback regulation, the HPT axis involves the synthesis and release of TRH in the hypothalamus, which stimulates the synthesis and secretion of TSH in the anterior pituitary. Then TSH promotes the thyroid to release T₄, which is later converted to active T₃ by deiodinase type I (Dio1) and type II (Dio2). In turn, excessive T₃ and T₄ inhibit the synthesis and secretion of both TRH and TSH. The decreased serum TH concentration was not accompanied by a rise of TSH or TRH during food deprivation, reflecting a deviation from the classic negative feedback regulation of the HPT axis.

Local TH metabolism is probably a determinant of net effects and presents a flexible and dynamic regulation, which plays an optimal role in the organism's adaptation to a range of environmental challenges (46). The expression changes of TH metabolism-related genes differ in the liver, thyroid, pituitary and hypothalamus of mice after 48 hours of fasting (47). Differential regulations of TRH metabolism by fasting depend on the age, and species of the animals (48, 49). Thus, the combination of the decrease of TH metabolism in peripheral tissues and stability in the central HPT axis maintains the basal physiological requirement and new energy metabolism homeostasis to adapt to long-term food scarcity. The tissue variability in regulatory pattern is manifested in the differential responsiveness of the central and peripheral HPT axis to fasting, especially the intracellular T₃ availability (50).

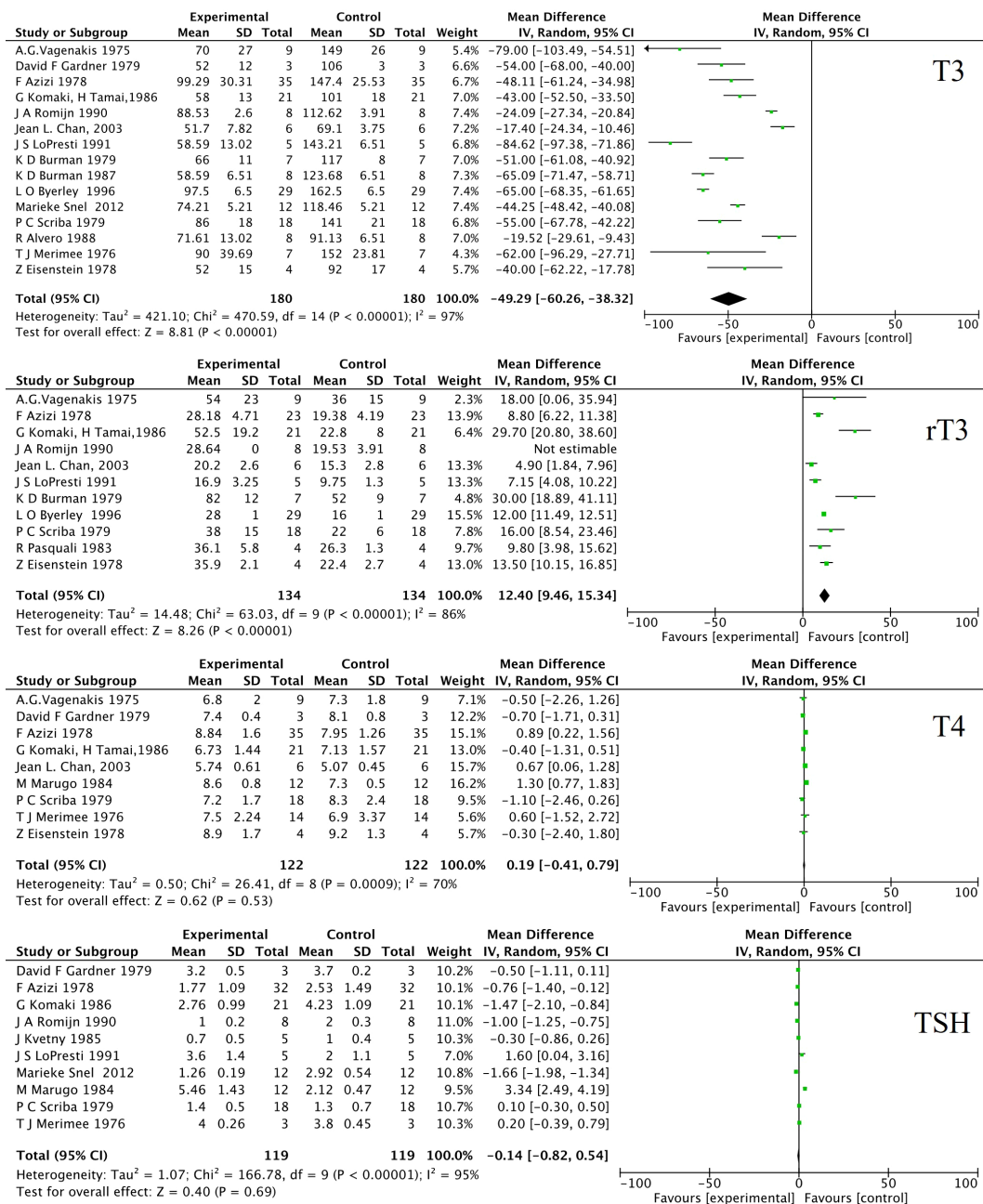


FIGURE 1

Forest plot of the effects of long-term fasting on T3, rT3, T4 and TSH. 16 human research papers were selected by manually review from PubMed and Web Of Science using the following term: "long-term fasting" or "prolonged fasting" and "thyroid hormone". The data were analyzed by RevMan 5.3 for MacOS (Cochrane Collaboration, Oxford, UK) as the standard protocol and parameters. The weighted mean differences (WMD) were applied for the comparison of continuous variables. The heterogeneity in studies was assessed through the chi-squared (χ^2) test and inconsistency index (I^2). χ^2 p value < 0.05 or $I^2 > 50\%$ were considered as significant heterogeneity. A random-effect model was used to estimate the combined WMD. Otherwise, the fixed-effect model was applied. SD, standard deviation; IV, inverse variance; CI, confidence interval; df, degree of freedom.

4.2 Fasting affects peripheral TH metabolism

During fasting, THs are shifted toward inactivated metabolism including the increase of rT3 conversion and decrease of T4 to T3 conversion. The decreased peripheral conversion of T4 to T3 is likely the primary mechanism responsible for circulating TH change, due to the lack of an appropriate TSH and TRH response

in serum. As previously described, a prominent feature of the fasting state is a significant decrease in circulating T3 levels and an increase in rT3. Total caloric deprivation appears to shift the peripheral metabolism of T4 from the activation pathway to the deactivation pathway (51), possibly due to the decreased generation of T3 and the metabolic clearance rate of rT3 (52). The constancy of T4 levels throughout the fasting period by administration of L-thyroxine could not restore the decrease in serum T3, indicating

that fasting decreases T3 production (41, 53). The enzyme activity for the conversion of T4 to T3 in fasted rat liver homogenate was reduced by 54%, primarily due to a reduction in enzyme concentration rather than co-factor availability (54). After a 48-hour fasting, only the transport of T3 into the perfused rat intracellular liver compartment decreased, but not the transport to the extracellular liver compartment (55). The clearance of rT3 is also affected by fasting. Both 7 and 13 days of fasting decreased rT3 clearance without changing rT3 production, compared to controls (56). However, Galton et al. found unchanged or even increased liver T3 and T4 concentrations within 16- or 36-hour fasting, with an increase in T3 clearance due to sulfation and UDP-glucuronidation (57). Some reports indicate that T3 glucuronidation was diminished, but T3 sulfation and subsequent deiodination were unaffected in the perfused rat liver after a 48-hour fasting (55). Similarly, Vries et al. also found that a 36-hour fasting did not alter intrahepatic T3 concentrations in rats (58), but a 48-hour fasting decreased both serum and liver T3 and T4 levels in mice (59). A gradual decrease of T3 and T4 concentrations in serum and liver during 12- to 48-hour fasting is confirmed in a recent review (43).

4.3 The potential alteration of central HPT response to fasting

Although fasting induces lower TH level, TSH and TRH secretion remains low, pointing to an altered hypothalamic setpoint of the HPT axis. Fasting, whether intermittent or prolonged, has complex effects on the response of the central HPT axis. The absence of an increase in serum TSH and TRH implies decreased negative feedback from the central part of the HPT axis. Although some reports suggest no change in serum TRH during fasting, LTF influences the secretion and function of TRH through mechanisms such as suppressing the activity of TRH neurons, reducing TRH gene expression, and inhibiting TRH secretion (45, 49). Previous animal studies have shown direct and indirect effects of decreased serum leptin contributing to the decrease of hypophysiotropic TRH neurons (40, 49). Leptin directly acts on hypophysiotropic TRH neurons that project to ME to regulate TSH production in the pituitary and regulates TRH neurons through pro-opiomelanocortin (POMC) and agouti-related peptide/neuropeptide Y (AgRP/NPY). However, recombinant methionyl human leptin only prevents the fasting-induced TSH decrease and pulsatility, but does not significantly affect the changes of the TH level (60).

Moreover, most studies report significant decreases in TSH response to TRH after fasting (60–62), but not all (63). Burman reported in 1980 that 10-day fasting impaired TSH secretion after TRH infusion (64). In the early phase (48 hours) of a 6-day fasting experiment, serum TSH still decreased in the control and TRH infused subjects (44). A 60-hour fasting decreases the mean 24-hour TSH concentration, associated with a decline in mean TSH amplitude, but not its frequency (65). Longer fasting results in a significantly lower TSH response than shorter fasting (66). Recently, Sinkó reported that fasting for 24 hours or 48 hours did not change TH action in the hypothalamic arcuate nucleus-ME region using

TH action indicator mice, coupled with decreased expression of TSH β and FT3 levels (67). Fasting promotes a 94% reduction in TSH β mRNA expression in the pituitary (47), suggesting a decrease in the response of the pituitary to the low serum levels of T4. Fasting induces an increase of Dio2 expression in the hypothalamus, followed by an upregulation of local T3 content, which is critical to augment the suppression of the central HPT axis and avoid a negative feedback TH elevation (68). These data suggest that there are more complex and fine-tuned regulatory mechanisms of the HPT axis to maintain its stability in the brain during fasting.

5 The fine regulation of tanycytes on HPT axis during fasting

Tanycytes play a crucial role in maintaining the homeostasis of the central HPT axis during fasting by controlling the supply and metabolism of THs. Tanycytes are specialized ependymogial cells in the third ventricle and are recognized as multifunctional players in energy metabolism through the organization of hormonal and nervous signals (69). Moreover, early studies indicated that Dio2 is absent in the PVN neurons (70). Additionally, it has been observed that a higher dose of T3 than the physiological level is necessary to reduce TRH expression in the PVN (71). This implies that the negative feedback loop involving T3 and TRH in the PVN may be less sensitive, allowing for a more nuanced regulation of the HPT axis.

5.1 Tanycytes regulate the bioavailability of THs to neurons

Acting as gatekeepers of the blood-hypothalamic barrier, tanycytes express the monocarboxylate transporter 8 (MCT8), OATP1c1 (organic anion-transporting polypeptide 1C1), and Dio2 enzyme. T4 is taken up from the cerebrospinal fluid into β 2 tanycytes and deiodinated into the active form T3 by the highly expressed Dio2. Then, T3 diffuses to neighboring neurons, supplying almost 80% of the adult brain's T3 (72–74). Interestingly, the modulator of cellular TH bioavailability μ -crystallin (CRYM), which has high affinity to T3 and T4, is relatively less expressed in β 2 tanycytes than in other subtype tanycytes (75). This differential expression adjusts the time-course of T3 interaction with its receptor or the efflux of T3, and the transcription of target genes (72). TSH induces transcriptional regulation of TH-gatekeeper genes through the Tshr/G α q/PKC pathway in tanycytes (76).

Axons from TRH-containing neurons of the paraventricular nucleus (PVN) project into the ME, where the neuron terminals make contact with the β 2 tanycytic end feet (77). Increased circulating TH upregulates the expression of pyroglutamyl peptidase II (PPII), a highly specific TRH inactivating enzyme, in tanycytes and enhances the degradation of extracellular TRH in the ME through glial-axonal association (77). Stimulation of the TRH receptor I increases the intracellular calcium in tanycytes via the G α q/11 pathway, which in turn increases the size of tanycyte end

feet and the expression of PPII, moderating the release of TRH (78). Moreover, the expression of PPII in β 2 tanycytes is also upregulated in response to elevated T3 levels, thereby forming a more refined negative feedback regulation. Thirdly, tanycytes are characterized by molecular signatures that sense and integrate nutrient/hormone signaling to modulate and maintain energy homeostasis (79). Indeed, β tanycytes form a “barrier” by expressing tight junction proteins and controlling the access of peripheral metabolites and hormones (80, 81). Many studies have described the metabolic sensor role of tanycytes for glucose, amino acids, leptin etc. Fasting can upregulate the facilitated glucose transporter and enhance blood-hypothalamus barrier plasticity through VEGF-dependent signaling (82, 83). Tanycytes also express receptors involved in the HPT axis and control TRH release (72, 78). Leptin has been identified as a key regulator of the central HPT axis by regulating pro-TRH expression in the PVN and could restore type III iodothyronine deiodinase (Dio3) expression in the liver (84, 85). Tanycytes regulate leptin's entry into the hypothalamus and play an important role in the supply of THs via the expression of TH transporters and deiodinases (81).

5.2 Tanycytes mediate a central TSH-TSHR feedback loop

Tanycytes have the high number of Tshr transcripts (86). TSH released from pars tuberalis (PT) acts locally on tanycytic TSHRs, resulting in increased Dio2 expression and TH synthesis (87). Both hypothalamic tanycytes and pituitary PT-specific cells can respond to different photoperiods and regulate circulating TH levels (88). Photoperiod-dependent seasonal variations are integrated by tanycytes via the detection of the TSH released from the PT in the anterior pituitary gland (89). The PT-derived TSH is distinctly glycosylated compared with pars distalis-derived TSH. Furthermore, PT-TSH, detectable in the circulation, does not stimulate the thyroid gland (90).

Although our understanding of the functional roles of tanycytes in the regulation of the central HPT during fasting is lacking, current evidences suggest that these local regulatory mechanisms form a more refined negative feedback regulation of TH metabolism, potentially contributing to the homeostasis of the central HPT axis during fasting.

5.3 The effects of fasting on the TH metabolism in tanycytes

Local hyperthyroidism in the hypothalamus suppresses TRH release, which helps avoid excessive TRH response that could affect the body's adaptability to fasting. Tracer kinetic studies have shown that serum T3 levels can accurately predict tissue T3 content and T3 signaling in most tissues, except the brain and pituitary gland (91). The uptake and conversion of THs are important for local T3 bioavailability and its action in the brain. Although MCT8 plays a pivotal role in the transfer of THs across the blood-brain barrier, fasting for 48 hours induces no significant alteration of MCT8 and

monocarboxylate transporter 10 (MCT10) in the hypothalamus and pituitary (47, 92). MCT10 expression was found to increase upon fasting, whereas MCT8 expression remained unchanged (58). Conversely, fasting significantly induced MCT8 expression in the ependymal layer and PVN in mice (93). The impact of fasting on MCT8 expression levels and regional distribution requires further thorough investigation.

Fasting indeed impacts the expression and activity of Dio2 in tanycytes. Researchers observed a 2-fold increase in the expression of Dio2 mRNA in the mouse hypothalamus, but noted no change in the expression of Dio3 and Thr β (47). In the initial phase of fasting, Dio2 mRNA expression and activity are robustly upregulated in tanycytes, leading to an increase in locally formed T3 and the suppression of TRH (68, 94). Coppola et al. observed that an appropriate induction of Dio2 activity during negative energy balance is dependent upon both leptin and glucocorticoid signaling (95).

Fasting influences the TRH release via tanycytes. Fasting temporarily increases the levels of PPII and Dio2 mRNA in tanycytes after 48 hours, followed by an increase of PPII activity in the ME and a partial reversion of the reduction in PVN pro-TRH mRNA levels and the number of TRH neurons. This delayed increase of ME PPII in fasted rats may facilitate the maintenance of the deep down-regulation of the HPT axis function (45).

6 Differential regulatory mechanisms

6.1 Different expression and activity of deiodinases in tissues

Despite the abundant clinical evidence, there is still a very limited understanding of how tissue-wide TH bioavailability and local TH action are regulated during development and fasting (96). Fasting decreases TH levels while keeping TSH concentrations unaltered, which is linked to local Dios expression and activity alterations in the brain hypothalamus and liver. The individual contributions of the three Dio isoenzymes to systemic and local TH provision are distinct during fasting and exhibit different regulatory mechanisms.

The main source of daily circulating T3 comes from the out ring deiodination of T4 by Dio1 and Dio2 in human. Thyroid gland contributes with about 20% of the daily T3 production, and the residual 80% is contributed outside the thyroid parenchyma, mainly from liver (97–99). The decrease in hepatic deiodination activity may be the primary factor for the decrease in circulating T3 during fasting. A 30-hour fasting decreases the activity of Dio1 in the liver and pituitary, with no changes in the kidney, and reduces the Dio2 activity in brown fat (57). However, fasting induces an increase in Dio2 expression in the hypothalamus (68). The decrease in Dio2 expression and activity was tissue-specific given that cerebral cortex Dio2 mRNA remains stable during fasting period, which leads to reduction in serum T3 levels, whereas serum T4 remains largely unaffected (98).

Dio3 is regarded as the major TH-inactivating enzyme catalyzing both T4 and T3 into rT3 and T2. Fasting results in

markedly decreased serum T3 concentration coupled with a marked decrease in liver T3 concentration. Fasting also increases Dio3 mRNA expression mediated by the CAR and mTOR pathway in mice liver and WAT (59). A 36-hour fasting increases Dio3 activity, without changing Dio1 activity (14, 57). Dio3 plays a role in the fasting-induced alteration of TH homeostasis because the decrease of serum T3 is partially blunted in the Dio3-knockout mice after a 30-hour fasting (57). Leptin stimulates hypothalamus TRH expression and plays an important role in the regulation of the HPT axis. Fasting elicits significant reductions of serum leptin concentrations as shown by meta-analysis (100). Leptin administration restores the fasting-induced increase of hepatic Dio3 expression in mice, while serum TH levels and liver TR β 1 expression remain low (85). It is confirmed that fasting decreases the expression of Dio2 in the liver and increases it in the hypothalamus, and increases the expression of Dio3 in the liver. However, further exploration is needed to understand the expression and activity of Dios in the liver and hypothalamus, as well as the molecular mechanisms underlying their regulation.

6.2 Different expression of transporters in tissues

Concentrations of TH in local tissues are mediated by the uptake of circulating THs via their transmembrane transporters. The transport of T3 (55) and T4 (101) to liver cells is decreased, presumably due to the depletion of ATP in a perfused rat liver model. TH transport is mediated by widely expressed MCT8, MCT10 or brain-specific OATP1C1. Both MCT8 and MCT10 mediate T3 transport, but MCT8 also transports rT3 and T4, which are not efficiently transported by MCT10 (102). MCT10 mRNA expression is upregulated in the fasted liver, but MCT8 is not affected (14, 59). In mice, a 48-hour fasting induces a 4-fold increase of MCT8 mRNA expression in WAT, while MCT10 mRNA expression remains unchanged, indicating an enhanced uptake of T3 by adipocytes (59). Decreased MCT8 and increased MCT10 transcription in the fasted liver are also observed in rats. Moreover, the expression of both transporters is increased in the fasted gastrocnemius muscle (103). In the hypothalamus, the expression of MCT8 was reduced, but MCT10 did not change after 48-hour of fasting in mice, and its down-regulation upon fasting may be involved in this feedback mechanism (92). Till now, there is little data and research about the effects of fasting on OATP1C1, which is low expression in primates, so it is not discussed in present review.

7 Conclusion and perspective

Taken together, prolonged fasting has an application potential in emergency rescue under some special environments with food shortage, but the inducing efficiency to hypometabolism and the health maintenance mechanism still need to be understood, especially in the TH metabolism which plays an important role in

energy expenditure regulation. Extended fasting significantly influences TH metabolism through localized and differentiated regulatory mechanisms in different tissues, involving the response of the HPT axis, reduced uptake and conversion of THs in the periphery, and nuanced regulation via tanycytes. The molecular mechanism of this local differentiation regulating deiodinated enzymes and transporters will be a top priority for future research.

Author contributions

XS: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. SJ: Investigation, Writing – original draft, Writing – review & editing. HZ: Writing – original draft, Writing – review & editing, Funding acquisition. FW: Writing – original draft, Writing – review & editing. HW: Writing – original draft, Writing – review & editing. CY: Funding acquisition, Writing – original draft, Writing – review & editing. YG: Funding acquisition, Writing – original draft, Writing – review & editing. LW: Conceptualization, Writing – original draft, Writing – review & editing. YL: Conceptualization, Writing – original draft, Writing – review & editing. ZD: Conceptualization, Funding acquisition, Investigation, Writing – original draft, Writing – review & editing.

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Conflict of interest

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A new LNC89/LNC60-Col11a2 axis revealed by whole-transcriptome analysis may be associated with goiters related to excess iodine nutrition

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Goiter related to excessive iodine nutrition remains a significant public health issue in some countries. There has been no reported study on long noncoding RNAs (lncRNAs) related to goiters. In this study, goiter was induced by drinking water with excess iodine for 10 or 20 weeks in Kunming mice. Whole transcriptome sequencing results showed that LNC89 expression increased in mice goiter tissues compared to normal thyroid tissues and higher in 20 weeks goiter tissues than in 10 weeks goiter tissues, which were identified by qRT-PCR. Cooperate with human-mouse homologous gene conversion, a new LNC89/LNC60-Col11a2 axis was predicted by LncTar and expression correlation analysis based on whole transcriptome sequencing results. Increased Col11a2 expression was also identified by qRT-PCR and Western blot in the mice goiter tissues. In the human normal thyroid cell line Nthy-ori-3 treated with KI₀₃, LNC60 and Col11a2 expression increased with promoted cell viability, which were reversed by siLNC60 treatment. Furthermore, LNC60 and Col11a2 mRNA levels were found increased in peripheral blood of nodular goiter patients from high water iodine areas of China and have high diagnostic values for nodular goiter while AUC of LNC60 and Col11a2 are 89.97% and 84.85%, respectively. In conclusion, the novel LNC89/LNC60-Col11a2 axis may be involved in the progression of goiter related to iodine excess, providing potential biomarkers and therapeutic targets in the future.

KEYWORDS

iodine excess, thyroid, goiter, Col11a2, lncRNA

Introduction

Water with high iodine content and iodine rich foods are two major factors related to iodine excess worldwide. In addition to Japan and Korea, which have iodine rich foods, high-water-iodine areas are primarily found in 13 countries and regions worldwide (1). According to China's 2017 water iodine monitoring results, 51 administrative counties including 25,317 administrative villages still had high water iodine levels exceeding 100 µg/L, affecting 40.65 million people (2). Although iodized salt supply has been halted, over 80% of surveyed individuals in high-water-iodine areas had a median urinary iodine level above 300 µg/L, indicating a risk of excessive iodine intake (3, 4).

Iodine excess so far was found related to various thyroid diseases, including goiter (5). Goiter prevalence was 6.3% to 11% in the areas with high water iodine levels, while goiter prevalence increased with water and urinary iodine levels (4, 6). Various theories have been proposed to explain the development of goiter, including the iodine blocking effect (Wolff–Chaikoff effect), inhibition of hormone secretion due to gland storage, inhibition of the sodium-iodine symporter (NIS), intrathyroidal redistribution of organic iodine, colloid retention, and others (5, 7). However, there is no consensus on the exact mechanisms behind goiter, and further research is needed.

The primary type of goiter caused by iodine excess is colloid goiter, also known as diffuse goiter (8, 9), which was not noticed at the early stage. However, as the damage gets worse, the thyroid volume gradually increases, possibly leading to breathing difficulties and difficulty swallowing. Patients may need treatments like radio frequency ablation, laser therapy, or surgery. These treatments can burden patients and harm their thyroid function permanently. Additionally, due to goiter can increase the risk of thyroid nodules and thyroid cancer, goiter patients may require regular ultrasound exams. Therefore, it is urgent to elucidate its mechanism and develop new therapeutic methods.

Long noncoding RNAs (lncRNAs) have emerged as a novel type of biomarker and play a crucial role in the diagnosis and treatment of various diseases. lncRNAs may be involved in the regulation of gene expression through transcription, chromatin modification, posttranscriptional regulation, and translation (10–12). To date, numerous lncRNAs have been discovered in thyroid cancer, such as FOXD3-AS1, BRM, HOTAIR, and MALAT1. However, there was no report on lncRNAs related to other thyroid disease (13–15).

In this study, based on the whole transcriptome sequencing, we hope to find a new lncRNA–mRNA co-expression network related to goiter and identify new molecular markers for goiter diagnosis and therapy.

Method

Animals

Eighty female Kunming mice (18 ± 4 grams and 4 weeks of age) were purchased from Vital River Laboratories Animal Technology Co., Ltd. (Beijing, China) and raised in specific pathogen free (SPF) conditions with constant temperature and humidity (temperature 23

± 1°C, humidity 45% ± 5%, 12-hour light, 12-hour dark cycle). The research committee certified that the study subjects were healthy and fit for the experiment. Five mice per cage were allowed free access to food, water, and commercial SPF mouse maintenance feed. After a week of acclimation to the diet, all mice were randomly assigned to four groups: Control 1, Control 2, Treatment 1 and Treatment 2. Control 1 and Treatment 1 were fed for 10 weeks, and Control 2 and Treatment 2 were fed for 20 weeks. Potassium iodate (KIO₃) solution was dissolved in double distilled water (DDW). DDW with 50 µg/L iodine is for control groups and 300 µg/L iodine is for the treatment groups. The chow iodine content was 305 ± 142 µg/kg, and approximately 5–8 g of food and 5–10 ml of water per mouse were considered in a day. The estimated iodine intake of the mice in the control groups was 1.5 µg/d and that of the mice in the treatment groups was above 4 µg/d. The committee of Harbin Medical University approved the animal experiment protocols. Experimental protocols and animal welfare guidelines were in accordance with the Care and Use of Laboratory Animals, National Institutes of Health (NIH). Ensure that the animal is provided with a proper living environment, behaves normally, has no irritable behavior, and the high iodine dose is not super high, and that gnawing stick is equipped. The animals were euthanized at the end of the study to ensure no additional suffering.

Sample preparation

During the feeding period, mice were observed daily, and their weights were measured weekly. Before euthanasia, 24-hour urine samples were collected from mice using metabolic cages. Urine was filtered through a 0.22 µm filter, centrifuged (3000×g, 5 minutes, room temperature), and then stored at -20°C for measuring urinary iodine levels. Mice were anesthetized with pentobarbital, cardiac blood was collected and centrifuged (4000×g, 15 minutes, room temperature), and the serum was stored at -80°C for measuring thyroid function and serum iodine levels. After euthanasia, the left thyroid lobes of six mice from each group were fixed in 2.5% glutaraldehyde (Sigma–Aldrich, USA) for TEM electron microscopy, and the corresponding right thyroid lobes were fixed in 4% paraformaldehyde (Biosharp, China) for paraffin section preparation. Fourteen mouse thyroid samples were snap-frozen in liquid nitrogen and stored at -80°C. Among them, three randomly selected thyroid tissue samples from the control groups and three randomly selected goiter tissues from treatment groups were used for whole transcriptome sequencing, while the remaining samples were used for real-time quantitative PCR (qRT–PCR) and Western blot (WB) experiments.

Measurement of urinary iodine, serum iodine, iodine in feed, and thyroid function (TSH and FT4)

Urinary iodine (UI), serum iodine (SI), and iodine in the feed were measured according to the standards WS/T 107.1-2016, WS/T 572-2017, WS 302-2008 and GB 5009.267-2020 (16–19). Thyroid-stimulating hormone (TSH) and free thyroxine (FT4) levels were measured using enzyme-linked immunosorbent assay (ELISA) kits

(Kenuodi, China CK-E20360 and CK-E20383) in 10ul serum according to the kit protocol. The detection range of TSH is 0.75 to 24mU/L with accuracy 0.1mU/L, of FT4 is 1.25 to 40 pmol/L with the accuracy 0.1pmol/L.

Transmission electron microscopy

Samples were prepared and dehydrated by laboratory personnel according to standard procedures and observed under a microscope (Hitachi 7700, Japan). Water is extracted from the tissues by passing them through the graded alcohol as 30%, 50%, 70%, 80%, 90%, 95%, absolute alcohol, and then absolute acetone.

Hematoxylin and eosin staining and immunohistochemistry analysis

Tissues were embedded and cut into 4 μ m sections. After deparaffinization, H&E staining and immunohistochemistry were performed according to standard methods. After section preparation, images were captured using a BX 53 microscope (Olympus, Japan).

RNA extraction and RNA sequencing

Total RNA was extracted using TRIzol reagent (TaKaRa, Japan), and RNA concentration and quality were detected using a NanoDrop spectrophotometer (NanoDrop Technologies, USA) and Invitrogen Qubit 3.0. A spectrophotometer (Thermo Fisher Scientific, USA) was used. RNA-seq was performed on the Illumina HiSeq 2500 platform (Genesky Biotechnologies, Inc., Shanghai, China) using a 2 $\times$ 150 bp paired-end sequencing strategy. All differentially expressed genes were selected based on the criteria of fold change $|\log_2FC| > 1$ and $P < 0.05$.

Human-mouse homologous gene conversion and Lnc RNA target mRNA analysis

The NONCODE website (<http://www.noncode.org/>) was accessed to retrieve gene sequences. NCBI BLAST was entered for sequence alignment to select highly homologous human lncRNAs. We used LncTar software for predicting RNA targets of lncRNAs (20). For a pair of lncRNA and mRNA which are predicted to have interaction, they also must have a strong co-expression. We set a minimum required Pearson correlation coefficient (PCC) of 0.5 between them for this purpose.

Quantitative real-time PCR

Total RNA was reverse-transcribed to obtain cDNA using the RT-PCR First-Strand cDNA Synthesis Kit (Roche, USA).

Quantitative real-time PCR was performed using the SYBR method (Roche, USA), and gene expression in triplicate experiments was compared using the $2^{-\Delta\Delta Ct}$ method. Mouse β -Actin was used as the internal reference, and the primers sequence can be found in [Supplementary Table 1](#).

Western blot

Total protein was extracted from the tissues using radioimmunoprecipitation assay buffer (RIPA) buffer (Beyotime, China) and quantified using a bicinchoninic acid (BCA) BCA protein assay kit (Beyotime, China). Twenty micrograms of protein from each sample were separated on a 15% polyacrylamide gel through electrophoresis and transferred to obtain immunoblot bands. Finally, the bands were visualized using a chemiluminescence imaging system (Tanon, China). The grayscale values of the bands were quantitatively analyzed using ImageJ software (NIH, Bethesda). Primary antibodies are rabbit anti-mouse Col11a2 antibody (YT1009, 1:500, Immunoway, China) and mouse anti-mouse β -actin antibody (TA811000, 1:2000, OriGene, USA) while mouse and rabbit second antibodies from Abbkine (A25012, 1:10000, China) and CST (14708, 1:2000, USA).

Cell line

The Nthy-ori-3-1 cell line is an immortalized human normal thyroid cell line provided by Professor Qiao Hong's research group in the Department of Endocrinology at the Second Affiliated Hospital of Harbin Medical University. The cells were authenticated using short tandem repeat (STR) analysis. The cells were cultured at 37°C with 5% CO₂ in complete medium consisting of 10% fetal bovine serum (Procell, China), 2% penicillin-streptomycin (Solarbio, China), and RPMI-1640 medium (Gibco, USA).

Cell viability detection

Cells were seeded in 96-well plates at a density of 3000 cells per well and cultured overnight in complete medium. Afterward, they were treated with different concentrations of potassium iodate for 24, 48, 72, and 96 hours. Cell viability was assessed using the Cell Counting Kit-8 (CCK-8, Biosharp) and the ethynyl deoxyuridine (EdU, RiboBio) assay, following the manufacturer's protocols. We investigated the influence of different iodine concentrations (0, 10⁻³ M, 10⁻⁵ M, 5 $\times$ 10⁻⁶ M, and 10⁻⁷ M) on cell viability in Nthy-ori-3 thyroid cells. Nthy-ori-3 cells were cultured to attain 70-80% confluency before being subjected to iodine treatments for 24 hours. An untreated control group was maintained for comparison. Subsequently, EdU (10 μ M) was introduced to the cells during the active cell profiling phase. Following cell harvesting, fixation with 4% paraformaldehyde, permeabilization with 0.3% Triton X-100, and Click reaction using Ruibo's EdU Click-iT™ kit, cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI). Microscopy and

flow cytometry analyses were conducted to quantify EdU-positive cells, providing insights into the impact of iodine concentration on cell profiling in Nthy-ori-3 cells. Statistical analysis was performed to determine the significance of the observed effects.

Cell transfection experiment

We used LipofectamineTM 3000 transfection reagent (Thermo Fisher) to transfect LNC60 siRNA into Nthy-ori-3 thyroid cells according to the manufacturer's protocol. The synthesis of siRNA targeting LNC60 (5'-CACUCAGGCUGCAAGCAGUTT-3' and 5'-ACUGCUUGCAGCCUGAGUGTT-3') and nontargeting controls (5'-UUCUCCGAACGUGUCACGUTT-3' and 5'-ACGUGACACGUUCGGAGAATT-3') were performed by Anhui Universal Biotech Co., Ltd.

Case control study of the nodular goiter patients

According to the national standards (GB 16005-2009 and GB/T 19380-2016), the areas with median water iodine (MWI) levels more than 100 µg/L were defined as high water iodine areas. From June 2019 to January 2020, an epidemiological survey was carried out in the towns of Malingang, Shiji, Gucheng and Wanfu of Heze city, Shandong province, where the MWI was more than 100 µg/L according to the 2017 Chinese national report on water iodine distribution at township level. A total of 1344 people were recruited, including 315 males and 1029 females age 18 to 70 years old. After ultrasound examination, male with thyroid volume greater than or equal to 23 ml and female with thyroid volume greater than or equal to 18 ml were diagnosed as goiter. Finally, we got 26 patients with goiter, and 26 healthy controls were selected according to age ($\pm$ 3 years) and sex, 1:1 matching. In these survey areas, the supply of iodized salt was stopped in 2009, therefore the high iodine content in local water resources can reflect the excess iodine nutrition of local residents. Population with other thyroid diseases, disabilities, mental disorders, abnormally low autoimmune function, have taken thyroid disease-related drugs, such as methimazole, propylthiouracil, levothyroxine tablets, and so on, have pregnancy or with incomplete research data, such as missing blood or demographic data were excluded from both cases and controls, and informed consent was signed. When the subjects were fasting, 15 ml of venous blood was collected by disposable vacuum sampling, and centrifuged at 20–30°C (3000 RPM, 10 minutes). Serum was collected and stored at -80°C in the laboratory for RNA extraction. cDNA synthesis and qRT-PCR detection of LNC 60 and human Col11a2 were performed as above. Human β -Actin was used as the internal reference, and the primers sequence can be found in [Supplementary Table 1](#).

Statistical analysis

Statistical analysis was performed using R software (version 4.3.1). The data normality distribution was validated using the

Kolmogorov–Smirnov test. For normally distributed data (such as body weight, thyroid organ coefficient), t tests were used, and data are represented as the mean $\pm$ standard deviation. For nonparametric tests, data are represented as the median with interquartile range (IQR) such as serum iodine, urinary iodine, FT4, TSH, and Mann–Whitney U test was used for comparison between groups. A bilateral test was used, with *P* value less than 0.05 indicating statistical significance. Evaluation of diagnostic value depends on receiver operating characteristic (ROC) curve. When the area under the curve (AUC) >0.7 with *P* <0.05 , it was considered to have diagnostic values. Data visualization was performed using R packages, including the ggplot2 package for scatter plots, box plots, and bubble plots and the ggVennDiagram package for Venn diagrams.

Results

Establishment of the goiter mouse model

At 10 weeks, the goiter group of mice had urinary iodine and serum iodine levels of 569.40 µg/L and 63.13 µg/L, respectively, which were significantly higher than those of the control group at the same period, which had levels of 171.60 µg/L and 26.76 µg/L (*P* <0.01 ; *P* <0.001). At 20 weeks, the high-iodine group of mice had urinary iodine and serum iodine levels of 312.5 µg/L and 62.83 µg/L, respectively, which were significantly higher than those of the control group at the same period, which had levels of 186.90 µg/L and 27.65 µg/L (*P* <0.01 ; *P* <0.001). At 10 weeks, the thyroid weight and organ coefficient of the high-iodine group of mice were 6.93 ± 1.76 mg and 0.171%, respectively, which were significantly higher than those of the control group at the same period, which were 4.92 ± 2.95 mg and 0.09%. At 20 weeks, the thyroid weight and organ coefficient of the high-iodine group of mice were 7.54 ± 2.78 mg and 0.163%, respectively, which were significantly higher than those of the control group at the same period, which were 5.00 ± 1.02 mg and 0.101% ([Table 1](#)).

Histological changes in the thyroid tissues of goiter mouse model

At 10 weeks and 20 weeks, the thyroid follicles of the control group ([Figures 1A1, B1](#)) mice were rich in colloid, and the thyroid epithelial cells had a cubic or low cubic shape with round cell nuclei and regular arrangement. Excess iodine can cause changes in the pathological structure of the thyroid. Compared to the control group, the 10 weeks high-iodine goiter group ([Figure 1C1](#)) exhibited enlarged follicles, increased colloid, flattened cell nuclei, and a small amount of inflammatory reactions in the HE staining. In addition to the pathological changes seen in the 10-week goiter group, the 20-week high-iodine goiter group ([Figure 1D1](#)) showed more severe pathological structural changes, such as empty follicles and thinning or disappearance of the follicle basement membrane. TEM results showed that the thyroid ultrastructure in the control group at 10 weeks and 20 weeks ([Figures 1A2, B2](#)) was normal, with round and

TABLE 1 Detection of serum iodine and thyroid function in mice.

Period (Weeks)	Group	Serum Iodine	Urine Iodine	FT4	TSH	Weight	Thyroid Weight	Organ Ratio
		(μg/L)	(μg/L)	(pmol/L)	(mU/L)	(g)	(mg)	(%)
10	Treatment1	63.13(55.76)a**	569.40 (78.10)a***	21.32(1.78)	3.18(0.74)	43.59 ± 3.20	6.93 ± 1.76a*	0.171(0.067)
10	Control1	26.76(14.70)	171.60(114.10)	21.87(1.04)	3.11(0.50)	43.67 ± 3.94	4.92 ± 2.95	0.09(0.0592)
20	Treatment2	62.83(33.43)b***	312.50 (84.40)b***	22.07(2.58)	3.44(0.96)b*	42.15 ± 5.57	7.54 ± 2.78b***	0.163(0.096)b***
20	Control2	27.65(13.80)	186.90(112.00)	21.87(1.05)	3.10(0.50)	44.30 ± 6.18	5.00 ± 1.02	0.1011(0.044)

a compared with Control1. b compared with Control2. ***P*<0.01 ****P*<0.001. Mann–Whitney *U* test.

smooth cell nuclei, deep colloid, normal morphology and structure of mitochondria, and endoplasmic reticulum and neat arrangement of microvilli. Compared to the 10-week control group, TEM results in the goiter group at the same time showed ultrastructural changes in the thyroid, including flattened nuclei, swollen mitochondria in thyroid epithelial cells, sparse microvilli, shallower colloid, and slight nuclear condensation (Figure 1C2). In addition to the ultrastructural changes observed in the 10-week high-iodine group, the 20-week high-iodine goiter group showed more severe cell apoptosis and necrosis (Figure 1D2).

Thyroid function analysis of the goiter mouse model

At 10 weeks, the levels of TSH and FT4 in the high-iodine goiter group of mice were 3.18 mU/L and 21.32 pmol/L, respectively, slightly higher than those in the control group (3.11 mU/L and 21.87 pmol/L), but the difference was not statistically significant. At 20 weeks, TSH in the high-iodine goiter group of mice was significantly higher than that in the control group (3.44 mU/L and 3.10 mU/L) (*P*<0.05); FT4 was slightly higher than that in the control group (22.07 pmol/L and 21.87 pmol/L), but the difference was not statistically significant (Table 1).

Differentially expressed lncRNAs were revealed by whole transcriptome sequencing in the goiter mouse model

Compared to the control group at 10 weeks, the goiter mice had 723 upregulated differential lncRNAs and 2005 downregulated lncRNAs (Figure 2A). Compared to the control group at 20 weeks, the high-iodine group of mice had 1322 upregulated differential lncRNAs and 1125 downregulated differential lncRNAs (Figure 2B). We chose differentially expressed lncRNAs, whose BaseMean are more than 50, the absolute values of log2Fold Change are more than three in the 20 weeks goiter, and their log2Fold Change are more than that in the 10 weeks Goiter. We got seven up regulated lncRNA and four down regulated lncRNA, and only six lncRNA can be designed qualified primers, as shown in Table 2 and Figure 2.

Validation of differential lncRNAs by qRT-PCR in the goiter mouse model

qRT-PCR results showed that the expression of LNCNONMMUG019446.2 (LNC19) was significantly downregulated in 10-week goiter tissues, but not in the 20-week goiter tissues (Figure 2F). The expression of NONMMUG011211.2 (LNC11) in the 20-week goiter tissues was significantly downregulated, but not in the 10-week goiter tissues (Figure 2D). The expression of LNC NONMMUG018089.2 (LNC89) was significantly upregulated both in the 10-week and 20-week goiter tissues, and the increase was more pronounced in the 20-week goiter group compared to the 10-week high-iodine group (Figure 2E). Analysis of the nucleotide sequences of these lncRNAs revealed that only LNC89 had highly homologous human lncRNAs, with the transcript ID being NONHSAT207060.1 (LNC60).

Construction and validation of the LNC89-Col11a2 regulatory axis

Subsequently, we screened differentially expressed genes in the sequencing results, and then, based on correlation analysis, coexpressed mRNAs of LNC89 were identified, and this study selected Col11a2, which had a correlation coefficient exceeding 0.9 with LNC89. The correlation results (*R*=0.93, *P*<0.05) are shown in Table 3.

WB results showed that compared to that in the 10-week control group, Col11a2 protein expression in the 10-week goiter tissues of mice was increased, but the difference was not statistically significant (Figure 3A). Compared to that in the 20-week control group, Col11a2 protein expression in the 20-week goiter tissues was significantly increased (Figure 3B). The qRT-PCR results showed that the Col11a2 mRNA was significantly increased both in the 10-week and 20-week goiter tissues (Figure 3C). IHC results showed that compared to the control group, Col11a2 in the 10-week and 20-week goiter tissues displayed significant brown staining (Figures 3D2-5), and the brown staining in the 20-week goiter tissues was more pronounced than that in the 10-week group. The integral optical density values (IOD) of Col11a2 immunohistochemistry in the goiter tissues were significantly higher than that in the control group (Figure 3D1).

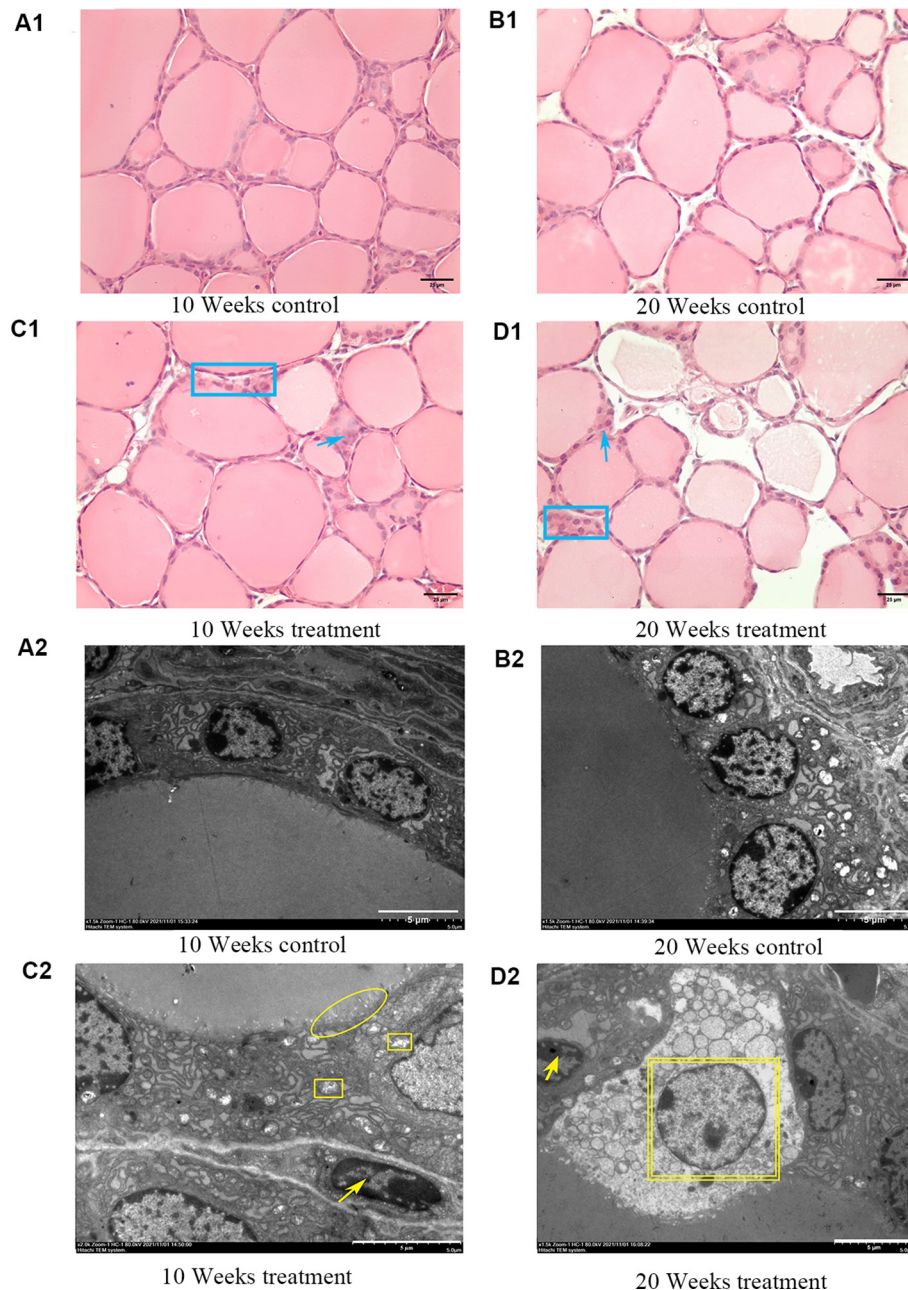


FIGURE 1

Pathological and TEM ultrastructural changes in thyroid of mice caused by iodine excess. The top image shows thyroid structure by HE staining.

(A1) 10 weeks control group. (B1) 20 weeks control group. (C1) 10 weeks treatment1 group. (D1) 20 weeks treatment group. Scale bar = 25 μm.

Blue rectangle: nuclei flat with different shapes or overlapping. Blue arrows: inflammatory response. Blue oval: follicular hyperplasia, follicular capsule thinning or even disappearance. The bottom image shows the ultrastructure of the thyroid glands by TEM. Scale bars = 5 μm. (A2) 10 weeks control1 group. (B2) 20 weeks control group. (C2) 10 weeks treatment1 group. (D2) 20 weeks treatment group. Scale bar = 5 μm. Yellow oval: sparse microvilli with or without microvilli ciliation. Yellow square: mitochondrial swelling, yellow arrow: nuclear condensation. Yellow double-layer square: necrotic scar bar=5 μm.

The expression of LNC60 and Col11a2 were induced by high-iodine treatment in Nthy-ori-3 cells

CCK8 and EdU assays were used to evaluate the cell viability of Nthy-ori-3 cells treated with different iodine concentrations (0, 10^{-7} M, 5×10^{-6} M, 10^{-5} M) for 72 hours. The CCK8 results showed that 5×10^{-6} M iodine promote Nthy-ori-3 cells viability (Figure 4A2).

EdU results showed that 5×10^{-6} M and 10^{-5} M KIO_3 promote Nthy-ori-3 cells viability (Figures 4B1–2). Then, Nthy-ori-3 cells were treated with different iodine concentrations for 72 hours. qRT-PCR results showed that compared to the control group, the expression of LNC60 and Col11a2 was significantly increased in the 10^{-5} M and the 5×10^{-6} M treatment groups (Figure 5A). WB results showed that compared to the control group, the 5×10^{-6} M treatment group had significantly increased Col11a2 protein expression (Figure 5B).

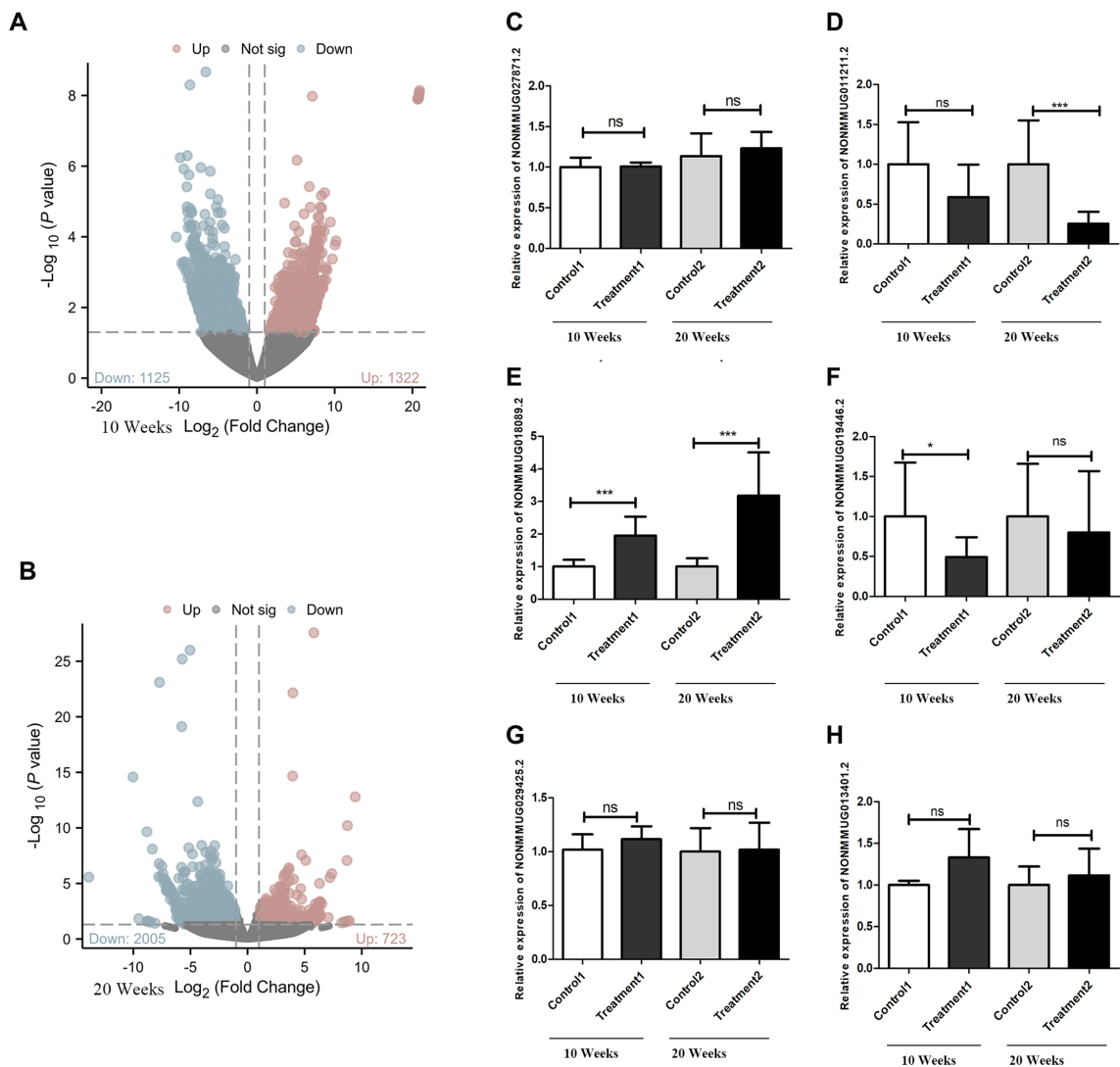


FIGURE 2 Differentially expressed lncRNAs and verification with qRT-PCR. **(A)** There were 2728 differentially expressed lncRNAs between the 10 weeks control group and treatment groups, 2005 of which were downregulated DElncRNAs and 723 of which were upregulated DElncRNAs, as shown by the volcano plot. **(B)** There were 2447 differentially expressed lncRNAs between the 20 weeks control group and Treatment groups, 1125 of which were downregulated DElncRNAs and 1322 of which were upregulated DElncRNAs, as shown by the volcano plot. **(C)** NONMMUG027871.2 expression level has no difference between treatment groups and control groups. **(D)** NONMMUG011211.2 expression decreased in both treatment groups, but only statistically significant in 20 weeks. **(E)** NONMMUG018089.2 expression levels increased in both treatment groups, with statistically significant. **(F)** NONMMUG019446.2 expression levels decreased in both treatment groups, but only statistically significant in 10 weeks. **(G)** NONMMUG029425.2 expression has no difference between treatment groups and control groups. **(H)** NONMMUG013401.2 increased in both the treatment groups, but with no statistically significant. **P* < 0.05. ****P* < 0.001.

TABLE 2 Differentially expressed lncRNA meeting the screening criteria.

ID	Base Mean	log2FC	P	Type
NONMMUG019446.2	86.15498009	-5.021611926	8.86177E-06	Down
NONMMUG013401.2	54.65454322	-4.072693926	0.002492893	Down
NONMMUG011211.2	79.19357743	-3.602198857	0.012952995	Down
NONMMUG029425.2	147.8639377	3.147507597	0.006733359	Up
NONMMUG018089.2	106.6304867	5.194913509	0.006409145	Up
NONMMUG027871.2	29.88260751	3.436631048	0.001566769	Up

The expression of Col11a2 was regulated by LNC60

After 72 hours transfection of siRNA-LNC60, the LNC60 expression level was significantly reduced (knockdown rate was 70.60%). After 72 hours of culture with 5×10⁻⁶ M KIO₃, the LNC60 expression level was significantly reduced in the siRNA-LNC60 group (knockdown rate was 74.10%) compared to the siRNA-NC group. qPCR and WB results showed that after knocking down LNC60, the expression of Col11a2 before and after iodine treatment was significantly reduced. Regardless of whether iodine treatment was administered, the siRNA-LNC60 group exhibited significantly

TABLE 3 co-expressed mRNA of NONMMUG018089.2.

lncRNA	lncRNA log ₂ FC	lncRNA Regulation	mRNA	mRNA log ₂ FC	mRNA Regulation	Cor
NONMMUG018089.2	5.19	Up	H2-Ke6	-1.20	Down	-0.81
NONMMUG018089.2	5.19	Up	Col11a2	1.31	Up	0.93**

lower Col11a2 expression than the siRNA-NC group, and iodine treatment may enhance the Col11a2 expression inhibition (Figures 5C, D). Furthermore, after knocking down siRNA-LNC60, the cell viability decreased compared to that of the control group after treatment with 5×10^{-6} M KIO₃ (Figure 5E).

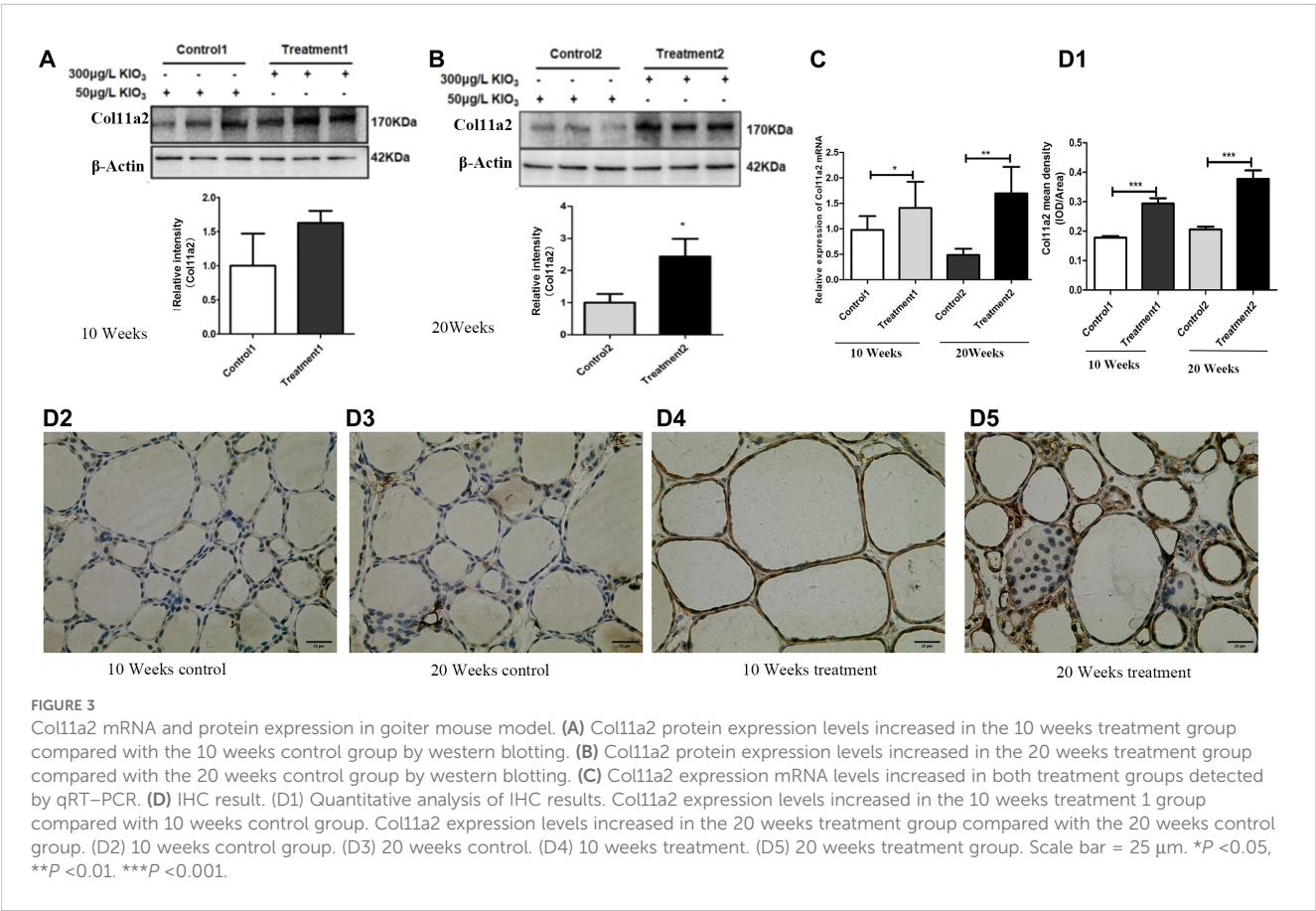
The expression levels of Col11a2 mRNA and LNC60 were increased in peripheral blood of nodular goiter patients from high water iodine areas of China

In this study, 26 patients with nodular goiter and 26 healthy control subjects were selected in high- iodine areas (water iodine content >100 μg/L). There were 5 males and 21 females in both groups, with ages of 44.80 ± 9.68 years and 45.80 ± 8.26 years, respectively. The thyroid volume of the patient group was 31.62 ± 7.95 ml, and that of the control group was 9.37 ± 3.22 ml. There was a significant difference in thyroid volume between the two groups

($P<0.05$). qPCR results showed that compared to the healthy control population, the LNC60 and Col11a2 mRNA levels in whole blood of thyroid goiter patients were significantly elevated ($P<0.001$ and $P<0.01$). Receiver operating characteristic (ROC) curve results showed that AUC of LNC60 was 89.97% (95% CI: 78.991%-98.96%), $P<0.001$. AUC of Col11a2 was 84.85% (95% CI: 72.75%-96.41%), $P<0.001$. Therefore, LNC60 and Col11a2 have high diagnostic values for nodular goiter patients from high water iodine areas of China (Figure 6).

Discussion

Iodine excess always induced colloid goiter, also known as diffuse goiter. In this study, thyroid in the 10-week high-iodine group exhibited pathological features such as enlarged follicles, increased colloid, flattened cell nuclei, and inflammatory responses. The 20-week high-iodine thyroid goiter group not only had more severe above pathological features, but also had other



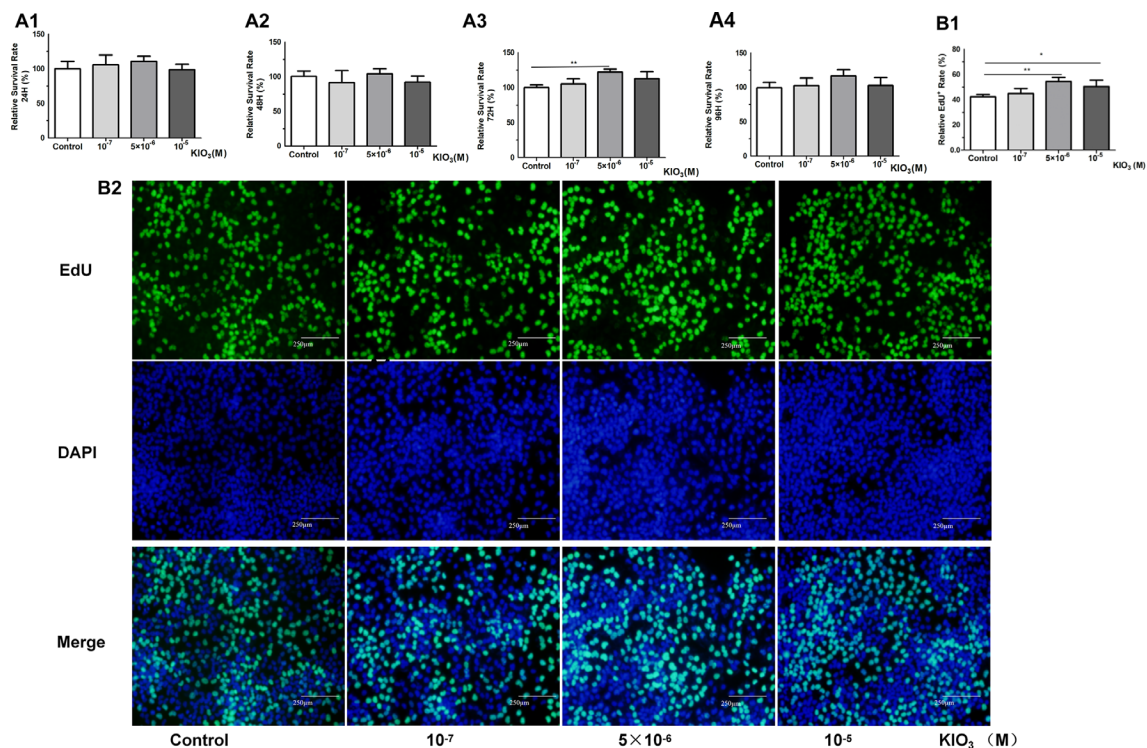


FIGURE 4

CCK8 and EdU assays were used to evaluate the cell viability of Nthy-ori-3 cells treated with different iodine concentrations. Analysis of cell viability using the CCK8 assay. **(A1)** After 24 hours of treatment, no significant cell viability promotion was observed. **(A2)** After 48 hours of treatment, no significant cell viability promotion was observed. **(A3)** After 72 h of 5×10⁻⁶ M KIO₃ treatment, cell viability was promoted. **(A4)** After 96 h of treatment, no significant cell viability promotion was observed. **(B1)** After 72 h of 5×10⁻⁶ M KIO₃ treatment, a significant viability promotion in EdU was observed. **(B2)** The lower figure shows Cell viability detected by EdU. Fluorescence microscopy images of EdU incorporation, illustrating the results. **P*<0.05, ***P*<0.01, *t* test.

more severe pathological structural changes, including thinning or loss of the basement membrane, overlapping cell nuclei, and other features. Therefore, the model of goiter was successfully constructed. Lei et al. found that the 12,000 µg/L KIO₃ group showed a decrease in mitochondria at seven months under TEM microscopy (21). Our TEM results showed that in addition to all the ultrastructural changes of the 10-week group, such as mitochondrial swelling, endoplasmic reticulum widening and cell nucleus flattening, microvilli ciliation, cell pyrosis and even cell necrosis occurred in the 20-week group of hyperiodinated goiter. However, there is no mitochondrial loss. The reason may be that our iodine excess is light.

Goiter is not necessarily accompanied by abnormal thyroid function. Previous studies found that mice drinking excessive iodine water (1500–3000 µg/L KIO₃) for 10 weeks to 7 months could cause colloid goiter, but there were serious thyroid function abnormalities at the same time (21–23). In order to better simulate the formation process of simple goiter caused by iodine excess in the population, to avoid severe thyroid function abnormality, this study chose 300 µg/L KIO₃ with 10 weeks and 20 weeks, respectively. The results showed that the TSH in the 300 µg/L high-iodine drinking water group at 10 weeks did not change, but that in the 20-week high-iodine drinking water group was significantly increased (*P*<0.05). However, there was no statistical difference in serum FT4 level compared with the control group. This suggests that 20

weeks of high iodine feeding may cause subclinical hypothyroidism in mice. The results of this experiment are similar to those of previous studies. Liu Shujun et al. fed Kunming mice with 300 µg/L high-iodine drinking water for 10 and 20 weeks, and found that the TSH level of mice was higher than that of the control group, but it is not statistically significant (24). It was also found that TT3, TT4 and TSH levels did not change significantly after feeding mice water with 300 µg/L and 600 µg/L iodine for three months (25). Rats may tolerate higher doses, and Teng et al. found that feeding Wistar rats with three and six times the dose of high iodine for 24 weeks did not affect TSH levels (26). Shan et al. fed Wistar rats with 50 times the dose of iodine for 12 weeks and 24 weeks, and found that although the serum TSH of the rats was increased, T3 and T4 were still not affected by high iodine (27). Therefore, whether iodine can cause goiter accompanied by abnormal thyroid function is related to iodine dosage and treatment time. The model established in this study will be more helpful to explore the mechanisms of goiter.

In recent years, many lncRNAs have been found to be closely related to thyroid cancer (28). However, there has been no report on lncRNAs related to goiter. In this study, we found that LNC89 was significantly upregulated in both 10-week-old and 20-week-old goiter mice, indicating its close association with goiter and suggesting its potential importance in the early diagnosis and screening of goiter. Human LNC60 is homolog to LNC89. So far, there is no report about LNC89 or LNC60. The cell viability

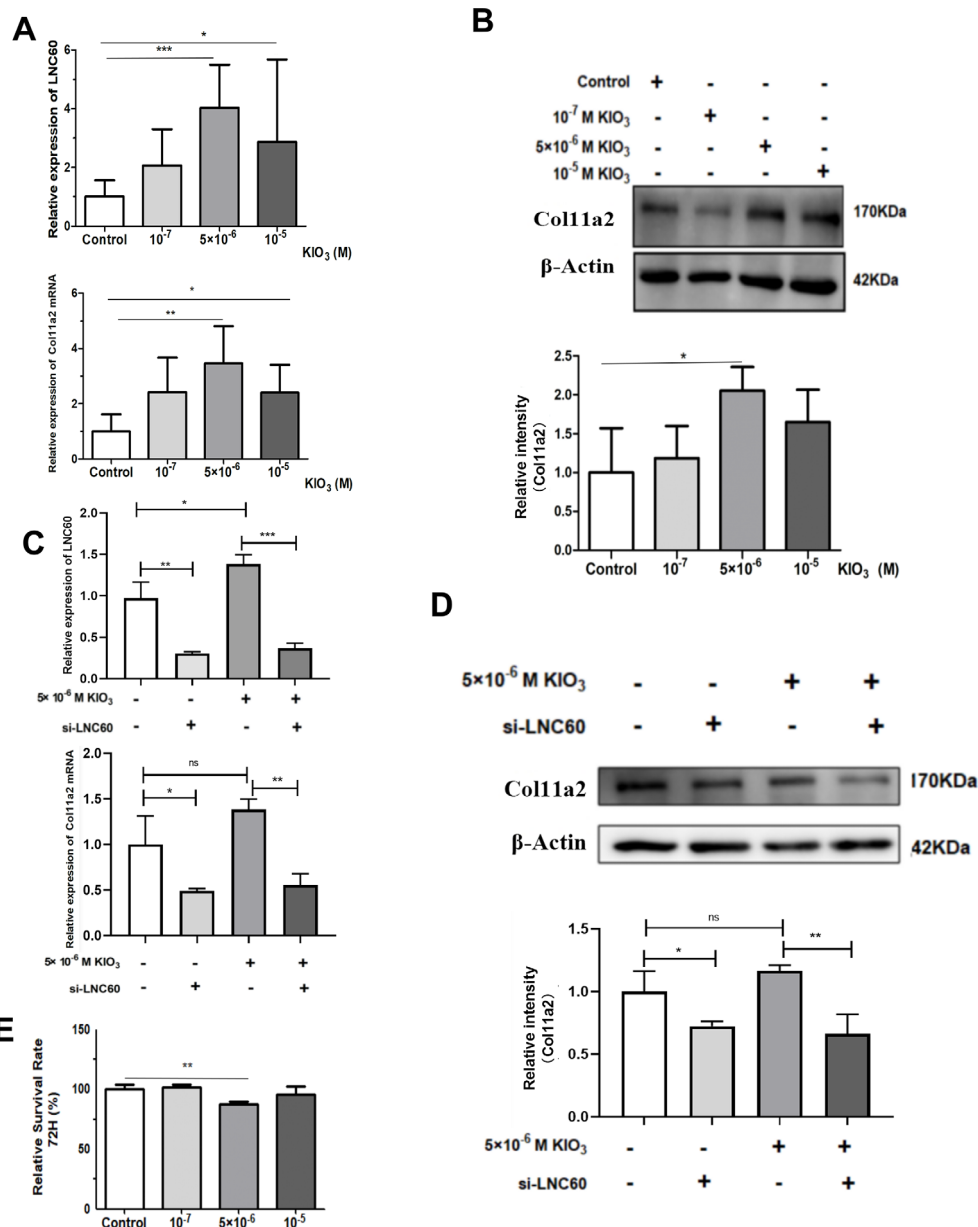


FIGURE 5

LNC60-Col11a2 axis in Nthy-ori-1 cells. (A) After treatment with 10^{-5} and 5×10^{-6} KIO_3 for 72 h, qPCR showed that LNC60 and Col11a2 mRNA levels were significantly increased compared with those in the control group. (B) With 5×10^{-6} KIO_3 treated for 72 h, Wb shows Col11a2 protein level was significant increased compared with the Control group. (C) After siLNC60, qPCR showed that LNC60 and Col11a2 were significantly decreased compared with those in the NC group. (D) After siLNC60, Wb showed that the Col11a2 protein level was significantly decreased compared with that in the NC group. (E) After siLNC60, the CCK8 assay showed a significant cell viability inhibition in the 5×10^{-6} M KIO_3 treatment group at 72 hours. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, t test.

increased in Nthy-ori-3 cells treated with 10^{-6} M KIO_3 , while LNC60 was significantly upregulated. Knocking down LNC60 resulted in Nthy-ori-3 cell viability inhibition with 10^{-6} M KIO_3 treatment, suggesting an association between LNC60 and cell viability. In this study, the expression of LNC60 in the whole blood of nodular goiter patients in high-iodine areas significantly increased. So that, LNC89/LNC60 may be related to iodine induced goiter or nodular goiter.

Constructing a lncRNA-mRNA coexpression network is a powerful method for inferring the function of lncRNAs (14). In

this study, a new LNC89/LNC60-Col11a2 target was constructed. The qPCR results demonstrated that Col11a2 was significantly upregulated in the 10-week and 20-week thyroid goiter mice, suggesting its association with goiter. When Nthy-ori-3 cells were treated with 10^{-6} M KIO_3 , cell viability improved, accompanied by increased Col11a2 expression. Inhibiting LNC60 reduced Col11a2 expression, particularly after iodine treatment, highlighting a more pronounced inhibitory effect. The expression of Col11a2 in the whole blood of thyroid goiter patients in high-iodine areas significantly increased, too. Cooperate with the LNC89/LNC60

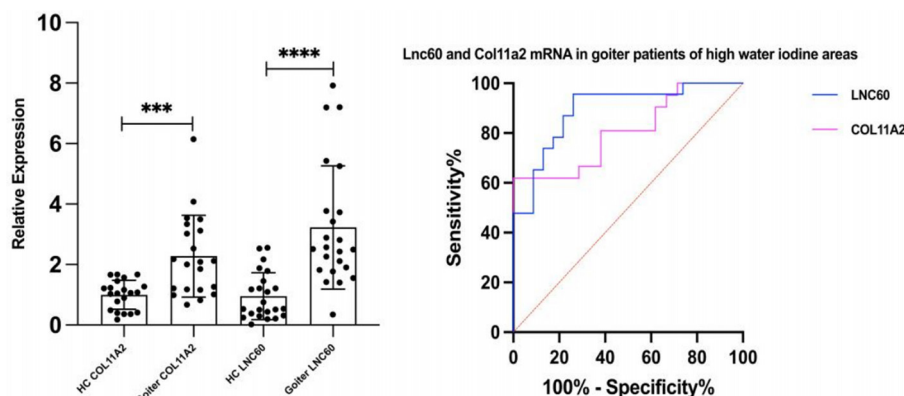


FIGURE 6

The expression of LNC60 and Col11a2 mRNA in the peripheral blood of Goiter patients and health control populations. *** $P < 0.001$, **** $P < 0.0001$, t test.

results, we proved that LNC89/LNC60-Col11a2 may be related to iodine induced goiter or nodular goiter.

Multiple studies have shown that collagen proteins, including Col11a2, are related to growth, differentiation, tissue remodeling, wound healing, and cell proliferation. Collagen genes are known to be associated with thyroid diseases, such as Col1a1, which is significantly upregulated in papillary thyroid carcinoma (PTC). Analysis of TCGA data has indicated that Col1a1 plays a crucial role in PTC recurrence and adverse prognosis. Col11a2 is a collagen gene whose function loss is associated with various syndromes involving deafness (29). We know that deaf is one of symptoms included in cretinism, which is related to iodine deficiency. This gene is also closely related to multiple fibrotic diseases, such as cardiac fibrosis, liver fibrosis, and lung fibrosis, and is a relatively common fibrotic disease gene (30–32). As shown above, we found Col11a2 up regulated in goiter mice groups. We also found a significant increase in Col11a2 expression in patients with nodular goiter. A small amount of fibrosis can be seen in the thyroid tissue of patients with high iodine-induced goiter (33, 34). Therefore, Col11a2 involved goiter progress may be related to fibrosis and iodine regulation.

However, this study has some limitations. First, cell function experiments need to be perfected, including overexpression of lnc60, over expression and knockdown of Col11a2. Second, the sample size of goiter patients is limited and should be expanded, and include multi-centers and areas with different iodine nutrition levels, making the lnc60-Col11a2 axis more meaningful. Thus, future research should enrich functional experiments and expand population studies to elucidate the mechanisms and assess the potential role of the LNC60-Col11a2 axis as diagnostic and prognostic markers for goiter including nodular goiter.

In summary, this study provides preliminary evidence for the involvement of the novel LNC89/LNC60-Col11a2 axis in the development of iodine-induced thyroid goiter, shedding light on early diagnostic biomarkers and therapeutic targets for thyroid goiter. More, this study not only enriches the lncRNA library, but

also provides a new regulatory mechanism for Col11a2-related other diseases.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: PRJNA1005249 (SRA).

Ethics statement

This study was conducted by the medical ethics committee of Harbin Medical University (20191102). The study was conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by the medical ethics committee of Harbin Medical University (20191102).

Author contributions

GN: Data curation, Formal Analysis, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. SL: Data curation, Validation, Writing – original draft. WZ: Data curation, Resources, Writing – original draft, Writing – review & editing. FM: Methodology, Writing – original draft. ZR: Writing – original draft, Data curation. JL: Writing – original draft, Data curation. DS: Funding acquisition, Resources, Software, Supervision, Visualization, Writing – review & editing. ML: Funding acquisition, Investigation, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fendo.2024.1407859/full#supplementary-material>

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Association between vitamin C intake and thyroid function among U.S. adults: a population-based study

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Background: Although some evidence suggests a role for vitamin C intake in thyroid diseases, the complex interplay between vitamin C intake and thyroid function remains incompletely understood. The objective of this study was to explore the relationship between vitamin C intake and serum thyroid function in the United States adults.

Methods: A total of 5,878 participants from the National Health and Nutrition Examination Survey (NHANES) between 2007 and 2012 were included in this study. Weighted multivariate linear regression models, subgroup analyses, and interaction terms were used to assess the association between vitamin C intake, evaluated as a continuous and categorical variable, and thyroid function. Additionally, restricted cubic spline (RCS) regression was employed to assess any nonlinear relationship that may exist between vitamin C intake and thyroid function.

Results: After adjusting for covariates, our research found a significant inverse correlation between vitamin C intake and total thyroxine (TT4) ($\beta = -0.182$, $P = 0.006$). Using subgroup analyses, the association was more pronounced among subjects with lower alcohol consumption ($\beta = -0.151$, $P = 0.013$, P for interaction = 0.043). In RCS regression, the correlation between vitamin C and TT4 exhibited a distinct reversed L-shaped curve pattern in total participants (P for nonlinear = 0.005) and male adults (P for nonlinear = 0.014). Additionally, we found an inverted U-shaped curve pattern in the relationship between vitamin C intake and FT4 (P for nonlinear = 0.029), while an U-shaped curve relationship was observed between vitamin C consumption and the FT3/FT4 ratio (P for nonlinear = 0.026).

Conclusion: The findings of our study have illustrated a notable correlation between vitamin C intake and thyroid function. A high level of vitamin C intake is associated with a decreased in TT4 levels among American adults, and the association was more pronounced among subjects with lower alcohol consumption. Furthermore, our analysis revealed a nonlinear correlation between the intake of vitamin C and the levels of TT4, FT4, and FT3/FT4 ratio.

Our findings support the rationale for making food-based dietary recommendations and maybe provide guidance for diet guidelines with thyroid dysfunction to a certain extent in the future.

KEYWORDS

vitamin C, thyroid function, alcohol, NHANES, cross-sectional analysis

1 Introduction

Thyroid, a crucial endocrine gland, regulates various functions in the body, such as metabolism, growth, and development through the secretion of thyroid hormones (1). The incidence of thyroid dysfunction varies by factors including age (2), sex (3), diet (4) geography (5), and genetics (6). For example, women are more likely to have thyroid problems at certain ages, such as puberty, pregnancy, and menopause, due to fluctuating hormone levels. In addition, factors such as environmental pollution (7), poor living habits (8), and trace elements (9, 10) may also increase the risk of thyroid disease. According to epidemiological data, the incidence of thyroid-related diseases has increased and become a health concern of global worldwide. A nationally representative cross-sectional study has revealed that the overall prevalence of thyroid dysfunction among adults stands at 15.17%. Specifically, subclinical hypothyroidism accounts for 12.93% of the cases, overt hypothyroidism comprises about 1.02%, subclinical hyperthyroidism constitutes 0.44%, and overt hyperthyroidism represents 0.78% of the total prevalence (11).

Vitamin C is an essential nutrient that plays a pivotal role in various biochemical processes within the body, including enzymatic activation, antioxidant reactions, and the maintenance of immune functions (12, 13). Unlike other animals, humans lack the ability to synthesize vitamin C internally. Therefore, we must obtain sufficient amounts of vitamin C from food or supplements to maintain good health. In recent years, there has been increasing interest in exploring the potential link between vitamin C and thyroid function and thyroid disorders. Several studies have shown that vitamin C kills thyroid cancer cells by inhibiting signaling pathways via distinct mechanisms (14–16). In addition, lack of vitamin C can make thyroid tissue more susceptible to oxidative stress, which can increase the risk of goiter development. And appropriate supplementation of vitamin C can effectively reduce this oxidative stress and lower the incidence of goiter (17).

The majority of existing studies have concentrated on the effects of vitamin C on specific thyroid disorders or its interaction with thyroid medications, rather than exploring its broader impact on thyroid function and hormone levels. Therefore, the objective of this study was to investigate the correlation between vitamin C intake and serum thyroid function among adults in the United

States, employing data obtained from the National Health and Nutrition Examination Survey (NHANES).

2 Methods

2.1 Study population

NHANES is a program of studies designed to assess the health and nutritional status of adults and children in the United States. Through a combination of interviews and physical examinations, NHANES provides insights into the prevalence of major diseases and conditions, dietary and nutritional patterns, and health-related behaviors in the United States. Conducted by the National Center for Health Statistics (NCHS) at the Centers for Disease Control and Prevention (CDC), all participants were required to sign the informed consent document prior to the commencement of the investigation.

For the current study, a total of 30,442 participants were identified and selected from the NHANES cycles between 2007 and 2012. Exclusion criteria were: (1) age of participants was < 20 years or participants were currently pregnant; (2) participants with thyroid cancer or receiving thyroid-related medication treatment; (3) participants without thyroid measurement data; (4) participants without vitamin C intake data; (5) participants with missing covariate values. Finally, 5,878 participants were incorporated into our analysis (Figure 1).

2.2 Serum thyroid measure

The thyroid panel comprises a series of tests intended for evaluating thyroid gland activity. This panel includes determinations of thyroid stimulating hormone (TSH), total thyroxine (TT4), free thyroxine (FT4), total triiodothyronine (TT3), free triiodothyronine (FT3), thyroglobulin antibodies (TgAb), and thyroid peroxidase antibodies (TPOAb). Thyroid blood specimens were processed, stored and shipped to University of Washington, Seattle, WA. Detailed specimen collection and processing instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM).

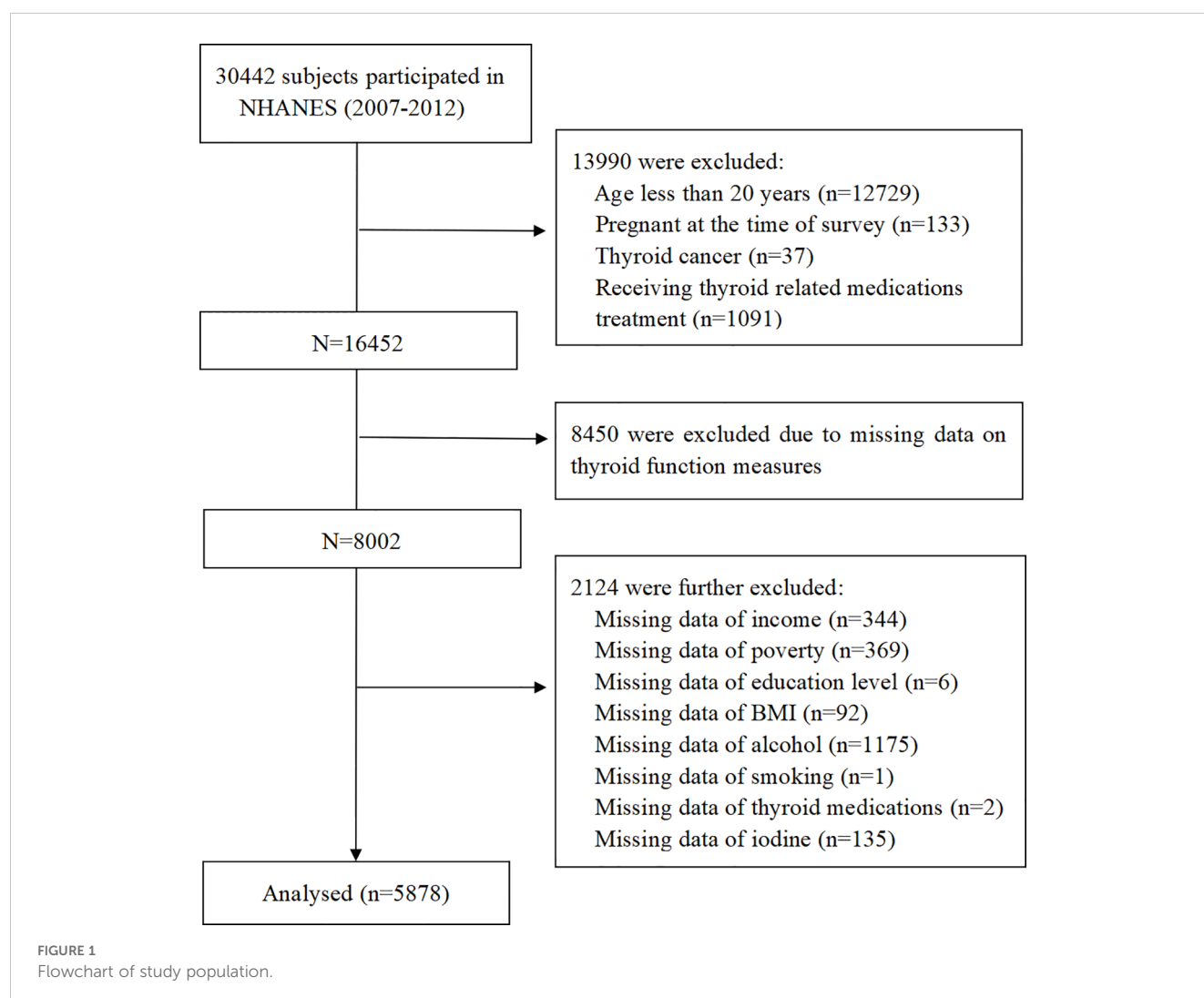
2.3 Dietary vitamin C intake assessment

Dietary vitamin C intake was derived from the Food and Nutrient Database for Dietary Studies (FNDDS), which is utilized to code and analyze dietary intakes for the What We Eat In America (WWEIA), NHANES. Dietary vitamin C intake was calculated based on the average of two 24-hour dietary recalls. The initially collected recall was obtained in-person at the Mobile Examination Center (MEC), while the second interview was conducted over the phone after an interval of 3 to 10 days. In this study, the total intake of vitamin C includes a variety of foods and vitamin C supplements. Furthermore, due to the non-normal distribution characteristics of the vitamin C intake data, we implemented logarithmic transformations in order to obtain normal distributions of the data (named $\ln V_c$), which was subsequently confirmed by the conduct of Shapiro-Wilk tests. The $\ln V_c$ were categorized into quartiles as follows: quartile 1 (0-1.508); quartile 2 (1.509-1.828); quartile 3 (1.829-2.074), and quartile 4 (2.075-3.037).

2.4 Covariates

According to the previous studies, we considered as confounding factors, information on demographics, socioeconomic status, diet, and health conditions, such as age, gender, race/ethnicity, education level, annual family income, alcohol consumption, poverty to income ratio (PIR), body mass index (BMI), urinary iodine concentration (UIC) and smoking (18). Considering the effects of other trace elements on the thyroid function, we also included dietary vitamin A (Va) (19), vitamin D (Vd) (20), vitamin E (Ve) (21), zinc (Zn) (22) and selenium (Se) (23) intake in this study.

Age was grouped into three groups: 20-40, 41-60 and more than 60 years. Education level was classified into three categories: less than high school, high school graduate, more than high school. Race was grouped into non-Hispanic white, non-Hispanic black, Mexican American, and others. Annual family income was divided into three groups (< 25,000, 25,000 to 74,999 and $\geq 75,000$). Poverty was defined as PIR less than 1.0, and PIR is further classified into four



categories(<1.0, 1.0-1.9, 2.0-3.9, ≥4.0). BMI was categories as <25.0, 25.0–29.9, and ≥30 kg/m². Alcohol consumption was defined as the average daily intake of any type of alcoholic beverage over the past year. A previous study on thyroid function have classified alcohol consumption into three categories of ≤50 g/day, 51 to 100 g/day, and > 100 g/day, and we adopt this classification method as well (24). UIC to determine iodine conditions in participants, which was classified as <100, 100-299 and ≥300 ug/L (25). Smoking habits were categorized as never, former, or now.

2.5 Statistical analysis

All statistical analyses were performed using the R package. Continuous variables were expressed in terms of the mean (standard deviation, SD), while categorical variables were

summarized as frequency and percentage. With regard to the baseline characteristics, categorical variables were analyzed using the chi-square test for comparison, and continuous variables were analyzed using the one-way analysis of variance.

Weighted multivariate linear regression models were implemented to analyze the potential correlation between lnVc consumption and thyroid function indices. Model 1 was unadjusted for any covariates, whereas Model 2 underwent adjustments for a comprehensive array of factors including age, gender, education level, race/ethnicity, PIR, BMI, UIC, alcohol consumption, annual family income and smoking habits. Based on Model 2, Model 3 further adjusted for additional confounders, including Va, Vd, Ve, Zn and Se. Subgroup analysis, interaction tests and restricted cubic spline (RCS) regression were employed to evaluate the nonlinear association between lnVc and thyroid function. P value < 0.05 was considered statistically significant.

TABLE 1 Baseline characteristics of the NHANES (2007–2012) study population in lnVc quartiles.

Characreristics	lnVc quartiles					P-value
	Overall	Q1	Q2	Q3	Q4	
N(%)	5878(100)	1472(25.788)	1467(24.916)	1469(24.604)	1470(24.691)	
lnVc	[0, 3.037]	[0, 1.508]	(1.508, 1.828]	(1.828, 2.074]	(2.074, 3.037]	
Vc(mg/d)	84.797(2.383) ^a	17.805(0.333)	47.770(0.432)	89.192(0.526)	187.751(3.193)	<0.0001
FT4(pmol/L)	10.116(0.069)	10.160(0.097)	10.129(0.087)	10.168(0.097)	10.005(0.080)	0.214
FT3(pg/mL)	3.189(0.011)	3.227(0.021)	3.168(0.017)	3.169(0.016)	3.188(0.016)	0.03
TSH(uIU/mL)	2.023(0.074)	1.954(0.087)	2.207(0.234)	1.988(0.050)	1.943(0.082)	0.729
TT3(nmol/L)	1.807(0.014)	1.847(0.020)	1.820(0.016)	1.770(0.024)	1.789(0.018)	0.016
TT4(ug/dL)	7.788(0.047)	7.900(0.068)	7.824(0.055)	7.785(0.056)	7.638(0.062)	< 0.001
TGAb(IU/mL)	7.661(1.625)	4.946(1.491)	9.760(2.990)	10.838(5.288)	5.212(1.240)	0.357
TPOAb(IU/mL)	19.442(1.762)	17.122(3.461)	19.045(2.572)	16.739(2.364)	24.959(5.512)	0.591
FT3/FT4	1.186(0.008)	1.193(0.011)	1.178(0.011)	1.175(0.012)	1.199(0.011)	0.21
Age(years)						0.092
20-40	2112(41.608) ^b	561(45.443)	489(38.258)	497(39.040)	565(43.543)	
41-60	1968(38.760)	525(38.304)	500(40.973)	471(38.543)	472(37.219)	
>60	1798(19.632)	386(16.253)	478(20.769)	501(22.417)	433(19.238)	
Gender						<0.0001
Female	2861(50.645)	743(52.933)	738(51.301)	769(54.939)	611(43.315)	
Male	3017(49.355)	729(47.067)	729(48.699)	700(45.061)	859(56.685)	
Race						0.029
White	2796(69.851)	797(73.619)	702(70.647)	684(70.267)	613(64.696)	
Black	1211(10.687)	290(9.886)	285(9.726)	287(9.918)	349(13.260)	
Mexican	912(8.317)	181(6.571)	247(8.871)	245(8.428)	239(9.472)	
other race	959(11.145)	204(9.924)	233(10.755)	253(11.387)	269(12.572)	
Family income(\$)						< 0.001
<25,000	1996(25.829)	606(31.851)	488(25.328)	454(22.571)	448(23.292)	

(Continued)

TABLE 1 Continued

Characreristics	lnVc quartiles					P-value
	Overall	Q1	Q2	Q3	Q4	
25,000-74,999	2530(42.582)	610(43.467)	654(42.846)	643(44.037)	623(39.944)	
>=75,000	1352(31.588)	256(24.682)	325(31.826)	372(33.392)	399(36.764)	
Education						<0.0001
<high school	1590(18.133)	488(23.147)	402(18.479)	355(15.965)	345(14.706)	
High School	1360(23.417)	394(28.443)	356(26.652)	305(19.691)	305(18.614)	
>high school	2928(58.451)	590(48.410)	709(54.869)	809(64.344)	820(66.679)	
PIR						< 0.001
<1.0	1216(15.079)	393(18.692)	292(14.134)	265(13.525)	266(13.807)	
1.0-1.9	1598(20.771)	446(25.129)	423(21.531)	372(18.054)	357(18.160)	
2.0-3.9	1545(27.285)	332(26.003)	392(26.771)	418(29.264)	403(27.173)	
>=4.0	1519(36.865)	301(30.177)	360(37.564)	414(39.157)	444(40.859)	
BMI (kg/m ²)						0.137
<25	1670(31.604)	408(29.062)	393(29.131)	414(33.658)	455(34.709)	
25-29.9	2024(33.456)	487(33.661)	523(33.783)	506(32.541)	508(33.825)	
=>30	2184(34.939)	577(37.278)	551(37.086)	549(33.800)	507(31.465)	
Alcohol (g/d)						0.178
<=50	5620(94.254)	1405(94.304)	1390(92.138)	1412(95.518)	1413(95.077)	
51-100	211(4.700)	50(4.915)	63(5.980)	49(3.640)	49(4.241)	
>100	47(1.046)	17(0.781)	14(1.883)	8(0.842)	8(0.683)	
Smoke						<0.0001
never	3113(53.919)	625(43.382)	775(50.188)	824(60.483)	889(62.147)	
former	1525(24.148)	355(23.148)	376(24.728)	418(24.423)	376(24.336)	
now	1240(21.933)	492(33.470)	316(25.084)	227(15.094)	205(13.517)	
UIC(ug/L)						0.426
<100	1888(33.542)	486(33.227)	482(35.444)	457(33.030)	463(32.461)	
100-299	2893(48.780)	703(48.474)	710(46.457)	754(51.771)	726(48.462)	
>=300	1097(17.679)	283(18.298)	275(18.099)	258(15.199)	281(19.078)	
Va(mg/d)	631.242 (12.921)	422.439 (14.572)	577.928 (14.488)	700.939 (19.612)	833.670 (21.192)	<0.0001
Vd(mg/d)	4.694(0.088)	3.542(0.164)	4.603(0.235)	4.998(0.203)	5.688(0.209)	<0.0001
Ve(mg/d)	7.916(0.156)	5.621(0.183)	7.223(0.155)	8.815(0.158)	10.115(0.307)	<0.0001
Se(mg/d)	112.986(1.216)	101.720(1.596)	112.924(2.466)	113.844(1.713)	123.959(2.136)	<0.0001
Zn(mg/d)	12.067(0.188)	10.462(0.405)	11.771(0.282)	12.467(0.199)	13.646(0.334)	<0.0001

^aWeighted mean (SD). ^bActual frequencies (weighted percentages). lnVc, Logarithm-transformed vitamin C; SD, standard deviation; Vc, vitamin C; FT4, free thyroxine; FT3, free triiodothyronine; TSH, thyroid stimulating hormone; TT3, total triiodothyronine; TT4, total thyroxine; TGAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; PIR, poverty to income ratio; BMI, body mass index; UIC, urinary iodine concentration; Va, vitamin A; Vd, vitamin D; Ve, vitamin E; Se, selenium; Zn, zinc.

3 Results

3.1 Baseline participant characteristics

This study included 5,878 US participants aged 20 years or older from NHANES (2007-2012). The baseline characteristics of participants grouped by lnVc quartiles are presented in [Table 1](#). The mean dietary vitamin C intake among these participants was 84.797(2.383) mg/day. For demographic, socioeconomic and dietary condition information, participants in different lnVc quartiles have different Va, Vd, Ve, Zn, Se, gender, race/ethnicity, PIR, annual family income, education level and smoking habits (all $P<0.05$). For thyroid function indices, differences in FT3, TT3, and TT4 were statistically significant (all $P<0.05$).

3.2 The correlation between lnVc and thyroid function

The correlation between lnVc and thyroid hormone levels was illustrated in [Table 2](#). In Model 1, a negative relationship was observed between lnVc and TT4 [$\beta = -0.208$, 95% CI= (-0.321, -0.094), $P<0.001$]. After adjusting for a comprehensive array of factors including age, gender, education level, race/ethnicity, PIR, BMI, UIC, alcohol consumption, annual family income and smoking habits in Model 2, this negative association was remained [$\beta = -0.204$, 95% CI= (-0.312, -0.096), $P<0.001$]. Further adjustments for additional confounders in Model 3, including Va, Vd, Ve, Zn and Se, the negative association also remained significant [$\beta = -0.182$, 95% CI= (-0.305, -0.059), $P= 0.006$].

TABLE 2 The associations between lnVc and thyroid function.

	Model 1		Model 2		Model 3	
	β (95%CI)	P	β (95%CI)	P	β (95%CI)	P
TT4 (ug/dL)						
lnVc	-0.208(-0.321,-0.094)	<0.001	-0.204(-0.312,-0.096)	<0.001	-0.182(-0.305,-0.059)	0.006
Categories						
Q1	ref		ref		ref	
Q2	-0.076(-0.217, 0.065)	0.285	-0.059(-0.193, 0.074)	0.368	-0.05(-0.186, 0.085)	0.445
Q3	-0.114(-0.234, 0.006)	0.061	-0.129(-0.253,-0.005)	0.041	-0.116(-0.242, 0.010)	0.070
Q4	-0.261(-0.375,-0.148)	<0.0001	-0.243(-0.361,-0.126)	<0.001	-0.226(-0.348,-0.105)	<0.001
p for trend		<0.0001		<0.001		0.001
TT3 (nmol/L)						
lnVc	-0.057(-0.100,-0.014)	0.011	-0.03(-0.065, 0.005)	0.093	-0.029(-0.074, 0.016)	0.192
Categories						
Q1	ref		ref		ref	
Q2	-0.027(-0.070, 0.016)	0.214	-0.004(-0.041, 0.033)	0.829	-0.005(-0.042, 0.032)	0.779
Q3	-0.077(-0.127,-0.027)	0.003	-0.044(-0.096, 0.009)	0.102	-0.044(-0.102, 0.014)	0.130
Q4	-0.059(-0.108,-0.009)	0.021	-0.034(-0.078, 0.011)	0.128	-0.035(-0.086, 0.016)	0.172
p for trend		0.004		0.053		0.097
FT4 (pmol/L)						
lnVc	-0.045(-0.211,0.120)	0.584	-0.141(-0.292, 0.011)	0.068	-0.11(-0.259, 0.039)	0.141
Categories						
Q1	ref		ref		ref	
Q2	-0.032(-0.226,0.162)	0.744	-0.052(-0.227, 0.123)	0.544	-0.041(-0.221, 0.139)	0.642
Q3	0.008(-0.203,0.219)	0.940	-0.06(-0.261, 0.141)	0.546	-0.048(-0.252, 0.156)	0.628
Q4	-0.155(-0.339,0.029)	0.096	-0.251(-0.429,-0.073)	0.008	-0.234(-0.395,-0.073)	0.007

(Continued)

TABLE 2 Continued

	Model 1		Model 2		Model 3	
	β (95%CI)	P	β (95%CI)	P	β (95%CI)	P
FT4 (pmol/L)						
p for trend		0.153		0.01		0.008
FT3 (pg/mL)						
lnVc	-0.028(-0.070,0.013)	0.171	-0.008(-0.044, 0.027)	0.631	-0.004(-0.041, 0.032)	0.816
Categories						
Q1	ref		ref		ref	
Q2	-0.058(-0.106,-0.011)	0.017	-0.034(-0.075, 0.006)	0.094	-0.033(-0.074, 0.007)	0.099
Q3	-0.058(-0.101,-0.015)	0.010	-0.014(-0.053, 0.024)	0.449	-0.012(-0.054, 0.029)	0.549
Q4	-0.039(-0.089, 0.012)	0.131	-0.026(-0.070, 0.018)	0.238	-0.022(-0.066, 0.021)	0.298
p for trend		0.12		0.356		0.466
TSH (uIU/mL)						
lnVc	-0.06(-0.250,0.129)	0.524	-0.084(-0.259, 0.091)	0.331	-0.036(-0.217, 0.144)	0.680
Categories						
Q1	ref		ref		ref	
Q2	0.253(-0.229,0.736)	0.296	0.232(-0.249, 0.713)	0.330	0.235(-0.250, 0.720)	0.325
Q3	0.034(-0.195,0.263)	0.767	-0.029(-0.260, 0.201)	0.795	0.007(-0.223, 0.237)	0.950
Q4	-0.01(-0.217,0.196)	0.919	-0.02(-0.203, 0.163)	0.825	0.035(-0.175, 0.245)	0.730
p for trend		0.539		0.413		0.721
TPOAb (IU/mL)						
lnVc	4.689(-5.465,14.843)	0.358	6.032(-6.342,18.406)	0.326	8.694(-6.584,23.971)	0.251
Categories						
Q1	ref		ref		ref	
Q2	1.923(-6.597,10.444)	0.652	1.885(-6.587,10.358)	0.651	3.142(-5.761,12.046)	0.470
Q3	-0.383(-9.269, 8.502)	0.931	-0.7(-9.996, 8.596)	0.878	1.324(-9.152,11.800)	0.795
Q4	7.837(-5.614,21.289)	0.247	9.637(-6.304,25.578)	0.225	12.544(-6.267,31.355)	0.180
p for trend		0.302		0.282		0.220
TGAb (IU/mL)						
lnVc	1.519(-4.200,7.237)	0.596	1.443(-5.134, 8.020)	0.656	3.186(-5.904,12.276)	0.475
Categories						
Q1	ref		ref		ref	
Q2	4.813(-1.857,11.483)	0.153	4.719(-2.304,11.743)	0.179	5.14(-2.369,12.650)	0.169
Q3	5.892(-6.206,17.989)	0.332	5.248(-8.046,18.542)	0.424	6.408(-8.377,21.192)	0.377
Q4	0.266(-3.669, 4.201)	0.892	0.47(-4.412, 5.352)	0.844	2.17(-5.368, 9.709)	0.555
p for trend		0.828		0.862		0.611

(Continued)

TABLE 2 Continued

	Model 1		Model 2		Model 3	
	β (95%CI)	P	β (95%CI)	P	β (95%CI)	P
FT3/FT4						
lnVc	-0.003(-0.024,0.017)	0.746	0.015(-0.005, 0.035)	0.140	0.012(-0.010, 0.033)	0.262
Categories						
Q1	ref		ref		ref	
Q2	-0.015(-0.039,0.010)	0.236	-0.004(-0.028, 0.019)	0.704	-0.006(-0.030, 0.018)	0.607
Q3	-0.017(-0.044,0.009)	0.188	0.006(-0.020, 0.031)	0.639	0.005(-0.022, 0.032)	0.715
Q4	0.007(-0.021,0.034)	0.633	0.022(-0.005, 0.050)	0.110	0.021(-0.007, 0.048)	0.130
p for trend		0.701		0.064		0.077

Model 1 was adjusted no covariates;
Model 2 was adjusted for age, gender, education level, race/ethnicity, PIR, BMI, UIC, alcohol consumption, annual family income, smoking habits.
Model 3 was further adjusted for Va, Vd, Ve, Zn and Se.
CI, confidence interval; Ref, reference.

Furthermore, when lnVc was categorized, the negative trend remained statistically significant (P for trend = 0.001). Compared with individuals in quartile 1(Q1), those in quartile 4(Q4) consistently displayed reduced TT4 levels in all models.

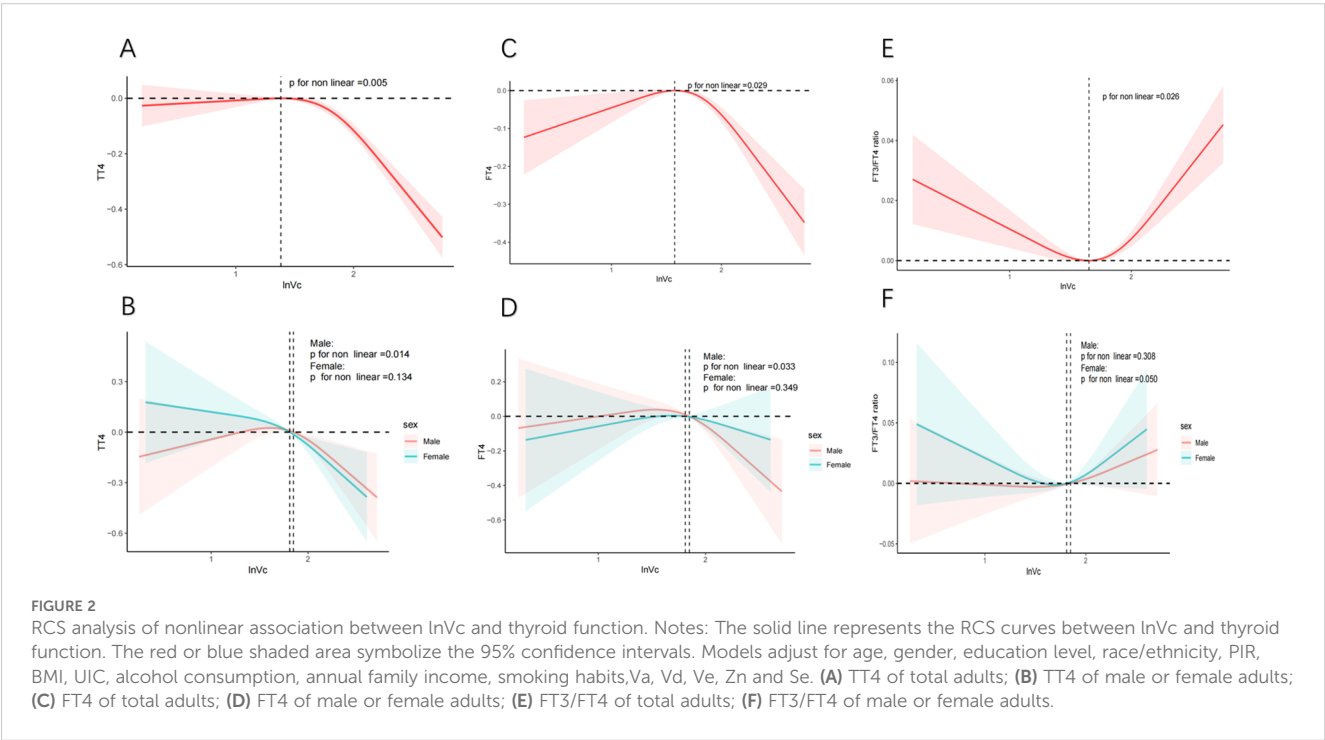
In Model 1, lnVc exhibited a negative correlation solely with TT3 [β = -0.057, 95% CI=(-0.100, -0.014), P =0.011]. When lnVc was treated as a categorical variable in Model 1, this inverse relationship remained significant (P for trend = 0.004). However, upon adjusting for potential confounders in Models 2 and 3, this association was no longer observed.

In contrast, when lnVc was used as a categorical variable, the participants in quartile 4(Q4) had a lower FT4 than those in quartile 1 (Q1) in Model 2 (P for trend = 0.01) and Model 3 (P for trend = 0.008).

3.3 RCS analysis

The relationship between lnVc and thyroid hormone levels was further scrutinized through the application of RCS curves. Initially, upon adjusting for potential confounding factors, we observed a reverse U-shaped curve association (P for nonlinear = 0.029) between lnVc and FT4 (Figure 2C). Regarding the strong inverted U-shaped relationship, Figure 2C showed an increase of FT4 levels with increasing lnVc levels, which reached the highest around 1.575 and then decreased thereafter. In addition, the inflection point for lnVc levels in females was 1.811 (P for nonlinear = 0.033) (Figure 2D).

Second, an U-shaped curve relationship was noticed (P for nonlinear = 0.026) between lnVc and FT3/FT4 ratio (Figure 2E).



When the lnVc level was 1.652, the FT3/FT4 ratio was the lowest. And, there was no significant difference between the genders (Figure 2F).

The correlation between lnVc and TT4 exhibited a distinct reversed L-shaped curve pattern in total participants(*P* for nonlinear = 0.005, Figure 2A) and male adults (*P* for nonlinear = 0.014, Figure 2B). However, we did not find a nonlinear correlation among TSH, FT3, TT3, TPOAb, TgAb, and lnVc (Supplementary Figures S2).

3.4 Subgroup analysis

Our research revealed a negative correlation between lnVc and TT4. To further explore this correlation among diverse population groups, we conducted a subgroup analysis utilizing multivariable linear regression. The outcomes of this stratified analysis were presented in Figure 3 and Table 3.

In the subgroup analysis (Figure 3), alcohol consumption plays a pivotal role in modulating the association between lnVc and TT4. Remarkably, this significant association was more apparent in subjects with lower alcohol consumption[β= -0.151, 95% CI=

(-0.267, -0.035), *P*=0.013, *P* for interaction = 0.042]. Furthermore, when lnVc was used as a categorical variable (Table 3), the groups with lower alcohol consumption (< 50 g/d) and higher lnVc consumption showed a more substantial reduction in TT4 levels compared to other groups[β= -0.186, 95% CI= (-0.304, -0.068), *P* = 0.002, *P* for interaction = 0.004].

4 Discussion

This cross-sectional study was conducted to investigate the potential association between vitamin C intake and thyroid function among 5,878 participants from the NHANES between 2007 and 2012. The findings of this study demonstrated a significant inverse correlation between vitamin C intake and TT4 levels. Furthermore, the subgroup analysis and interaction terms suggested that the association was more pronounced in subjects with lower alcohol consumption. In addition, our investigation also uncovered a nonlinear relationship between vitamin C and thyroid function markers, including TT4, FT4, and the FT3/FT4 ratio.

In previous studies, most scholars focused on studying the relationship between vitamin C intake and thyroid cancers (16,

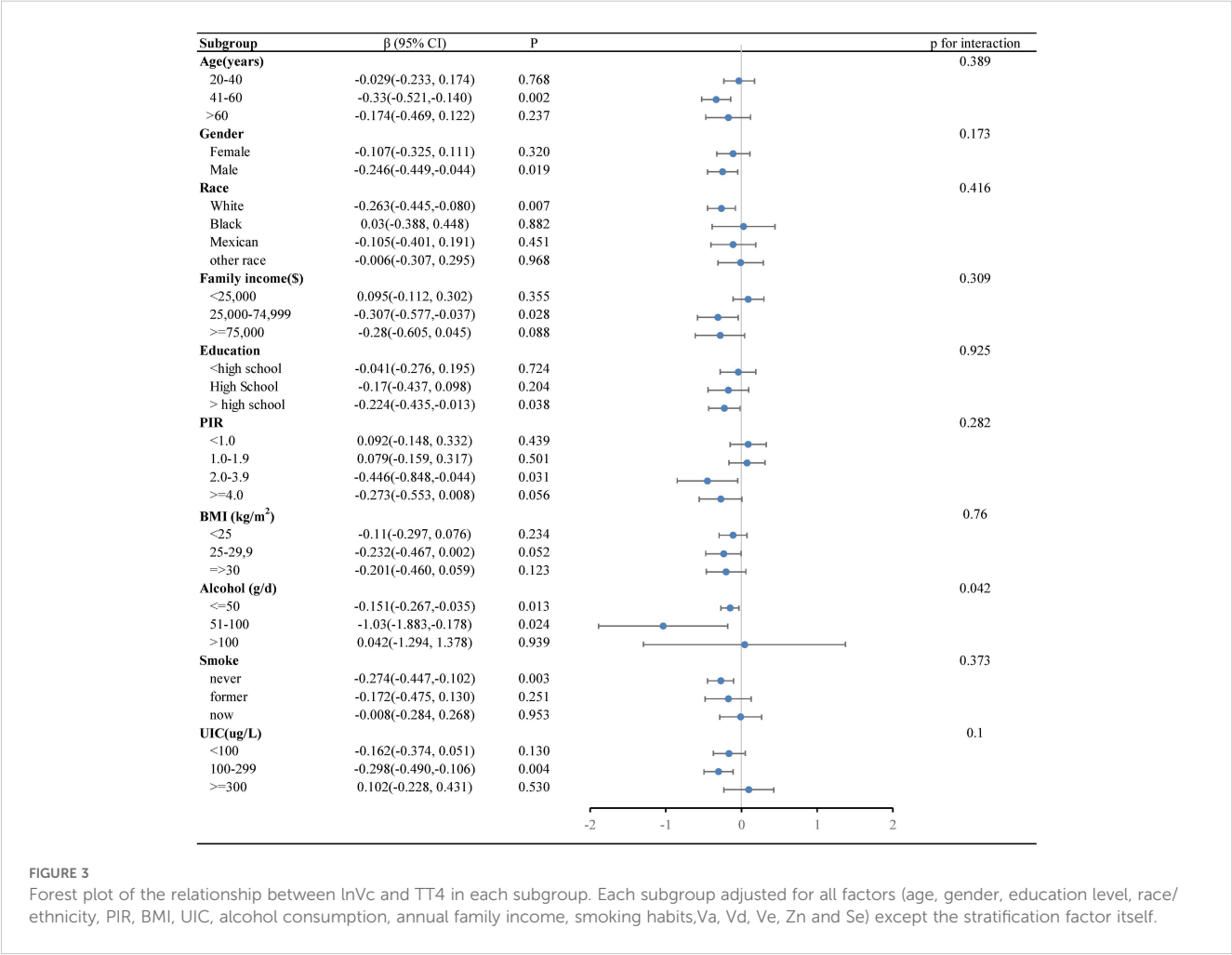


TABLE 3 Relationship between lnVc and TT4 in each subgroup.

Characeristics	lnVc quartiles					
	Q1	Q2	Q3	Q4	p for trend	p for interaction
Age(years)						0.291
20-40	ref	0.176(-0.066, 0.418)	0.066(-0.211, 0.343)	-0.074(-0.303, 0.156)	0.395	
41-60	ref	-0.216(-0.439, 0.008)	-0.285(-0.557,-0.014)	-0.373(-0.631,-0.115)	0.016	
>60	ref	-0.171(-0.445, 0.103)	-0.188(-0.451, 0.075)	-0.197(-0.515, 0.120)	0.224	
Gender						0.347
Female	ref	0.045(-0.165, 0.255)	-0.117(-0.329, 0.096)	-0.165(-0.373, 0.042)	0.068	
Male	ref	-0.138(-0.354, 0.079)	-0.112(-0.304, 0.079)	-0.273(-0.513,-0.033)	0.045	
Race						0.124
White	ref	-0.101(-0.274, 0.072)	-0.196(-0.366,-0.027)	-0.354(-0.525,-0.183)	<0.001	
Black	ref	-0.246(-0.594, 0.103)	0.097(-0.299, 0.492)	0.003(-0.437, 0.444)	0.616	
Mexican	ref	0.116(-0.255, 0.488)	0.06(-0.267, 0.387)	0.033(-0.328, 0.393)	0.983	
other race	ref	0.306(-0.225, 0.836)	0.055(-0.306, 0.416)	0.095(-0.238, 0.428)	0.998	
Family income(\$)						0.782
<25,000	ref	0.039(-0.265, 0.344)	0.015(-0.300, 0.331)	0.01(-0.297, 0.318)	0.97	
25,000-74,999	ref	-0.109(-0.362, 0.145)	-0.095(-0.366, 0.176)	-0.32(-0.608,-0.031)	0.055	
>=75,000	ref	-0.035(-0.436, 0.365)	-0.25(-0.600, 0.100)	-0.295(-0.682, 0.093)	0.047	
Education						0.665
<high school	ref	0.006(-0.288, 0.300)	0.144(-0.189, 0.477)	-0.087(-0.394, 0.220)	0.91	
High School	ref	-0.129(-0.466, 0.207)	-0.034(-0.280, 0.212)	-0.217(-0.541, 0.107)	0.254	
> high school	ref	-0.047(-0.239, 0.145)	-0.205(-0.421, 0.011)	-0.26(-0.473,-0.048)	0.01	
PIR						0.944
<1.0	ref	0.06(-0.375, 0.494)	0.061(-0.403, 0.524)	0.082(-0.319, 0.482)	0.713	
1.0-1.9	ref	0.087(-0.194, 0.368)	0.086(-0.212, 0.384)	-0.075(-0.333, 0.184)	0.7	
2.0-3.9	ref	-0.226(-0.547, 0.096)	-0.263(-0.630, 0.104)	-0.332(-0.809, 0.146)	0.172	
>=4.0	ref	-0.009(-0.335, 0.318)	-0.185(-0.483, 0.112)	-0.321(-0.664, 0.022)	0.022	
BMI (kg/m ²)						0.692
<25	ref	0.069(-0.201, 0.338)	0.014(-0.185, 0.213)	-0.159(-0.378, 0.060)	0.11	
25-29,9	ref	-0.182(-0.499, 0.135)	-0.261(-0.554, 0.031)	-0.238(-0.515, 0.039)	0.075	
=>30	ref	-0.068(-0.335, 0.198)	-0.103(-0.354, 0.148)	-0.292(-0.581,-0.003)	0.056	
Alcohol (g/d)						0.004
<=50	ref	0.018(-0.126, 0.162)	-0.093(-0.214, 0.028)	-0.186(-0.304,-0.068)	0.002	
51-100	ref	-1.117(-2.038,-0.195)	-0.788(-1.887, 0.312)	-1.011(-2.206, 0.184)	0.173	
>100	ref	-0.775(-1.922, 0.371)	0.772(-0.431, 1.975)	-0.135(-1.783, 1.512)	0.908	
Smoke						0.937
never	ref	-0.084(-0.293, 0.125)	-0.163(-0.384, 0.058)	-0.276(-0.484,-0.069)	0.012	
former	ref	-0.014(-0.308, 0.281)	-0.17(-0.444, 0.104)	-0.168(-0.513, 0.178)	0.212	
now	ref	-0.011(-0.296, 0.274)	0.058(-0.283, 0.399)	-0.151(-0.518, 0.216)	0.599	

(Continued)

TABLE 3 Continued

Characeristics	lnVc quartiles					
	Q1	Q2	Q3	Q4	p for trend	p for interaction
UIC						0.734
<100	ref	-0.048(-0.354, 0.258)	-0.1(-0.324, 0.123)	-0.223(-0.519, 0.073)	0.122	
100-299	ref	-0.132(-0.346, 0.083)	-0.188(-0.409, 0.033)	-0.312(-0.534,-0.090)	0.009	
>=300	ref	0.129(-0.261, 0.519)	0.068(-0.318, 0.455)	0.016(-0.343, 0.376)	0.971	

Data are presented as β (95% CI). Each subgroup adjusted for all factors (age, gender, education level, race/ethnicity, PIR, BMI, UIC, alcohol consumption, annual family income, smoking habits, Va, Vd, Ve, Zn and Se) except the stratification factor itself. CI, confidence interval; Ref, reference.

26), goitre (17), benign thyroid diseases (27), hyperthyroidism and hypothyroidism (28), with few exploring the effects of vitamin C intake on thyroid function in normal individuals. Karimi et al. (29) showed that the concentration of anti-thyroid peroxidase antibodies significantly decreased in autoimmune thyroiditis patients treated with vitamin C compared to those receiving a placebo. Jubiz et al. (30) evaluated the effect of vitamin C administration in hypothyroid patients with elevated TSH levels, showing that vitamin C can increase serum T4, T3, and decrease TSH. The findings of these studies support the potential role of vitamin C in the treatment of thyroid-related diseases.

Our research revealed a significant inverse correlation between vitamin C intake and TT4, but we did not observe significant differences in TPOAb and TSH levels between the four groups. This could be attributed to several factors. First, it is possible that the participants in our study did not have sufficient vitamin C intake. Low levels of vitamin C in the body may limit its beneficial effects on thyroid function, even with supplementation. Furthermore, the dose and duration of vitamin C intake in our study may have been insufficient to observe significant changes in TPOAb and TSH levels. In the studies mentioned, participants were administered 500mg of vitamin C daily for months. In contrast, our study only used the average vitamin C intake over a period of two days. Longer-term and higher-dose vitamin C supplementation may be necessary to observe significant improvements in thyroid function and auto-antibody levels. Moreover, our study population consisted of individuals with normal thyroid function. It is possible that the effects of vitamin C on thyroid function may be more pronounced in individuals with pre-existing thyroid conditions.

In our study, we found an inverted U-shaped curve relationship between vitamin C and FT4, while an U-shaped curve relationship was noticed between vitamin C and FT3/FT4 ratio. An increased of FT4 levels with increasing lnVc levels, which reached the highest around 1.575 and then decreased thereafter. When the lnVc levels were less than 1.652, the FT3/FT4 ratio decreased with the increase of lnVc levels. The Food and Drug Administration (FDA) increased the vitamin C recommendations to 90 mg/day for men and 75 mg/day for women. And the study suggests that raising the existing daily recommended amount of vitamin C to 200 milligrams per day would have a positive impact on the function of the immune system

(31). Excessive intake of vitamin C may also have adverse side effects, such as kidney stones, nausea, and diarrhea (32, 33).

The subgroup analysis and interaction terms indicated that the association between vitamin C intake and TT4 levels was more apparent in subjects with lower alcohol consumption. This finding suggests that alcohol consumption plays a pivotal role in modulating the association between vitamin C and thyroid function. Previous studies have shown that vitamin C levels are decreased in alcoholics, excessive alcohol consumption can interfere with the absorption and utilization of vitamin C, leading to its depletion in the body (34–37).

Moreover, gender-specific subgroup analysis showed that women are more likely to be affected from vitamin C intake in maintaining healthy thyroid function. However, no significant interaction effects were observed. Previous studies have demonstrated that the prevalence of thyroid disorders, including hypothyroidism, hyperthyroidism (38), thyroid nodules (39) and thyroid cancer (40), are vary significantly between men and women. These finding could be attributed to gender-related differences in thyroid physiology and metabolism, as well as dietary habits and hormonal status (41–43). Metsios et al. (44) found that a brief exposure to moderate passive smoke significantly increased the TT3 and FT4 levels compared to the control group. Gruppen et. al (45) studies also demonstrated that smokers with ≤ 20 cigarettes per day were associated with higher serum FT4, FT3 and lower TSH compared to nonsmokers. In our study, subgroup analysis revealed that the impact of vitamin C on thyroid function was particularly evident among non-smokers. This suggests that smokers may benefit more from increasing their intake of vitamin C to maintain healthy thyroid function.

However, there are also limitations to using the NHANES database for this research. Firstly, the vitamin C intake relies on self-reported dietary intake data, which may be subject to inaccuracies and biases. This can affect the reliability and validity of the findings. Secondly, even though we have tried our best to eliminate potential confounders, there are still some factors that cannot be fully controlled. These factors may have an impact on the relationship between vitamin C and thyroid function. Finally, we are unable to firmly establish the precise causal relationship between vitamin C intake and thyroid function due to the retrospective cross-sectional nature of this study.

5 Conclusion

In conclusion, this cross-sectional study suggested the association between vitamin C and thyroid function. A high level of vitamin C intake is associated with a decrease in TT4 levels among adults in the United States, and the association was more pronounced in subjects with lower alcohol consumption. In addition, our analysis revealed a nonlinear correlation between the intake of vitamin C and the levels of TT4, FT4, and FT3/FT4 ratio. In the future, more studies are needed to further explore the causal relationship.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving humans were approved by the National Center for Health Statistics. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JW: Conceptualization, Data curation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. CJ: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. QW: Formal analysis, Investigation, Methodology, Writing – review & editing. XL: Data

curation, Funding acquisition, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fendo.2024.1462251/full#supplementary-material>

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Association between night eating frequency and thyroid function and sensitivity: a cross-sectional study from the NHANES database

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Thyroid function is closely linked to circadian rhythms, but the relationship between the frequency of night eating and thyroid function remains unclear. Our study aimed to investigate the association between night eating frequency and its impact on thyroid function and sensitivity. This study included 6093 participants from the U.S. National Health and Nutrition Examination Survey (2007–2012). Night eating behavior was assessed through 24-hour dietary recall, with night eating frequency calculated on the basis of food intake between 10:00 PM and 4:00 AM. The thyroid hormone indices examined included T3, T4, FT3, FT4, TSH, TGA, Tg, and TPOAb, whereas thyroid hormone sensitivity was assessed via indices such as the FT3/FT4, TSHI, TT4RI, and TFQI. The associations between night eating frequency and thyroid function were analyzed via weighted univariate and multivariate linear regression analyses. Subgroup analyses and interaction test analyses were also employed to test this correlation. Compared with individuals who did not eat at night, those who ate more frequently at night had higher levels of Tg (OR 1.223 [95% CI 1.048, 1.429], p trend=0.015) but lower levels of T3 (OR 0.728 [95% CI 0.611, 0.868], p trend=0.235) and TPOAb (OR 0.728 [95% CI 0.611, 0.868], p trend=0.235). Subgroup analysis indicated that this association between Tg and night eating was stronger in the DM group (Tg: OR 1.49 [95% CI 1.15, 1.93]), p interaction=0.022) and that the association between TPOAb and night eating was stronger in the group without DM (TPOAb: OR 0.9 [95% CI 0.82, 0.97]), p interaction=0.003). Our findings suggest a significant association between night eating frequency and thyroid function. However, no statistically significant differences were found in thyroid sensitivity based on night eating frequency. Despite these findings, the hormone fluctuations observed were within normal clinical ranges. Further rigorously designed studies are needed to establish a causal relationship between night eating frequency and thyroid function.

KEYWORDS

thyroid function, thyroid sensitivity, circadian rhythms, night eating, cross-sectional study 31 32

1 Introduction

Thyroglobulin (Tg) is synthesized and stored within the thyroid follicular lumen (1). When the body requires thyroid hormone synthesis, thyroglobulin undergoes iodination and enzymatic digestion, leading to the production of thyroxine (T4) and triiodothyronine (T3). Thyroid peroxidase (TPO) is the key enzyme responsible for this biosynthesis (2). T3 and T4 are essential for human growth, development, and metabolism; they act on multiple target organs and play crucial roles in thermogenesis, protein synthesis, glucose metabolism, and nervous system development (3). The biologically active forms of these hormones are free T3 (FT3) and free T4 (FT4). The secretion of thyroid hormones, which includes thyroid-stimulating hormone (TSH) and thyrotropin-releasing hormone (TRH), is regulated by the hypothalamic–pituitary–thyroid axis. In addition to primary hormones, several calculated indices are commonly used to assess thyroid sensitivity: FT3/FT4 for evaluating peripheral thyroid hormone sensitivity and the thyroid feedback quantile-based index (TFQI), TSH index (TSHI), and thyrotroph T4 resistance index (TT4RI) for assessing central thyroid hormone sensitivity (4–6).

Research on circadian rhythms indicates that in metabolically healthy adults, various metabolic cycles, insulin secretion and sensitivity, and energy expenditure follow a rhythmic pattern (7). Maintaining alignment with circadian rhythms in sleep and diet can reduce the risk of diseases (8). On the other hand, night eating has been shown to impair glucose tolerance (9), trigger mental disorders (10), and accelerate weight gain (11) by disrupting these circadian rhythms. The consumption of foods, such as high-fat, high-sugar, high-salt snacks at night, can further increase total cholesterol and low-density lipoprotein (LDL) cholesterol levels while reducing fat oxidation (12, 13). Therefore, night eating may disrupt the balance of endocrine hormone secretion.

Several studies have demonstrated that disruptions in circadian rhythms can interfere with TSH secretion, thereby affecting thyroid function (14). However, no studies have specifically examined the impact of night eating frequency on thyroid indicators. To address this gap, our study explored the relationships between night eating frequency and both thyroid function and thyroid sensitivity.

2 Materials and methods

2.1 Study population

The data from 2007–2008, 2009–2010, and 2011–2012 were used for this study ($n = 30442$). We excluded 20039 individuals whose dietary recall data were unreliable and thyroid hormone indices were missing, 1366 participants younger than 18 years of age, 67 pregnant participants, 1043 participants with heart failure and cancer, 348 participants whose BMI and waist circumference data were missing, and 348 participants with thyroid disease. Overall, our sample consisted of 6093 participants (Figure 1).

2.2 Dietary assessment

The baseline dietary intake data from 2007–2012 were gathered from the initial 24-hour dietary recall interview. This first recall was conducted in person by trained personnel at the NHANES mobile examination centers. Standardized protocols and measurement tools were used to assess the volume and dimensions of the food. During the interviews, the participants were asked to provide details about the quantity and timing of each food and beverage they consumed. The nutrient values were calculated via the Food and Nutrient Database for Dietary Studies (FNDDS).

2.3 Main exposures

The main exposure was the frequency of night eating. Night eating was defined as food consumption between 10:00 PM and 4:00 AM, on the basis of the natural light cycle rhythm in this study. The frequency of night eating was categorized into three groups: “No night eating”, “One time”, and “Two times and over”.

2.4 Thyroid function and sensitivity

In this study, the levels of TT3, FT3, and TT4 were measured via a competitive binding immunoassay. Free T4 was determined through a two-step enzyme immunoassay. Sensitive human thyroid-stimulating hormone was detected via a two-site “sandwich” immunoassay. TgAb (TGA) and TPOAb (IU/mL) levels were evaluated via a sequential two-step immunoassay “sandwich” assay, whereas Tg levels (ng/ml) were measured via a simultaneous one-step “sandwich” assay. Detailed instructions for sample collection and processing are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). The following is the calculation method for sensitivity to thyroid hormone indices:

FT3/FT4 is achieved by $FT3/FT4 \text{ ratio} = FT3/FT4$, TSHI is achieved by $TSHI = \ln TSH + 0.1345 \times FT4$, TT4RI is achieved by $TT4RI = FT4 \times TSH$, TFQI is achieved by $TFQI = \text{cumulative distribution function (cdf} FT4) - (1 - \text{cdf} TSH)$.

2.5 Covariates

The covariates included age (years), sex (male/female), race/ethnicity (Mexican American/non-Hispanic Black/non-Hispanic White/other), education (below high school/high school/above high school), income (poor/not poor), smoking status (never/former/now), drinking status (yes/no), body mass index ($<25/25\sim29/\geq30$), physical activity (yes/no), hours of sleep ($<6h/6\sim8h/>8h$), hyperlipidemia (yes/no), hypertension (yes/no), diabetes (DM, yes/no), obesity (yes/no), abdominal obesity (Aobesity, yes/no), UIC (urinary iodine concentration, <100 (deficient), $100\sim299$ (normal), ≥300 $\mu\text{g/L}$ (excess)) (15), glycohemoglobin (%), TG

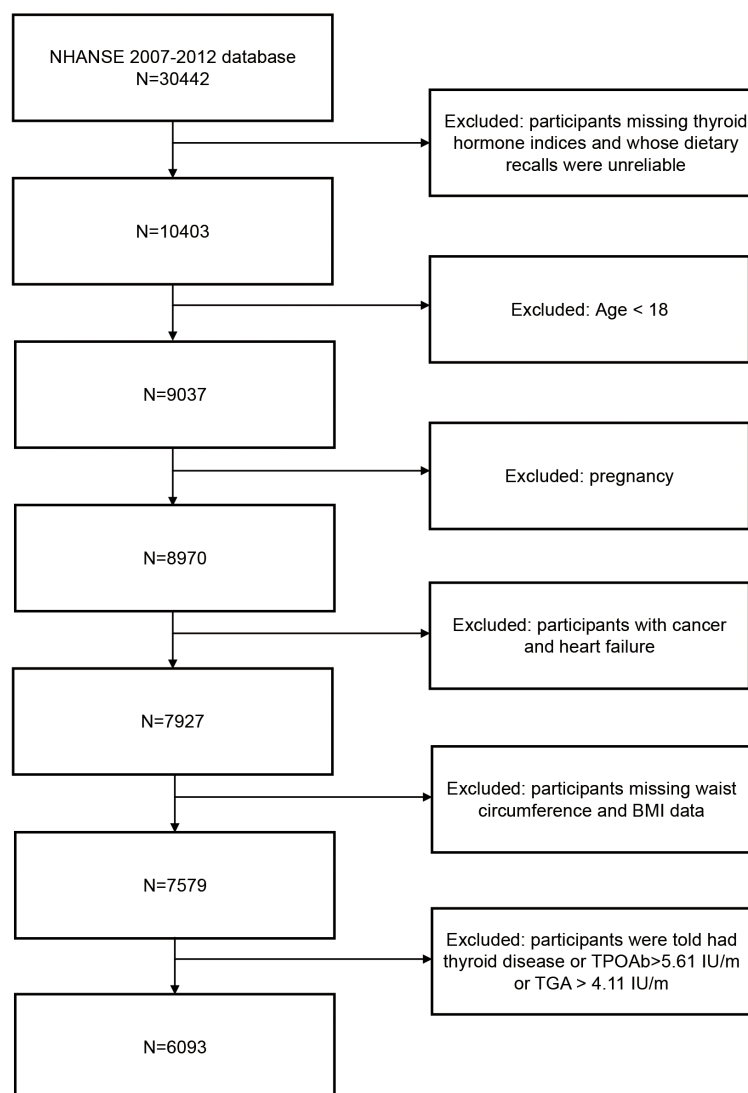


FIGURE 1
Flowchart of the study population.

(mmol/L), Ur (mmol/L), Cr (μ mol/L), TC (mg/dL), LDL (mg/dL), HDL (mg/dL), neutrophil-lymphocyte ratio (NLR). Obesity participants were those whose BMI ≥ 30 (16). Poor participants were those whose ratio of family income to poverty < 1 . For the definition of drinking status, all participants needed to answer this question, "Had at least 12 alcohol drinks/1 yr?". Those who answered "No" were labeled as "No drinking," while those who answered "Yes" were labeled as "drinking". Waist circumference (WC) < 102 cm was considered normal for men, and WC ≥ 102 cm was considered Aobesity. WC < 88 cm was considered normal for women, and WC ≥ 88 cm was considered Aobesity (17).

Hypertension was defined as a diagnosis of hypertension, the use of antihypertensive drugs, a systolic blood pressure ≥ 140 mm Hg, or a diastolic blood pressure ≥ 90 mm Hg. DM was defined as self-reported, diagnosed diabetes, glycohemoglobin $\geq 6.5\%$, or fasting plasma glucose ≥ 126 mg/dL. Hyperlipidemia was defined

as taking antihyperlipidemic drugs, TG ≥ 200 mg/dL, TC ≥ 150 mg/dL, HDL < 40 mg/dL for males and < 50 mg/dL for females, and LDL ≥ 130 mg/dL. Heart failure participants were those who were informed that they had heart failure. The cancer participants were those who had been told that they had cancer. Thyroid disease participants were those who were told that they had cancer-related thyroid disease, or thyroid antibody abnormalities were defined as TPOAb ≥ 5.61 IU/ml or TGA ≥ 4.11 IU/ml.

The Dietary Inflammatory Index (DII) is a credible measure of the extent to which dietary factors contribute to an individual's inflammatory response (18). The DII score of night eating was calculated from the 24-h dietary recall data according to the calculation method described in the R package dietaryindex (19). In this study, we used total alcohol, beta-carotene, caffeine, carbohydrate, cholesterol, energy, fat, fiber, folic acid, vitamins (A, C, E, B1, B12), iron, zinc, magnesium, monounsaturated,

polyunsaturated and saturated fatty acids, niacin, ALA (octadecatrienoic acid), EPA (alpha-linolenic acid), protein, selenium and zinc to calculate DII scores.

2.6 Statistical analyses

The complex survey design factors involved in the NHANES, including weights, clustering, and stratification, were all considered as recommended by the NCHS analytical guidelines. Data analysis was performed via R version 4.3.0. Weighted multiple linear regression model were applied to evaluate the associations of the frequency of night eating with thyroid function and sensitivity, including FT3, FT4, TT3, TT4, TSH, TG, TGA, TPOAb, FT3/FT4, TSHI, TT4RI and TFQI (no-night eating group as a reference), in three different model. The results are expressed as OR with 95% confidence intervals (CIs). We adjusted for no variable in Model 1. Model 1 is the unadjusted model. Model 2 was adjusted for baseline sex, age, BMI, race, marital status, family income and education. Model3 were further adjusted for baseline alcohol consumption, smoking status, physical activity, the DII of night eating, urinary iodine concentration, DM status, hypertension status, obesity status, hyperlipidemia status, abdominal obesity status and =UIC=. Owing to the presence of outliers in the data, some data were log-transformed, including T3, T4, TSH, Tg, TGA, TPOAb and TT4RI. We performed subgroup analyses with categorical

variables, including sex, age, BMI, race, alcohol consumption, smoking status, physical activity, DII of night eating, UIC, DM, hypertension, obesity, hyperlipidemia, and Aobesity. We performed interaction term tests to check for heterogeneity between subgroups. Weighted multiple linear regression model were applied to investigate the correlations among the frequency of night eating and biochemical variables, including serum urea nitrogen (Ur), serum creatinine (Cr), HDL, LDL, TC, TG, and the neutrophil-lymphocyte ratio (NLR). The results are expressed as OR values with 95% CIs. Three model (model 1, 2, and 3) were adjusted as described above. A two-tailed p value of less than 0.05 was considered significant. The baseline characteristics are expressed as the means $\pm$ Standard error of mean (SME) or numbers (percentages).

3 Results

3.1 Baseline characteristics of the participants

This study included 6093 participants from NHANES 2007–2012. The baseline characteristics of the participants across night eating frequencies are shown in [Table 1](#). In terms of the frequency of night eating, compared with participants who did not eat at night, those who had more frequent night eating behavior were more

TABLE 1 Baseline characteristics of the participants.

	Overall (n=6093)	No night eating (n=4829)	One time (n=515)	Two times and over (n=522)	p value
Age					
18~34	2143(35.18)	1606(33.26)	215(41.81)	268(51.46)	<0.001
35~64	3332(54.68)	2693(55.77)	255(49.61)	241(46.14)	
≥65	618(10.14)	530(10.97)	44(8.57)	13(2.4)	
BMI					
<25	2068(33.94)	1593(32.98)	187(36.35)	204(39)	0.219
25~29	1694(27.8)	1371(28.4)	124(24.04)	130(24.96)	
≥30	2331(38.26)	1865(38.62)	204(39.61)	188(36.04)	
Gender					
Male	3313(54.38)	2556(52.93)	295(57.26)	335(64.09)	0.001
Female	2780(45.62)	2273(47.07)	220(42.74)	187(35.91)	
Race					
Mexican American	563(9.24)	465(9.62)	40(7.83)	39(7.43)	<0.001
Non-Hispanic White	3991(65.5)	3218(66.64)	329(63.88)	314(60.09)	
Non-Hispanic Black	737(12.1)	554(11.48)	72(14.07)	96(18.3)	
Other	801(13.15)	592(12.26)	73(14.22)	74(14.19)	
Marital					
Yes	3699(60.71)	3020(62.54)	270(52.47)	256(49.02)	<0.001

(Continued)

TABLE 1 Continued

	Overall (n=6093)	No night eating (n=4829)	One time (n=515)	Two times and over (n=522)	p value
Marital					
No	2394(39.29)	1809(37.46)	245(47.53)	266(50.98)	
PIR					
Poor	891(14.63)	662(13.71)	92(17.87)	96(18.4)	0.027
Not poor	5202(85.37)	4167(86.29)	423(82.13)	426(81.6)	
Education					
Below high school	388(6.37)	324(6.7)	24(4.65)	16(3.1)	0.005
High School	779(12.79)	621(12.85)	75(14.53)	55(10.58)	
Above high School	4926(80.84)	3885(80.45)	416(80.82)	451(86.31)	
Smoke					
Never	3437(56.41)	2782(57.6)	272(52.8)	257(49.25)	0.001
Former	1285(21.09)	1032(21.38)	108(20.95)	95(18.15)	
Now	1372(22.51)	1015(21.02)	135(26.26)	170(32.6)	
Drink					
Yes	4331(71.08)	3487(72.2)	379(73.64)	375(71.89)	0.842
No	1762(28.92)	1342(27.8)	136(26.36)	147(28.11)	
Activity					
No	3244(53.24)	2569(53.19)	296(57.45)	251(48.08)	0.053
Yes	2849(46.76)	2260(46.81)	219(42.55)	271(51.92)	
Sleep time					
<6 h	869(14.27)	647(13.39)	91(17.67)	98(18.72)	0.021
6~8 h	4848(79.56)	3880(80.35)	394(76.56)	390(74.77)	
>8 h	376(6.17)	302(6.26)	30(5.76)	34(6.51)	
Hypertension					
Yes	1869(30.67)	1501(31.09)	156(30.27)	138(26.41)	0.243
No	4224(69.33)	3328(68.91)	359(69.73)	384(73.59)	
Hyperlipidemia					
Yes	3892(63.87)	3129(64.8)	303(58.77)	305(58.35)	0.032
No	2201(36.13)	1700(35.2)	212(41.23)	217(41.65)	
Obesity					
Yes	1970(32.34)	1580(32.71)	181(35.14)	150(28.66)	0.169
No	4123(67.66)	3249(67.29)	334(64.86)	372(71.34)	
AObesity					
Yes	4132(67.81)	3330(68.96)	337(65.44)	327(62.55)	0.065
No	1961(32.19)	1499(31.04)	178(34.56)	195(37.45)	
DM					
Yes	598(9.81)	465(9.62)	60(11.58)	40(7.65)	0.269
No	5495(90.19)	4364(90.38)	455(88.42)	482(92.35)	

(Continued)

TABLE 1 Continued

	Overall (n=6093)	No night eating (n=4829)	One time (n=515)	Two times and over (n=522)	p value
UIC					
Deficient	2020(33.15)	1624(33.62)	169(32.79)	167(32)	0.679
Normal	1960(32.17)	1572(32.55)	156(30.29)	163(31.27)	
Excessive	2113(34.68)	1634(33.83)	190(36.92)	192(36.73)	
DII	0.48 ± 0.02	0 ± 0	2.17 ± 0.09	3.62 ± 0.04	<0.001
T3	1.83 ± 0.01	1.83 ± 0.01	1.81 ± 0.02	1.83 ± 0.02	0.756
T4	7.74 ± 0.02	7.75 ± 0.02	7.64 ± 0.06	7.71 ± 0.07	0.195
FT3	3.23 ± 0.01	3.22 ± 0.01	3.26 ± 0.02	3.32 ± 0.02	<0.001
FT4	0.79 ± 0	0.79 ± 0	0.79 ± 0.01	0.79 ± 0.01	0.97
TSH	1.75 ± 0.01	1.75 ± 0.02	1.83 ± 0.07	1.64 ± 0.04	0.044
TGA	0.66 ± 0	0.67 ± 0.01	0.64 ± 0.01	0.65 ± 0.01	0.094
Tg	14.83 ± 0.45	14.81 ± 0.55	14.25 ± 0.67	15.74 ± 0.71	0.004
TPOAb	0.86 ± 0.01	0.88 ± 0.01	0.77 ± 0.03	0.77 ± 0.04	0.07
FT3/FT4	4.2 ± 0.01	4.19 ± 0.01	4.24 ± 0.03	4.31 ± 0.03	0.006
TSHI	1.76 ± 0.01	1.77 ± 0.01	1.78 ± 0.03	1.7 ± 0.03	0.041
TT4RI	173.36 ± 1.45	174.38 ± 1.59	179.02 ± 6.44	161.85 ± 4.48	0.008
TFQI	-0.06 ± 0	-0.07 ± 0	-0.06 ± 0.01	-0.07 ± 0.01	0.882
Ur	4.46 ± 0.02	4.49 ± 0.02	4.31 ± 0.07	4.29 ± 0.06	0.079
Cr	76.62 ± 0.28	76.56 ± 0.32	78.33 ± 0.92	77.2 ± 0.71	0.04
Glycohemoglobin	5.53 ± 0.01	5.53 ± 0.01	5.53 ± 0.04	5.49 ± 0.04	0.008
TC	5.04 ± 0.01	5.07 ± 0.02	4.94 ± 0.05	4.89 ± 0.05	0.002
TG	1.78 ± 0.02	1.74 ± 0.02	1.83 ± 0.07	1.9 ± 0.08	0.799
LDL	2.97 ± 0.01	2.99 ± 0.01	2.91 ± 0.04	2.79 ± 0.04	0.038
HDL	1.34 ± 0.01	1.35 ± 0.01	1.3 ± 0.02	1.3 ± 0.02	0.031
NLR	2.13 ± 0.01	2.12 ± 0.02	2.18 ± 0.05	2.06 ± 0.05	0.068

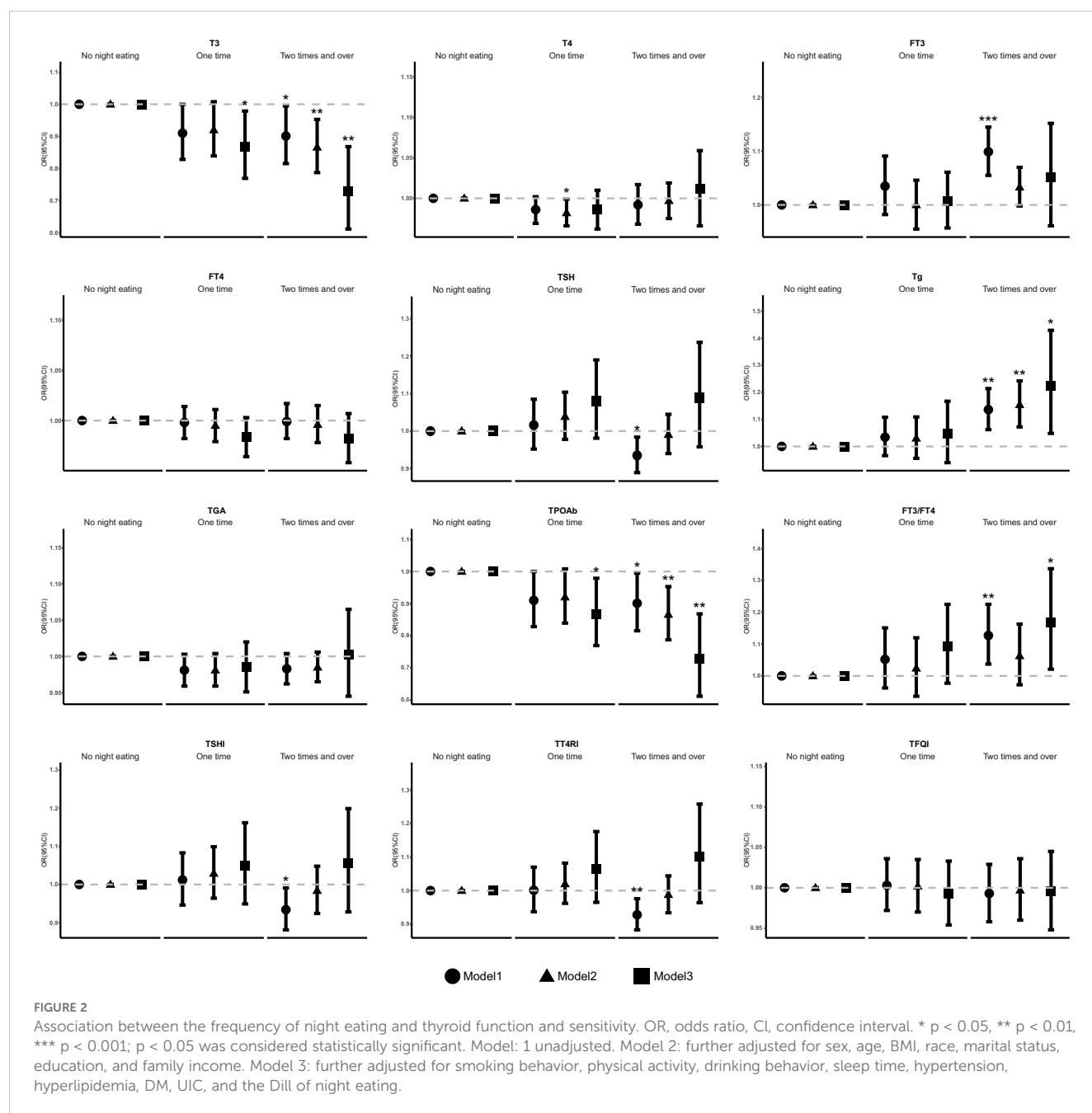
Continuous variables are presented as the means ± SEM, and p values were calculated via survey-weighted linear regression analysis. The categorical variables are presented as percentages, and p values were calculated via the survey-weighted chi-square test. BMI, body mass index; PIR, poverty income ratio; BMI, body mass index; Aobesity, abdominal obesity; DM, DM; UIC, urinary iodine concentration; DII, dietary Inflammatory Index of night eating; TT3, total triiodothyronine; TT4, total thyroxine; FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid-stimulating hormone; Tg, thyroglobulin; TGA, thyroglobulin antibodies; TPOAb, thyroid peroxidase antibodies; FT3/FT4, FT3-FT4 ratio; TSHI, thyroid-stimulating hormone; TT4RI, thyrotroph thyroxine resistance Index; TFQI, thyroid feedback quantile-based index; Ur, serum urea nitrogen; Cr, serum creatinine; TC, total cholesterol; TG, total triglycerides; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; NLR, neutrophil-lymphocyte ratio.

likely to be young, be male, Non-Hispanic Black, single, and poor, have higher levels of education, like smoking, sleep less than 6 hours, be less likely to have hyperlipidemia, have higher DII levels of night eating, have higher FT3, have lower TSH, have higher Tg, higher FT3/FT4, lower TSHI, lower TT4FRI, higher Cr, lower glycohemoglobin, lower TC, lower LDL and lower HDL at baseline.

3.2 The association between frequency of night eating and thyroid function and thyroid hormone sensitivity

The association of frequency of night eating with thyroid function and thyroid hormone sensitivity were evaluated

(Figure 2, Supplementary Table S1). Compared with people who had no night eating, individuals who had frequent night eating were negatively associated with T3 levels in model 1, which was adjusted for nothing (OR 0.901 [95% CI 0.815, 0.995], p trend=0.104)]. This association remained significant after further adjusting for sex, age, BMI, race, marital status, education, and family income in model 2 (OR 0.866 [95% CI 0.787, 0.953], p trend=0.064). Model 3, which was further adjusted for smoking behavior, physical activity, drinking behavior, sleep time, hypertension, hyperlipidemia, DM, UIC, and the DII of night eating, remained significant (OR 0.728 [95% CI 0.611, 0.868], p trend=0.235). For TPOAb, the results showed a similar trend to that at T3. In model 1, the OR was 0.901 (95% CI 0.815, 0.995), with a p trend of 0.104. In model 2, the OR was 0.866 (95% CI 0.787, 0.953), with a p trend of 0.064. In model 3,



the OR was 0.728 (95% CI 0.611, 0.868), with a p trend of 0.235. Conversely, there was a positive association with Tg levels in model 1 (OR 1.36 [95% CI 1.026, 1.214], p trend=0.047), model 2 (OR 1.154 [95% CI 1.072, 1.242], p trend=0.088) and model3 (OR 1.223 [95% CI 1.048, 1.429], p trend=0.015).

Compared with people who had no night eating, individuals who had more frequent of night eating were positively associated with FT3 (OR 1.099 [95% CI 1.055, 1.145], p trend=0.011) and negatively associated with TSH (OR 0.935 [95% CI 0.889, 0.984], p trend=0.025) in model 1. Moreover, compared with people who had no night eating, individuals who consumed one time at night were negatively associated with T3 (OR 0.867 [95% CI 0.769, 0.979], p

trend=0.235) in model 3 and T4 (OR 0.982 [95% CI 0.966, 0.999], p trend=0.266) in model 2.

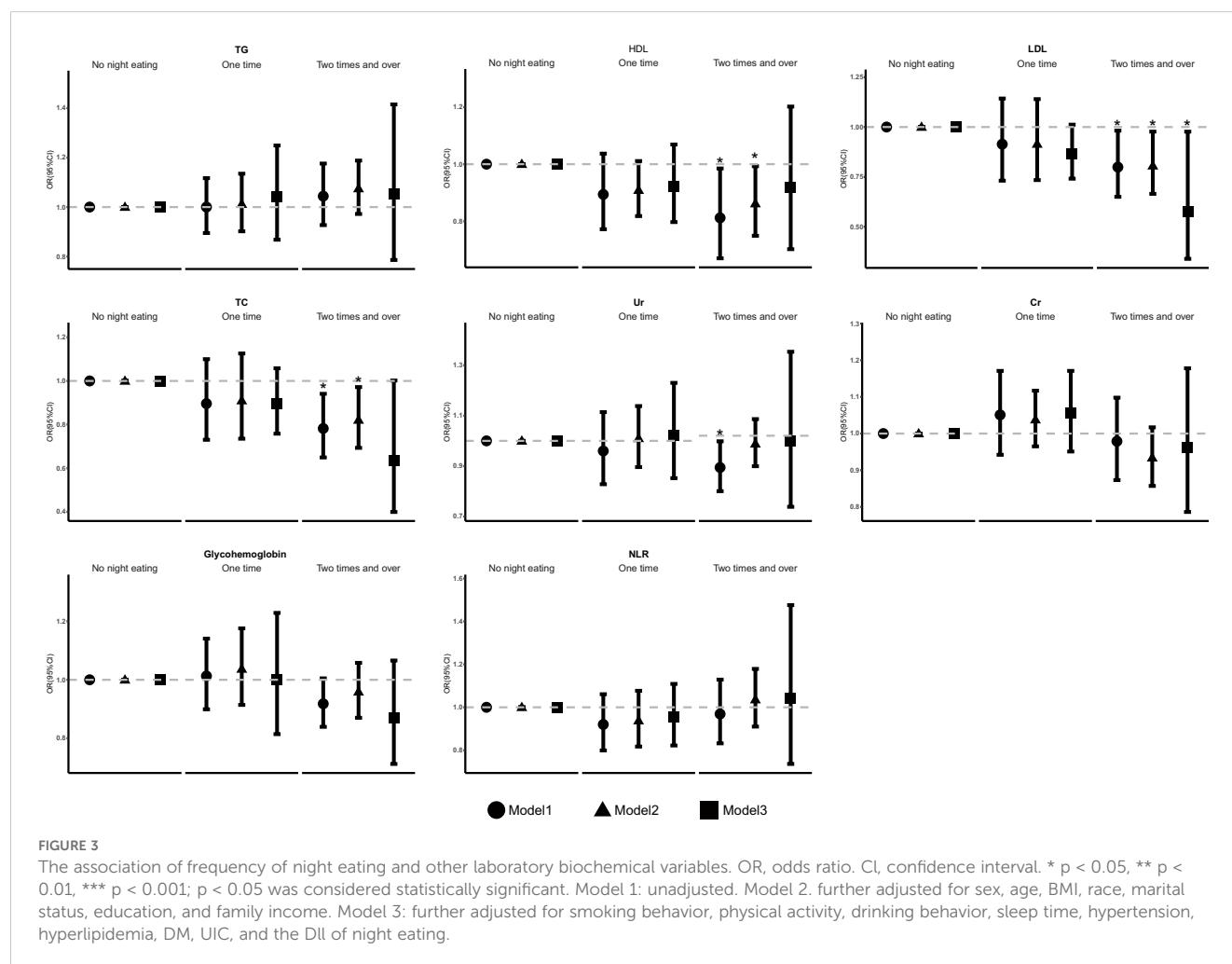
Further analyses of thyroid sensitivity revealed that, compared with no night eating, more frequent night eating behavior were positively associated with FT3/FT4 levels in model 1 (OR 1.127 [95% CI 1.037, 1.225], p trend=0.05) and model3 (OR 1.168 [95% CI 1.021, 1.337], p trend=0.516), but this association was not statistically significant in model 2. Conversely, a significantly negative association with the TSHI (OR 0.934 [95% CI 0.881, 0.991], p trend=0.051) and TT4RI (OR 0.928 [95% CI 0.883, 0.976], p trend=0.015) was detected in model 1, but no statistically significant association was detected in the other model.

3.3 The association of frequency of night eating and other laboratory biochemical variables

Furthermore, weighted linear regression analysis was applied to investigate the relationships between the frequency of night eating and other biochemical variables (Figure 3). Statistical differences were found in laboratory tests for blood lipids between those who had higher eating frequency at night and those who did not. Our results indicate that, compared with people who did not eat at night, individuals who consumed at least one night were negatively associated with HDL levels in model 1 (OR 0.812 [95% CI 0.670, 0.985]) and model 2 (OR 0.862 [95% CI 0.749, 0.993]). Moreover, it was also negatively associated with LDL levels in model 1 (OR 0.799 [95% CI 0.650, 0.982]), model 2 (OR 0.806 [95% CI 0.665, 0.977]) and model 3 (OR 0.575 [0.339, 0.977]) and with TC levels in model 1 (OR 0.782 [95% CI 0.649, 0.941]) and model 2 (OR 0.821 [95% CI 0.693, 0.972]) and with Ur levels in model 1 (OR 0.874 [95% CI 0.780, 0.978]). The laboratory parameters Cr, glycohemoglobin, NLR, and TG were not significantly different among the model.

3.4 Subgroup analysis

Subgroup analyses were performed to examine the associations between eating frequency and T3, Tg, TGA, and TPOAb levels in various populations that were categorized according to age, BMI, sex, race, smoking status, alcohol consumption, physical activity, sleep time, hypertension, hyperlipidemia, obesity, Aobesity, DM, and UIC (Figures 4–7). The results revealed that none of the subgroups mentioned above affected the association between the frequency of night eating and T3 (each p interaction > 0.05). For Tg levels, it is noteworthy that a significant positive association between the frequency of night eating and the number of participants with (OR 1.49 [95% CI 1.15, 1.93]), p interaction=0.022) or without (OR 1.13 [95% CI 1.04, 1.22]) DM was found. Moreover, an interaction effect between smoking and one time night eating on Tg levels was also found (OR 0.86 [95% CI 0.79, 0.98], p interaction=0.025). For TGA levels, a significant positive association with higher eating frequency at night was found in participants aged 65 years and older (OR 1.15 [95% CI 1.03, 1.27]), p interaction=0.03). Conversely, a significant negative association with higher eating frequency at night eating was found in the participants with UIC (OR 0.94 [95% CI 0.9, 0.98]),



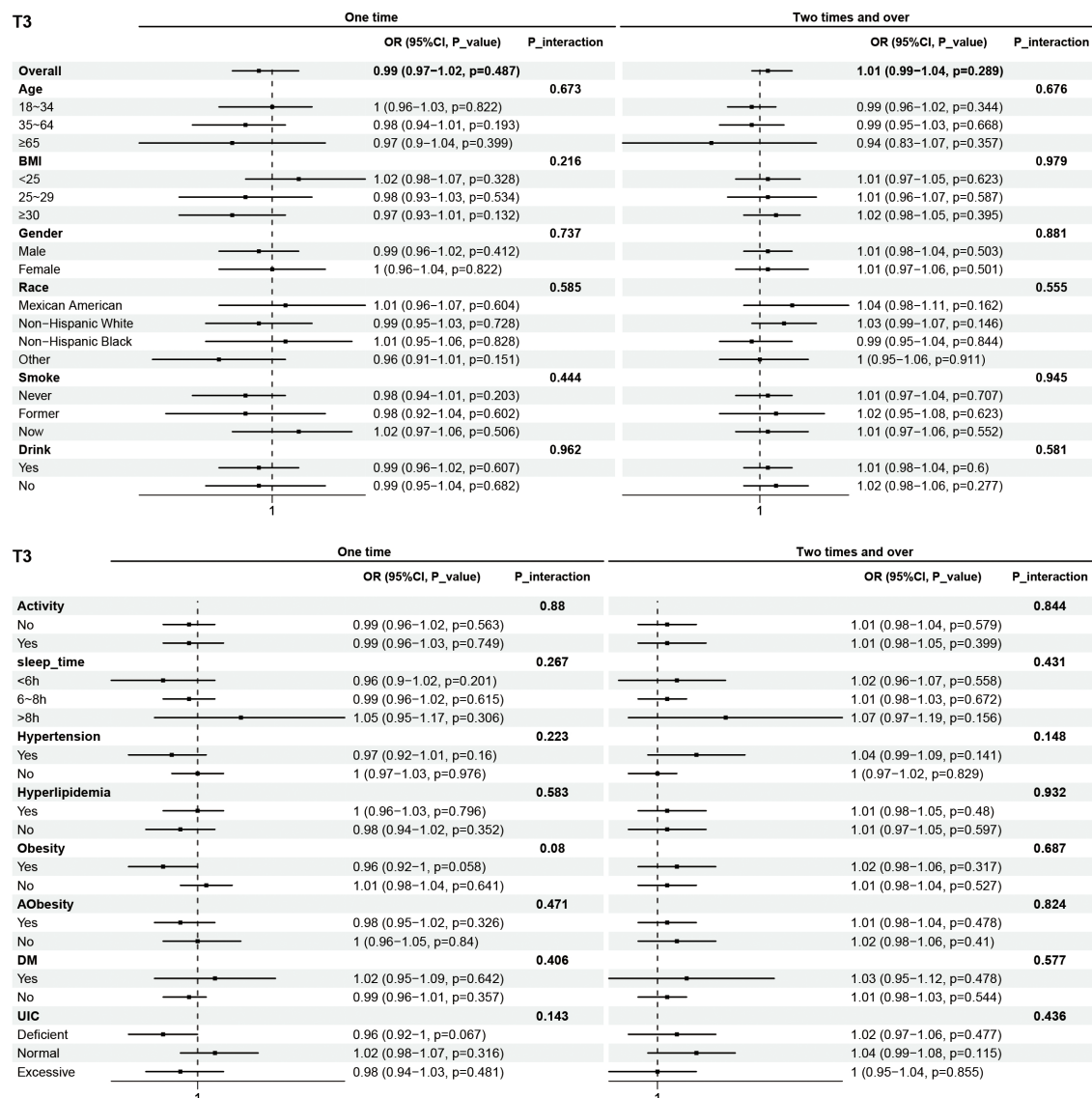


FIGURE 4

Subgroup analysis of association between night eating frequency and T3 levels. The "No night eating" group was used as a reference.

p interaction=0.008). Furthermore, TPOAb was first found to be negatively associated with one-time (OR 0.88 [95% CI 0.8, 0.96]), p interaction=0.014) or more-time (OR 0.9 [95% CI 0.82, 0.97]), p interaction=0.003) night eating in participants without DM. Conversely, a significant positive association with more night eating was found in the participants with DM (OR 1.34 [95% CI 1.03, 1.72]), p interaction=0.003).

4 Discussion

This study expands on existing research regarding night eating frequency and is the first, to our knowledge, to examine its relationship with thyroid function and sensitivity. To investigate this association, we analyzed data from a nationally representative

cohort of U.S. adults. To reduce potential confounding factors, we excluded participants who were under 18 years of age, had cancer, had heart failure, were pregnant, or had thyroid disease. Our findings revealed a strong correlation between thyroid function indices and night eating frequency. Specifically, more frequent night eating was associated with higher levels of Tg and lower levels of T3 and TPOAb. Additionally, our study revealed that, compared with not eating at night, more frequent night eating was linked to lower LDL levels. Subgroup analysis, stratified by DM status, indicated that among participants with DM, increased night eating frequency might lead to more pronounced increases in Tg and TPOAb levels. Conversely, in healthy participants, frequent night eating was associated with a decrease in TPOAb levels. Notably, we observed that among participants aged 65 years and older, frequent night eating led to a relative increase in TGA levels, whereas the opposite

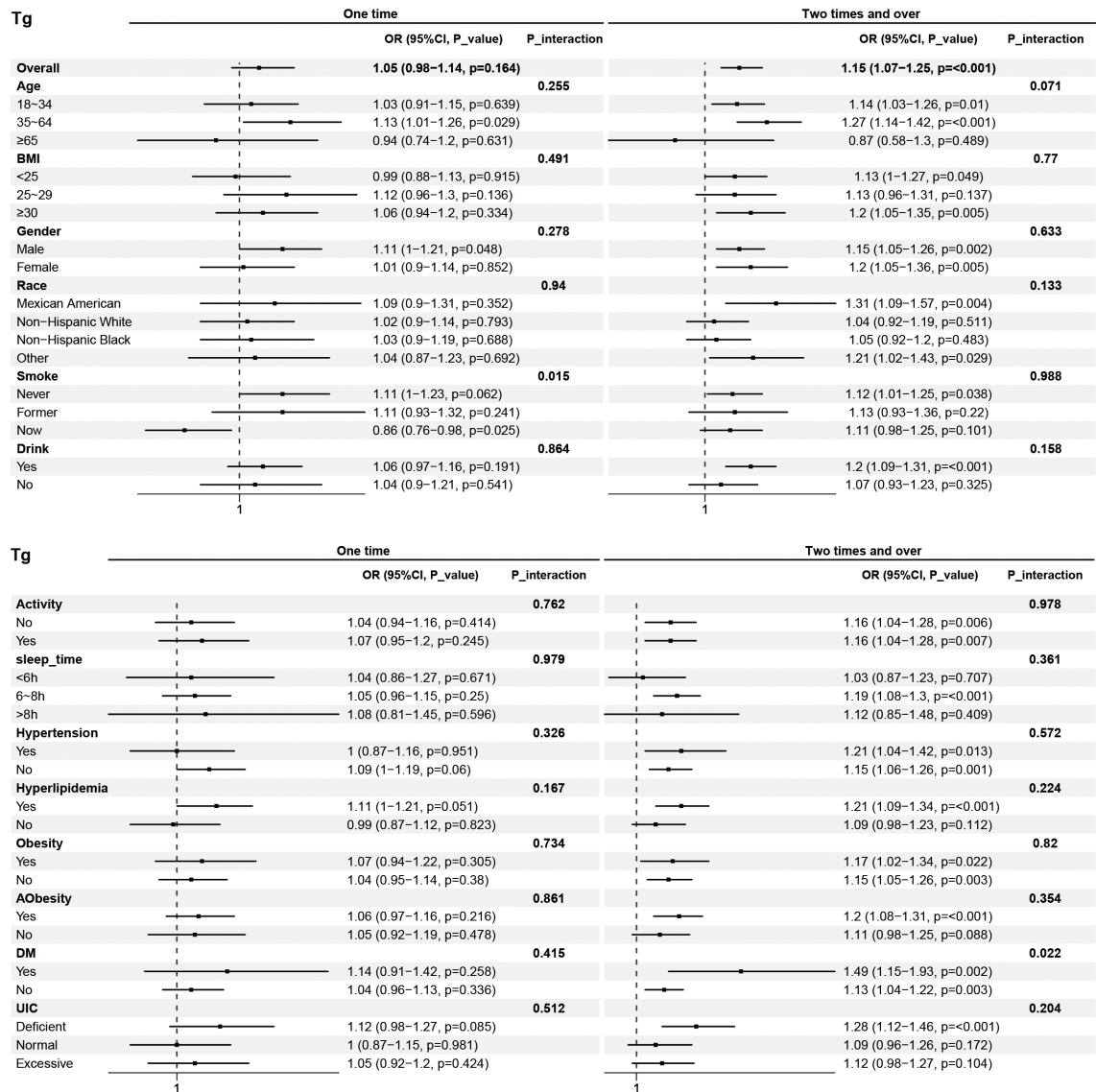


FIGURE 5 Subgroup analysis of association between night eating frequency and Tg levels. The "No night eating" group was used as a reference.

effect was observed in those with UIC deficiency. Importantly, our results did not reveal a statistically significant difference between thyroid sensitivity and night eating frequency.

Regulating the timing of food intake throughout the day can influence the rhythmicity and regularity of certain aspects of the circadian system and related behaviors (20). Organisms encounter different rhythmic occurrences, such as daily and seasonal cycles. To effectively cope with these cyclic changes in their environment, organisms have developed biological clocks. Almost every cell in the body possesses an intrinsic circadian clock lasting approximately 24 hours, which meticulously governs physiological processes and hormonal rhythms (21). Studies on rodents have shown that consuming a high-fat diet during the rest phase (the light phase for nocturnal rodents) leads to more significant weight gain than does consuming the same diet during the active phase (22). Furthermore, nocturnal light exposure increases body weight by

altering the timing of food intake (23). In addition, many previous studies have suggested that disruptions in circadian rhythms may affect the endocrine system, potentially leading to obesity, DM and cardiovascular disease (24–26). The hypothalamus-pituitary-thyroid axis is regulated by the circadian rhythm through the central circadian pacemaker located in the suprachiasmatic nuclei of the anterior hypothalamus (27). Moreover, the literature has reported that the expression profiles of circadian clock genes are abnormal in well-differentiated thyroid cancer but not in benign nodules or healthy thyroids (14). Disruptions in circadian rhythms have also been associated with elevated TSH levels, increasing the risk of thyroid nodules and autoimmune thyroiditis (AIT) (14, 26, 28). AIT, also known as Hashimoto's thyroiditis (HT) or chronic lymphocytic thyroiditis, is characterized by excessive production of TGA and TPO and infiltration of lymphocytes into thyroid tissue. Notably, a previous study demonstrated a broad disruption of clock

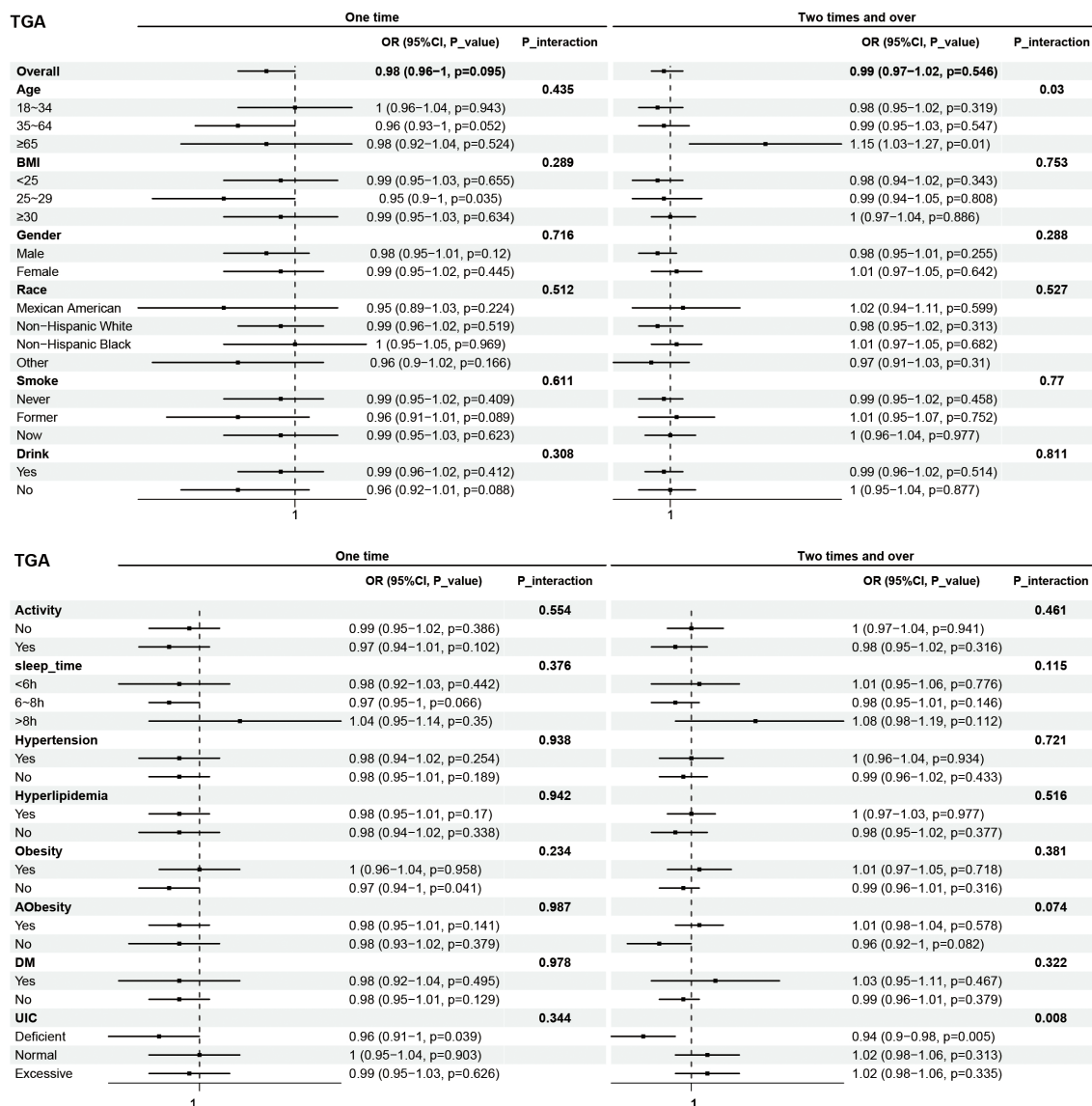


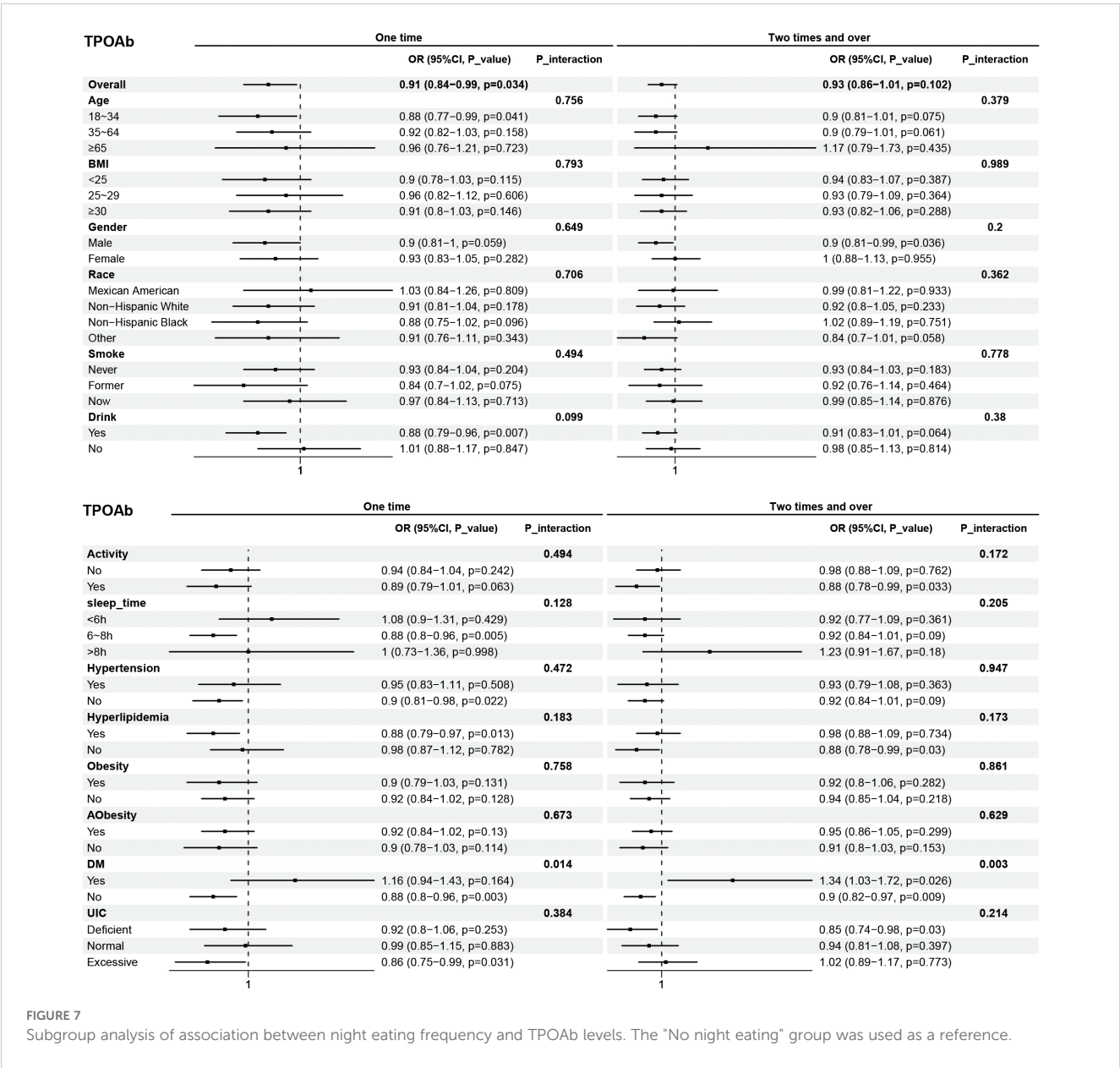
FIGURE 6

Subgroup analysis of association between night eating frequency and TGA levels. The "No night eating" group was used as a reference.

gene profiles within thyroid samples from patients with autoimmune thyroiditis (28). Interestingly, numerous studies have reported the effects of Ramadan fasting on thyroid function in Muslim populations. During Ramadan, Muslims are required to abstain from food and drink between dawn and sunset as part of their religious obligations. This practice not only disrupts eating patterns and sleep schedules but also disturbs circadian rhythms. Current research indicates that in healthy adults, TSH levels increase significantly throughout the fasting month, while fT4 and fT3 levels remain unchanged (29). However, in individuals with hypothyroidism, Ramadan fasting worsens thyroid function (30, 31). Shift workers, whose circadian rhythms are significantly disrupted, also exhibit variations in thyroid hormone levels under different dietary influences (32). This suggests a dual impact of nighttime eating and circadian rhythm disturbances on thyroid

function. Our study revealed that more frequent night eating resulted in a decrease in T3 and TPOAb levels and an increase in Tg levels, but TSH concentrations were not significantly different from no night eating participants, which suggested a complex interaction between circadian rhythms and thyroid dysfunction.

Importantly, we evaluated the night eating DII. The DII, proposed by J.R. Hébert et al. (33), is a literature-derived dietary tool for measuring individual dietary inflammation. Many studies have shown that higher increased levels of IL-6 and CRP (34-36), which results in increased FT3 and TT4 levels (37, 38) and increased risk of thyroid carcinoma (39). Our results revealed that those who ate two or more times at night had higher DII scores. This may be because multiple eating episodes resulted in a higher inflammation score. Therefore, we speculate that in addition to rhythm disruption, dietary inflammation may also be a contributing



factor. Specifically, we hypothesize that when diurnal eating rhythms are disrupted and night eating promotes inflammation, thyroid function may be more severely impaired.

Thyroid hormones are reportedly related to pancreatic β -cell development and influence glucose metabolism through several organs (38). Therefore, thyroid disease and DM are two closely associated disorders. Compared with the general population, type 1 diabetes(T1DM) patients develop thyroid disease at an early age (40). The prevalence of thyroid disease is significantly greater among patients with type 2 diabetes (T2DM) than in the general population (41). In individuals with DM and normal thyroid function, the nocturnal TSH peak has been found to be absent or weak, and the TSH response to TRH is also impaired (42). Although the prevalence of thyroid dysfunction was greater in the T2DM group, TPOAb and TGA were more common in the healthy group (43). In addition, a study reported a greater prevalence of thyroid disease in subjects in the United States with DM than in those without DM, especially in

patients who were TPOAb positive. Surprisingly, our results revealed that frequent night eating may be one of the reasons for the increased production of TPOAb in diabetic patients, even if the effects of glucose metabolism disorders are not as significant as those caused by circadian rhythm disruptions.

A higher prevalence of thyroid disorders has been documented in people with DM than in normoglycemic individuals, whereas patients with both endocrinopathies have poorer glycemic control and are more vulnerable to the development of complications. We speculate that the reason for the elevated Tg levels may be that night eating caused an increase in blood glucose levels, prompting the secretion of thyroid hormones to participate in glucose homeostasis, which also positively affected the Tg level. Therefore, owing to the interaction effects, the Tg levels in DM participants increased more than those in participants who did not eat at night.

As we all know, iodine intake significantly affects thyroid function. Chronic exposure to excess iodine intake induces

autoimmune thyroiditis, partly because highly iodinated Tg is more immunogenic (44). Conversely, iodine deficiency may decrease the level of iodinated Tg and lead to a decrease in TGA levels. This situation is more pronounced in participants with iodine deficiency, who eat more times at night. Furthermore, our results indicate that the elevation in Tg levels is more pronounced in the elderly population, particularly those who eat more times at night. One study reported that the level of TGA gradually increased with increasing age in females, whereas TGA levels peaked in males aged 50–59 years (42), which is consistent with our results.

Additionally, we focused on laboratory indicators such as renal function, glycated hemoglobin, blood lipids, and the NLR, in addition to thyroid function, with the intention of identifying any associations between these indicators and impaired thyroid function. Thyroid hormones are known to affect energy metabolism. Many patients with metabolic syndrome have subclinical or clinical hypothyroidism and vice versa (45). A study of railway workers revealed that irregular work schedules, which affect their circadian rhythm of sleep, may lead to dyslipidemia (46). Another study indicated that men who slept less than 7 h had a greater probability of dyslipidemia than day workers did (47). One study reported that subjects consumed a specified snack (192.4 ± 18.3 kcal) either during the 10:00AM or 23:00PM for 13 days. Compared with the daytime group, the night eating group presented increased LDL levels (12). In addition, the results of a meta-analysis indicated that increased TSH levels were significantly associated with increased TC and LDL levels (48). The metabolically unhealthy non-obese (MUNO) phenotype is significantly associated with hypothyroidism in individuals with thyroid autoimmunity, with a pronounced prevalence in women (49). The above studies revealed the intricate relationships among lipid metabolism, thyroid hormones, and the circadian rhythm. Notably, our results revealed that more frequent night eating was associated with lower levels of LDL, and these findings are almost consistent with those reported in another study (50). However, in the subgroup analyses of obesity, Aobesity, BMI, and hyperlipidemia, no interactions were found between these indicators and T3, Tg, TPOAb, or TGA. The common perception is that circadian rhythm disruptions lead to an increase in LDL, typically regarded as a “bad” lipid parameter, which in turn increases the risk of developing cardiovascular diseases and disrupts thyroid hormone secretion (51). However, our research revealed the opposite findings. These results, not only stem from variations in study methods, subjects, and populations, but also suggest the presence of potential underlying mechanisms that warrant further investigation.

Our study also had some limitations. First, the participants included in the study did not represent the entire population. Second, the frequency of night eating was obtained through dietary recall, the accuracy of which cannot be reliably estimated. Additionally, night eating information is only a single dietary recall and cannot represent long-term habits. Third, unknown and unmeasured confounding factors are likely present, so we cannot make strong causal inferences. Fourth, we could not determine whether participants were taking any medications or supplementation. Given the limitations mentioned above, the present results still need further confirmation by longitudinal prospective large cohort studies with accurate information.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: <https://wwwn.cdc.gov/nchs/nhanes/Default.aspx>.

Ethics statement

The studies involving humans were approved by National Center for Health Statistics. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

YZ: Conceptualization, Methodology, Software, Writing – original draft. SZ: Investigation, Software, Writing – review & editing. SL: Validation, Visualization, Writing – review & editing. YW: Investigation, Writing – review & editing. HZ: Software, Writing – review & editing. JW: Validation, Writing – review & editing. LW: Writing – review & editing. XW: Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fendo.2024.1489459/full#supplementary-material>

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The lymphocyte levels of Hashimoto thyroiditis patients were significantly lower than that of healthy population

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Background and purpose: Hashimoto thyroiditis (HT) is an autoimmune disease without infectivity. We compared the differences of blood lymphocytes levels between HT patients and healthy people.

Patients and methods: This study included a total of 84 HT patients whose thyroid function was normal and 60 HT patients with abnormal thyroid function. A corresponding number of medical examination population in our hospital were randomly selected as the control groups. White blood cell count, neutrophil count, neutrophil percentage, lymphocyte count, and lymphocyte percentage were compared between HT patients and healthy population. The correlations between TSH, FT4 and above parameters were further tested.

Results: We found significant differences between HT with normal thyroid function group and control group in lymphocyte count ($P < 0.001$), lymphocyte percentage ($P < 0.001$) and neutrophil percentage ($P < 0.001$), but no differences in sex ($P = 0.134$), age ($P = 0.200$), white blood cell count ($P = 0.315$) and neutrophil count ($P = 0.790$). Significant differences were observed in neutrophil count ($P = 0.032$), neutrophil percentage ($P = 0.010$), lymphocyte count ($P = 0.010$) and lymphocyte percentage ($P < 0.001$) between HT with abnormal thyroid function group and control group, but not in sex ($P = 0.769$), age ($P = 0.060$) and white blood cell count ($P = 0.156$) between the two groups. There were significant differences in white blood cell count ($P = 0.009$) and neutrophil count ($P = 0.032$) between HT patients in the normal thyroid function group and HT patients in the abnormal thyroid function group. Neither FT4 nor TSH was associated with lymphocyte levels or neutrophil levels.

Conclusions: The lymphocyte levels in HT patients were significantly lower than healthy population. The neutrophil count in HT patients with regular thyroid function was lower than those in abnormal thyroid function HT patients.

KEYWORDS

Hashimoto thyroiditis, lymphocyte count, lymphocyte percentage, neutrophil count, lymphocyte migration

1 Introduction

Hashimoto thyroiditis (HT), also known as chronic lymphocytic thyroiditis, is characterized by painless, diffuse goiter and the presence of high titer autoantibodies in the serum, and some patients will eventually develop hypothyroidism. In recent years, the prevalence and incidence of HT have been increasing. In China, the rate of thyroid autoimmune antibody elevation is about 14.19% (1) and women aged 30 to 50 are the highest incidence group. HT is a non-infectious autoimmune disease with unknown etiology and interaction between genetic and autoimmune factors. Based on the interactions of environmental factors and genetic background, the autoimmune manifestations of HT occur, causing an imbalance between the self-tolerance mechanisms maintained by regulatory T lymphocytes and B lymphocytes (2–4). The main cause of HT is the activation of cellular and humoral immune responses against autoantigens. The main thyroid antigens that induce antibody-mediated responses are thyroglobulin (Tg), thyroid peroxidase antigen (TPO) and thyroid stimulating hormone receptor (TSHR) (5). Thus, in patients with HT, changes in thyroid hormone levels and metabolism can be observed, leading to corresponding clinical symptoms. About 20–30% of HT patients may develop hypothyroidism (6–8). Thyroid lymphocyte infiltration (especially T cell infiltration) is the main feature of HT. The thyroid is gradually replaced by lymphocytes, which can lead to fibrosis and atrophy of thyroid cells (6). Currently, the diagnosis of HT is determined through clinical manifestations, thyroid ultrasound results and the detection of serum anti-thyroid antigen antibodies (anti-thyroid peroxidase antibodies and thyroglobulin antibodies) (7).

It is lymphocyte migration that is the mechanism causing lymphocyte infiltration in thyroid tissue of HT patients. Lymphocyte migration, being a homing mode of lymphocyte, involves a series of molecules in the specific migration of lymphocytes from blood to various tissues and organs. Studies have verified that lymphocyte migration is also implicated in the pathogenesis of other diseases, such as inflammatory bowel disease, rheumatoid arthritis and systemic lupus erythematosus (9–13). Whether the migration of a large number of lymphocytes in the blood to the thyroid tissue due to lymphocyte migration leads to a decrease in the count and percentage of lymphocyte in the blood and whether there are differences in blood lymphocyte count and percentage between HT patients with normal thyroid function and HT patients with hypothyroidism remain to be determined. Consequently, this study aimed to assess the differences in blood lymphocyte levels between HT patients and healthy population.

2 Methods

2.1 Ethical considerations

The research adhered to the Declaration of Helsinki and the local Ethics Committee approved the study.

2.2 Patients and study design

The study encompassed 144 HT patients. HT patients who met the diagnostic criteria and did not undergo thyroid surgery were included. The following conditions are excluded: (1) HT patients with blood system diseases, tumor or infection, (2) HT patients who declined blood tests, (3) prior treatment with corticosteroids, (4) patients with hyperthyroidism. When the patients were first admitted to the hospital, their gender, age were recorded. On the second day of being hospitalized, venous blood was taken to examine leukocyte count, lymphocyte count, neutrophil count, lymphocyte percentage and neutrophil percentage, thyroid function. Blood routine and thyroid function were detected automatically by machine. Hypothyroidism refers to elevated thyroid stimulating hormone (TSH) and decreased free thyroid hormone 4 (FT4), and subclinical hypothyroidism refers to elevated TSH and normal FT4 (14, 15).

To compare the differences of leukocyte count, lymphocyte count, neutrophil count, lymphocyte percentage and neutrophil percentage between HT patients and healthy people, 84 medical examination population in our hospital were randomly chosen as the control group. Their corresponding indicators were also recorded.

Firstly, the differences in white blood cell count, neutrophil count, neutrophil percentage, lymphocyte count, lymphocyte percentage between HT patients with abnormal thyroid function, HT patients with normal thyroid function, and healthy controls were compared. Abnormal thyroid function included hypothyroidism (elevated TSH and decreased FT4) and subclinical hypothyroidism (elevated TSH and normal FT4). Normal thyroid function meant that FT4, FT3 and TSH are normal. If there was a difference, then the correlations between TSH, FT4 and these different parameters were further tested.

2.3 Statistical analysis

Descriptive data were shown as median (interquartile) or percentage. The normality of all numerical variables was examined. For nonparametric tests, the Mann-Whitney U test was used, and for categorical variables, the Chi-square test or Fisher's exact test was applied. The correlation among the data was calculated by Pearson correlation analysis or Spearman rank correlation analysis. SPSS 21.0 software was utilized for statistical analysis, and a P-value less than 0.05 indicated statistical significance.

3 Results

3.1 The lymphocyte levels in the HT patient group were markedly decreased compared to those in the control group

According to thyroid function, HT patients were divided into normal thyroid function group and abnormal thyroid function group. 60 HT patients with irregular thyroid function as well as 84 HT patients with regular thyroid function were present. We found

significant differences between HT with normal thyroid function group and control group in lymphocyte count ($P<0.001$), lymphocyte percentage ($P<0.001$) and neutrophil percentage ($P<0.001$), but no differences in sex ($P=0.134$), age ($P=0.200$), white blood cell count ($P=0.315$) and neutrophil count ($P=0.790$). Significant differences were observed in neutrophil count ($P=0.032$), neutrophil percentage ($P=0.010$), lymphocyte count ($P=0.010$) and lymphocyte percentage ($P<0.001$) between HT with abnormal thyroid function group and control group, but not in sex ($P=0.769$), age ($P=0.060$) and white blood cell count ($P=0.156$) between the two groups. Both lymphocyte count and percentage in HT patients group were significantly lower than that in control group, whether thyroid function is normal or not (Table 1).

3.2 Analysis of the differences between HT with regular thyroid function group and HT with irregular thyroid function group

No statistically significant differences were found in sex ($P=0.686$), age ($P=0.843$), lymphocyte count ($P=0.854$), lymphocyte percentage ($P=0.107$) and neutrophil percentage ($P=0.463$) when comparing the two groups. However, significant differences existed in white blood cell count ($P=0.009$) and neutrophil count ($P=0.032$) between the two groups. Neutrophil count in HT with normal thyroid function group was significantly lower than HT with abnormal thyroid function group (Table 2).

3.3 Correlations between FT4, TSH and neutrophil count, neutrophil percentage, lymphocyte count, lymphocyte percentage in the 144 HT patients

According to Table 1, no significant differences was observed in lymphocyte count, neutrophil count, lymphocyte percentage and neutrophil percentage between HT patients and control group. In

this study, there were no linear correlations between FT4 and lymphocyte count ($r=0.176$, $P=0.034$), lymphocyte percentage ($r=0.215$, $P=0.010$), neutrophil count ($r=-0.134$, $P=0.108$), neutrophil percentage ($r=-0.127$, $P=0.128$). Similarly, there were no linear correlations between TSH and lymphocyte count ($r=-0.057$, $P=0.499$), lymphocyte percentage ($r=-0.170$, $P=0.041$), neutrophil count ($r=0.177$, $P=0.034$, neutrophil percentage ($r=0.152$, $P=0.070$) (Figure 1).

4 Discussion

HT is an autoimmune disease in which the destruction of the thyroid is the result of lymphocyte infiltration. It affects approximately 160 million people worldwide, and women are 4-10 times more susceptible than men (7, 16). In HT, a cellular immune response with high inflammatory load and apoptosis occurs, resulting in tissue destruction and thyroid dysfunction. Generally, it is thought that HT is the outcome of multiple environmental factors causing immune dysfunction on the basis of genetic predisposition. Nevertheless, the molecular mechanism by which immune dysfunction gives rise to the destruction of thyroid tissue still remains unclear (17–20). Susceptibility genes associated with HT include PTPN22, HLA-DR3, FOXP3, CD40, IL-2R α and CTLA-4 (20–23).

The massive infiltration of lymphoid cells in the thyroid tissue of HT patients may be due to lymphocyte migration. Through a series of molecular mediations, lymphocyte migration from blood to thyroid tissue leads to lymphocyte infiltration in thyroid tissue. Studies have shown that Th1 cytokines such as interleukin-1, interleukin-6 and tumor necrosis factor- α (TNF- α) can mediate the infiltration and accumulation of antigen-producing B lymphocytes, cytotoxic T cells, and macrophages into thyroid tissue (24, 25). Macrophage migration inhibitory factor (MIF) can promote the production of inflammatory Th1 cytokines, including IL-2, TNF- α and IL-6 (26). A study published in 2014 showed that MIF levels were significantly higher in HT patients than in healthy people and were associated with

TABLE 1 Analysis of the differences between HT with normal thyroid function group and Control group, HT with abnormal thyroid function group and Control group.

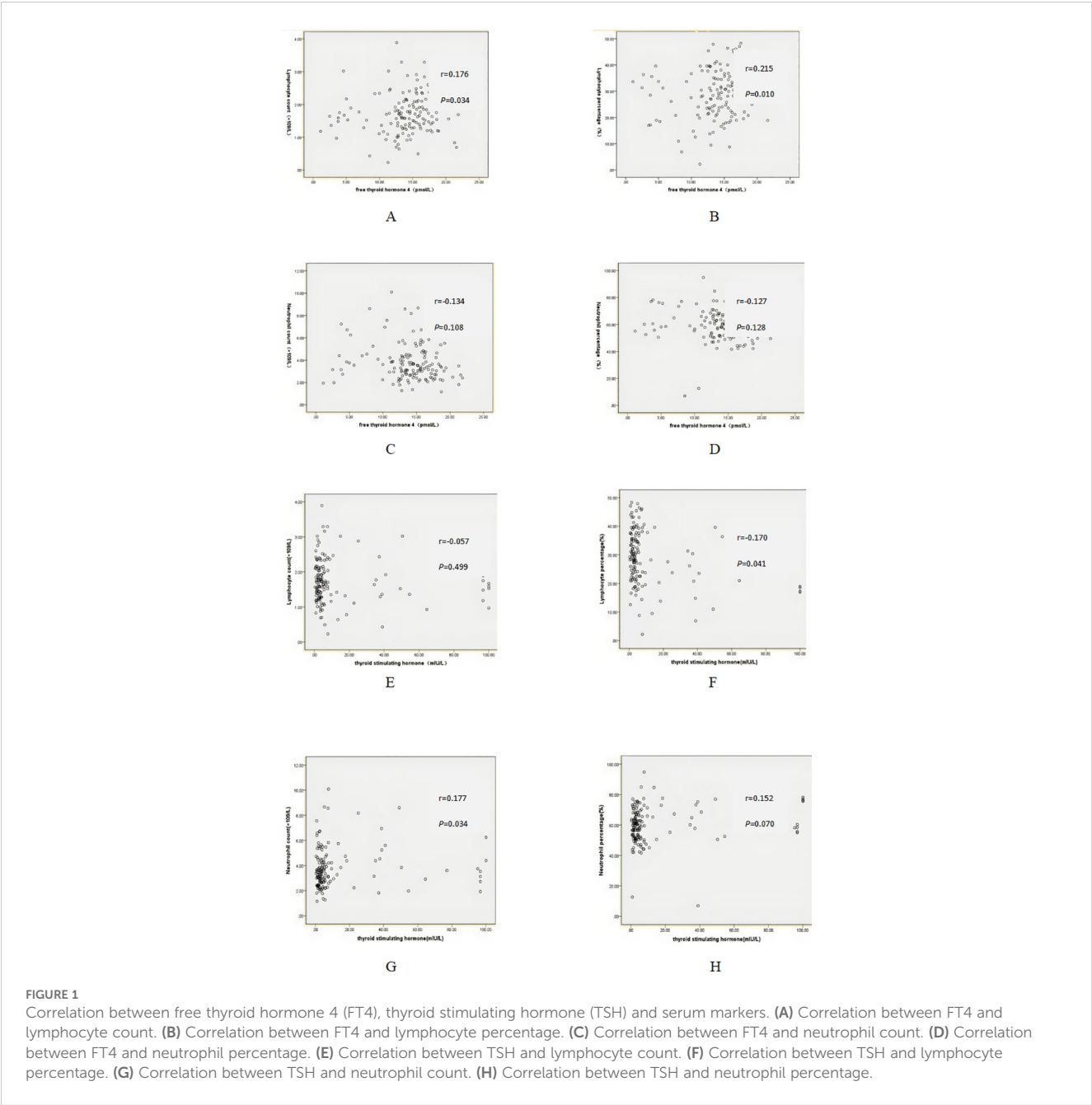
	HT with normal thyroid function group, n=84	Control group, n=84	P-value	HT with abnormal thyroid function group, n=60	Control group, n=60	P-value
Sex:female, n (%)	78 (92.86%)	72 (85.71%)	0.134	54 (90.00%)	53 (88.33%)	0.769
Age (years): M (QR)	39.00 (31.00-51.00)	36.50 (23.25-48.00)	0.200	41.00 (29.00-53.00)	35.5.00 (25.25-40.60)	0.060
White blood cell: M (QR), $\times 10^9/L$	5.80 (4.50-6.50)	5.90 (4.90-6.90)	0.315	6.35 (5.21-7.63)	6.00 (4.90-6.98)	0.156
Lymphocyte count: M (QR), $\times 10^9/L$	1.70 (1.30-2.10)	2.10 (1.70-2.50)	<0.001	1.65 (1.30-2.17)	2.05 (1.70-2.50)	0.010
Lymphocyte percentage: %	30.20 (20.20-35.60)	35.10 (30.70-43.00)	<0.001	29.20 (19.20-36.70)	35.10 (30.45-40.20)	<0.001
Neutrophil count: M (QR), $\times 10^9/L$	3.30 (2.50-4.20)	3.30 (2.60-4.10)	0.790	3.79 (2.82-5.14)	3.35 (2.60-4.28)	0.032
Neutrophil percentage: %	60.10 (50.40-65.20)	55.25 (51.13-59.40)	<0.001	60.25 (50.65-70.57)	56.00 (51.10-59.38)	0.010

M, median; QR, Quartile Range; HT, Hashimoto thyroiditis.

TABLE 2 Analysis of the differences between HT with normal thyroid function group and HT with abnormal thyroid function group.

	HT with normal thyroid function group, n=84	HT with abnormal thyroid function group, n=60	P-value
Sex:female, n (%)	78 (92.86%)	54 (90.00%)	0.686
Age (years): M (QR)	39.00 (31.00-51.00)	41.00 (29.00-53.00)	0.843
White blood cell: M (QR), ×10 ⁹ /L	5.80 (4.50-6.50)	6.35 (5.21-7.63)	0.009
Lymphocyte count: M (QR), ×10 ⁹ /L	1.70 (1.30-2.10)	1.65 (1.30-2.17)	0.854
Lymphocyte percentage: %	30.20 (20.20-35.60)	29.20 (19.20-36.70)	0.107
Neutrophil count: M (QR), ×10 ⁹ /L	3.30 (2.50-4.20)	3.79 (2.82-5.14)	0.032
Neutrophil percentage: %	60.10 (50.40-65.20)	60.25 (50.65-70.57)	0.463

M, median; QR, Quartile Range; HT, Hashimoto thyroiditis.



hypothyroidism (27). This result indirectly reflected that lymphocyte migration may be involved in the pathogenesis of HT. In addition, many studies have confirmed that lymphocyte migration plays an important role in the pathogenesis of IBD (28, 29). Studies also confirmed the existence of lymphocyte infiltration in exocrine gland tissue of Sjogren's syndrome (SS), which leads to the injury of SS exocrine gland (30–32). As lymphocyte migrated from the blood to the thyroid tissue, we expected that the levels of lymphocyte in the blood of HT patients may be lower than that of healthy population. Therefore, 84 HT patients with regular thyroid function and 60 HT patients with irregular thyroid function were included to compare the differences of blood lymphocytes levels between the two groups and healthy population. The results indicated that although the total leukocyte count in HT patients had no significant difference compared to that of the healthy population, the lymphocyte count and percentage were notably lower than those of the healthy population, while neutrophil percentage was significantly higher than that of healthy population. A study published in 2020 showed that neutrophil/lymphocyte ratio was statistically higher than healthy population, a result that is consistent with this study (33). In addition, this study also found that the neutrophil count and white blood cell count in HT patients with abnormal thyroid function were significantly higher than those with normal thyroid function. A study showed that over activation and over recruitment of neutrophils at the site of inflammation are thought to contribute to the pathogenesis and progression of IBD (34). The oxidative stress damage caused by the production of reactive oxygen species is involved in the pathogenesis and progression of IBD, and neutrophil cells are an important source of reactive oxygen species. In the inflammatory microenvironment, reactive oxygen species can damage biological macromolecules such as deoxyribonucleic acid, proteins and lipids, thus causing tissue cell damage. Given that the neutrophil count in HT patients with abnormal thyroid function is considerably higher than that in HT patients with normal thyroid function, could it be that the activation of neutrophils aggravates the destruction of thyroid tissue, thereby resulting in hypothyroidism? The mechanism of this remains to be further studied. This study also studied whether FT4 and TSH were correlated with lymphocyte and neutrophil levels, but the results showed that there was no linear correlation. The reason may be that neutrophils only initiate the destruction of thyroid tissue, but not associated with severity. The severity of thyroid tissue destruction may be due to other factors. Just as the level of serum amylase can only be used to diagnose acute pancreatitis, and does not reflect the severity of acute pancreatitis.

This study has certain drawbacks. First, in this study, HT patients with hypothyroidism and subclinical hypothyroidism were collectively referred to as thyroid dysfunction, and were not analyzed separately. Secondly, while this study demonstrated that the neutrophil count in HT patients with irregular thyroid function exceeded that in HT patients with regular thyroid function, it unfortunately did not further dig into the possible mechanism underlying this phenomenon. Specifically, the sample size within this study was relatively limited and it was only a single-center investigation. Consequently, it is anticipated that more comprehensive studies will

be carried out in the future, incorporating a larger number of patients to enhance the validity and generalizability of the findings. Whether monitoring lymphocyte levels can be used in the diagnosis and treatment of HT and whether neutrophil activation causes hypothyroidism remain to be investigated.

To sum up, the findings of this study possess significant clinical importance. The lymphocyte levels in HT patients were significantly lower than normal, and the neutrophil counts in abnormal thyroid function HT patients were higher than those in normal thyroid function HT patients.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of Suqian City. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

HX: Writing – original draft. RX: Formal analysis, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Association between *Helicobacter pylori* infection, serum thyroid-stimulating hormone, and thyroxine in the National Health and Nutrition Examination Survey 1999–2000

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Background: *Helicobacter pylori* has been increasingly implicated in extra-gastric diseases. Current evidence regarding the association between serum thyroid-stimulating hormone (TSH), thyroxine (T4), and *H. pylori* infection remains inconclusive. Consequently, this study aimed to explore the correlation between TSH and T4 levels and *H. pylori* infection in a US-based population sample.

Methods: Data from the US National Health and Nutrition Examination Survey (NHANES), comprising 971 participants aged 30–85 years from 1999 to 2000, were analyzed. Binary logistic regression was employed to analyze the correlation between *H. pylori* and TSH and T4 levels. The impact of TSH and T4 on *H. pylori* infection was further assessed using restricted cubic spline (RCS) analysis. In addition, subgroup analyses stratified by sex and age were conducted.

Results: Subjects with *H. pylori* seropositivity demonstrated lower serum TSH levels and higher serum T4 levels compared to those with *H. pylori* seronegativity. A significant positive correlation was identified between *H. pylori* seropositivity and T4 levels with increasing quartiles of hormonal levels in both univariate regression models (Q4 vs. Q1: OR = 1.483; 95% CI, 1.033–2.129) and multivariate regression models (Q4 vs. Q1: OR = 1.004; 95% CI, 0.981–1.026). Conversely, a negative correlation was observed between *H. pylori* seropositivity and TSH levels with increasing quartiles of hormonal levels in univariate regression models (Q4 vs. Q1: OR = 0.579; 95% CI, 0.403–0.831) and in multivariate regression models (Q4 vs. Q1: OR = 0.580; 95% CI, 0.389–0.866). In stratified analyses, the adjusted association of serum T4 levels with *H. pylori* seropositivity was statistically significant among men (T4: Q4 vs. Q1: OR = 2.253; 95% CI, 1.311–3.873), age over 68 years in TSH levels (Q4 vs. Q1: OR = 0.434; 95% CI, 0.206–0.911), and age 41–54 years in T4 levels (Q4 vs. Q1: OR = 4.965; 95% CI, 2.071–11.903). RCS analysis revealed a non-linear

relationship between TSH levels and *H. pylori* infection. Notably, when TSH < 0.98 IU/ml, the likelihood of *H. pylori* infection significantly increased.

Conclusions: Lower TSH and higher T4 levels were associated with *H. pylori* infection, particularly among men and elderly individuals.

KEYWORDS

Helicobacter pylori infection, thyroid stimulating hormone, thyroxine, NHANES, CDC

1 Introduction

Helicobacter pylori is a bacterium that preferentially colonizes in the gastric epithelium, often causing a spectrum of digestive disease, including peptic ulcer, chronic gastritis, and even gastric cancer. It has been reported that approximately half of the global population is infected with *H. pylori* (1), with a prevalence rate of approximately 35.6% in the US (2). Furthermore, numerous extra-gastric diseases have been proven to be associated with *H. pylori* infection, such as dermatosis, thyroid diseases, and metabolic, cardiovascular, and neurological diseases (3–5).

Thyroxine (T4) is a thyroid hormone (TH) synthesized and secreted by the thyroid gland (6). The regulation of THs is governed by the complex hypothalamic–pituitary–thyroid (HPT) axis. Thyroid-stimulating hormone (TSH), produced by the anterior pituitary gland, promotes the synthesis and release of THs, primarily through negative feedback mechanisms within the HPT axis (7). Evidence indicates that *H. pylori* infection plays a pivotal role in thyroid disease (8), particularly autoimmune thyroid diseases (ATDs), owing to its ability to mimic the antigenic profile present on thyroid cell membranes (9). Vincenzo Bassi et al. discovered a heightened prevalence of *H. pylori* exclusively among patients with hyperthyroid Graves' disease (GD), in contrast to those with Hashimoto thyroiditis (HT) (10). Additionally, De Luis et al. reported markedly elevated levels of anti-*H. pylori* immunoglobulin G antibodies in patients with subclinical hyperthyroidism, compared to the control cohort (11). Furthermore, a recent prospective study highlighted a substantial association between *H. pylori* infection and the likelihood of subclinical hyperthyroidism in Chinese women, independent of dietary factors (12). Both TSH and T4 are considered sensitive biomarkers for evaluating thyroid function, reflecting conditions of either hypothyroidism or hyperthyroidism (13). At present, the relationship between *H. pylori* infection and plasma levels of TSH and T4 in the general population remains insufficiently investigated and contentious (14–16).

Since the T4 data were incorporated in October 2023, it is now particularly intriguing to explore the relationship between TSH, T4 levels, and *H. pylori* infection based on data from the 1999–2000 National Health and Nutrition Examination Survey (NHANES).

2 Materials and methods

2.1 Study design and sample

NHANES is a publicly accessible database managed by the Centers for Disease Control and Prevention (CDC), providing extensive data regarding the health and nutritional status of the non-institutionalized U.S. population. The survey encompasses information derived from questionnaires, demographic profiles, laboratory tests, and physical examinations (17, 18). The data analyzed in this study were from the 1999–2000 NHANES cycles, encompassing participants who had both *H. pylori* infection and measurements of plasma TSH and T4 levels, with T4 data being added to the database in October 2023.

The exclusion criteria were as follows: (1) individuals aged <30 or >85 years; (2) missing data for *H. pylori* serology, TSH, or T4 levels; and (3) individuals with thyroid disease or taking thyroid medications. Participants possessing relevant laboratory data and demographic variables of interest were incorporated into this investigation, culminating in a total sample size of 971 individuals aged between 30 and 85 years old. The schematic representation of the participant selection process is illustrated in Figure 1.

2.2 *Helicobacter pylori* status

In accordance with the NHANES protocol (19), *H. pylori*-specific immunoglobulin G (IgG) levels were quantified utilizing the *H. pylori* IgG enzyme-linked immunosorbent assay (ELISA) developed by Wampole Laboratories (Granbury, N) (20). Standard ELISA cutoff values were applied to classify participants as seropositive [optical density (OD) value ≥ 1.1] or seronegative (OD value < 0.9) for *H. pylori*. Indeterminate results (OD values between 0.9 and 1.1) were excluded to avoid potential biases in the statistical analysis of this investigation (21).

2.3 Thyroid-stimulating hormone and thyroxine

The dependent variable analyzed in this study was *H. pylori* seropositivity, while the primary independent variables of interest

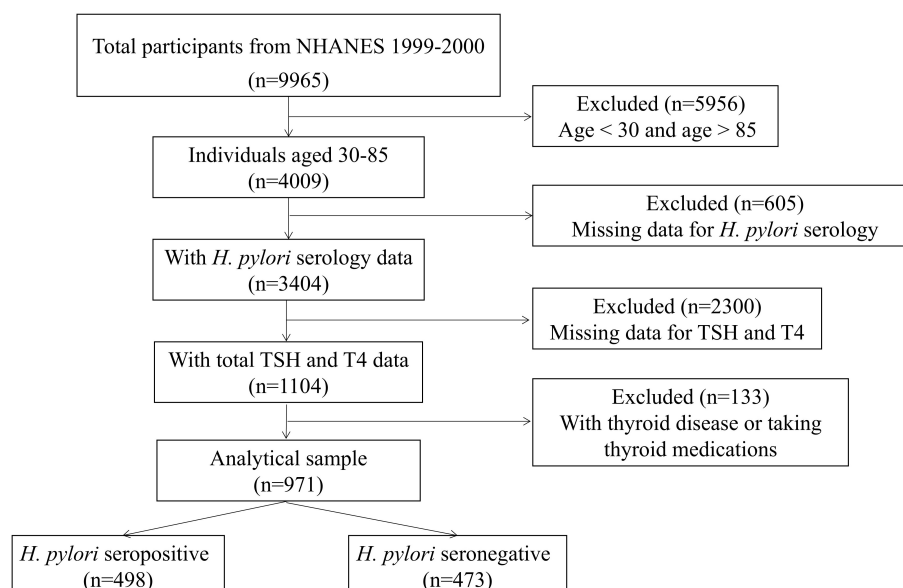


FIGURE 1
The flowchart of sample selection.

were plasma levels of TSH and T4. Both serum TSH and T4 were sourced from the NHANES laboratory dataset, designated as LAB18T4, which was updated in October 2023. Thyroid medications have been identified in the prescription drug medication document.

2.4 Covariates

The covariates examined in this research encompass age, gender, race, education level, body mass index (BMI), smoking behavior, alcohol behavior, and homocysteine levels. These variables were selected based on existing evidence linking them to both *H. pylori* serostatus and thyroid function (18, 21, 22). Among the covariates, age, TSH, T4, and homocysteine were categorized as continuous variables, while sex, race, education level, BMI, smoking behavior, and alcohol behavior were classified as categorical variables.

2.5 Statistical analyses

For continuous variables, independent *t*-test or Mann–Whitney test was employed to analyze the differences between groups. Depending on the normality of distribution, continuous variables were presented as mean $\pm$ SD; otherwise, they were presented as Median. Categorical variables were assessed using the chi-square test and reported as counts and percentages. Levels of TSH and T4 were categorized into quartiles (Q1 to Q4). Multiple regression analysis was conducted to identify the factors influencing TSH and T4 levels. Furthermore, the relationship between TSH, T4, and *H. pylori* was examined through restricted cubic spline (RCS) analysis, with knots positioned at the 5th, 35th, 65th, and 95th percentiles.

Statistical analyses were carried out using SPSS (version 26.0) and R software (version 4.1.3), with a significance threshold set at $p < 0.05$, where both the p for overall and the p for non linear relationship in RCS were less than 0.05.

3 Results

3.1 Characteristics of included subjects

A total of 971 participants were included in this study, with 498 classified as *H. pylori* IgG seropositive and 473 as *H. pylori* IgG seronegative. Notable differences were detected between the two groups ($p < 0.05$) in terms of age, race, educational level, and serum TSH and T4 levels. The baseline characteristics of the study subjects are detailed in Table 1.

3.2 Association between *H. pylori* seropositivity and TSH and T4

3.2.1 Multiple regression model

The outcomes of different multivariate linear regression models are summarized in Tables 2, 3: Model 1 is unadjusted, model 2 is adjusted for age and sex, and model 3 is further adjusted for race and educational level.

In the unadjusted model, a negative association was identified between *H. pylori* seropositivity and TSH levels across increasing quartiles of hormonal levels (Q4 vs. Q1: OR = 0.579; 95% CI, 0.403–0.831, $p = 0.003$). This negative relationship persisted after adjustments for confounding factors in model 2 (Q4 vs. Q1: OR = 0.544, 95% CI, 0.377–0.786; $p = 0.001$) and model 3 (Q4 vs. Q1: OR = 0.580, 95% CI, 0.389–0.866; $p = 0.008$). Conversely, a significant positive association

TABLE 1 Baseline characteristics of the study subjects.

	<i>H. pylori</i> seropositive (<i>n</i> = 498)	<i>H. pylori</i> seronegative (<i>n</i> = 473)	<i>p</i> -value
Age (years)	56.0 (42.0–68.0)	52.0 (39.0–67.5)	0.037
Sex			0.168
Male	260 (52.2%)	226 (47.8%)	
Female	238 (47.8%)	247 (52.2%)	
Race			<0.001
Mexican American	197 (39.5%)	55 (11.6%)	
Other Hispanic	39 (7.8%)	9 (1.9%)	
Non-Hispanic white	132 (26.5%)	335 (70.8%)	
Non-Hispanic black	111 (22.2%)	65 (13.7%)	
Other races	19 (3.8%)	9 (1.9%)	
Educational level			<0.001
Less than high school	277 (55.6%)	108 (22.8%)	
High school	92 (18.4%)	120 (25.3%)	
More than high school	128 (5.7%)	244 (51.5%)	
Others	1 (0.2%)	1 (0.2%)	
BMI	27.78 (24.58–31.59)	27.27 (24.06–31.96)	0.317
Homocysteine (μmol/L)	8.14 (6.45–10.20)	7.69 (6.29–9.88)	0.055
Serum TSH (IU/mL)	1.48 (1.02–2.15)	1.64 (1.13–2.43)	0.008
Serum T4 (nmol/L)	97.80 (84.90–113.30)	92.70 (83.70–108.10)	0.013
Smoking behavior			0.21
Never	203 (40.8%)	194 (41.0%)	
Some days	113 (22.7%)	116 (24.5%)	
Every day	182 (36.5%)	163 (34.5%)	
Alcohol behavior			0.749
Yes	332 (66.7%)	322 (68.1%)	
No	166 (33.3%)	151 (31.9%)	

Other race/ethnicity includes all race/ethnicity other than Mexico-American, non-Hispanic white, and black. BMI, body mass index; TSH, thyroid-stimulating hormone; T4, thyroxine. Bold represents statistically significant.

TABLE 2 Association of thyroid-stimulating hormone level with *Helicobacter pylori* seropositivity.

TSH	Model 1		Model 2		Model 3	
	OR (95% CI)	<i>p</i> -value	OR (95% CI)	<i>p</i> -value	OR (95% CI)	<i>p</i> -value
Q1	Ref		Ref		Ref	
Q2	0.731 (0.511–1.046)	0.087	0.715 (0.499–1.026)	0.068	0.701 (0.477–1.032)	0.072
Q3	0.780 (0.545–1.117)	0.175	0.758 (0.528–1.087)	0.132	0.775 (0.525–1.140)	0.195
Q4	0.579 (0.403–0.831)	0.003	0.544 (0.377–0.786)	0.001	0.580 (0.389–0.866)	0.008

Model 1: no covariates were adjusted; model 2: age and sex were adjusted; model 3: age, sex, race, and educational level were adjusted. OR, odds ratio. Q1: <1.09 mIU/L, Q2: 1.09–1.57 mIU/L, Q3: 1.57–2.30 mIU/L, Q4: >2.30 mIU/L.

TABLE 3 Association of thyroxine level with *Helicobacter pylori* seropositivity.

T4	Model 1		Model 2		Model 3	
	OR (95% CI)	<i>p</i> -value	OR (95% CI)	<i>p</i> -value	OR (95% CI)	<i>p</i> -value
Q1	Ref		Ref		Ref	
Q2	0.865 (0.601–1.245)	0.434	0.885 (0.614–1.278)	0.515	0.886 (0.597–1.316)	0.549
Q3	1.161 (0.810–1.666)	0.416	1.211 (0.840–1.747)	0.305	1.252 (0.842–1.862)	0.267
Q4	1.483 (1.033–2.129)	0.033	1.552 (1.076–2.238)	0.019	1.004 (0.981–1.026)	0.048

Model 1: no covariates were adjusted; model 2: age and sex were adjusted; model 3: age, sex, race, and educational level were adjusted. OR, odds ratio. Q1: <83.7 nmol/L, Q2: 83.7–95.2 nmol/L, Q3: 95.2–110.7 nmol/L, Q4: >110.7 nmol/L.

was observed between *H. pylori* and T4 levels across increasing quartiles of hormonal levels (Q4 vs. Q1: OR = 1.483; 95% CI, 1.033–2.129, *p* = 0.033). This positive connection remained significant after adjustments for confounding factors in model 2 (Q4 vs. Q1: OR = 1.552; 95% CI, 1.076–2.238, *p* = 0.019) and was marginally significant in model 3 (Q4 vs. Q1: OR = 1.004; 95% CI, 0.981–1.026, *p* = 0.048), as presented in [Table 3](#).

3.2.2 Subgroup analyses

In the sex-stratified subgroup analyses, a positive correlation was discovered between the *H. pylori* seropositivity and T4 levels in men (Q4 vs. Q1 OR = 2.253; 95% CI, 1.311–3.873; *p* = 0.003). However, no significant relationship was observed between TSH levels and *H. pylori* seropositivity in men, as the positive correlation disappeared after adjusting for confounding variables in the multivariate regression model (Q4 vs. Q1 OR = 0.636; 95% CI, 0.340–1.188; *p* = 0.156). Among women, neither TSH nor T4 levels exhibited any association with *H. pylori* seropositivity. Detailed findings are provided in [Tables 4, 5](#).

In the age-stratified subgroup analyses, a negative correlation was identified between *H. pylori* seropositivity and TSH levels among participants aged over 68 years (Q4 vs. Q1: OR = 0.434; 95% CI, 0.206–0.911; *p* = 0.027). Furthermore, no significant association was observed between TSH levels and *H. pylori* seropositivity in other age groups, as detailed in [Table 6](#). Additionally, there was a positive association between *H. pylori* seropositivity and T4 levels among participants aged 41–54 years (Q4 vs. Q1: OR = 4.965; 95% CI, 2.071–11.903; *p* < 0.001). However, T4 levels showed no significant relationship with *H. pylori* seropositivity in other age groups, as outlined [Table 7](#).

3.2.3 Non-linear relationship between TSH, T4, and *H. pylori* infection

An RCS model was employed to assess the association between TSH levels and *H. pylori* infection. As illustrated in [Figure 2](#), when TSH levels fall below 0.98 IU/mL, the risk of *H. pylori* infection rises significantly (*p* < 0.05). Conversely, there was no linear relationship between T4 levels and *H. pylori* infections.

TABLE 4 Association of thyroid-stimulating hormone level with *Helicobacter pylori* seropositivity based on subgroup of sex.

Subgroup	TSH	Model 1		Model 2		Model 3	
		OR (95% CI)	<i>p</i> -value	OR (95% CI)	<i>p</i> -value	OR (95% CI)	<i>p</i> -value
Male	Q1	Ref		Ref		Ref	
	Q2	0.621 (0.373–1.035)	0.068	0.614 (0.368–1.024)	0.062	0.611 (0.339–1.013)	0.102
	Q3	0.595 (0.355–0.997)	0.049	0.584 (0.348–0.980)	0.042	0.675 (0.370–1.233)	0.202
	Q4	0.494 (0.294–0.831)	0.008	0.465 (0.273–0.792)	0.005	0.636 (0.340–1.188)	0.156
Female	Q1	Ref		Ref		Ref	
	Q2	0.845 (0.508–1.406)	0.518	0.830 (0.498–1.385)	0.830	0.830 (0.449–1.436)	0.459
	Q3	1.012 (0.613–1.671)	0.963	0.974 (0.587–1.614)	0.918	1.248 (0.691–2.256)	0.463
	Q4	0.672 (0.405–1.116)	0.125	0.633 (0.379–1.058)	0.081	0.799 (0.440–1.452)	0.461

Model 1: no covariates were adjusted; model 2: age and sex were adjusted; model 3: age, sex, race, and educational level were adjusted. OR, odds ratio. Q1: <1.09 mIU/L, Q2: 1.09–1.57 mIU/L, Q3: 1.57–2.30 mIU/L, Q4: >2.30 mIU/L.

TABLE 5 Association of thyroxine level with *Helicobacter pylori* seropositivity based on subgroup of sex.

Subgroup	T4	Model 1		Model 2		Model 3	
		OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Male	Q1	Ref		Ref		Ref	
	Q2	0.917 (0.570–1.477)	0.722	0.937 (0.580–1.514)	0.791	1.169 (0.671–2.037)	0.582
	Q3	1.258 (0.773–2.047)	0.356	1.303 (0.795–2.135)	0.294	1.617 (0.913–2.864)	0.100
	Q4	2.184 (1.278–3.734)	0.004	2.253 (1.311–3.873)	0.003	2.061 (1.093–3.887)	0.025
Female	Q1	Ref		Ref		Ref	
	Q2	0.819 (0.459–1.461)	0.499	0.812 (0.454–1.454)	0.812	0.576 (0.298–1.112)	0.100
	Q3	1.097 (0.626–1.924)	0.746	1.142 (0.648–2.012)	0.645	0.938 (0.493–1.783)	0.844
	Q4	1.211 (0.702–2.088)	0.491	1.278 (0.737–2.216)	0.382	0.848 (0.447–1.610)	0.614

Model 1: no covariates were adjusted; model 2: age and sex were adjusted; model 3: age, sex, race, and educational level were adjusted. OR, odds ratio. Q1: <83.7 nmol/L, Q2: 83.7–95.2 nmol/L, Q3: 95.2–110.7 nmol/L, Q4: >110.7 nmol/L.

4 Discussion

This study utilized newly updated NHANES data to explore the relationship between TSH and T4 levels and *H. pylori* infection. In summary, *H. pylori* seropositivity demonstrated a positive correlation with T4 levels and a negative correlation with TSH levels. In stratified analyses, the adjusted association between serum T4 levels and *H. pylori* seropositivity was statistically significant in men but not in women, with significant correlations observed for serum TSH in participants over 68 years of age and for T4 in those aged 41–45 years.

As is widely acknowledged, thyroid function is composed of TSH and T4, with these serum markers often exhibiting a negative correlation. Our research identified variations in TSH and T4 levels between populations positive and negative for *H. pylori*. The non-linear connection revealed an enormous rise in the likelihood of *H. pylori* infection when TSH levels < 0.98 IU/mL. This suggests that individuals exhibiting hyperthyroid tendencies are more susceptible to *H. pylori* infection, corroborating findings from prior studies (10).

H. pylori testing should be considered for individuals undergoing treatment with medications known to be affected by this infection, such as T4, as outlined in the Houston Consensus (23). Therefore, this suggests an implied relationship between *H. pylori* infection and thyroid function. Nonetheless, the impact of thyroid function disorders, including hyperthyroidism or hypothyroidism, on *H. pylori* infection remains a contentious issue. Larizza et al. demonstrated that *H. pylori* could provoke an immune response against thyroid cells (24). A notable association has also been observed between Cag-A-positive *H. pylori* strains and GD, regardless of the patients' hormonal status (25). The association may be attributed to cross-reactivity between antibodies against the *H. pylori* Cag-A protein and the follicular

cells of the thyroid gland (26). Additionally, conflicting findings from other research have indicated a positive correlation between *H. pylori* infection and autoimmune atrophic thyroiditis (27, 28).

The mechanisms by which elevated T4 levels and suppressed TSH levels heighten the susceptibility to *H. pylori* infection remain incompletely understood; however, several lines of evidence may shed light on this relationship. Firstly, the discovery of a homologous 11-residue peptide shared by both gastric parietal cell antigens and thyroid peroxidase suggests a common epitope (29), implying that antibodies generated during *H. pylori* infection may cross-react with thyroid antigens, potentially contributing to hyperthyroidism (30). Secondly, studies have shown a strong correlation between IgG anti-*H. pylori* antibodies and thyroid autoantibodies, along with a reduction in thyroid autoantibody levels following the successful eradication of *H. pylori* infection (31). Thirdly, previous investigations have demonstrated that *H. pylori* strains can express fucosylated Lewis determinants, which are commonly found in various host tissues and may trigger an autoimmune response that could impair thyroid function (32). Typically, thyroid diseases, particularly ATDs, are influenced by a variety of autoimmune mechanisms. It has been reported that *H. pylori* infection plays a role in the pathogenesis of HT, the leading cause of hypothyroidism (28). In addition, in GD, humoral autoimmunity characterized by a TH2 profile is prevalent. Once infected with *H. pylori*, cytokines such as interleukin (IL)-4, IL-5, and IL-6 are activated, initiating a cascade of humoral immune responses that may modify the expression of adhesion molecules on the gastric mucosa, thereby contributing to the hyperthyroidism observed in GD (10, 33). The mechanism is believed to be associated with molecular mimicry between *H. pylori* antigens and thyroid constituents. Collectively, these findings offer compelling explanations for the observed correlation between *H. pylori* infection and hyperthyroidism.

TABLE 6 Association of thyroid-stimulating hormone level with *Helicobacter pylori* seropositivity based on subgroup of age.

Subgroup	TSH	Model 1		Model 2		Model 3	
		OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
<41	Q1	Ref		Ref		Ref	
	Q2	0.729 (0.367–1.448)	0.366	0.730 (0.367–1.452)	0.730	0.409 (0.176–0.950)	0.038
	Q3	0.759 (0.397–1.591)	0.517	0.796 (0.397–1.593)	0.796	0.799 (0.339–1.880)	0.606
	Q4	0.622 (0.284–1.363)	0.235	0.621 (0.283–1.362)	0.235	0.658 (0.248–1.746)	0.401
41–54	Q1	Ref		Ref		Ref	
	Q2	0.806 (0.403–1.612)	0.541	0.804 (0.397–1.628)	0.544	1.551 (0.652–3.691)	0.321
	Q3	0.731 (0.358–1.493)	0.731	0.738 (0.357–1.527)	0.413	1.304 (0.529–3.212)	0.564
	Q4	0.505 (0.243–1.046)	0.066	0.570 (0.282–0.800)	0.139	1.198 (0.472–3.042)	0.704
54–68	Q1	Ref		Ref		Ref	
	Q2	1.031 (0.497–2.317)	0.953	1.041 (0.501–2.161)	0.914	0.911 (0.381–2.178)	0.835
	Q3	0.737 (0.356–1.526)	0.411	0.740 (0.357–1.533)	0.418	0.518 (0.210–1.279)	0.154
	Q4	0.650 (0.312–1.351)	0.248	0.811 (0.488–1.349)	0.420	0.712 (0.281–1.803)	0.474
>68	Q1	Ref		Ref		Ref	
	Q2	0.383 (0.171–0.861)	0.020	0.391 (0.174–0.880)	0.023	0.428 (0.178–1.030)	0.058
	Q3	0.722 (0.333–1.568)	0.411	0.719 (0.331–1.564)	0.406	1.153 (0.490–2.718)	0.744
	Q4	0.422 (0.202–0.884)	0.022	0.434 (0.206–0.911)	0.027	0.645 (0.283–1.470)	0.297

Model 1: no covariates were adjusted; model 2: age and sex were adjusted; model 3: age, sex, race, and educational level were adjusted. OR, odds ratio. Q1: <1.09 mIU/L, Q2: 1.09–1.57 mIU/L, Q3: 1.57–2.30 mIU/L, Q4: >2.30 mIU/L.

A notable correlation was found between persistent *H. pylori* infection and ATDs in female patients, regardless of thyroid gland functional status. Specifically, this association was observed in relation to both HT and GD across multiple studies (10, 12), whereas no such

connection was noted for non-autoimmune thyroid disorders. Although this association was primarily observed in women, a trend suggesting a similar relationship was noted in men (34). Because of the limitations of the NHANES database, the present study was unable to

TABLE 7 Association of thyroxine level with *Helicobacter pylori* seropositivity based on subgroup of age.

Subgroup	T4	Model 1		Model 2		Model 3	
		OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
<41	Q1	Ref		Ref		Ref	
	Q2	0.436 (0.190–0.997)	0.049	0.434 (0.190–0.995)	0.049	0.288 (0.106–0.784)	0.015
	Q3	1.714 (0.792–3.712)	0.172	1.730 (0.797–3.758)	0.166	1.395 (0.539–3.612)	0.493
	Q4	1.062 (0.503–2.239)	0.875	0.921 (0.530–1.602)	0.835	0.749 (0.290–1.933)	0.550

(Continued)

TABLE 7 Continued

Subgroup	T4	Model 1		Model 2		Model 3	
		OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
41–54	Q1	Ref		Ref		Ref	
	Q2	2.500 (1.068–5.849)	0.035	2.974 (1.235–7.162)	0.015	2.711 (0.968–7.596)	0.058
	Q3	2.049 (0.911–4.610)	0.083	2.317 (1.006–5.336)	0.048	2.171 (0.782–6.030)	0.137
	Q4	4.083 (1.759–9.477)	0.001	4.965 (2.071–11.903)	<0.001	3.986 (1.346–11.798)	0.013
54–68	Q1	Ref		Ref		Ref	
	Q2	0.605 (0.292–1.256)	0.178	0.633 (0.302–1.324)	0.224	0.618 (0.252–1.513)	0.292
	Q3	0.646 (0.315–1.324)	0.233	0.683 (0.329–1.419)	0.307	0.853 (0.353–2.061)	0.724
	Q4	1.059 (0.515–2.178)	0.876	1.157 (0.546–2.454)	0.703	0.924 (0.360–2.373)	0.870
>68	Q1	Ref		Ref		Ref	
	Q2	1.026 (0.537–1.961)	0.937	0.975 (0.506–1.877)	0.939	1.134 (0.558–2.307)	0.728
	Q3	1.205 (0.590–2.459)	0.609	1.120 (0.542–2.316)	0.759	1.269 (0.584–2.759)	0.547
	Q4	1.564 (0.770–3.178)	0.216	1.446 (0.702–2.979)	0.318	1.306 (0.590–2.890)	0.510

Model 1: no covariates were adjusted; model 2: age and sex were adjusted; model 3: age, sex, race, and educational level were adjusted. OR, odds ratio. Q1: <83.7 nmol/L, Q2: 83.7–95.2 nmol/L, Q3: 95.2–110.7 nmol/L, Q4: >110.7 nmol/L.

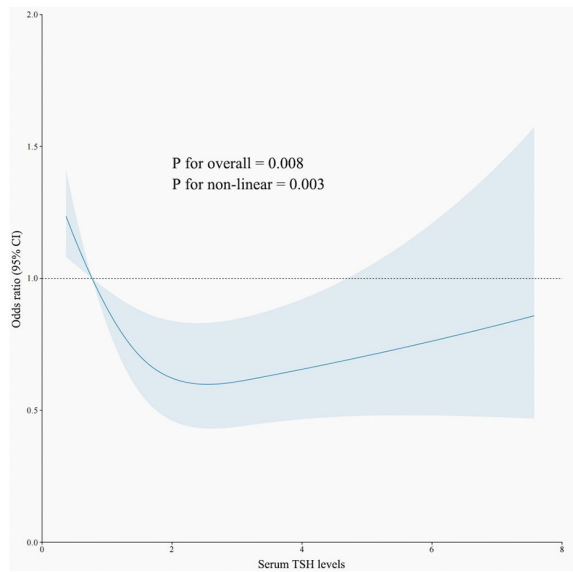


FIGURE 2
The restricted cubic spline curve for the relationship between serum TSH levels and *H. pylori* infection. The blue lines represent odds ratios, and blue areas represent 95% confidence intervals.

include thyroid autoantibodies such as thyroglobulin antibody (TGAb) and thyroid peroxidase antibodies (TPOAbs), which may have resulted in a weakened association between *H. pylori* seropositivity and TSH levels as well as T4 levels within gender cohorts.

Previous studies have suggested that the successful colonization of *H. pylori* in the gastric environment is influenced by age-related physiological factors and host characteristics, with the incidence of *H. pylori*-related diseases increasing with age (35, 36), a finding consistent with our results. Furthermore, additional factors such as socioeconomic status, geographical location, and ethnicity may also contribute to the rates of *H. pylori* infection (37). Breckan et al. reported that the prevalence of *H. pylori* is age-dependent, rising from adolescence and reaching its peak between the ages of 60 and 70 years (38).

It has to be recognized that there are several limitations in this study. First, *H. pylori* infection was defined based on serological testing, which cannot distinguish between past and present infections. Second, the NHANES 1999–2000 dataset lacked certain relevant information, such as TGAb and TPOAb, which weakens the association between *H. pylori* and thyroid autoimmunity, thus hindering a more thorough exploration of the underlying mechanisms. Should future NHANES updates include these data, more detailed classification comparisons could be conducted again in the future. Third, as a cross-sectional study, it does not allow for the establishment of causative relationships between TSH and T4 levels and *H. pylori* seropositivity, nor does it provide temporal data to differentiate between past and present *H. pylori* infections. Further longitudinal studies are needed to make interpretations of observed associations in the future. Lastly, while we accounted for the influence of certain medications, other factors, such as long-term contraceptive use in women, may affect thyroid function (39), as well as dietary factors like “high-salt” intake (40) or a high dietary inflammatory index (41), which are likely to influence the prevalence of *H. pylori* infection.

5 Conclusion

Overall, serum TSH and T4 levels were found to be associated with the risk of *H. pylori* infection, particularly among men, individuals with hyperthyroidism, and elderly adults. These people should pay close attention to *H. pylori* screening, as *H. pylori* infection is closely related to gastric cancer.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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Ethics statement

The studies involving humans were approved by Ethics Committee of Ningbo Medical Center Li Huili Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because the data were obtained from public databases and were exempted from ethical review by the Ethics Committee of Ningbo Medical Center Li Huili Hospital.

Author contributions

TL: Writing – review & editing, Data curation, Formal Analysis, Investigation, Writing – original draft. SL: Data curation, Formal Analysis, Writing – review & editing. JL: Data curation, Writing – original draft. XS: Data curation, Writing – review & editing. DC: Data curation, Writing – review & editing. JS: Conceptualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Does the island area also need to insist on salt iodization to prevent iodine deficiency disorders? a cross-sectional survey in Hainan Province, China

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Background: To investigate the epidemiology and related factors of iodine deficiency disorders (IDD) in Hainan Province, to know the iodine nutrition status and thyroid disease status of residents, and to explore whether salt iodization should be adopted to prevent and control IDD in island areas. To provide a basis for the effective implementation of scientific iodine supplement prevention and control strategy.

Methods: All 21 cities, counties (districts) in the province were investigated. Superficial soil, residential drinking water, common food, urine samples of children, adults and pregnant women, household salt samples, thyroid B-ultrasound of adults and pregnant women, adult blood samples were collected. Soil iodine, water iodine, food iodine, urine iodine, daily salt intake, salt iodine, thyroid volume, nodules and thyroid function were measured.

Results: The median iodine content in soil was 4.37mg/kg, the median iodine content in drinking water was 6.0μg/L, the iodized salt coverage rate was 98.6%, and the consumption rate of qualified iodized salt was 97.9%. The median urinary iodine concentration (MUIC) in children and adults was 180.3μg/L and 151.6μg/L, respectively, which was the adequate level of iodine. The median urinary iodine in pregnant women was 144.6μg/L, which was slightly lower than the adequate level. The main source of iodine intake was salt iodine, which contributed 59.8% to total dietary iodine. Kelp, milk and seaweed, whose contribution rates were 8.2%, 7.3% and 6.6%, respectively. The total iodine contribution rate of other foods was 18.1%, among which the contribution rate of fish, shrimp and crabs was only 2.4%. The overall prevalence of thyroid diseases was 27.01%. The detection rates of subclinical hypothyroidism and subclinical hyperthyroidism in males were significantly higher than those in females, and the detection rates of thyroid nodules and goiter were significantly lower than those in females. The

detection rates of thyroid nodules in central mountainous areas were significantly higher than those in eastern and western coastal areas.

Conclusions: At present, the iodine nutrition level in Hainan Province is generally in a suitable state, and the iodine intake of residents mainly comes from iodized salt. The strategy of salt iodization prevention and control of IDD should be adhered to in island area.

KEYWORDS

island area, iodine status, salt iodization, iodine deficiency disorders, thyroid function

1 Introduction

Iodine deficiency disorders (IDD) is a general term for a group of diseases and hazards manifested by insufficient iodine intake, mainly including endemic goiter and endemic cretinism, overt hypothyroidism (1–3). Similarly, excessive iodine intake can also cause goiter, autoimmune thyroiditis, and increased detection rate of subclinical hypothyroidism (4, 5). Since 1995, China has implemented the policy of universal salt iodization for prevention and treatment of IDD, which has gradually solved the problem of iodine malnutrition and IDD in China for a long time. Since 2010, China has continued to eliminate IDD, providing experience for the prevention and treatment of IDD in other countries (6). Hainan Province is the southernmost provincial administrative region in China, surrounded by the sea, with a permanent population of 10.43 million. In recent years, there has been the spread of the opinion that “living by the sea is not short of iodine and excessive iodine supplement by salt causes increasing in thyroid diseases”. There are misunderstandings about the knowledge about iodine and thyroid diseases, and some residents have the mentality of “fearing salt iodization”, which has a certain impact on the prevention and treatment of IDD in China.

In order to understand the iodine intake level of residents in island province, the iodine nutrition status of the population, the relationship between iodine and thyroid disease, and whether to continue to eat iodized salt, we conducted a cross-sectional survey in all districts and counties of Hainan Province, providing scientific basis for the prevention and control of IDD in island areas, and providing new ideas for the prevention and control of IDD in coastal areas, so as to truly implement local conditions, classified guidance and scientific iodine supplementation.

Abbreviations: DRIs, Dietary nutrients; FT3, Free triiodothyronine; FT4, Free thyroxine; IDD, Iodine deficiency disorders; I-FFQ, Iodine-specific food Frequency Questionnaire; MUIC, Median urinary iodine concentration; RNI, Recommended nutrient intake; TGAb, Thyroglobulin antibody; TPOAb, Thyroid peroxidase antibody; TSH, Thyroid stimulating hormone; UL, Tolerable upper intake level; USI, Universal salt iodization.

2 Materials and methods

2.1 Survey areas

All the 21 cities and counties (districts) were selected in Hainan Province, and each survey county was divided into 5 sampling areas according to the east, west, south, north and middle, and 1 township/street is randomly selected in each area.

2.2 Survey content and sample collection

Currently, all Chinese people no longer adopt other specific measures to supplement iodine, such as taking iodized oil pills. So, two soil samples (5 ~ 20cm) and two peripheral water samples were randomly selected from each survey site. Each district selected 20 permanent residents over 18 years old (non-same household, male and female half), 20 pregnant women, 40 children (boy and girl half). Urine sample (10mL), salt sample (50g), thyroid B-ultrasound detection in adults and pregnant women, adult blood sample (5mL), urine iodine, salt iodine, thyroid volume and nodule detection, and thyroid function detection in adults were collected. Including free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH), thyroglobulin antibody (TGAb), thyroid peroxidase antibody (TPOAb) determination.

2.3 Investigation of iodine content in food

Grains (rice, noodles), meat (pork, beef, mutton, chicken, duck), milk (milk, yogurt), eggs (eggs, duck eggs, salted duck eggs), fish (freshwater fish, shellfish, sea fish, shrimp, crabs), bean products (tofu, bean curd, dried beans), seaweed (kelp, laver, nori), pickled products (Chinese sauerkraut, tuber mustard, etc.), processed food (sausage, ham sausage) 9 categories, each category 2 different kinds of samples sold in the market are collected in each county (district). Collect 5 servings of different kinds of fresh vegetables and 5 servings of different kinds of fresh

fruits that are commonly eaten by local residents. No less than samples of various types of food were collected from each city, county (district) to determine the iodine content.

2.4 Survey of total iodine intake in population

Per capita daily salt intake, per capita daily water intake and Iodine-specific food Frequency Questionnaire (I-FFQ) were measured using 3-day weighing method to calculate the daily food intake of adults and pregnant women.

According to the following formula: Total dietary iodine intake = $[(\Sigma \text{ intake of various types of food} \times \text{iodine content of various types of food}) + (\text{drinking water} + \text{water consumption for cooking food}) \times \text{iodine content of water} + \text{salt intake} \times \text{salt iodine content}] \times (1 - \text{cooking loss rate}) \times 92\% \text{ bioavailability}$. The WHO defines the cooking loss rate of iodized salt as 20%.

2.5 Detection method

The salt iodine content was detected by direct titration method of “Determination of iodine by General Test Method for Salt Industry”. The urinary and food iodine content was determined using the As-Ce catalytic spectrophotometry method. Determination of soil iodine content according to “Regional geochemical Sample analysis method Part 24 Inductively coupled Plasma mass spectrometry”. Thyroid function test was determined using the direct chemiluminescence method. Thyroid measurement was performed using B-ultrasound with a probe frequency of 7.5 MHz or higher.

2.6 Diagnostic criteria

Participants were randomly selected. The diagnostic criteria for thyroid diseases were listed in [Table 1](#).

2.7 Statistical analysis

Excel software was used to establish the database and organize the data. Statistical analyzes of this study were performed using IBM SPSS v20.0 (IBM Corporation, USA). Normal distribution test was carried out for continuous data, normal distribution data was represented by mean, and T-test was used for comparison between groups. Non-normal data were represented by median. Mann-whitney test was used for comparison between two groups, and Kruskal-Wallis test was used for comparison between multiple groups. Statistical data were expressed as numbers and percentages, and χ^2 test was used for comparison between groups. Bilateral test was used in all cases, and $P < 0.05$ was considered statistically significant.

TABLE 1 Diagnostic criteria for the thyroid diseases included in this study.

Thyroid disease	Diagnostic criteria*
Goiter	Thyroid volume >25 ml (male) or >18 ml (female) (14)
Nodule	Goiter with nodule >5 mm in diameter
Overt hyperthyroidism	FT4 > 19.05 pmol/L, TSH < 0.35 μ IU/mL
Subclinical hyperthyroidism	FT4 within the normal range, TSH < 0.35 μ IU/mL
Overt hypothyroidism	TSH > 4.94 μ IU/mL, FT4 < 9.01 pmol/L
Subclinical hypothyroidism	TSH > 4.94 μ IU/mL, FT4 within the normal range
Thyroid autoimmunity	TPOAb > 5.61 IU/mL; TGAb > 4.11 IU/mL, or both

*Reference ranges: FT3, 2.43–6.01 pmol/L; FT4, 9.01–19.05 pmol/L; TSH, 0.35–4.94 μ IU/mL; TPOAb, 0–5.61 IU/mL; TgAb, 0–4.11 IU/mL. Reference ranges were provided by the fourth affiliated hospital of Harbin Medical University.

3 Results

3.1 Basic information

According to the geographical location of Hainan Province, the 21 districts and counties are divided into western coastal areas (Chengmai, Changjiang, Ledong, Danzhou, Dongfang, Lingao), central mountainous areas (Baoting, Tunchang, Baisha, Qiongzong, Wuzhishan, Ding ‘an) and eastern coastal areas (Xiuying District, Longhua District, Qiongzong District, Meilan District, Qionghai, Sanya, Wanning, Wenchang, Lingshui). The median water iodine concentration in the western coastal, central mountainous, and eastern coastal areas was below 10.0 μ g/L. Additionally, the coverage of iodized salt and the rate of consumption of qualified iodized salt were both over 95%. Basic information is shown in [Table 2](#).

3.2 Iodine content of food in different regions

A total of 632 food samples were collected, among which the iodine content of laver was the highest (4424.76 μ g/100g), and that of condiment was the lowest (2.41 μ g/100g). The difference analysis of iodine content of various foods between the western coastal area, the central mountainous area and the eastern coastal area showed that there was no statistical difference in iodine content of other foods except for the significant difference in fruit ($H=6.656$, $P=0.036$). The results are showed in [Table 3](#).

3.3 Dietary iodine intake of different populations in different regions

A total of 4227 people were investigated, including 2120 adults and 2107 pregnant women. The food intakes of adults and pregnant

TABLE 2 Basic information of the survey areas.

Survey areas	Survey object	Water Iodine (μg/L)	Soil Iodine (mg/kg)	Urinary Iodine (μg/L)	Household salt iodine content (mg/kg)	Iodized salt coverage(%)	Qualified iodized salt consumption rate(%)
West coast	children	6.1 (672)	2.82 (60)	179.1(1238)	24.9 (1868)	98.2	96.3
	adults			141.6(603)			
	pregnant women			139.4(610)			
Central area	children	3.8 (634)	5.13 (60)	186.0(1236)	25.0 (1840)	99.2	98.0
	adults			148.4(602)			
	pregnant women			141.4(604)			
East coast	children	6.0 (1260)	5.17 (90)	172.8(1827)	24.1 (2733)	98.3	95.6
	adults			155.4(913)			
	pregnant women			132.3(906)			

(): number

women in western coastal areas, central mountainous areas and eastern coastal areas were 117.0 μg/d, 102.8 μg/d and 106.2 μg/d, respectively. Except soybean products, fruits and processed foods, iodine intake of other foods had statistical significance among different regions ($P<0.05$). The results are listed in [Table 4](#).

3.4 Iodine intake in water of different populations in different regions

The median iodine concentration in total water was 6.0 μg/L, and the iodine intake through drinking water was 6.5 μg/d.

The daily iodine intake in western coastal area, central mountainous area and eastern coastal area was 6.1 μg, 3.6 μg and 7.6 μg, respectively. There were statistically significant differences in water iodine intake between adults and pregnant women in different regions ($P<0.05$). The results are shown in [Table 5](#).

3.5 Iodine intake in salt of different populations in different regions

A total of 2120 adults and 2107 pregnant women were investigated. The median salt iodine content was 24.6 mg/kg, and there was no

TABLE 3 Iodine content of food in different regions(μg/100g).

Food	Western		Central		Eastern		Total		<i>H</i>	<i>P</i>
	N	IC	N	IC	N	IC	N	IC		
Staple food	11	4.62	12	2.89	14	3.82	37	3.76	2.948	0.229
Bean products	15	13.11	13	10.39	23	7.56	51	9.91	4.315	0.116
Meat	24	6.75	27	6.76	24	5.60	75	6.44	3.112	0.211
Eggs	15	25.87	14	26.44	14	23.62	43	25.32	0.619	0.734
Dairy	12	24.23	11	25.21	17	23.03	40	23.99	0.148	0.929
Seafood	17	32.43	20	32.15	29	23.96	66	28.16	2.270	0.321
Vegetables	33	3.31	27	4.99	45	4.49	105	3.86	3.040	0.219
Fruits	31	2.50	28	3.06	44	4.10	103	3.34	6.656	0.036
Nori	6	4386.7	6	4880.0	9	4688.9	21	4424.8	1.212	0.545
Wet kelp	6	1606.0	6	2785.0	5	2570.2	17	2305.7	0.500	0.779
Salted products	14	8.11	9	8.41	20	7.22	43	7.76	2.439	0.295
Processed food	9	45.29	6	49.48	13	47.88	28	46.42	0.115	0.944
Condiment	2	2.41	2	2.41	1	2.41	5	2.41	1.800	0.407

IC, iodine concentration.

TABLE 4 Dietary iodine intake of different populations in different regions (μg/d).

Food	Western		Central		Eastern		Total		H	P
	A	PW	A	PW	A	PW	A	PW		
Staple food	11.1	9.0	13.8	11.6	10.5	9.6	11.8	10.1	47.034	<0.001
Bean products	10.0	1.5	1.0	1.2	0.9	1.1	4.0	1.3	52.687	<0.001
Meat	2.0	1.9	2.3	2.3	2.2	2.2	2.2	2.1	6.322	0.012
Eggs	5.5	7.1	5.7	7.3	6.6	7.5	5.9	7.3	82.861	<0.001
Dairy	13.7	28.2	10.4	28.7	15.5	28.2	13.2	28.4	497.320	<0.001
Seafood	7.8	8.7	5.0	5.3	4.8	8.9	5.9	7.6	7.780	0.005
Vegetables	10.0	9.6	10.8	11.2	11.0	10.8	10.6	10.5	1.594	0.207
Fruits	4.1	5.9	4.0	5.9	4.8	5.5	4.3	5.8	209.566	<0.001
Nori	24.1	21.8	16.3	15.3	16.2	18.5	18.9	18.5	4.345	0.037
Wet kelp	24.8	24.3	20.8	24.4	19.2	26.6	21.6	25.1	6.823	0.009
Salted products	0.4	0.3	0.4	0.3	0.2	0.2	0.3	0.3	35.482	<0.001
Processed food	0.6	0.5	0.5	0.4	0.3	0.3	0.5	0.4	8.895	0.003
Condiment	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	14.730	<0.001
Total	114.2	118.9	91.1	114.0	92.3	119.5	99.2	117.5	152.015	<0.001
Utilization rate	84.1	87.5	67.0	83.9	67.9	88.0	73.0	86.5	152.024	<0.001

A, adults; PW, pregnant women; Utilization rate= Total× (1- 20%cooking loss rate) ×92% bioavailability.

statistical difference in salt iodine content between different regions. The total salt iodine intake was 172.2 μg/d, and the daily salt iodine intake was 173.2 μg, 172.2 μg and 169.4 μg in western coastal area, central mountainous area and eastern coastal area, respectively, with no significant difference. The results are listed in Table 6.

3.6 Total iodine intake and distribution of different populations in different regions

Excluding 20% cooking loss rate and considering 92% bioavailability, the average total iodine intake of residents was 211.5 μg/d, that of adults 204.8 μg/d, and that of pregnant women 217.9 μg/d. The total daily iodine intakes were 218.1 μg, 205.0 μg and 208.4 μg in the western coastal region, the central mountainous region and the eastern coastal region, respectively.

There were statistically significant differences in total iodine intake among different populations in different regions ($P<0.05$). The results are shown in Table 7.

3.7 Urinary iodine status of different populations in different regions

The overall median urinary iodine levels in children, pregnant women and adults were 180.3 μg/L, 144.6 μg/L and 151.6 μg/L, respectively, and the difference was statistically significant ($P<0.05$). The median urinary iodine values of children in the central mountainous area were 190.8 μg/L, 149.4 μg/L and 161.0 μg/L, respectively, which were significantly higher than those in the western coastal area and the eastern coastal area. The results are shown in Table 8.

TABLE 5 Iodine intake in water of different populations in different regions (μg/d).

Area	WI	Adults			PW			Total			H	p
		N	WII	TII	N	WII	TII	N	WII	TII		
Western	6.5	501	1001	6.5	502	910	5.9	1003	931	6.1	11.736	0.001
Central	3.3	701	1221	4.0	704	1042	3.4	1405	1083	3.6	8.863	0.003
Eastern	6.5	918	1187	7.7	901	1180	7.7	1819	1175	7.6	0.186	0.666
Total	6.0	2120	1153	6.9	2107	1056	6.3	4227	1087	6.5	14.046	<0.001

WI, water iodine; WII, water iodine intake; TII, Total iodine intake.

TABLE 6 Iodine intake in salt of different populations in different regions (μg/d).

Area	SI	Adults			Pregnant women			Total			H	p
		N	SII	TII	N	SII	TII	N	SII	TII		
Western	24.4	601	6.9	168.4	602	6.9	168.4	1203	7.1	173.2	0.006	0.938
Central	24.6	601	7.0	172.2	604	6.9	169.7	1205	7.0	172.2	1.316	0.251
Eastern	24.2	918	7.1	171.8	901	7.3	176.7	1819	7.0	169.4	1.766	0.184
Total	24.6	2120	7.0	172.2	2107	7.0	172.2	4227	7.0	172.2	2.061	0.151

SI, salt iodine; SII, Salt iodine intake; TII, Total iodine intake.

3.8 Prevalence of thyroid diseases in adults in different region

A total of 2078 adults were surveyed, including 940 males and 1138 females. The detection rates of different thyroid diseases were: thyroid nodule (25.41%), subclinical hyperthyroidism (3.66%), subclinical hypothyroidism (2.60%), goiter (2.45%), over hypothyroidism (1.36%) and over hyperthyroidism (0.77%). The positive rates of TgAb and TPOAb were 26.95% and 20.40%, respectively. There were significant differences in thyroid nodules in the western coastal area, the central mountainous area and the eastern coastal area. No statistical difference was found in different regions for other diseases. The results are shown in Table 9.

4 Discussion

The average iodine content in soil and the median iodine content in water were 4.37 mg/kg and 6.0 μg/L respectively. According to the Standard for the Definition and demarcation of iodine deficient areas and iodine adequate areas (WS/T 669-2020) (7) all cities, counties and towns in Hainan Province are iodine-deficient areas. The iodine content in soil and water was low, and there was no correlation between the two.

According to the results, the coverage rate of iodized salt was 98.6%, and the consumption rate of qualified iodized salt was 97.9%. The iodine content of various foods in Hainan Province is similar to the results of the Iodine Supplement Guide for Chinese Residents, and the average dietary iodine intake of residents is 211.5 μg/d (Adults 204.8 μg/d, pregnant women 217.9 μg/d), slightly lower than the 231.22 μg/d in coastal areas of Tianjin (8) and slightly

higher than the 198.3 μg/d in coastal areas of Fujian (9). According to the Reference Intake of Dietary Nutrients for Chinese Residents (2023 edition), the dietary iodine intake of the general population of Hainan residents is within the UL (Tolerable upper intake level) (600 μg/d), while that of pregnant women is slightly lower than RNI (Recommended nutrient intake) (230 μg/d).

The iodine intake of Hainan residents mainly comes from salt iodine, and the contribution rate of salt iodine to total dietary iodine is 59.9% (172.2/287.4), which is lower than that of Tianjin (8) (69.0%) and Fujian (9) (63.8%). The food with high iodine intake is kelp, whose contribution rate is 8.1% (23.4/287.4), and the total contribution rate of other foods is 32%. Among them, the contribution rate of fish, shrimp and crabs and other seafood is only 2.4%, ranking seventh among the dietary iodine contribution rates of Hainan residents. The contribution rates of processed food, pickled products and condiments were only 0.2%, 0.1% and 0 respectively, mainly because the processed food and pickled products of Hainan residents generally used non-iodized salt, and soy sauce was the main condiment.

Hainan Province is located in the coastal zone, seafood sources are rich, and the overall seafood intake of residents is higher, but the contribution rate of aquatic products to the dietary iodine of Hainan residents is far lower than that of iodized salt, kelp, seaweed. It can be seen that in coastal areas, although the iodine intake in some foods is higher than that in inland areas, the iodine nutrition obtained from food alone still does not reach the recommended intake, This is consistent with other research findings (10). Compared with the reference intake of dietary nutrients (DRIs), if there is no salt iodine intake, 90.1% of Hainan residents' dietary iodine intake is less than RNI, and 9.9% is between RNI and UL. When salt iodine intake was added, the dietary iodine intake was less than the RNI in 34.7% of the

TABLE 7 Total iodine intake and distribution of different populations in different regions (μg/d).

Area	Western			Central			Eastern			Total		
	A	PW	Total	A	PW	Total	A	PW	Total	A	PW	Total
Food	114.2	118.9	117	91.1	114	102.8	92.3	119.5	106.2	99.2	117.5	108.7
Water	6.5	5.9	6.1	4	3.4	3.6	7.7	7.7	7.6	6.9	6.3	6.5
Salt	168.4	168.4	173.2	172.2	169.7	172.2	171.8	176.7	169.4	172.2	172.2	172.2
Total	289.1	293.2	296.3	267.3	287.1	278.6	271.8	303.9	283.2	278.3	296.0	287.4
Utilization rate	212.8	215.8	218.1	196.7	211.3	205.0	200.0	223.7	208.4	204.8	217.9	211.5

A, adults; PW, pregnant women; Utilization rate= Total× (1- 20%cooking loss rate) ×92% bioavailability.

TABLE 8 Median urinary iodine status of different populations in different regions.

Area	Children	Pregnant women	Adults	Z	P
Western	184.2	137.0	144.9	117.92	<0.05
Central	190.8	149.4	161.0	121.42	<0.05
Eastern	174.3	145.6	149.8	80.051	<0.05
Total	180.3	144.6	151.6	314.8	<0.05
Z	14.364	7.633	11.354		
P	0.001	0.022	0.003		

individuals. If no iodized salt was consumed, the dietary iodine intake of the vast majority of adults (85.0%) and pregnant women (95.1%) could not reach the RNI. After adding iodized salt, 97.7% of the adult dietary iodine intake in Hainan Province was between RNI and UL, and 67.5% of the pregnant women's dietary iodine intake was lower than the RNI formulated by the Chinese Nutrition Society. Due to the special physiological needs of pregnant women, RNI is higher than the general population, under the same salt iodine level, cannot meet the needs of the body, pregnant women should be guided to increase the intake of iodine-rich food.

As a key group of iodine supplementation, it is of great significance for children to maintain appropriate iodine nutrition status, and the median urinary iodine of children is often used as an indicator of iodine nutrition evaluation of the whole population. The survey shows that the median urinary iodine of children in Hainan Province is 180.3 $\mu\text{g/L}$, which is at an appropriate level, the ratio of urinary iodine < 50 $\mu\text{g/L}$ is 4.3%, and the median urinary iodine of adults is 151.6 $\mu\text{g/L}$, which meets the elimination standard of IDD in China, indicating that through the cooperation of relevant departments and joint efforts in various aspects, with the intervention of comprehensive prevention and control measures based on USI (Universal salt iodization), the iodine nutrition level of Hainan residents remained in a suitable state. It also confirmed the rationality of using the urinary iodine level of children as the iodine nutrition evaluation index of the general population in the national iodine deficiency elimination monitoring system.

The monitoring results showed that the median urinary iodine of pregnant women in the province was 144.6 $\mu\text{g/L}$, slightly lower than the recommended standard, and there was a risk of iodine deficiency, which should be paid great attention to. The median urinary iodine was lower than the Kerver JM's result (11). The median urine iodine in 6 of the 21 districts or counties was greater than 150.0 $\mu\text{g/L}$, and the median urine iodine in the other 15 cities or counties was between 100.0 and 149.0 $\mu\text{g/L}$. Pregnant women, as a special needs population, may need other iodine supplementation measures to meet their own needs and those of their offspring. Some studies have also shown that the median urinary iodine between 100 and 149 $\mu\text{g/L}$ can meet the iodine needs of pregnant women, and the difference in urine volume during different pregnancy may affect the median urinary iodine. Given the high iodized salt coverage rate, scientific research on the appropriate median urinary iodine should be further strengthened.

The overall prevalence rate of adult thyroid disease in this survey was 27.01% (550/2036), which was similar to that of the study conducted by Weiping Teng (12, 13). The prevalence of subclinical hypothyroidism and subclinical hyperthyroidism in males was significantly higher than that in females, while the detection rate of thyroid nodules and goiter was significantly lower than that in females, this is similar to Liu's findings (14). The detection rate of thyroid nodules was significantly different among different regions, and the central mountainous area was significantly higher than the eastern and western coastal areas.

TABLE 9 Prevalence of thyroid diseases in adults in different region (%).

Diseases	Western (n=897)		Central (n=586)		Eastern (n=897)		TDR	χ^2	p
	N	DR	N	DR	N	DR			
over hypothyroidism	2	0.34	4	0.68	10	1.11	0.77	2.699	0.237*
subclinical hypothyroidism	14	2.35	13	2.22	27	3.01	2.6	1.076	0.584
over hyperthyroidism	6	1.01	3	0.51	12	1.34	1.01	2.417	0.299
subclinical hyperthyroidism	22	3.7	24	4.1	30	3.34	3.66	0.571	0.752
thyroid nodules	139	23.36	197	33.62	192	21.4	25.41	29.739	<0.001
goiter	10	1.68	12	2.05	29	3.23	2.45	4.164	0.125
positive TPOAb	122	20.5	111	18.94	191	21.29	20.40	1.212	0.546
positive TgAb	171	28.74	157	26.79	232	25.86	26.95	1.513	0.469

DR, detection rates.

There was no regular trend in the detection rates of other diseases in the three regions, and no statistical difference was observed in all the three regions, which could not be attributed to the fact that urinary iodine in the central mountainous region was significantly higher than that in the other two regions (161.0 $\mu\text{g/L}$ VS 149.8 $\mu\text{g/L}$, 144.9 $\mu\text{g/L}$), and the median urinary iodine in the three regions was within the appropriate range. The pathogenesis of thyroid diseases is complicated and varied, and may be affected by heredity, endocrine dysfunction, immune dysfunction, mental stress, environmental pollution and other factors (15–18). Currently, the detection rate of thyroid diseases in Hainan Province is at a normal level.

However, this research still has some limitations. For example, the small size of the analyzed population groups, the heterogeneity of the evaluated samples, the variability of foods in the diet of the locals included in the study, etc.

In conclusion, the level of iodine in ambient water and soil is low in all districts or counties of Hainan Province, all of which are iodine deficient areas, and the iodine intake of residents mainly comes from salt. The iodine nutrition level of children and adults is in an appropriate state, and the median urine iodine of pregnant women is slightly lower than the recommended standard. Pregnant women can appropriately add iodine-rich foods such as kelp and laver when eating iodized salt. The overall detection rate of thyroid disease was in a reasonable range, and no statistical difference was found between different regions except for the detection rate of thyroid nodules. Therefore, salt iodization should be insisted on preventing IDD in all island regions of China.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by Ethics Review Committee of Hainan Provincial Center for Disease Control and Prevention. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Prevalence of goitre and associated factors among school-aged children in Guraferda District, Southwest Ethiopia

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Introduction: Goitre is a significant public health problem, particularly in underdeveloped countries like Ethiopia. Iodine Deficiency Disease is the leading cause of preventable brain injury in children, resulting in poor academic performance.

Objectives: To determine the prevalence of goitre and associated factors among children aged 6–12 years in Guraferda District, Southwest Ethiopia in 2024.

Methods: A community cross-sectional survey was conducted with 949 children ages 6–12 years who lived in selected kebeles in Guraferda District. Structured questionnaires, physical examinations, and iodized salt tests were all used to collect data. To identify factors related with goitre, a logistic regression analysis was performed using SPSS version 26. Statistical significance was determined at $p < 0.05$ using adjusted odds ratios and 95% confidence intervals.

Results: The prevalence of Goitre among school-age children in this study was 37.6, 95% CI: 34.4, 40.8%. Female gender (AOR = 1.614, 95% CI: 1.199, 2.172), mothers with non-formal education (AOR = 1.93, 95% CI: 1.437, 2.592) (AOR = 1.93, 95% CI: 1.44, 2.592), rural residence (AOR = 2.291, 95% CI: 1.162, 3.239), storing salt near heat sources (AOR = 1.407, 95% CI: 1.042, 1.900), low food diversification status (AOR = 4.928, 95% CI: 3.332, 7.289), and consuming cabbage at least once a week (AOR = 2.874, 95% CI: 2.012, 4.106) were positively associated with Goitre, while consuming milk at least once a week (AOR = 0.217, CI: 0.145, 0.324) was negatively associated with Goitre.

Conclusion: The study findings indicate a high prevalence of Goitre in the area. Factors such as being female, living in rural areas, mothers with no formal education, storing salt near heat sources, consuming cabbage, and low food diversification were associated with increased odds of Goitre. Therefore, it is recommended to ensure universal access to iodized salt and raise awareness in the community about the importance of using iodized salt.

KEYWORDS

child, goitre, iodine deficiency, associated factors, prevalence, cross-sectional study, Ethiopia

Introduction

Goitre, characterized by an unusual swelling of the thyroid gland, is among the most prevalent endocrine issues affecting children and adolescents (1). It represents one of the most severe outcomes of prolonged iodine deficiency in the human body, particularly in children residing in areas with low iodine levels (2). The condition is typically triggered by a significant intake of goitrogenic foods such as millet, sweet potatoes, corn, and cabbage, along with a minimal consumption of iodine-rich foods (2, 3). Iodine rich foods like Seaweed (nori, kelp, kombu, wakame) Fish, shellfish (cod, canned tuna, oysters, shrimp) are not commonly consumed in this study area (4).

The diagnosis of goitre depends on the visibility of the thyroid gland and the degree of its enlargement or the presence of nodules inside it. As a result, in 1979, it was advised that the palpation technique be employed as the most precise and reliable way for detecting endemic goitre and grading its severity (5). Iodine deficiency is caused by either insufficient iodine consumption in the diet or the intake of goitrogens (6). A total goitre rate (TGR) of 5% or higher is suggested as the threshold to signify a public health issue according to the consensus reached by major international organizations, including the WHO (7). Goitre classified based on WHO/UNICEF/ICCIDD classification scheme as follows: Grade 0: None or no goitre (palpable or visible) Grade 1 or palpable: A goitre that is palpable but not visible when the neck is in the normal position, (i.e., the thyroid is not visibly enlarged). Grade 2 or visible: A swelling in the neck that is clearly visible when the neck is in a normal position and is consistent with an enlarged thyroid when the neck is palpated. The total goiter rate (TGR) is the percentage of people in a population who have a goiter, which is an enlarged thyroid gland. TGR is calculated by dividing the number of people with a goiter by the total number of people examined (8).

In 2020, globally, 21 countries still have insufficient iodine in their diets (9). The most recent global estimate estimates that 1.88 billion individuals, including 241 million school-age children, do not get enough dietary iodine (10). According to the WHO, as of 2004, 42.6% of Africa's population suffers from low iodine intake (11). Over a 10-year period from 1993 to 2003, trend analyses showed that the prevalence of Goitre rose from 15.6 to 28.3% (12).

Furthermore, half of the world's 736 million extremely poor people live in only five countries: India, Nigeria, the Democratic Republic of the Congo, Ethiopia, and Bangladesh. India (93% HHIS, mUIC in women of reproductive age 178 µg/L), Nigeria (93% HHIS, mUIC in SAC 130 µg/L), Democratic Republic of Congo (82% HHIS, mUIC in SAC 249 µg/L), and Ethiopia (86% HHIS, mUIC in SAC 104 µg/L) are all iodine-sufficient at the national level (9).

The prevalence of goitre among children aged 6–12 years varies according to numerous research conducted around the world. It was recorded as 20.5% in India, 11.4% in Rajasthan, 32% in Portugal, 24.2% in Iran, 22.3% in southern Sudan (12–16). In Ethiopia a systematic review reported a pooled prevalence of goitre among school-age children was 42.9% (95% CI: 38.8–46.9). The highest prevalence of goitre (46.7%) was observed in Oromia region and the lowest (26.3%) was observed in Benishangul-Gumuz region (17).

Ethiopia began its iodized salt program in the 1990s, and the government has achieved tremendous success in iodized salt coverage, resulting in sharply enhanced iodine intake across the country. The country has achieved and sustained greater than 89% Household (HH) iodized salt coverage from its lowest point of 15% coverage and subsequently improved iodine intake across its population (18). Based on the most recent available median urinary iodine concentration (UIC) data from 194 WHO Member States Ethiopia has adequate Iodine intake (19). In addition, iodine levels in salt surpassing 15 ppm were found in 42.7% of families. Additionally, 23.2% of households had salt levels that met the national threshold of 20–40 ppm (20). Despite the Ethiopian government's efforts to implement mandated salt iodization over the past decade, an iodine shortage persists in the country (21). The prevalence of iodine deficiency among school age children, with mean urinary iodine concentration below the cut-off, was 48% (22). Systematic review and meta-analysis done reported the pooled prevalence of iodine deficiency among school-age children in Ethiopia was found to be 58% (95%CI 44.00–77.00) (23). Furthermore, several places have reported endemic cases linked to goitrogens present in drinking water, which may include specific chemical components that affect the synthesis of thyroid hormones (24). Goitrogens are chemicals that are toxic to the thyroid or that break down to produce toxic chemicals. Goitrogens are present in various foods, such as cassava, cabbage, turnips, and rutabagas. Cassava, for example, is a staple in Africa (25). Goitrogens such as fluoride, found in water, and thiocyanates, found in cassava and cruciferous vegetables, can interfere with iodine uptake. Foods are considered goitrogenic if they contain substances that inhibit thyroid function or iodine utilization (26).

The aim of this study is to assess the prevalence of goitre and associated factors among school age children (6–12 years of age) in Guraferda District, Southwest Ethiopia.

Materials and methods

Study design, setting and population

A cross-sectional study based in the community was carried out from February 1 to March 30, 2024, in Guraferda District, located in the Bench-Sheko Zone, which comprises one of the six Districts in this zone. The total population in the study area was estimated to be 51,016 (25,253 males and 25,763 females) according to the 2007 Gregorian calendar (G.C) Census conducted by the Central Statistics Agency (CSA), with a projected 10,411 households in 2024. Guraferda District is geographically located between 6°51'0" north and 35°4'0" east of the equator. It covers an area of 2,565.40 km² and is at an elevation of 501–2,500 m above sea level. The mean annual temperature in the zone is 20–32°C, with an average rainfall of 700–1,500 mm. Guraferda District is made up of four urban kebeles (It is the smallest administrative unit in Ethiopia) and 27 rural kebeles, totalling 31 kebeles. The district contains five high schools, 48 primary schools, and three government-operated kindergartens alongside three private ones. Data from the Guraferda District Education office indicates that there are 9,307 school-age children residing in the district. The

primary agricultural products produced in the district include maize, rice, millet, cassava, cabbage, godere (is a staple food in all Kebeles of the Guraferda District which was source of most of the daily food intake for large rural populations), honey, and various animal products. The study included all school-aged children (6–12 years) living in the area, excluding only children in the same age group with neck swelling unrelated to Goitre.

Sample size determination and sampling procedures

Sample size determination

In order to establish the sample size for this study, a literature review was conducted for both objectives. After calculating the sample sizes for each objective individually, the larger sample size was selected for this study. This study has two specific objectives as follows:

- 1 To determine the prevalence of goitre and
- 2 To identify factors associated factors with Goitre.

The sample size determination for the 1st objective

The sample size for determining the prevalence of goitre was calculated using a single population proportion formula, based on the following assumptions: a confidence level of 95%, a margin of error of 5%, and a goitre prevalence of 28.37% (27) among school-age children.

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}}\right)^2 * P(1-p)}{d^2}$$

Where, n = sample size, $Z_{\alpha/2}$ = Critical value = 1.96, P = prevalence of goitre (28.37%), δ = precision (marginal error) = 0.05.

Then, $n = \frac{(1.96)^2 (0.2837)(1-0.2837)}{0.04^2} \cong 488$, by taking design effect 1.5 and adding 10% non-response rate the total sample size will be $= \left(488 * 1.5\right) + \left(\frac{488 * 1.5 * 10}{100}\right) = 805$

Finally, the sample size for the 1st objective was 805.

The sample size determination for the 2nd objective

The sample size for factors associated with goitre in school-aged children (6–12 years) was calculated from different studies (28, 29), using EPI info version 7.1 with a double population proportion formula to calculate the OR of the factors (Table 1). The assumptions were: 80% Power, 95% confidence level (z) and Ratio exposed of unexposed is 1:1.

Finally, the sample size obtained was the largest sample size from the two objectives, which was 994.

Sampling procedures

Guraferda District comprises a total of 31 kebeles. A simple random sampling method, specifically a lottery system, was used to select 30% of these kebeles. A survey was carried out in the chosen kebeles to assess the total number of school-age children living there. The overall sample was allocated proportionally among the selected kebeles. Households containing at least one school-age child were identified using family folder codes, which were then compiled to establish a sample frame. These codes were entered into Microsoft Excel, where random numbers were generated to select households. In cases where there were multiple school-age children (ages 6–12) in a selected household, one child was randomly chosen using a lottery method. If the household head was not present during data collection, a follow-up visit was arranged for the next day. If the household head remained absent after the second visit, a third visit was planned for the subsequent day. If the household head is still not available after three visits, the next household with at least one school-age child would be included in the study (Figure 1).

Variables

Outcome variable

Goitre (presence or absence).

Explanatory variables

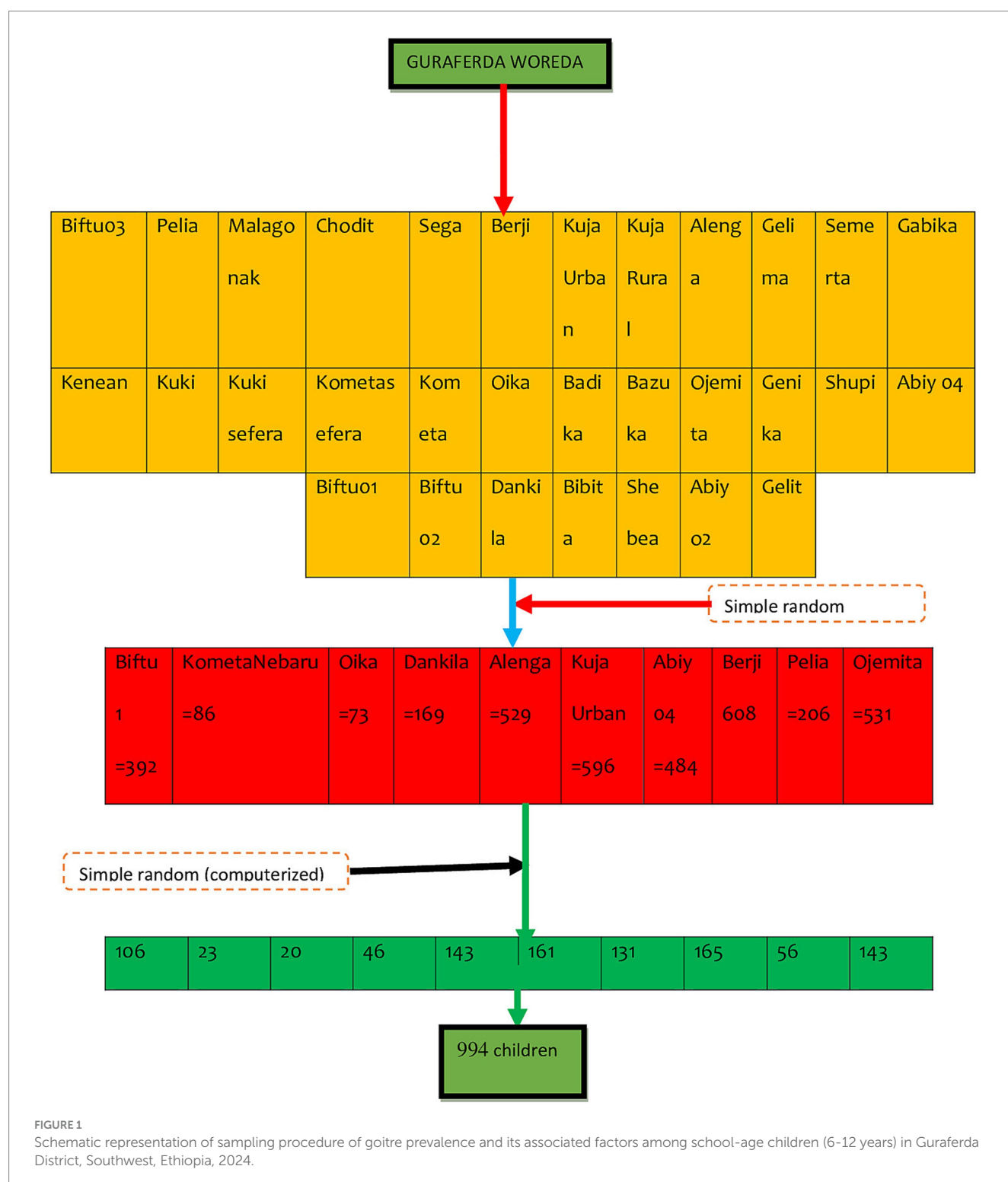
After a thorough review of the literature regarding factors that affect goitre prevalence, the study incorporated seventeen (17) explanatory variables identified in prior research. These variables included child age, child gender, parental educational background, parental occupations, marital status, household wealth level, source of water, place of residence, family size, rooms in the house, types of food consumed, types of salt used, dietary diversification, family history of goitre, and knowledge about goitre and its prevention (3, 28–32).

Data collection tools and procedures

Data were gathered through structured questionnaires administered by interviewers, physical examinations, and a quick iodized salt test. The questionnaires were adapted from existing literature (3, 27–33) and translated into the local language (Amharic) by a certified translator. The translation process followed a forward-backward translation method to ensure accuracy order to reflect the context of study area. Initially, the questionnaires were created in Amharic, then translated into English, and subsequently translated back into Amharic to ensure consistency. To verify the questionnaires' accuracy, a pretest was carried out with 5% of the participants in the study. Physical examination for determining the status of goitre among school children, the participants were instructed to bring a

TABLE 1 Sample size for the second objective.

S. no.	Factors	AOR	% Outcome among unexposed	Sample size	Design effect (1.5)	Non response rate (10%)	Final sample size
1	Using non iodized salt	2.20	18.28	302	$302 * 1.5 = 453$	46	499
2	Cabbage consumption	2.52	4.9	602	$602 * 1.5 = 903$	91	994
3	Living with family in a single room	2.30	42.26	204	$204 * 1.5 = 306$	31	337



handful of salt used by their home. The iodine content of the sample salt was tested by rapid iodized salt test kit. The salt sample was taken in a teaspoon and then a drop of the test solution was poured on the salt.

Physical/thyroid examination

The presence of goitre was evaluated through physical assessments of school-aged children (ages 6–12) and categorized using the WHO goitre staging system into grade 0, grade 1, or grade 2. Goitre was

classified as absent (grade 0) if there was neither palpable nor visible swelling, as grade 1 when it was palpable but not visible, and as grade 2 when it was visible on the neck. Ultimately, goitre was deemed present if a child exhibited either grade 1 or grade 2 goitre, or both (8, 34).

The iodine content of the salt

In order to assess the presence of adequately iodized salt among the households sampled, the interviewer requested each household to provide a teaspoon (Approximately 5 g) of the salt used for food

preparation the night before. The iodine content of the salt sample was measured using a rapid iodized salt testing kit (MBI test kit; MBI Kits International, Tamil Nagar, India). This kit included a stabilized starch-based solution that induces a chemical reaction observable through a color change. The salt sample was then placed in a small cup and spread out flat, as instructed with the testing kit. Two drops of the test solution from the white ampule were added onto the salt's surface, and the resulting color was compared against a color chart within 1 min to gauge the iodine concentration (intense color). If there was no color change observed on the salt after a minute, additional test solution was applied to a new sample, adding up to five drops of recheck solution from the red ampule, followed by two drops of the test solution on the same spot for comparison with the color chart. Ultimately, the results were classified as either 0 ppm (no iodine in the salt), <15 ppm (light blue and insufficient iodine), or ≥ 15 ppm (deep blue and sufficient iodine content). This assessment utilized the Improved Iodized Salt Field Test Kit, Batch No. 014 MF FEB. 2020, EXP NOV. 2022, specifically for salt fortified solely with potassium iodide. An unopened ampule was utilized, and the kit was validated for visual detection of potassium iodide concentration with a detection limit of 15 ppm, yielding reliable results. The test kit was sourced from UNICEF via the Guraferda District Health Office. In the analysis, households showing iodine levels below 15 ppm, as well as those with no detectable iodine, were deemed inadequate, while households with iodine concentrations exceeding 15 ppm were classified as adequate, in accordance with findings from a previous study (35).

Household wealth status

Assets of the household were gathered through structured questionnaires modified from the 2019 Ethiopian District Health Survey (EDHS). The resulting data underwent analysis using Principal Component Analysis (PCA). Ultimately, Household wealth status was classified into quintiles based on a composite score derived from ownership of assets, housing characteristics, and access to services, as per the Ethiopian Demographic and Health Survey (EDHS) methodology in the order of Lowest, second, middle, fourth and highest (36).

Dietary diversification

To evaluate the dietary diversity levels, information about the food items consumed within the last 24 h by school-age children (ages 6–12) was gathered using a dietary diversity questionnaire tailored from criteria for measuring household and individual dietary diversity. A list of commonly consumed local food items was compiled by consulting key informants in the kebele. These food items were subsequently categorized into common food groups. School-age children from the surveyed households were interviewed regarding the foods they had eaten in the preceding 24 h using the 24-h food recall method. The food items ingested in that timeframe were classified into 12 distinct food groups. Ultimately, the data was divided into categories reflecting low dietary diversity (≤ 3 food groups), medium dietary diversity (4 and 5 food groups), and high dietary diversity (≥ 6 food groups) (37).

Data quality control

To ensure the quality of the data, the supervisor and data collector participated in a two-day training focused on tools and methodologies.

A pretest of the instrument was administered to 5% of samples from kebeles that were excluded from the study. A standardized checklist, adapted from guidelines for assessing household and individual dietary diversity, was utilized (37). The reliability of the instruments was assessed by measuring both intra-observer and inter-observer agreement using the Kappa coefficient. A Kappa coefficient value of 0.75 or higher is deemed satisfactory. For this evaluation, 20 questionnaires were used for both inter-observer and intra-observer reliability, resulting in a Kappa coefficient of 0.86, which satisfied the acceptable standards. During the data collection period, the supervisor and principal investigators reviewed each questionnaire every morning to confirm completeness. Furthermore, to guarantee the validity and reliability of goitre assessment, two data collectors examined each school-age child. In instances where the first two examiners disagreed on goitre ascertainment, a third examiner was consulted to evaluate, and the two consistent findings were utilized for diagnosis.

Data management and analysis

The data was carefully examined and cleaned to address any missing or anomalous values. Data entry and analysis were performed using Epi Data version 4.6 and SPSS version 26.0, respectively. Descriptive statistics were computed for various variables as necessary. To assess the household wealth index, Principal Component Analysis (PCA) was employed, ensuring all prerequisites were satisfied. Given that the outcome variable was categorical, both binary and multivariable logistic regression analyses were utilized to control for potential confounding factors and to identify associations with the outcome variable. The independent variables in the logistic regression model were included through Stepwise multiple regression using forward selection. The Hosmer & Lemeshow Goodness of Fit Test was conducted to evaluate the appropriateness of the variables in predicting the dependent variables, resulting in a value of 0.795. Binary logistic regression was performed utilizing simple Logistic Regression where contributions to the model were considered significant at $p < 0.25$. All independent variables that showed $p < 0.25$ in bivariate analyses were incorporated into the multivariate model. Diagnostics for multicollinearity indicated collinearity between cabbage and cassava consumption, with Variance Inflation Factor (VIF) values of 34.28 and 34.67, respectively. Because cassava consumption exhibited the highest VIF value, it was excluded from the model. Ultimately, only variables with a VIF < 10 among the independent variables were retained in the model, with minimum and maximum VIF values of 1.01 and 1.60, respectively. The Adjusted Odds Ratio with a 95% Confidence Interval was reported, with the significance level established at $p < 0.05$. The findings were presented using text, frequency, and statistical summaries.

Ethical approval

The study was carried out after receiving ethical approval with reference number of #I/O/H/I/R/B/024/14 from Bule Hora University's Institutional Review Board. Permission was also obtained from the town administration office in Mizan-Aman. All study participants were provided with brief explanations about the purpose and benefits of the study. Written consent were obtained from parents

or guardians in exchange for their full cooperation. Names and other personal information that could compromise the confidentiality of the respondents were not used. The confidentiality and privacy of the participants' information were protected, and their right to withdraw or not participate was respected. Feedback forms were prepared for participants, providing an overview of the results from the physical measurements. Finally, those with goitre were referred to the nearest health facility

Results

Socio-demographic and economic characteristics

Among the 994 expected school-age children, 949, accompanied by their mothers or caregivers, took part in the study, achieving a response rate of 95.47%. The average age of the participants was 8.08 years (SD = 1.696). Regarding their place of residence, 687 (72.4%) were living in rural areas. Most of the children, 678 (71.4%), came from families consisting of five members or more, while 274 (28.9%) fell into the second quintile of wealth status. Nearly two-thirds (590, 62.2%) of the mothers/caregivers were engaged in farming occupations, and 477 (50.26%) as well as over half, 523 (55.11%), of the children's mothers and fathers lacked formal education, respectively (Table 2).

Nutritional characteristics of school age children

The results from the rapid iodine test indicate that 141 (14.9, 95% CI: 12.6, 17.2%) of the household salt samples tested were non-iodized. Furthermore, 409 (43.1, 95% CI: 39.8, 46.3%) samples of household salt contained insufficient iodine levels (1–15 ppm), while only 399 (42, 95% CI: 38.9, 45.3%) exhibited adequate iodine levels (≥ 15 ppm). Regarding the dietary diversification status of children, 254 (26.8, 95% CI: 23.9, 29.5%) had the lowest dietary diversity, 326 (34.4, 95% CI: 31.4, 37.3%) had medium dietary diversity, and 369 (38.9, 95% CI: 35.9, 42.1%) achieved high dietary diversity (Figure 2).

Dietary habit and goitrogenic food consumption by children

A significant majority of the children (82.8%) had regularly included eggs in their diet. Millet was the most frequently eaten goitrogenic staple, with 438 children (46.2%) reporting its consumption. Among the vegetables and dairy items, sweet potato and milk were consumed by 523 (55.1%) and 663 (69.9%) of the children, respectively (Table 3).

Knowledge and practice of caregiver toward goitre

Among the total caregivers/households surveyed, 665 (70.1%, CI: 67.3, 72.8%) demonstrated a good knowledge of goitre, whereas 284 (29.9%, CI: 27.2, 32.7%) displayed a poor knowledge. Furthermore,

384 (40.5%, CI: 37.4, 43.9%) of the caregivers indicated that they utilize unpacked salt (Table 4).

Prevalence of goitre

The overall prevalence of goitre among children of school age in this research area was found to be 37.6% (95% CI: 34.8, 40.5%). Out of the total number of children with goitre, 238 (66.7, 95% CI: 61.2, 71.2%) presented with grade one goitre, while 119 (33.3, 95% CI: 28.8, 38.8%) had grade two. Furthermore, the prevalence of goitre was recorded at 36% for 6–8-year-olds and 42% for those aged 9–12 years. The occurrence of goitre was 44.2% among females and 30.04% among males. Among the participants in the study, 139 (14.6, 95% CI: 12.6, 16.9%) reported having a family history of goitre (Figure 3).

Factors associated with goitre

In the analysis using multivariable logistic regression, factors linked to goitre in school-age children included the child's sex, dwelling location, maternal education level, consumption of milk, consumption of cabbage, keeping salt near heat sources, and the diversity of food intake (p -value < 0.05). The likelihood of goitre occurrence was 1.578 times greater in female children compared to males (AOR = 1.578, 95% CI: 1.155, 2.157). Children whose mothers or caregivers had no formal education had nearly double the odds of developing goitre than those with mothers or caregivers who had formal education (AOR = 1.93, 95% CI: 1.44, 2.592). Moreover, children residing in rural areas had odds of goitre that were almost twice as high as those living in urban areas (AOR = 2.291, CI: 1.62, 3.24).

In addition, the odd of getting goitre was 1.4 times more likely in children whose families stored salt near heat than in those whose families kept salt away from heat sources (AOR = 1.41, 95% CI: 1.042, 1.90). Conversely, children with the lowest food diversification status faced a fivefold increase in goitre risk when compared to children with the highest food diversification status (AOR = 4.93, 95% CI: 3.332, 7.29). Likewise, children who consumed cabbage at least once weekly had nearly three times the odds of developing goitre compared to those who never ate cabbage (AOR = 2.874, 95% CI: 2.012, 4.106). Conversely, the chances of goitre were 78% lower in children who consumed milk at least weekly compared to those who did not consume milk (AOR = 0.217, CI: 0.145, 0.392) (Table 5).

Discussion

Ethiopia has achieved and sustained greater than 89% Household (HH) iodized salt coverage from its lowest point of 15% coverage and subsequently improved iodine intake across its population (18). Despite the Ethiopian government's efforts to implement mandated salt iodization over the past decade, an iodine shortage persists in the country (21).

The study revealed that 37.6% of school-aged children (ages 6–12) in the analyzed region were afflicted by goiter, designating it as an endemic area per the World Health Organization (WHO), which defines any region with a goiter prevalence exceeding 5% (8), which states that any area with a goitre prevalence rate greater than 5% is considered endemic. The finding corresponds with a research conducted in northwest Ethiopia involving children of the

TABLE 2 Socio-demographic characteristics of school-age children and their parents/caregivers in Guraferda District, Southwest Ethiopia, 2024 (*n* = 949).

Variables	Categories	Frequency	Percentage
Sex of child	Male	454	47.8
	Female	495	52.2
Age of child	6–8 years	704	74.2
	9–12 years	245	25.8
Religion of the mother's/caregivers	Muslim	232	24.4
	Orthodox	328	34.6
	Protestant	376	39.6
	Others	13	1.4
Residence	Urban	262	27.6
	Rural	687	72.4
Marital status	Married	817	86.1
	Divorced	85	9.0
	Widowed	47	5.0
Mother's educational level	Cannot read and write	221	23.3
	Read and write	256	27.0
	Primary	380	40.0
	Secondary	54	5.7
	College/University	38	4.0
Father's educational leve	Cannot read and write	223	23.5
	Read and write	300	31.6
	Primary	351	37.0
	Secondary	51	5.4
	College/University	24	2.5
Mother's occupation	House wife	129	13.6
	Farmer	590	62.2
	Government employee	61	6.4
	Private employee	39	4.1
	Merchant	130	13.7
Father occupation	Farmer	606	63.9
	Government employee	114	12.0
	Private employee	15	1.6
	Merchant	214	22.6
Households wealth status in quintile	Lowest	179	18.9
	Second	274	28.9
	Middle	166	17.5
	Fourth	179	18.9
	Highest	151	15.9
Family size	<5 family	271	28.6
	5 and above family	678	71.4

same age group and is also consistent with the national prevalence observed in studies undertaken in Debre Tabor town and Chole District (3, 27, 29, 30). However, the results of this study were lower than those from Anchar District, which found a goitre prevalence of 51.8%, Shebe Senbo District, which revealed a

prevalence of 59.1%, and Northeast Ethiopia, which indicated a prevalence of 62.1% (28, 30, 32, 38). The variation in results can be explained by variations in annual precipitation and elevation across the study sites, which could lead to the loss of iodine-rich topsoil (39, 40).

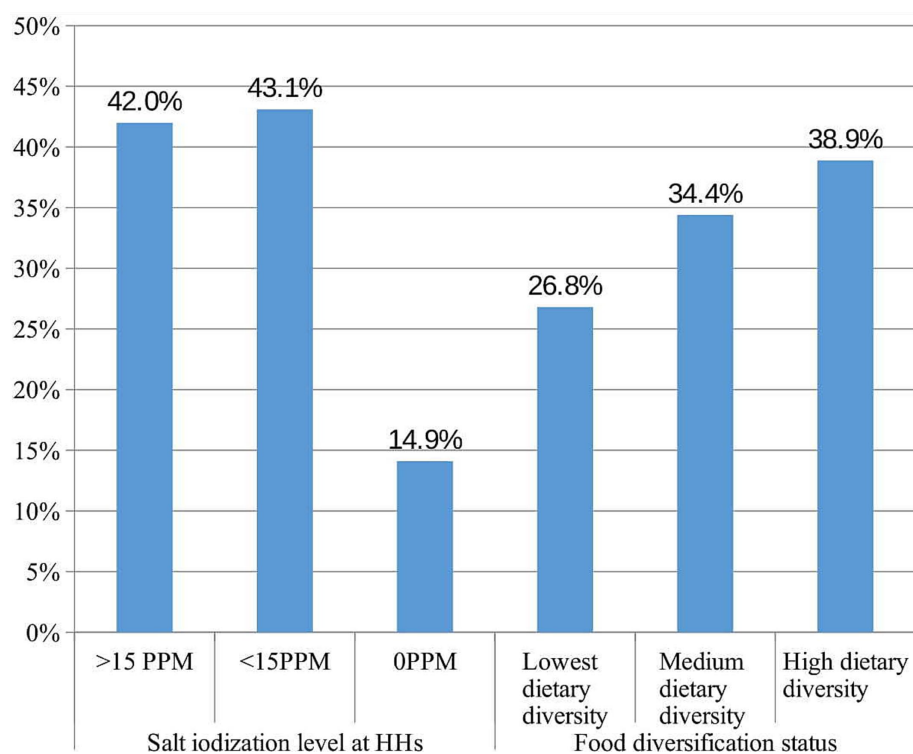


FIGURE 2

Nutritional characteristics of school age children in Guraferda District, Southwest Ethiopia, 2024.

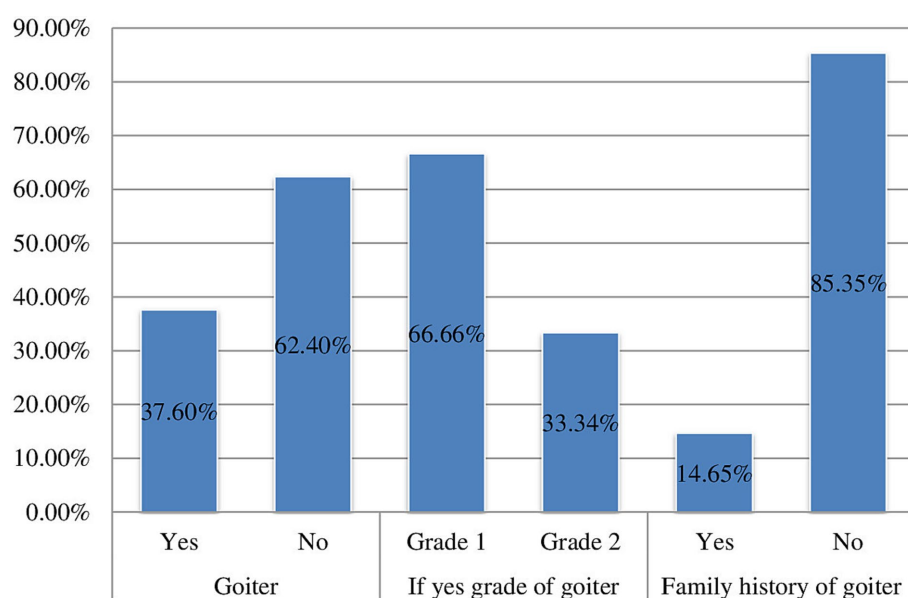


FIGURE 3

Prevalence of goitre among school age children (6–12 years) in Guraferda District, Southwest, Ethiopia, 2024.

The finding of this study is also lower than the study conducted in Butajira Town which reported a prevalence of 49.65% among pregnant women (41). The possible explanation for this difference might be attributed Iodine requirement will increase during pregnancy. Prevalence of Goitre in this study is much higher than a systematic

review conducted in Ethiopia (42). This disparity could be explained by a systematic review study that pooled the prevalence of goitre from various studies, with individual prevalence ranging from 5 to 56.2%. The finding of this study is also lower than a systematic review and meta-analysis done in Ethiopia which reported the pooled prevalence

TABLE 3 Goitreogenic and non goitreogenic food feeding status of school age children (6–12 years) in Guraferda District, Southwest Ethiopia, 2024.

Variables	Categories	Frequency	Percent
Have you ever eaten cabbage	Never	522	55.0
	Once a week	77	8.1
	Two times a week	67	7.1
	3 and above times a week	283	29.8
	Never	522	55.0
Have you ever eaten cassava	Never	520	54.8
	Once a week	98	10.3
	Two times a week	148	15.6
	3 and above times a week	183	19.3
Have you ever eaten millet	Yes	438	46.2
	No	511	53.8
Have you ever eaten rice	Yes	793	83.6
	No	156	16.4
Have you ever eaten maize	Yes	433	45.6
	No	516	54.4
Have you ever eaten potato	Yes	523	55.1
	No	426	44.9
Have you ever eaten godere	Yes	792	83.5
	No	157	16.5
Have you ever eat acho	Yes	547	57.6
	No	402	42.4
Have you ever eat honey	Yes	566	59.6
	No	383	40.4
Have you ever drink milk	Yes	663	69.9
	No	286	30.1
Have you ever eat eggs	Yes	786	82.8
	No	163	17.2

of IDD to be 58% among school age children (23). The difference could be explained by the fact that the Iodine test method employed in our investigation differed from that used in the previously reported study.

The rate of goitre found among the school-aged children involved in this study was also greater than the prevalence noted in studies carried out in Pakistan and Iran which reported 35.0 and 24.2% (16, 43, 44). The greater incidence of goitre in children observed in our research, when compared to results from analogous studies, might be linked to the region's mountainous terrain and the long-term poor soil conservation practices. This could have resulted in the depletion of the topsoil that is rich in iodine.

Millet was the most frequently eaten staple food in the study, with 438 children (46.2%) reporting its consumption. It is known for its goitrogenic effect (45) which might have increased the risk of developing goitre among study participants.

Female children had twice odds of developing goitre compared to male. This observation aligns with earlier research carried out in the Arsi Zone and North West Ethiopia (3, 28, 29). The increased occurrence of goitre in females could be attributed to the greater iodine needs of female children compared to their male counterparts,

TABLE 4 Knowledge and practice of care giver towards goitre prevention in Guraferda District, Southwest Ethiopia, 2024.

Variables	Categories	Frequency	Percent
Level of knowledge about goitre in HH	Good knowledge	665	70.1
	Poor knowledge	284	29.9
Type of salt used in the HH	Un packed	384	40.5
	Packed	565	59.5
Place where you commonly buy salt	Open market	53	5.6
	Shops	896	94.4
Do you expose salt to sun light?	Yes	96	10.1
	No	853	89.9
Place of salt stored in the house?	Near to fire	398	41.9
	Away from fire	551	58.1
Type of salt container used in the HH	Storage without cover	349	36.8
	Storage with cover	600	63.2
When did you add iodized salt during cooking?	After cooking	98	10.3
	During cooking	851	89.7

HH, Household.

particularly at the onset of puberty (46). The results of our research oppose the findings of a study carried out in the Kohat District of Pakistan, which indicated that the prevalence of goitre was greater in males than in females (43). The differences could stem from genetic variations between the two populations being studied.

Children who ate cabbage at least once a week, and especially those who ate it more frequently, had three times the likelihood of developing goitre compared to those who had never eaten cabbage. This observation aligns with earlier research carried out in different areas of the country, including southwest Ethiopia, Chole District, and Arsi Zone (3, 29, 33). One potential reason for this might be that cabbage is a naturally occurring goitrogenic food with thiocyanate and isothiocyanate, which prevent the transport of iodine to the thyroid gland. This blocking of iodide transport in the body could result in the swelling of the thyroid gland (3).

Children who drank milk at least once a week were less likely to develop goitre than those who did not. Our results align with studies carried out in Chole District and Anchar District in Eastern Ethiopia (29, 32). This discovery aligns with information from multiple sources suggesting that milk offers beneficial nutrients that help prevent goitre (47). Although animal-based foods contain less iodine compared to plant-based foods, milk and dairy products are still considered a decent source of iodine (47).

The odd of developing goitre was five times greater in children who had the least diverse diets compared to those who had the most varied food options. This observation aligns with research carried out in the Amhara regional state, Adama city, and Bale, Ethiopia (48–51). One potential reason for this observation could be that a variety of foods facilitated better absorption of iodine by the body. Sufficient intake of dietary iodine is one approach to combat iodine deficiency disorders. Groups that primarily consume a uniform diet centered around cereals frequently experience shortages in iodine, along with other essential nutrients like vitamin A and iron (48, 52, 53).

TABLE 5 Multivariable analyses of factors associated with goitre among school age children (6–12 years) in Guraferda District, Southwest Ethiopia, 2024 (*N* = 949).

Variables	Goitre			<i>p</i> value	COR (95% C.I.)	<i>p</i> value	AOR (95% C.I.)
	Categories	Yes <i>N</i> (%)	No <i>N</i> (%)				
Child sex	Female	219 (44.24)	276 (55.56)	0.000	1.817 (1.391, 2.374)	0.002*	1.614 (1.199, 2.172)
	Male	138 (30.4)	316 (69.6)	1			
Maternal educational level	Non formal education	219 (45.9)	258 (54.1)	0.000	2.054 (1.572, 2.686)	0.001*	1.930 (1.437, 2.592)
	Formal education	138 (29.24)	334 (70.76)	1			
Residence of households	Rural	290 (42.2)	397 (57.8)	0.000	2.126 (1.550, 2.917)	0.001*	2.291 (1.620, 3.239)
	Urban	67 (25.6)	195 (74.4)	1			
Have you ever eat cabbage	Yes	149 (34.9)	278 (65.1)	0.117	1.236 (1.15, 1.611)	0.001*	2.874 (2.012, 4.106)
	No	208 (39.85)	314 (60.15)	1			
Have you ever eat milk	Yes	287 (43.9)	376 (56.1)	0.000	0.425 (0.311, 0.579)	0.001*	0.217 (0.145, 0.324)
	No	70 (24.5)	216 (75.5)	1			
Place of salt stored in the house?	Near to fire	161 (40.45)	237 (59.55)	0.126	1.230 (1.14, 1.61)	0.026*	1.407 (1.042, 1.900)
	Away from fire	196 (35.6)	355 (64.4)	1			
Food diversification status children	Lowest dietary diversification	145 (57)	109 (43)	0.000	4.302 (3.012, 6.4)	0.001*	4.928 (3.332, 7.289)
	Medium dietary diversification	77 (23.6)	249 (76.4)	0.000	2.306 (1.664, 3.2)	0.001*	2.540 (1.778, 3.629)
	Highest dietary diversification	135 (36.6)	234 (63.4)	1			

*indicates statistically significant variables at the *p*-value < 0.005.

Children aged 6–12 residing in rural regions had 2.29 times greater odds of developing goitre compared to their counterparts living in urban areas. This aligns with results from a survey conducted in Portugal and United states of America which indicated that endemic goitre predominantly impacts the rural population (15, 54, 55). The noted disparity could stem from the reality that individuals residing in rural regions might possess less awareness regarding the causes and prevention of goitre, along with restricted access to healthcare services in these communities. Additionally, challenges in obtaining salt when required may result in the rural population not utilizing iodized salt as effectively as those in urban areas. Moreover, factors such as sex, education level, place of birth, family income, site of residence, knowledge, dietary intake, unprotected water consumption, consumption of goitrogenic foods, and family history can affect the prevalence of goiter (3, 56, 57).

The research indicated that participants whose mothers or caregivers had non-formal education were twice as likely to develop goitre. Children whose mothers or caregivers possessed less education faced a greater risk of developing goitre compared to those with mothers who received formal education. Evidence from various areas of the country reinforces the finding that children of less educated mothers are more susceptible to developing goitre (27, 32). A study conducted in Khoramabad indicated that as the educational level of parents rises, the prevalence of goitre decreases (16). A potential reason for the finding mentioned is that mothers or caregivers without formal education might not be utilizing iodized salt correctly, resulting in improper practices.

Additionally, our research revealed that keeping salt close to a fire was notably linked to the occurrence of goitre. School-age children whose families or caregivers keep salt near a fire had 1.4 times greater

odds of developing goitre compared to those whose families or caregivers store salt away from a fire. This result aligns with findings from Jimma Town, national-level study conducted in Ethiopia and Iran (30, 58, 59). One potential explanation for this observation could be that keeping salt close to a fire reduces the amount of iodization in salt at the household level (58, 60).

In this study, there was no association of iodine content with whether the salt was packed or unpacked, in contrast to earlier studies in Gondar, North West Ethiopia (61), Lalo Assabi District, West Ethiopia (16), and Robe town, South Central Ethiopia (62). Loss of iodine due to environmental factors from non-packaged salt was higher than from packaged salt in a previous study (63). The salt samples collected in this study were iodized using potassium iodate, rather than potassium iodide. As iodates are less soluble and more resistant to oxidation than iodide, the salt iodine content remains relatively constant under different environmental conditions (moisture, heat, and sunlight) even in unpacked salt (64, 65).

Limitations of the study

In this study, the urinary iodine levels of the subjects were not tested due to resource constraints, which could have revealed their recent iodine intake status and helped with treatment and monitoring. Goitre was diagnosed using WHO goitre staging methods, but the gold standard diagnosis of goitre is ultrasound, which may result in misclassification of subclinical stages of cases. Using only a 24-h questionnaire (it does not represent individual variability and requires recall) (66).

Conclusion

The prevalence of goitre in this study area was found to be 37.6%, classifying it as a goitre endemic area according to the World Health Organization (WHO), which states that any area with a goitre prevalence rate greater than 5% is considered endemic (8). Factors positively associated with goitre in this study included being female, mothers with no formal education, storing salt near fire, children with low food diversification, children living in rural areas, and children consuming cabbage at least once a week. We recommend that future studies in this region incorporate child growth and development measures to provide a more comprehensive understanding of the impact of IDD.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Anteneh Fikrie, Bule Hora University, Girma Tufa, Bule Hora University, Wako Dedecha, Bule Hora University, Zelalem Jabessa, Bule Hora University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

GT: Supervision, Validation, Writing – original draft, Writing – review & editing. WM: Conceptualization, Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft. KK: Conceptualization, Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft. SE: Conceptualization, Data curation, Formal analysis, Methodology,

Software, Supervision, Visualization, Writing – original draft. MG: Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

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Abbreviations

AOR - Adjusted Odds Ratio

CI - Confidence Interval

PCA - Principal Component Analysis

COR - Crude Odds Ratio

CSA - Central Statistics Agency

DDS - Dietary Diversity Score

EDHS - Ethiopian Demographic Health Survey

EPHI - Ethiopian Public Health Institute

GP - General Practitioner

GC - Gregorian calendar

HH - Households

ICCIDD - International Council for the Control of Iodine Deficiency Disorders

IDD - iodine Deficiency Disorder

OR - Odds Ratio

PPM - Parts Per Million

SNNPR - South Nation and Nationalities of people Region

SAC - School age children

SPSS - Statistical Package for the Social Sciences

TGR - Total Goitre Rate

TSH - Thyroid Stimulating Hormone

UNICEF - United Nations International Children Emergency Fund

WHO - World Health Organization



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Beyond thyroid dysfunction: the systemic impact of iodine excess

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As an essential micronutrient, iodine plays a crucial role in several physiological systems, particularly in the production of thyroid hormone. While deficiency is widely recognized, the consequences of iodine excess (IE) are less studied. IE, which may be caused by iodine-rich diets, supplements, iodinated contrast media and salt iodization, has been implicated in a range of adverse outcomes on thyroid and systemic health. Examples include autoimmune thyroid diseases like Graves' disease and Hashimoto's thyroiditis, driven by immune cell polarization and gut microbiota alterations. Furthermore, excessive iodine intake is associated with increased risks of cardiovascular diseases, including hypertension and atherosclerosis, due to oxidative stress, inflammation, and endothelial dysfunction. It contributes to the development of thyroid cancer, particularly papillary thyroid cancer, through genetic mutations such as BRAF mutations and enhanced cancer cell proliferation. Excess iodine intake has been implicated to have neurotoxic effects, significantly impairing learning and memory, negatively impacting neonatal brain development, and potentially contributing to the progression of neurodegenerative conditions. It also has a potential role in renal dysfunction in vulnerable populations, due to overload from povidone-iodine in sterile equipment. This mini-review aims to collate the adverse effects of IE, beyond its effect on thyroid health, through investigation of the cardiovascular, nervous, and renal systems. Through our consolidation of the current literature, we hope to raise awareness and contribute to the understanding of the multifaceted impact of excessive iodine intake.

KEYWORDS

iodine, iodine excess, thyroid, thyroid hormones, systemic health

Introduction

Iodine is an essential micronutrient obtained from food or supplements, primarily known for its role in thyroid hormone (TH) production. Once absorbed as iodide or converted from iodate in the gastrointestinal tract, iodine is primarily taken up by the thyroid gland and other tissues via the Na^+/I^- symporter, where it is oxidized and incorporated into thyroglobulin to produce THs (T4 and T3) (1, 2). These hormones are critical in regulating metabolism, growth, and development, while iodine also functions as

an antioxidant, enhances antioxidant enzyme expression, triggers apoptotic pathways in cancer cells and modulates the immune response (3).

The World Health Organization (WHO) recommends a daily intake of 120 µg for school-aged children, 150 µg for adults, and 250 µg for pregnant or breastfeeding women (4–6). As of 2019, iodine deficiency affected approximately 2.4% of the global population (7). Iodine deficiency has historically been more prevalent in inland and mountainous regions, particularly in areas with low natural iodine levels in the soil and water, such as the Great Lakes, Appalachians and the northwestern U.S., which were once known as the “goiter belt” (8–10). Before the introduction of iodized salt in the 1920s, goiter was highly prevalent, affecting up to 70% of children in these regions (10). Despite national salt iodization programs, iodine intake has declined in recent years in countries like the U.S., UK and Australia, largely due to changes in dietary habits, reduced consumption of iodized salt, and the increased use of non-iodized salt in processed foods (11). While many nations have adopted mandatory iodization policies, in the U.S. fortification remains voluntary, leading to inconsistent iodine intake, particularly among pregnant and lactating women who require higher iodine levels for fetal brain development (12). Inadequate iodine status results in clinical consequences such as goiter, hypothyroidism and, in severe cases, intellectual and developmental impairments (13). Between 2003 and 2020, the number of countries with sufficient iodine intake nearly doubled from 67 to 118; however, 21 countries still face iodine deficiency, and 13 experience excess intake due to either high groundwater iodine or over-iodized salt (14).

The pharmaceutical and food industries have played an instrumental role in addressing iodine deficiency globally,

particularly through the promotion of iodine supplementation and iodized salt. The International Council for the Control of Iodine Deficiency Disorders (ICCIDD), established in 1985, was pivotal in leading efforts to implement universal salt iodization, with coordinated action from governmental and multilateral organizations like WHO, UNICEF and the UN (15). The collaboration between these entities led to the formation of the Iodine Global Network (IGN) in 2014, continuing its mission of improving iodine nutrition worldwide (15). The strategic efforts of these organizations in partnership with the salt industry have been crucial in promoting salt iodization and iodine supplementation, addressing global iodine deficiency, and supporting public health initiatives.

However, while addressing iodine deficiency has been crucial, the overuse of iodine supplements and the increased availability of iodized salt have raised concerns about the potential risks of iodine excess (IE). Excessive iodine concentrations can lead to the Wolff-Chaikoff effect, as shown in Figure 1, which temporarily reduces the synthesis of T3 and T4, preventing excessive TH production (16). This effect occurs through the generation of inhibitory substances like intrathyroidal iodoaldehydes that impact thyroid peroxidase (16). However, the Wolff-Chaikoff effect is transient, typically lasting 1–2 days, after which normal TH synthesis resumes. This is followed by a reduction in sodium-iodide symporter expression, which limits iodide uptake and restores euthyroid function. In some cases, failure of this effect can lead to the Jod-Basedow phenomenon, characterized by excessive TH production, particularly in individuals with thyroid nodules or impaired thyroid regulation.

Excessive iodine intake, whether from iodine-rich diets, supplements or iodinated contrast media, can lead to TD. Excess

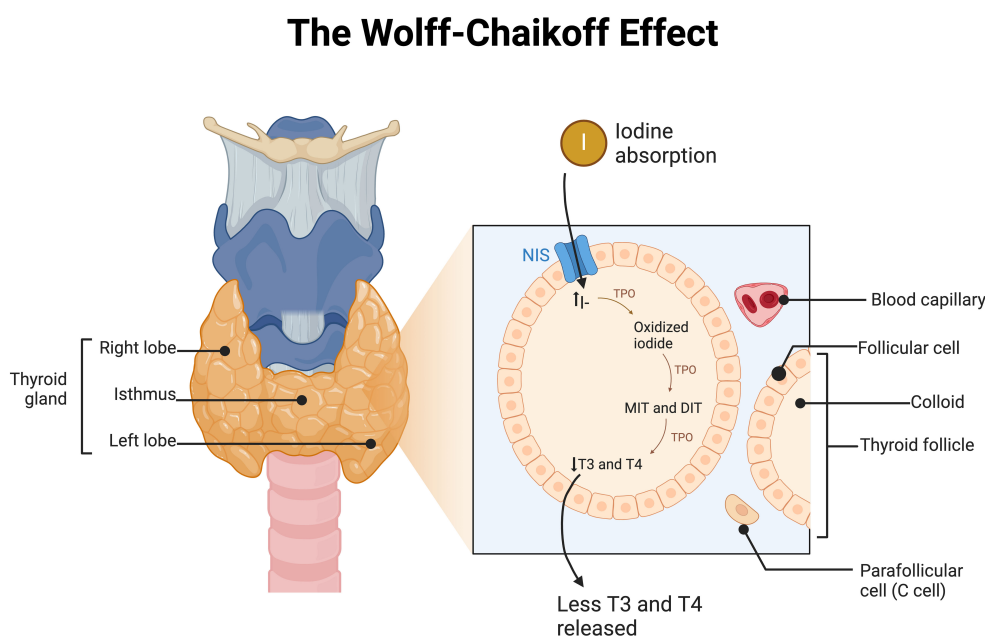


FIGURE 1

Schematic of the Wolff-Chaikoff effect in the thyroid follicle of the thyroid gland. NIS, Sodium/Iodide Symporter; I, Iodide, TPO, Thyroid Peroxidase; MIT, Monoiodotyrosine; DIT, Diiodotyrosine; T3, Triiodothyronine; T4, Thyroxine. Made with www.biorender.com.

iodine is usually regulated by the sodium-iodide symporter, limiting the transport of iodide into thyroid cells (17). However, in some individuals, such as those with autoimmune thyroid conditions, this regulation may fail, leading to chronic hypothyroidism or iodine-induced hyperthyroidism, especially in people with preexisting goiters (18, 19). Furthermore, iodine excess (IE) has been associated with the development of Grave's disease (GD) in iodine-sufficient regions, with a higher incidence observed in places like Iceland (4). Excess iodine may also contribute to papillary thyroid carcinoma (PTC) through increased BRAF mutations (20).

Beyond TD, excessive iodine has been shown to adversely affect other systems. Studies suggest that it can impair cardiovascular health by directly affecting endothelial function and contributing to both hypo- and hyperthyroidism, which increases the risk of heart failure and metabolic disorders (21–24). Additionally, excess iodine has been linked to neurological damage, disrupting neurotransmitter balance, triggering apoptosis and impairing brain structures, particularly in the hippocampus, leading to deficits in learning and memory (25, 26). This may also contribute to neurodegenerative diseases, such as Parkinson-like symptoms, by disrupting dopamine function (27). Furthermore, exposure to iodine contrast media in imaging procedures poses a risk to kidney function, especially in vulnerable populations like neonates and those with renal impairment, potentially causing TD and renal damage (28, 29).

This review aims to provide a comprehensive overview of the adverse effects of IE on thyroid health, while also examining its impact on other critical systems, including the nervous, cardiovascular and renal systems. By exploring recent evidence, we hope to contribute to a deeper understanding of how excessive iodine intake and exposure can influence these systems, ultimately guiding future research, clinical practice and public health strategies.

Search strategy

For this mini-review, a literature search was performed between December 2024 and January 2025. PubMed and Google scholar was used to find relevant articles. Keywords included “iodine excess”, “iodine”, “iodine excess and systemic disease”. Only articles written in the English language were used. Articles were selected based on relevancy.

Iodine excess and its impact on autoimmune thyroid diseases

Iodine plays a crucial role as a catalyst in TH synthesis, exerting a significant influence on various aspects of health. Its effects are intricately linked to the underlying functionality of the thyroid gland. IE, in vulnerable patients, can cause subclinical or overt TD. In fact, iodine toxicity may lead to thyroiditis, hypothyroidism, hyperthyroidism and PTC (30–34). While ID poses significant

health risks, efforts to address it through supplementation have introduced their own challenges, particularly the potential for inducing thyroid autoimmunity.

Iodine supplementation through iodine-enriched salt, implemented to prevent ID disorders, has been linked to the development of thyroid autoimmunity, often referred to as the ‘autoimmune thyroid phenomenon’ (35). Several studies have documented the progression of thyroid autoimmunity markers following iodine prophylaxis. A study in Sri Lanka examined thyroid autoantibodies in schoolgirls before and after three years of iodine supplementation (36). While antibody prevalence declined over time, there was a shift from predominantly thyroglobulin antibody (TgAb) positivity to a greater proportion having both TgAb and thyroid peroxidase antibodies (TPOAb). Since TPOAb is more strongly linked TD, this change could explain the higher prevalence of subclinical hypothyroidism observed in the later phase of the study (36). Similarly, an iodoprohylaxis program in Denmark was associated with an increased prevalence of thyroid autoantibody positivity and subclinical hypothyroidism after 4–5 years (37).

On a cellular level, research has identified multiple mechanisms through which excessive iodine intake influences the pathogenesis of autoimmune thyroid diseases (AITD), such as GD and Hashimoto's thyroiditis (HT). Elevated iodine has been linked to macrophage polarization imbalance, with gene set enrichment analysis (GSEA) revealing a role for M1 macrophage hyperpolarization in AITD. *In vitro* and *in vivo* studies confirmed that high iodine intake promotes this shift, alters cytokine expression and is mediated by the metabolic gene hexokinase 3 (HK3), suggesting that targeting HK3 could help mitigate these effects (38). Additionally, excessive iodine intake has been associated with rising HT incidence, partially through its suppression of autophagy and induction of apoptosis in thyroid follicular cells (TFCs). This was evidenced by reduced levels of the autophagy-related protein LC3B-II and increased caspase-3 expression in thyroid tissues from HT patients (39). Mechanistically, IE suppresses autophagy via downregulation of transforming growth factor beta 1 (TGF- β 1) and activation of the Akt/mTOR pathway, while also increasing reactive oxygen species (ROS) production and apoptosis in TFCs. Restoring autophagy mitigates these effects, highlighting its critical role in maintaining thyroid health and the need for a balanced approach to iodine supplementation.

Excessive iodine intake has also been shown to disrupt gut microbiota composition and metabolic processes, contributing to the pathogenesis of HT through the microbiota-gut-thyroid axis. A study involving human participants and a mouse model revealed significant differences in butanoate metabolism, with reduced butyric acid levels and butyrate-producing bacteria, such as *Clostridia*, in HT patients compared to healthy controls, as shown in Figure 2 (40). At the genetic level, varying iodine exposure was found to influence DNA methylation in genes related to natural killer cells, with killer cell lectin-like receptor C1 (KLRC1) showing hypomethylation and high expression in areas with both ID and excess, suggesting iodine's role in modulating immune function (41). The gut microbiota's production of short-chain fatty acids (SCFAs) and lipopolysaccharides plays a role in thyroid function by inducing

Excessive Iodine Effect on Gut Microbiota

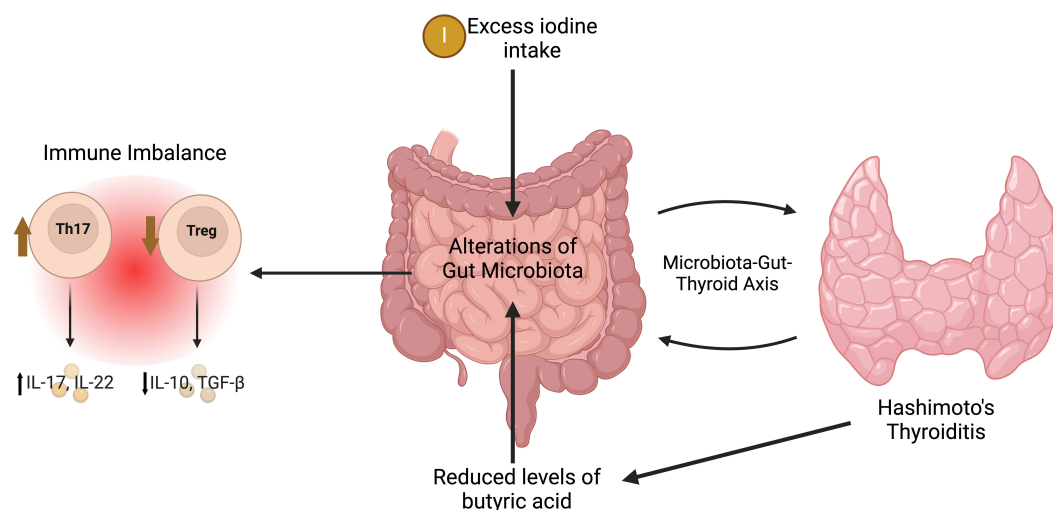


FIGURE 2

The effects of excess iodine on gut microbiota in Hashimoto's thyroiditis. Th17, T helper 17; Treg, Regulatory T cell; IL, interleukin; TGF- β , Transforming growth factor beta. Made with www.biorender.com.

iodine uptake, mediated by a sodium-iodide symporter. In particular, altered SCFA release may be a critical factor in how gut bacteria impacts sodium-iodide symporter expression, ultimately affecting thyroid iodine metabolism (42, 43). Additionally, Lactobacilli and Bifidobacteria, components of the normal intestinal flora that induce SCFA production, are implicated in the pathogenesis of AITD via molecular mimicry, a process driven by structural homology between their protein sequences and thyroid peroxidase/thyroglobulin (44). Molecular mimicry can drive the polarization of immune cells towards a pro-inflammatory phenotype, amplifying the autoimmune response (45). A mouse study using a random-effects model showed that AITD was associated with increased pathogenic gut bacteria and a decrease in beneficial bacteria like Lactobacillus and Bifidobacterium, both of which are known for their immunomodulatory effects (46). However, the translatability of these findings to humans requires further investigation, as differences in microbiota composition between species may influence outcomes. Taken altogether, these findings emphasize the multifaceted role of IE in autoimmune diseases, affecting both immune cell function and the microbiota, highlighting the need for careful management of iodine intake.

Building on these findings, further concerns have arisen about the potential risks of IE. For example, the incidence of thyrotoxicosis increased during mandatory salt iodization in Spain compared to periods without supplementation (47). However, not all thyroid disorders are significantly influenced by IE. A study demonstrated that excessive iodine intake does not affect the clinical outcomes of GD in iodine-replete areas, suggesting that dietary iodine restriction may not be necessary for its management (48). The increasing evidence pointing to a causal link between IE and potential harm necessitates careful consideration. Therefore, further studies are needed, and caution

should be exercised in managing iodine intake to mitigate potential risks. While iodine is essential for thyroid health, its excess can disrupt immune function, alter thyroid cell processes, and contribute to AITD, emphasizing the importance of balanced iodine intake to maintain thyroid function and overall health.

Impact of iodine excess on cardiovascular health

Excessive iodine intake has been linked to various cardiovascular disorders, as research suggests it may disrupt vascular function and promote inflammatory responses. A recent study using a mouse model demonstrated that excessive iodine intake impairs endothelial function by elevating inflammatory markers such as tumor necrosis factor α (TNF α), interleukin-6 (IL-6) and C-reactive protein (CRP) (49). These findings revealed reduced levels of key endothelial proteins, including endothelin-1 (ET-1), von Willebrand factor (VWF) and thrombomodulin (THBD), alongside increased inflammatory cytokine expression in iodine-exposed mice. This suggests that IE may contribute to vascular dysfunction by promoting inflammation, potentially compromising overall cardiovascular health.

Additionally, human studies have further underscored the detrimental effects of excessive iodine on cardiovascular health. A cross-sectional survey conducted in China, found that adults in iodine-excess areas exhibited elevated blood glucose levels, increased systolic and diastolic blood pressure and decreased high-density lipoprotein (HDL) cholesterol (50). These changes suggest that excessive iodine intake may lead to increased risk factors for hypertension and diabetes, further highlighting the cardiovascular risks associated with IE.

Excess iodine may also cause oxidative damage, a known contributor to cardiovascular disease. In a study utilizing Wistar rats, high iodine intake led to increased lipid peroxidation and altered antioxidant defense mechanisms in the thyroid gland, liver tissue and blood (51). This pro-oxidant effect can damage blood vessels, suggesting that oxidative stress (OS) induced by excessive iodine intake may contribute to vascular injury and cardiovascular dysfunction.

Moreover, another investigation involving rat aorta endothelial cells demonstrated that excess iodine exposure impaired cell proliferation, increased apoptosis and promoted OS (52). These changes were accompanied by altered expression of adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), which are associated with vascular injury and atherosclerosis development. This study further supports the notion that excessive iodine may directly damage the vascular endothelium, leading to long-term cardiovascular consequences. However, while these findings suggest a potential role of iodine in endothelial injury, *in vitro* studies cannot fully replicate the complexity of human vascular pathology.

Population-based studies have also highlighted the vascular effects of excessive iodine intake. A study in China revealed that residents in areas with high iodine levels had significantly higher rates of carotid intima-media thickening, a marker for atherosclerosis (53). This finding, even after adjusting for other factors such as age, gender and BMI, highlights the potential for IE to act as a risk factor for carotid artery damage and the development of atherosclerosis, further reinforcing the negative impact of IE on cardiovascular health.

Together, these studies demonstrate a consistent correlation between IE and cardiovascular risk. Excess iodine intake appears to impair vascular function through various mechanisms, including inflammation, OS, and endothelial dysfunction. While experimental studies provide useful mechanistic insights, the translatability of these findings to clinical practice remains a key limitation, emphasizing the need for further human-based research. These findings highlight the need for careful management of iodine levels to prevent potential cardiovascular damage and associated diseases.

Impact of iodine excess on thyroid cancer

In addition to the adverse effects of iodine excess on thyroid function, IE has also been linked to an increased risk of PTC. A 2023 hospital-based case-control study found that higher urinary iodine concentration (UIC) was associated with greater PTC risk, particularly in individuals under 45 years old (54). In Korea, where BRAF mutations are present in over 80% of PTC cases, research revealed that both low (UIC <300 µg/L) and excessively high (UIC ≥500 µg/L) iodine levels increased the likelihood of BRAF mutations, suggesting that extreme iodine levels contribute to PTC development through genetic alterations (55, 56). Similarly, a 14-year study in Poland observed a rise in BRAF-positive PTC cases following iodine supplementation, accompanied by smaller tumor

sizes and increased microcarcinomas, implicating iodine intake as an environmental factor (57).

A meta-analysis of case-control studies found that excessive iodine intake (UIC ≥300 µg/L) significantly increased PTC risk, while adequate intake (UIC 100–200 µg/L) had a protective effect (58). However, no strong association was found between iodine levels and BRAF mutations or lymph node metastasis (LNM) in PTC patients. The analysis highlighted the need for standardized UIC measurement methods to enhance the reliability of findings.

Mechanistic studies have explored how high iodine levels stimulate thyroid cancer (TC) growth. One study found that excessive iodine accelerated cell cycle progression in TC cells, promoting proliferation through a protein kinase B (AKT)-mediated pathway involving Wee1 and cyclin-dependent kinase 1 (CDK1). Blocking AKT phosphorylation reversed these effects, suggesting a potential therapeutic target (59).

While many studies suggest a link between IE and PTC, others provide mixed results. For instance, an analysis of 1,170 patients with thyroid nodules found both low (UIC <300 µg/L) and very high iodine levels (UIC ≥2500 µg/L) were associated with higher cancer risk, with male gender identified as a significant factor (60). This indicates variability in the iodine-cancer relationship across populations.

Animal studies offer further insights into iodine's role in thyroid carcinogenesis. While both ID and excess can promote thyroid tumors when combined with carcinogen exposure, excessive iodine alone does not significantly increase cancer incidence. For example, rats fed excessive iodine diets showed TC rates similar to those with adequate iodine intake, suggesting that IE is not inherently carcinogenic (61, 62). However, in models combining excessive iodine with radiation exposure, both deficiency and excess promoted cancer development, with ID showing more pronounced effects (63). These findings, while valuable, should be interpreted cautiously, as animal models may not fully replicate human thyroid physiology and carcinogenic responses.

Overall, excessive iodine intake appears to correlate with TC risk, particularly through genetic and cellular pathways. However, its carcinogenic impact seems contingent on other factors, such as environmental exposures or genetic predispositions. Further research is necessary to clarify the complex relationship between iodine and TC.

Impact of iodine excess on the nervous system

Iodine is essential for normal brain function and fetal neurodevelopment, with both deficiency and excess impacting the nervous system. Maternal iodine deficiency during pregnancy leads to hypothyroxinemia, characterized by low T4 levels, which deprives the fetus of critical thyroid hormones necessary for brain development (64–66). Severe iodine deficiency can result in cretinism, a condition associated with profound intellectual disability, motor impairments, and hearing and speech deficits (67). Moreover, even moderate iodine deficiency in pregnant

women has been linked to developmental delays in children, underscoring the critical role iodine plays in early brain development (68). However, while much attention has been given to ID, recent studies have also highlighted the potential neurotoxic effects of IE, particularly on brain development and function.

Cui et al. demonstrate that long-term exposure to excess iodine can harm the nervous system by impairing learning and memory, potentially through activation of the mitochondrial apoptosis pathway (25). In offspring rats exposed to excess iodine, hippocampal cell structure was altered, and proteins associated with apoptosis, such as Poly (ADP-ribose) polymerase (PARP), tumor protein p53 (p53) and Cleaved Caspase-3, were upregulated, while B-cell leukemia (Bcl2) expression was reduced. Additionally, IE affected monoamine neurotransmitters in the hippocampus differently between genders.

Similarly, another study revealed that maternal iodine intake, both too low and excessively high, negatively impacts brain development in offspring (26). Offspring from the low iodine and the 50-fold high iodine groups showed poorer performance in the Morris water maze, indicating impaired learning and memory. Additionally, reduced expression of neurotrophic proteins, including brain-derived neurotrophic factor (BDNF) and neuroendocrine-specific protein-A (NSP-A), was observed in the offspring's brains, further suggesting that both ID and IE disrupt thyroid function and neurodevelopment. A related study also found that 3-fold high iodine levels in female Wistar rats before pregnancy resulted in reduced BDNF levels and increased NSP-A levels in the hippocampus of their pups, which contributed to mild learning and spatial memory deficits (69). These findings highlight how elevated iodine intake can affect cognitive function, further emphasizing the delicate balance required for optimal brain development. However, as these findings are based on animal models, their direct applicability to human neurodevelopment requires further clinical validation.

Excess iodine, in the form of 3-iodo-L-tyrosine, an intermediate in TH synthesis, has also been shown to impair dopamine biosynthesis and induce Parkinson-like symptoms in mice (27). High concentrations of this molecule caused motor deficits, α -synuclein aggregation and damage to dopaminergic neurons in the substantia nigra. This study suggests that excess iodine may disrupt dopamine function and contribute to neurodegenerative conditions similar to Parkinson's disease.

In conclusion, while iodine is essential for brain function, the established correlation indicates that both deficiency and excess can have profound and lasting effects on neurological health, emphasizing the need for careful regulation of iodine intake. Animal studies provide valuable mechanistic insights, but further human research is necessary to establish the clinical relevance of these findings.

Impact of iodine excess on renal function

Excessive iodine intake can significantly impact renal and systemic health, especially in vulnerable populations such as

neonates, children with kidney disease and individuals with impaired renal function. Case reports highlight its role in hypothyroidism development in children undergoing peritoneal dialysis (PD). The use of povidone-iodine in sterile PD equipment was identified as a source of iodine overload, leading to elevated serum iodine levels and TD despite initially normal thyroid function. This reinforces the need for monitoring iodine exposure in pediatric nephrology (70).

In newborns with chronic kidney disease, exposure to iodine during voiding cystourethrography has been linked to transient hypothyroidism. Two cases demonstrated that intra-vesical iodine injection for diagnosing posterior urethral valves led to hypothyroidism, emphasizing the necessity of thyroid function monitoring post-procedure in such high-risk groups (71). Similarly, iodinated contrast media, widely used in diagnostic imaging, poses a risk for TD, particularly in neonates, fetuses, and patients with renal insufficiency (28). Clinical cases of acute kidney injury, such as tubular necrosis following iodine-based antiseptic use, further highlight iodine's nephrotoxic potential (72). While these clinical observations suggest a link between iodine exposure and renal dysfunction, controlled human studies are needed to confirm causality and identify safe exposure thresholds.

Experimental studies have shown that prolonged excessive iodine intake (1200-2400 $\mu\text{g/L}$) causes renal damage. Altered biochemical markers, such as elevated blood urea nitrogen and serum creatinine, and histological abnormalities, including glomerular vacuolation, highlight the toxic effects of iodine on renal structure and function. These findings point to iodine-induced OS and disruption of cellular homeostasis (29). However, as these studies were conducted in animal models, further research is necessary to establish their relevance to human kidney health. The correlation between iodine excess and renal dysfunction necessitates awareness of iodine's risks and careful monitoring is essential to mitigate its impact on renal and systemic health.

Conclusion

In summary, while iodine is essential, excess intake can have detrimental effects on various physiological systems. IE is associated with increased inflammatory markers, OS, and vascular dysfunction, contributing to cardiovascular issues such as hypertension and atherosclerosis. Additionally, high iodine intake may elevate the risk of TC, particularly through genetic mutations and cellular mechanisms promoting cancer cell proliferation. Iodine-induced autoimmunity, including conditions like GD and HT, also underscores the negative impact of excessive iodine on immune health, with mechanisms involving immune cell polarization and gut microbiota changes. Moreover, elevated iodine levels give rise to neurotoxic effects, affecting brain development and dopamine function. In addition, excess iodine can have a nephrotoxic effect, leading to the risk of acute kidney injury and damage to the renal system. Given the potential for

long-term health consequences, further research is crucial to fully understand the systemic effects of IE and to establish safe intake levels for optimal health.

Author contributions

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Conflict of interest

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From deficiency to excess: the impact of iodine excess on reproductive health

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Iodine is a critical trace element in the human body. It is primarily obtained through dietary sources such as dairy products, seafood, fish, eggs and certain vegetables. Iodine plays an essential role in various bodily functions, most notably in producing the thyroid hormones, triiodothyronine and thyroxine. Additionally, it influences the immune, cardiovascular, reproductive and gastrointestinal systems. Historically, iodine deficiency has been a significant global health issue; however, over the past decade, there has been a rise in iodine excess. This surge has been primarily attributed to inadequate monitoring and over-iodization of salt. Despite the well-documented consequences of iodine deficiency, the ramifications of excessive iodine intake remain underexplored. In view of rising global infertility rates, excess iodine has been linked to significant reproductive health effects. These include decreased sperm count, motility and morphology in males, as well as adverse pregnancy outcomes in females, such as maternal thyroid dysfunction and congenital hypothyroidism. This mini-review aims to collate and analyze current literature pertaining to the effects of iodine excess on reproductive health and shed light on its increasing incidence worldwide. Further research on the biological and clinical effects of iodine excess is required to derive a better understanding of this issue. Given the rising prevalence of iodine excess, it is crucial to raise awareness and implement proactive measures to prevent it from escalating into a major public health crisis in the future.

KEYWORDS

iodine, iodine excess, thyroid, thyroid hormones, reproductive health, infertility

Introduction

What is iodine

Iodine is a trace element in the human body, found at levels of 15–20 mg in a healthy adult, with the majority (70–80%) contained within the thyroid gland; it is acquired solely through dietary sources (1–7). Iodine is naturally present in foods such as dairy, seaweed, fish, eggs, broccoli, spinach and peas. Iodine plays several roles in the human body,

primarily in the synthesis of thyroid hormones such as triiodothyronine (T_3) and thyroxine (T_4) (1). This element is implicated in thyroid function and exerts profound effects on immune system modulation, the cardiovascular and reproductive systems, and gastrointestinal disorders, among others (1, 8, 9).

Daily recommended intake

The daily recommended intake for iodine according to the World Health Organization (WHO) is different for schoolchildren, at 120 μg , versus adults at 150 μg , with increased levels recommended for pregnant/lactating women at 250 μg (10–12). Historically, iodine deficiency has been a major concern, prompting countries such as the United States and Switzerland to introduce iodized table salt in the 1920s, which helped improve iodine levels (1, 13, 14). By the 1990s, these salt iodization programs were adopted globally, leading to a decrease in the prevalence of iodine deficiency worldwide (1, 13, 14). As of 2019, global iodine deficiency rates were at 2.4% (14).

Search strategy

A literature search was conducted between October 2024 to January 2025 utilizing Google Scholar and PubMed databases. The search employed keywords, including “iodine excess”, “iodine toxicity”, and “iodine overload”, among others. Only articles written in the English language were considered. Articles were included based on relevancy.

From deficiency to excess

Within the past decade, the number of countries with iodine deficiency has reduced from 54 to 30, while those with adequate levels have increased from 67 to 112 (15). Meanwhile, the number of countries with excessive iodine intake (defined as a median urinary iodine level over 300 $\mu\text{g/L}$) has doubled from 5 to 10: Brazil, Colombia, Somalia, Uganda, Armenia, Benin, Georgia, Honduras, Paraguay, Uruguay (15). Poor monitoring and over-iodization of salt have been the primary factors contributing to excessive iodine levels in these countries (15). The American Thyroid Association (ATA) sets the upper tolerable limit of iodine at 1100 μg and advises against daily intake exceeding 500 μg for children, adults and pregnant or lactating individuals (16). Meanwhile, the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) has proposed a maximum upper limit of 1 mg/day of iodine for the general population (10–12). Other organizations such as the former Scientific Committee on Food (SCF) of the European Commission and the UK Expert Group on Vitamins & Minerals (EVM) propose upper tolerable limit values for adults at 600 $\mu\text{g/day}$ and 500 $\mu\text{g/day}$ respectively (17, 18).

It is important to recognize that individuals can differ significantly in terms of their tolerance to iodine intake due to a multitude of factors. Diet plays an important role, as varying amounts and sources of iodine with differing eating patterns contribute to inter-individual variability (19). Furthermore, those with pre-existing

thyroid disease may be more susceptible to excessive iodine intake (10). Genetic mutation can additionally impact the normal physiological processes of the thyroid to iodine (20). Consequently, these factors should be considered when interpreting studies.

Although the impact of iodine deficiency is well-studied, iodine excess has received much less attention regarding its effects on human health. The literature contains a paucity of articles assessing the impacts of excess iodine especially in terms of reproductive health; however, some studies have shown excess iodine affects reproductive health and pregnancy by impairing spermatogenesis, hindering infant neurodevelopment, increasing the risk of infertility and contributing to gestational diabetes mellitus (GDM) and hypertensive disorders during pregnancy (HDP) (21–24). In this mini-review, we aim to highlight the impact excess iodine has on the reproductive health of males and females.

The body's reaction to excessive iodine exposure

Historically, one of the earliest mentions of the reaction of the thyroid gland to acute excessive iodine concentrations was described by Dr. Jan Wolff and Dr. Israel Chaikoff, an observation now known as the Wolff-Chaikoff effect (25, 26). By reducing the synthesis of T_3 and T_4 , this effect acts to block the excessive amounts of iodide in the body from generating large amounts of thyroid hormone (27–29). One proposed mechanism of the Wolff-Chaikoff effect is the production of inhibitory substances that impact thyroid peroxidase, namely intrathyroidal iodoaldehydes, iodolactones or iodolipids (25, 30).

However, the Wolff-Chaikoff effect is transient, usually lasting only one to two days, after which an escape occurs where regular synthesis of thyroid hormones resumes (25, 27, 31). Reduced expression of the sodium-iodide symporter has been theorized to diminish iodide uptake and the subsequent generation of thyroid hormones, thereby maintaining a euthyroid state (25, 27, 31).

Notably, excess amounts of daily iodide ranging from 30 mg to 2 g are typically well tolerated in individuals with normal thyroid glands. Although laboratory alterations are seen, such as a reduction in serum T_4 by 25%, T_3 by 15% and a 2 mU/L rise in TSH, these values remain within the normal ranges without clinical signs of thyroid dysfunction (32).

A failure of the Wolff-Chaikoff effect can give rise to the Jod-Basedow phenomenon, typically seen in patients with impaired thyroid regulation, including those with thyroid nodules. Rather than becoming hypothyroid, these patients may generate an excessive amount of thyroid hormone (25, 28, 31, 33, 34).

Although the effect of iodine on thyroid pathophysiology is well described, the role that excess iodine has independent of thyroid hormone in regard to infertility has not been well documented (35). Infertility, defined as a couple's inability to conceive after one year of regular unprotected intercourse, is a significant health issue impacting 50–80 million individuals worldwide (36–38). Infertility can be subcategorized into primary and secondary infertility, the former being when a woman has never conceived, and the latter being when a woman has had at least one successful previous conception but now has inability to conceive according to the WHO (39–41). Categories of infertility include ovulatory dysfunction, tubal occlusion, diminished ovarian reserve, endometriosis, uterine and

male factors (42). Furthermore, lifestyle and environmental factors such as sexual violence, sexually transmitted diseases, stress and nutritional insufficiency among many others have also been associated with reduced fertility (43). An identifiable cause can be found in 85% of infertile couples, whereas the remaining 15% are classified as unexplained infertility (42, 44). Furthermore, infertility rates in men have been rising over the past decades, with a notable 76.9% increase since 1990 (45). Additionally, 20–30% of infertility cases are due to males, while male factors contribute to 50–70% of infertile couples (46). Therefore, it is imperative to highlight the possible role that excess iodine may have on exacerbating this global health crisis (47).

Iodine excess and its impact on male reproductive health

Recent studies have shown that excessive iodine levels correlate with several adverse effects on male reproductive health, particularly with regard to semen quality. A study investigating the association between semen quality and iodine intake in 1,098 fertile Chinese men found that participants with iodine excess, defined as a urinary iodine concentration (UIC) of $\geq 200 \mu\text{g/L}$, exhibited a 5% higher semen volume, 26% lower semen concentration, 20% fewer semen counts, and a 1.48 month longer time to pregnancy in comparison to those with an optimal iodine intake ($100 \leq \text{UIC} < 200 \mu\text{g/L}$) (48). Although these findings were statistically significant, there was no significant effect on sperm motility (48). A strength of this paper is its relatively large multi-province sample of 1,098 couples, enhancing external validity; however, a limitation is its cross-sectional design which does not allow definitive establishment of causality. Furthermore, its sample inclusion of only fertile men, excluding infertile men, may underestimate the true effect of iodine on semen quality.

Another study conducted in Spain analyzing 96 couples with a median UIC of $97.6 \mu\text{g/L}$ undergoing infertility evaluation found a higher rate of altered spermatozoa morphology and a lower motile sperm count in those with higher semen iodine levels and urinary iodine levels respectively (35). Moreover, males who had not been able to achieve pregnancy with their partner for more than three years had a higher urinary iodine level than those trying for fewer than three years (35). In contrast to the previously mentioned study, this paper observed no association in certain semen characteristics such as volume, total sperm count and percentage of spermatozoa with progressive motility, after adjusting for age, smoking and BMI (35). Interestingly, the urinary iodine level was negatively correlated with the total motile sperm count (35). Moreover, males with abnormal sperm morphology possessed a higher median semen iodine level (35). Additionally, those who reported consuming iodized salt had significantly higher median semen iodine levels ($16.5 \mu\text{g/L}$) compared to those who consumed non-iodized salt ($11.7 \mu\text{g/L}$). A strength of this study is that it was the first study to measure semen iodine concentration in association with semen quality; a limitation is the small sample size. Although a causal relationship could not be established within the study due to cross-sectional design, the findings highlight the need for additional clinical research to investigate whether excess dietary iodine impacts spermatozoal morphology and other parameters, which could affect fertility.

The hypothalamic-pituitary-thyroid axis is key in reproductive health, especially in the context of iodine excess. Since iodine excess in certain susceptible individuals can lead to thyroid dysfunction (hypothyroidism or hyperthyroidism) (10). Hypothyroidism in humans can lead to reductions in total serum testosterone, luteinizing hormone (LH), follicle stimulating hormone, sex-hormone binding globulin and alterations in sperm quality (49–52). Similarly, adverse effects on fertility are seen in hyperthyroidism, such as alterations in sex steroid levels and spermatozoal DNA damage and motility abnormalities (53–55). Iodine excess' effect on fertility is theorized to be a consequence of oxidative stress on the testis (53).

A randomized control trial studying the effects of iodine excess in adult male rats evaluated parameters including testicular morphology, steroidogenic enzyme activity, and sperm count, viability and morphology (56). They found that male rats administered 100 times and 500 times the recommended iodine level saw dose- and duration-dependent impairment of reproductive function compared with controls (56).

Interestingly, the role of iodine in male reproductive health is substantiated by the immunohistochemical confirmation of the sodium-iodide symporter and pendrin (57, 58). One proposed mechanism is reactive oxygen species (ROS) generation following excessive iodine accumulation within the testes, adjudged by an alteration in levels of pro-oxidant and antioxidants (catalase, superoxide dismutase, glutathione peroxidase) as well as increased levels of lipid peroxidation (56). Under normal physiological states, ROS is generated as part of the steroidogenesis process, however, as a result of iodine-induced alteration in antioxidant levels, this leads to the inability of the testes to counteract the harmful effects caused by ROS (56, 59) causing cellular damage to testicular germ cells (56, 60). Furthermore, this leads to a decrease in testosterone synthesis via inhibition of the enzymes Δ^5 3 β -hydroxysteroid dehydrogenase (HSD) and 17 β -HSD (56). Since testosterone is an important regulator of spermatogenesis, these processes eventually result in functional and structural alterations in the testes (56, 61). Furthermore, prolonged ROS generation disrupts the hypothalamic-pituitary-adrenal axis by upregulating the adrenocortical stress signaling pathway thereby increasing corticosterone. This effect leads to a downstream inhibition of LH release further decreasing testosterone synthesis (56). Moreover, a protracted state of iodine excess can directly precipitate a hypothyroid state, eventually decreasing testosterone levels (56). While this study provides mechanistic insights, it was performed in rats, limiting translational relevance to humans.

Another study by Chakraborty et al. used rat models to evaluate the role of excess iodine in spermatogenesis. Similar to the previous findings, these authors observed a significant reduction in both sperm motility and sperm count (21). Furthermore, molecular investigations revealed that oxidative stress, increased apoptosis, reduced expression of blood-testis barrier (BTB) proteins, decreased regulators of spermatogenesis, and reduced expression of markers related to germ cell proliferation and differentiation all played a role in causing impaired spermatogenesis in the study (21). Ultimately, the mechanisms the authors propose that lead to spermatogenesis impairment is an interplay between cytoskeleton and BTB

disruption as well as oxidative stress (21). Another paper confirmed that excess iodine, by inducing ROS generation, led to spermatozoal cell apoptosis thereby affecting male fertility (62).

Although these findings are based on rat models, they provide valuable insight into how iodine may impact human reproductive health (Figure 1). Hence, it is crucial to develop effective strategies to mitigate iodine-induced male infertility to combat the rise in infertility rates over the past decades.

Iodine excess and its impact on female reproductive health

Iodine excess and female fertility

Unexplained infertility (UI) is the diagnosis given when a couple has been actively trying to conceive for more than a year unsuccessfully, and medical testing cannot identify a cause for infertility (44). For women who have low fertility, hysterosalpingography (HSG) is historically the most common first-line diagnostic modality to assess the uterine cavity and tubal patency (63). However, a study using an oil-soluble contrast medium (OSCM) in conjunction with HSG caused iodine excess, leading to the development of subclinical hypothyroidism in approximately 40% of women, and later-onset hyperthyroidism in 5% of women (64). Additionally, women who conceive successfully after HSG surgery may be at risk of excess iodine, as previous studies showed that women who underwent HSG before pregnancy remained in a prolonged state of high iodine throughout the gestational and postpartum periods (65). Moreover, another study confirmed persistently high levels of iodine after OSCM HSG, and iodine excess did not correlate to improved fertility. However, it was noted that 30% of their population was iodine deficient, and the OSCM

HSG corrected that deficiency, which aided in improving fertility for those patients (66). Thus, it is essential to recognize the impact of iodine excess on differing populations, as baseline iodine levels have the potential to influence outcomes of OSCM HSG.

Research on the impact of excess iodine on female reproductive physiology is limited. However, a study by Mahapatra et al. provides some insights. Rats were separated into three groups were given varying levels of iodine in the form of potassium iodide for an extended period of time, and demonstrated that extended exposure to iodine in excess exerted a biphasic mode of action, causing either a hypofunctioning or a hyperfunctioning ovary, with a fertility index of zero at both doses (67). Furthermore, a study evaluating ovarian and uterine histological changes following prolonged iodine excess demonstrated an alteration in the structure and number of ovarian follicles and corpus luteums (68). In addition, uterine changes were also observed which further contributed to the negative alteration of female rat reproductive function (68). Despite current findings, further research is essential to gain a comprehensive understanding of the effects of excess iodine on ovarian structure and function.

Iodine excess and pregnancy

The physiological demands of pregnancy necessitate an approximately 50% increase in iodine requirements, rendering this population vulnerable to iodine level imbalances (69). A median UIC below 100 $\mu\text{g/L}$ for nonpregnant women and children defines an iodine-deficient population (70). For pregnant women, the WHO defines UIC levels of 150–249 $\mu\text{g/L}$ as adequate iodine status, UIC values below 150 $\mu\text{g/L}$ indicating iodine deficiency, levels between 250 and 500 $\mu\text{g/L}$ being classified as more than adequate, and values

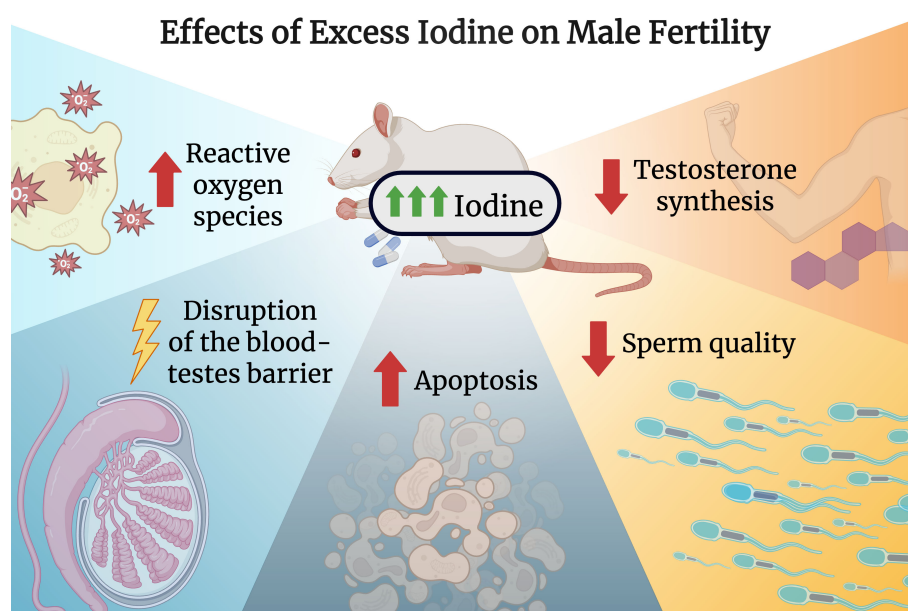


FIGURE 1
Effects of excess iodine on male fertility. Created with biorender.com.

exceeding 500 µg/L considered to be iodine excess (70). Iodine deficiency may be exacerbated by avoiding iodine-rich foods, stemming from concerns about heavy metal contamination in these foods, and managing nausea and vomiting (71), so iodine supplementation has become popular. However, pregnant women are at risk of iodine excess due to the intake of water, food, nutritional supplements or additional medications (10). A new study challenges the current WHO recommendation by suggesting a lower limit for iodine intake during pregnancy (72).

Excessive iodine during pregnancy can increase serum thyroid-stimulating hormone (TSH) concentrations, negatively impacting maternal thyroid function and potentially leading to further health risks, as seen in Figure 2. Findings from an observational study indicate that pregnant women consuming high-dose iodine supplements (>200 µg/day) may have a higher risk of serum TSH elevation compared to with women whose supplemental iodine intake was lower at <100 µg per day (73). Elevated maternal serum iodide can potentially lead to reduced fetal thyroid hormones, fetal skeletal development, placentation and preterm delivery (74). The ingestion of excess maternal iodine tablets during pregnancy has led to several cases of congenital hypothyroidism being reported (75).

Beyond these hormonal effects, excessive iodine intake has also been linked to thyroid autoimmunity, which has been associated with increased infertility rates, polycystic ovary syndrome, premature ovarian insufficiency and endometriosis (76, 77). Excess iodine may trigger immune dysregulation, contributing to autoimmune thyroid diseases such as Hashimoto's thyroiditis and Graves' disease, both of which have been associated with excess long-term morbidities in children and adolescence (78). Since immune balance is crucial for implantation, ovarian function and maternal-fetal interactions, these findings suggest that iodine-

related immune disturbances could contribute to reproductive challenges (79–81). While direct studies on iodine excess and reproductive immunology remain limited, further research is needed to clarify these potential mechanisms.

In addition to supplements, excess iodine may also be due to diet. For mothers who consumed excessive amounts of seaweed during both pregnancy and lactation, hypothyroidism was diagnosed in their offspring (82, 83). A Finnish study revealed a positive association between elevated serum iodide levels and risk of preterm birth (84). Furthermore, iodine excess during pregnancy was found to be linked to a significant increase in the occurrence of adverse mother and fetus outcomes, including eclampsia, umbilical cord wrapping around the neck, abnormal blood glucose and lipid levels (85).

Moreover, a study involving 214 pregnant women determined that those with UIC between 250–499 µg/L exhibited higher rates of GDM (20.3%) compared to the group with UIC 150–249 µg/L (9.7%) (24). Additionally, HDP, encompassing pre-eclampsia and gestational hypertension, occurred with a prevalence of 33.3% in those with UIC ≥ 250 µg/L compared to 4.3% in those with 150–249 µg/L (24). Neonatal growth parameters, such as head circumference, femur length, and estimated fetal weight were inversely associated with a state of iodine excess (86). Additionally, a cross-sectional study of 7,190 pregnant women in China found that both deficient and excessive iodine intake were associated with an increased risk of maternal hypothyroxinemia, hypothyroidism prevalence and thyroid autoimmunity (87). Despite its clinical significance, there are limited studies concentrating specifically on iodine excess during pregnancy, and maternal and neonatal outcomes. However, with the current research, the potential risks associated with even mild iodine excess during pregnancy warrant careful consideration.

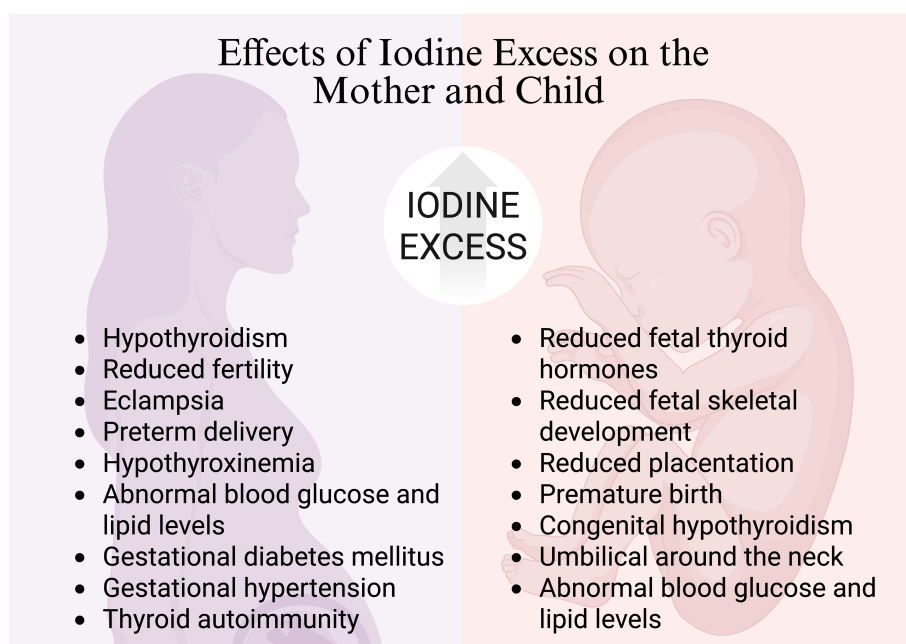


FIGURE 2
Effects of iodine excess on mother and child. Created with biorender.com.

Endocrine-disrupting chemicals and thyroid function

According to the Endocrine Society, Endocrine-disrupting chemicals (EDCs) are defined as a mixture of chemicals or an exogenous chemical that can interfere with any aspect of hormone action (88). They include a wide range of compounds such as fungicides, plasticizers, pesticides and industrial chemicals, among others (89). Certain EDCs, namely thiocyanate and perchlorate, affect thyroid metabolism by inhibiting the sodium-iodide symporter (90, 91). Additionally, compounds such as flame retardants may disrupt thyroid physiology.

A recent 2024 review examining flame retardants and thyroid function revealed conflicting evidence, with some studies finding a positive association with TSH while others noted a negative one (92). Similar relationships were found between flame retardants and total T3 and total T4 (92). The authors emphasize the need for long-term studies to more conclusively determine the effects flame retardants have on thyroid function (92).

As EDCs affect thyroid physiology, they can potentially impact iodine levels, making them a factor to consider in infertility. As a result, further research into the long-term effects of EDCs on thyroid function, iodine levels and reproductive health is crucial for mitigating possible future fertility challenges worldwide.

Conclusion

Excess iodine intake is an emerging concern with potential implications for reproductive health. While iodine is essential for thyroid function and overall well-being, an imbalance, whether deficiency or excess, can lead to significant health consequences. This review highlights evidence suggesting that excessive iodine exposure may impair male and female reproductive health by altering semen quality, disrupting hormonal balance, and contributing to conditions such as infertility, GDM, and HDP. Given the rising number of countries experiencing excessive iodine intake due to over-iodization and poor monitoring, it is crucial to implement strategies that ensure a balanced intake. Future research should further investigate the underlying mechanisms and long-term reproductive outcomes associated with iodine excess to refine public health recommendations. By addressing these gaps, we can

develop more precise dietary guidelines and policies to safeguard reproductive health while maintaining optimal iodine nutrition.

Author contributions

AiK: Conceptualization, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. AhK: Conceptualization, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. SN: Conceptualization, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. HH: Conceptualization, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. AB: Conceptualization, Project administration, Supervision, Writing – review & editing.

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The author(s) declare that no Generative AI was used in the creation of this manuscript.

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Modern challenges of iodine nutrition: vegan and vegetarian diets

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Vegetarian diets are gaining popularity worldwide, especially among women and in the younger part of the population, rising some concerns for the risk of inadequate iodine requirements if the diet is not correctly planned. Indeed, subjects under vegetarian dietary regimens, mainly the vegan ones, are at risk of developing both iodine deficiency and excess, due to lack of animal-derived foods on the one hand, and to the use of vegan alternatives (such as seaweed) and over-the-counter supplements on the other hand. Women in childbearing age and children are particularly vulnerable to the adverse thyroid consequences. Thus, this paper aims to provide some practical information to achieve adequate iodine intake and avoid adverse effects on thyroid in this population.

KEYWORDS

iodine deficiency, vegetarian diet, vegan diet, iodine supplementation, thyroid

Introduction

In recent years there is growing awareness of the potential benefits of plant-based diets, and vegetarian diets, that avoid some or all foods of animal origin, have gained in popularity, so that increasing number of individuals at any age is turning to vegetarianism supported by ethical/religious and ecological motivations, health concerns, and taste preferences (1, 2).

The umbrella term “vegetarianism” covers a range of dietary choices, whose common distinctive features are: i.) consumption of a wide variety of plant foods; ii.) Exclusion of flesh foods: all types of meat, meat products (sausages, salami, etc.), and fish (including sushi, mollusks and crustaceans). There are two main types of vegetarian diet: the lacto-ovo-vegetarianism (LOV), that includes dairy products, eggs, and honey (further subdivided in the subcategories lacto-vegetarianism which excludes eggs, and ovo-vegetarianism which excludes dairy products); and the veganism, that excludes animal foods at all (3, 4). There are also growing numbers of people in Western countries that have chosen to reduce their animal product consumption, and adopted a plant-base diet, ranging from pescatarian (excluding meat/poultry, but including fish/seafood) regimens, to demi-

vegetarian (or flexitarian) that occasionally include meat/poultry/fish (less than once a week) (2). Consequently, the nutritional profiles of vegetarian and plant-based diets can vary considerably. Compared with omnivorous diets, vegetarian diets contain more folate, fibers, antioxidants, sterols, phytochemicals and carotenoids, and less saturated fatty acids, animal protein and cholesterol, but may be weak in some minerals, such as iron, calcium and iodine, and vitamins (mainly B12) from animal source foods, if not carefully planned and well balanced according to current guidelines (2, 5).

Moreover, the rising demand for exclusively plant-based products has increased the commercial offer for alternative products, such as plant-based milk- and meat-substitutes, but also highly processed, ready-to-eat foods (6). Although the variety of foods available for vegetarians has increased in the last years, and most commercial plant-based foods are enriched in vitamins and micronutrients (iron, calcium, B12, or vitamin D), there remain some concerns as for the difficulties in reaching adequate amounts of iodine (7). The risk of iodine shortfall is of particular concern in children and pregnant/breastfeeding women.

Iodine nutrition and vegan/vegetarian diets

Iodine is a rate-limiting element for the synthesis of thyroid hormones, and it becomes a determinant of thyroid disease when not adequately supplied. This trace element can be obtained exclusively through the diet or through iodine supplements, and cannot be replaced by any other nutrient. Iodine is naturally present in some foods, especially milk and seafood, mainly as an iodide salt, but also in other chemical forms, including iodate, molecular iodine or monoatomic iodine incorporated in proteins. Iodide is almost completely absorbed in the stomach and duodenum, and enters the circulation. The thyroid gland concentrates it in appropriate amounts for thyroid hormone synthesis due to a specific iodide-sodium symporter (NIS), expressed on thyroid cell surface, and most of the remaining amount is excreted in the urine (8). Urinary iodine reflects dietary intake directly because more than 90% of dietary iodine is excreted in the urine (9). Median urinary iodine concentrations of 100–199 µg/L in children and adults, 150–249 µg/L in pregnant women and >100 µg/L in lactating women indicate adequate iodine intakes (10). The Recommended Dietary Allowances (RDA) of iodine are 150 µg/day for adults and 250 µg for pregnant/lactating women, according to the World Health Organization (WHO) (11) (Table 1). Similarly, dietary reference values have been provided by the European Food Safety Authority (EFSA), indicating as average requirement (AR) 150 µg/day for adults, 70–130 µg/day for infants aged 7–11 months and for children, and 200 µg/day for pregnant women. A value of 600 µg/day was also proposed as a Tolerable Upper Intake Level (UL) for adults, including pregnant and lactating women (12).

The iodine content of foods is highly variable between food categories as well as within each category, as shown in Table 2. Good food sources of iodine include fish and other seafood as well

TABLE 1 Iodine recommended intakes and nutritional status evaluation in different populations.

IODINE NUTRITION STATUS ASSESSMENT BY UIC		
Children and adults		
<20 µg/L	Insufficient	Severe ID
20–49 µg/L	Insufficient	Moderate ID
50–99 µg/L	Insufficient	Mild ID
100–299 µg/L	Adequate	Sufficient
≥300 µg/L	Excessive	Excess iodine
Pregnancy		
<150 µg/L	Insufficient	ID
150–249 µg/L	Adequate	Sufficient
≥500 µg/L	Excessive	Excess iodine
Lactation		
<100 µg/L	Insufficient	ID
≥ 100 µg/L	Adequate	Sufficient
≥500 µg/L	Excessive	Excess iodine
RECOMMENDED DIETARY ALLOWANCES (RDAs) FOR IODINE		
Birth to 6 months	110 µg/day	
7–12 months	130 µg /day	
1–8 years	90 µg/day	
9–13 years	120 µg/day	
14–19+ years	150 µg/day	
Pregnancy and Lactation	250 µg/day	
TOLERABLE UPPER INTAKE LEVELS (ULS) FOR IODINE		
1–3 years	200 µg/day	
4–8 years	300 µg/day	
9–13 years	600 µg/day	
14–18 years	900 µg/day	
19+ years	1100 µg/day *	

ID, iodine deficiency; UIC, urinary iodine concentrations. In bold the upper thresholds of UIC for iodine excess.

*Pertinent references:

World Health Organization. United Nations Children’s Fund & International Council for the Control of Iodine Deficiency Disorders. Assessment of iodine deficiency disorders and monitoring their elimination. 3rd ed. Geneva, Switzerland: WHO, 2007 (10).

Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zincexternal link disclaimer. Washington, DC: National Academy Press, 2001 (53).

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on Dietary Reference Values for iodine. EFSA Journal 2014; <https://doi.org/10.2903/j.efsa.2014.3660> (12).

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*The American Thyroid Association advises against 500 µg iodine daily for both children and adults and during pregnancy and lactation².

TABLE 2 Iodine content in common foods.

Description	Iodine (mcg/serving)	LactoOvo-Vegetarian	Vegan
Baked Cod	146.3		
Nori seaweed, dried	115.8		
Nonfat Yogurt*	100.1		
Whole Milk *	81.8		
Iodized Salt	78.2		
Fish sticks, oven-cooked	56.8		
Eggs, hard-boiled*	30.5		
Pasta, boiled in water with iodized salt	29.6		
Fresh bluefin tuna, cooked	19.6		
Mozzarella*	15.3		
Shrimps	12.9		
Salmon fillets, baked	10.9		
Canned tuna	7.4		
Veggie burger, soy based, baked	5.3		
Parmesan Cheese *	4.1		
Spinach, boiled	3.5		
Beef roast	3.3		
Soy Beverage	3.2		
Lamb chop, pan-cooked	2.1		
White bread *	1.2		
Chicken breast, roasted	1.0		
Butter	0.6		
Broccoli, boiled	0.4		
Cucumber	0.4		
Peach	0.3		
Rice, cooked	0.3		
Margarine	0.2		
Tomato	0.2		
Soy sauce	0.1		
Apple	0.1		
Avocado	0.1		
Lettuce	0.1		
Corn flakes	0.1		
Sea salt, non-iodized	0.02		

(Continued)

TABLE 2 Continued

Description	Iodine (mcg/serving)	LactoOvo-Vegetarian	Vegan
Olive Oil	0		
Potato, boiled	0		
Beans, boiled	0		
Lentils, cooked	0		
Tofu	0		
Almonds	0		

*Iodine content can vary based on country-specific iodine food enrichment policies, iodine concentration in soil and in fodder. Green color indicates foods included in LactoOvo-Vegetarian and Vegan Diets. Adapted from the USDA, FDA and ODS-NIH Database for the Iodine Content of Common Foods per Serving, Release 4, October 2024 (https://www.ars.usda.gov/ARSUserFiles/80400535/Data/Iodine/IODINE_DATABASE_RELEASE_4_PER_SERVING.pdf).

as eggs and milk, as well as their derivatives, given that iodine content of milk and eggs is influenced by animal feeding and hygienic practices. Meat, animal products and dairy products, contain iodine in a variable amount depending on whether farm animals have received iodine fortified feeds. Anyway, milk represents a relevant source of iodine, mainly in children (13), whereas plant-based beverage used as milk substitutes, such as soy and almond beverages, contain relatively small amounts of iodine unless fortified (14). Most fruits and vegetables contain little amounts of iodine, depending on the iodine content of the soil, and fertilizer use. Similarly, cereals and bread are poor food sources of iodine unless fortified (15). Despite seaweed (such as kelp, nori, kombu, and wakame) are among the best food sources of iodine, the iodine amounts in different seaweed species vary greatly, and may result in iodine excess. Iodine can be added to salt, and it is accepted that 30 ppm (30 mg of potassium iodate per kilogram of salt) is the lowest level that will ensure the provision of 100 µg of iodine per day (10). Iodine fortification of salt has been implemented in several countries on a mandatory or voluntary basis. Finally, iodine is available as a dietary supplement, and its content must not exceed 225 µg daily. A supplement of 150 µg/day iodine in the form of potassium iodide is advisable in subjects at risk of inadequate iodine intake (11).

A U-shaped relationship exists between iodine nutritional status and thyroid disorders, indicating that either too little or too much iodine may be harmful to the gland (16). The interval between inadequate and a more-than-adequate iodine intake is relatively narrow, so that even small changes may have profound effects on the prevalence of thyroid dysfunction and goiter (17). Iodine deficiency (ID), defined as a median urinary iodine concentration (UIC) <100 µg/L, is a major cause of hypothyroidism worldwide, mainly when severe (UIC <20 µg/L) or moderate (UIC <50 µg/L), and it also favours the development of goitre and thyroid nodules (18). Nevertheless, also an excessive iodine intake, due to inappropriate supplementation or excess intake of iodine-rich food, can be harmful to the thyroid, increasing the risk for thyrotoxicosis, hypothyroidism and autoimmunity (16, 17).

In general, an adequate iodine supplementation, reaching a population median UIC between 100 and 300 $\mu\text{g/L}$, is considered safe, with the benefits largely outweighing the potential risks. Universal salt iodization (USI) represents the best and safest strategy to implement iodine intake and prevent/eradicate ID in the general population, according to the WHO (10).

Nowadays in most industrialized countries, the main sources of iodine are iodized salt, fortified foods (such as bread), and animal-derived foods (cow milk, dairy products, fish, seafood, eggs) (19), while plant-derived foods contain low-quantities of iodine, unless fortified, with the only exception of seaweed (20). In addition to iodized salt and seafood, milk and dairy products are among the major sources of iodine in Mediterranean and Western diets, due to supplementation of iodine in animal feed (21).

As vegetarian diets are becoming more and more popular worldwide, especially among women and young people, concerns for iodine nutrition have raised (22). Indeed, plant-based foods have a lower iodine content than animal-derived ones, and vegan diets exclude many iodine-rich foods, including dairy, eggs, and/or fish (7). Since cow milk has demonstrated a significant role in contributing to the total iodine intake, especially in children, as demonstrated in UK and Norway-based studies, vegan diets eliminating milk-derived food are particularly at risk for iodine deficiency (23). Several studies have highlighted that subjects under vegan diet, who strongly restrict consumption of iodine-rich foods and are dependent only on iodine deriving from plants are at high risk of iodine deficiency (24). Iodine nutrition has been demonstrated to be inadequate among vegetarians, when assessed both as median UIC (25–28) and estimated iodine intake (25–29), and is particularly poor among vegans. Iodine intake in vegans appears to be even lower among women and in countries lacking USI programs, compared to countries in which iodine intake derives mainly from iodized salt or fortification of bread (30). Some studies also suggest that both vegetarians and vegans, especially women, also tend to consume less iodized salt (31). A further risk for vegetarians would stem from the combined risk of having also iron and selenium deficiency, which would increase the detrimental effect of iodine deficiency on thyroid function (25). For this reason, the most important Scientific Societies of Nutrition and Vegetarian/Vegan Nutrition recommend iodized salt and/or iodine containing supplements (150 $\mu\text{g/d}$) in childbearing age vegan women who do not consume iodized salt (28). Moreover, a LOV diet, that include milk, dairy products and eggs, provide adequate iodine sources, given that iodized salt is always recommended (23).

Finally, in vegetarian diets, an alternative source of iodine is represented by seaweeds (such as macroalgae, nori and kelp). However, even seaweed has a highly variable content of iodine, according to the species and processing of food, and can even expose users to iodine excess, so that uncontrolled consumption of seaweed should be discouraged (32).

Nutritional advices for mothers and children

Iodine nutrition is particularly crucial in women in fertile age, since during pregnancy ID can cause an increased risk of growth

retardation and brain damage for the fetus, and induce both maternal and fetal goiter (18, 33, 34). The iodine requirements increase during pregnancy, due both to an increased maternal thyroid hormone synthesis, greater urinary iodine loss due to an increased glomerular filtration rate and iodine transfer to the fetus (35). Several studies suggest that pregnant and lactating women may be at high risk of having inadequate iodine levels, even in countries where salt iodization programs are available (36, 37). Since iodized salt may not be a sufficient source of iodine to meet the minimum requirements of this vulnerable group, several international authorities recommend a daily supplement of 150 μg of iodine for pregnant and lactating women (38). This appears even more relevant since childbearing age women are generally more likely to follow a plant-based diet, especially in the 24–39 years age bracket (39). A recent Australian study specifically aimed at assessing iodine nutrition in women of childbearing age showed that vegan women had lower UIC and iodine intake when compared to omnivores, even if both groups had median UIC below the threshold for iodine deficiency (27).

Another recent Australian study highlighted that pregnant women following a vegetarian diet were more likely to report multivitamin use, even if urinary iodine levels were similar between multivitamin users and non-users (40).

It should be noted that a recent case report described a case of transient hypothyroidism with goiter due to severe ID in the child of a vegan mother with a severely restricted iodine intake from the UK (41). Nevertheless, studies regarding the possible impact of iodine deficiency in vegetarian/vegan mothers on pregnancy outcomes are lacking. Indeed, several studies show that a plant-based diet could reduce the risk of several pregnancy complications, such as pre-eclampsia, gestational diabetes and pre-term birth. In conclusion, vegetable based diets can be safe during pregnancy, but they may require a dedicated nutritional intervention to provide a balanced intake of key nutrients, including iodine, and to prevent maternal undernutrition (42).

Also breastfeeding mothers have an increased requirement of iodine, and some recent evidence suggest that vegan mothers could be at risk of not providing enough iodine to their children with breast milk. A recent study performed on lactating women showed that the breast milk iodine content was lower in a small sample of vegan and vegetarian mothers when compared with omnivorous ones, and that most samples indicated an inadequate iodine content (43). A comparison of the micronutrient composition of breastmilk of vegan mothers compared to that of omnivorous breast milk donors showed lower iodine content, even if in the normal range, in the vegan group. UIC was lower in vegan mothers when compared with omnivorous ones, even if the estimated iodine intake was similar (44). Nevertheless, a recent study correlating the maternal dietary choices to the iodine status of the offspring did not find any correlation between vegetarian practice and whether or not the mothers included dairy products in their diets and the toddlers UIC (45).

The risk of ID due to a plant-based diet is relevant also in children, since in vegetarian/vegan households children's eating behavior depends on parents, who often share eating habits setting

an example and wanting their children to share their beliefs. Moreover, several establishments such as kindergartens, colleges, schools and hospitals are providing vegetarian options for children for educational, health and environmental reasons. While plant-based diets provide a protection towards non-communicable diseases such as cardiovascular ones, initial data suggest that they could cause stunted growth (especially among vegan children) and alterations in body composition (46). In this context, data on iodine nutrition in vegetarian children are scarce. A recent German study found that both vegan and vegetarian children had lower iodine intake when compared to omnivores (47). On the contrary, a similar UIC was found by a Finnish study among vegan children when compared to omnivores (48). A recent study from the Czech Republic showed a trend towards lower UIC values in vegetarians and vegan children when compared to omnivores, even if median values were above the threshold for defining ID in all dietary groups. Vegan and vegetarian children also had a higher rate of AbTg positivity (49). Another possible source of concern is the widespread use of plant-based substitutes of cow milk for vegetarian and vegan newborns and toddlers: these drinks are usually not fortified with iodine and almost completely lack in this nutrient (50). Moreover, seaweed consumption is generally contraindicated in children, due to a higher risk of iodine overload and heavy metals contamination (51).

Given the high risk of ID in children following a plant-based diets, it seems reasonable to suggest the use of iodized salt with a varying amount according to fortification regimens (52). In vegetarian/vegan women, a reasonable approach would be to start low-dose iodine supplements optimally 3 months prior to pregnancy, and to consume iodized salt adding an oral supplement of 150 – 225 µg per day of iodine, in the form of potassium iodide during pregnancy and breastfeeding (53).

The supplement paradox: more isn't always better

Paradoxically, subjects under vegan and vegetarian diet are also at risk of developing complications from excess iodine, since vegan alternatives, such as seaweed, contain an excess amount of iodine that may disrupt thyroid homeostasis. Indeed, the availability of iodine from seaweed is largely variable, and it can provide amounts up to a hundred times the RDAs (54). Furthermore, dietary supplements marketed to improve iodine nutrition and thyroid function in vegan and vegetarians, often contain iodine and kelp products in amounts that largely exceed not only the RDA (150 µg per day in adults) but also the daily tolerable upper limit for iodine (TUL, 1000 µg per day) (55). An even lower threshold for safe iodine intake has been proposed by EFSA (600 µg iodine daily) (12) and by the American Thyroid Association (ATA) (500 µg iodine daily) (56). A recent

review by Farebrother et al., showing data from a survey of U.S. multivitamin supplements, found significant discordance between label information and laboratory assay, most products containing excess iodine from kelp. These easily accessible dietary supplements potentially expose users to the risk of thyroid dysfunction (57). People should be aware that products deemed as “natural” are not automatically healthy and safe, and many iodine supplements contain kelp, thus providing excess amounts. Mostly, children and pregnant and breastfeeding women are more susceptible to adverse effects of excess iodine, as well as the elderly and individuals with preexisting thyroid diseases. For this reason, the American Thyroid Association (ATA) advises against the ingestion of iodine and kelp supplements containing in excess of 500 µg iodine daily for both children and adults and during pregnancy and lactation (56).

As a source of iodine, vegetarians should use iodized salt, and, if convenient, supplements containing potassium iodide in adequate amounts (150 µg/day). As regarding the use of iodized salt, it should be mentioned that concerns exist on the cardiovascular risks deriving from excess salt consumption. There is evidence of a causal relationship between sodium intake and blood pressure, and excessive sodium consumption (>5 g sodium per day, e.g. one small teaspoon of salt per day) has been shown to be associated with an increased prevalence of hypertension with age (58). Accordingly, the 2020 International Society of Hypertension Global Hypertension Practice Guidelines (59), the 2018 European Society of Cardiology Hypertension Guidelines (60) and the WHO 2023 statement (61) recommend that sodium intake should be limited to <2 g per day (equivalent to <5 g salt per day) in the general population as well as in hypertensive patients. Beyond reducing the quantity of salt added as above reported, they recommended to limit the consumption of high salt containing foods, such as fast foods, soy sauce and processed foods, encouraging consumption of fresh vegetables and fruits. It is also worth of mention that, currently, there is no convincing evidence, from randomized controlled trials, that low salt intake (<2 g/day) is more effective than moderate intake (<5 g/day) in terms of reduction of cardiovascular risks and adverse outcomes (62). Thus, adding no more than 5 grams/d of iodized salt to foods provides 150 µg/die of iodine (that is the RDA) without increasing the risk of hypertension due to excess sodium intake (3, 63).

The recommended use of added iodized salt (no more than 5 gr/day) is useful to the thyroid function and safe with regard to the risk of hypertension and related CVD. Thus, it should not represent a limitation to promote iodine supplementation in the general population (63). We suggest iodized salt to ensure iodine sufficiency both in vegetarians/vegans and in subjects following a Mediterranean diet. In vegetarian/vegan subjects, iodine nutrition should be improved also by assuming pharmacological supplements with a titrated dose of 150 µg/day, and by using enriched foods (see

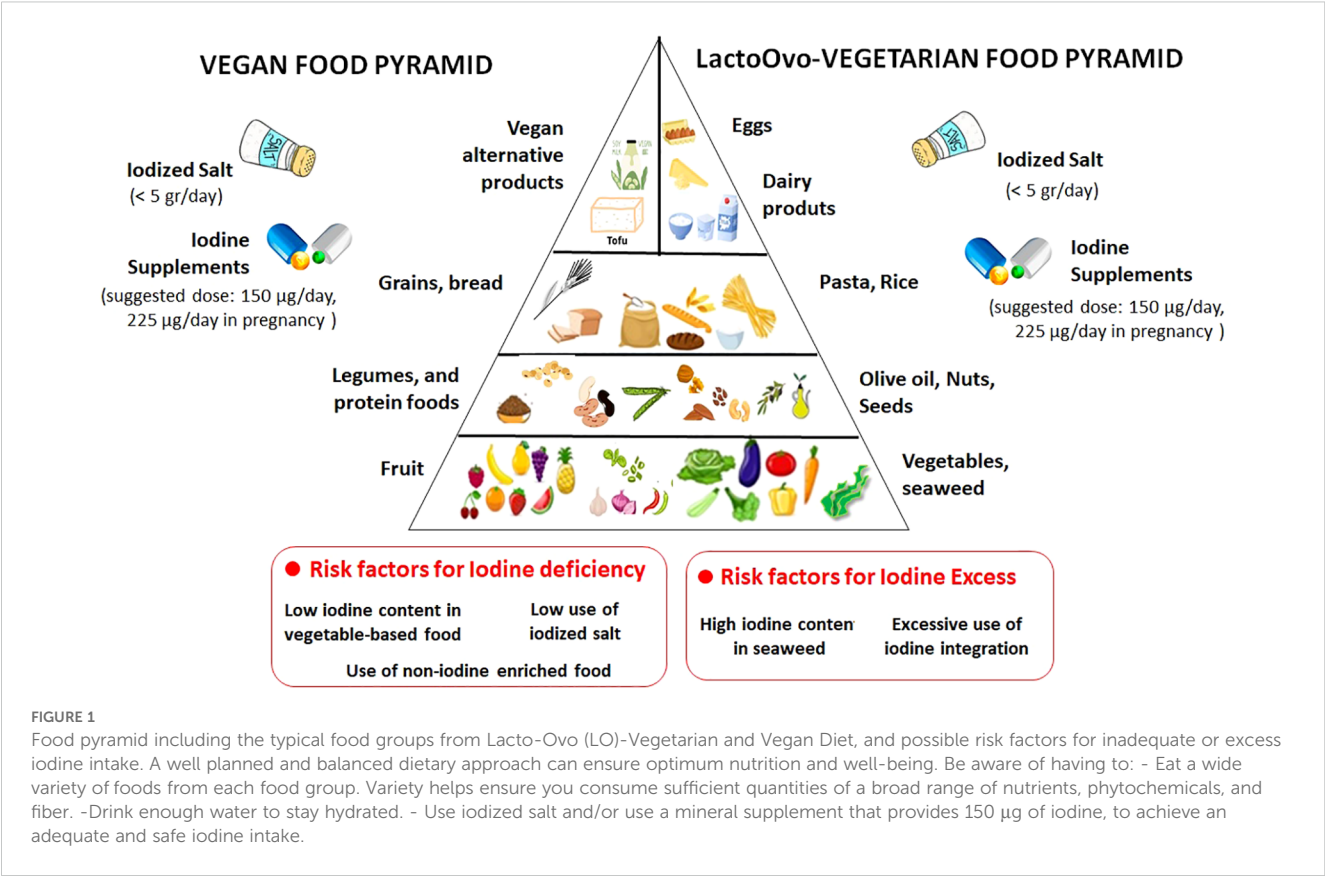


Table 2 for nutritional content of iodine in foods and vegan alternatives). Also iodine-exclusive supplements deriving from algae (mainly kelp) with a titrated dose (200-250 µg) of iodine are available, but the use of kelp products should be discouraged, according to the ATA recommendations (56). Also, they cautioned against taking iodine supplements with more than 150 µg in a daily dose, that represents RDA for adults.

Conclusions

In conclusion, a well-planned vegetarian diet can provide health benefits while aligning with ethical and environmental principles, provided that potential nutritional inadequacies should be taken into account. A healthy and nutritionally adequate vegetarian diet can be obtained including a wide variety of plant foods traditionally consumed in Mediterranean diets (cereals, pulses, vegetables, fruits, seeds, nuts, olive oil), and the necessary supplements (for instance, a reliable source of vitamin B12). Iodine nutrition is of concern since vegan diet are at risk of ID and guidelines on vegetarian/vegan diets often provide neutral advice on supplementing certain nutrients with plant sources without mentioning any potential risks. Iodized

salt represents an effective, safe and inexpensive tool to improve iodine nutrition and it should be recommended in subjects under vegetarian regimens of any grade and at any age, as well in subjects following a Mediterranean diet. To further implement iodine intake, vegans should use iodine-fortified plant-based milk alternatives, while consumption of sea vegetables should be discouraged, to prevent excess iodine intake. A daily supplement containing 150 µg of iodine could be suggested, mainly to childbearing women (Figure 1). Improved awareness of iodine sources to people under vegetarian/vegan diets is necessary to avoid both inadequate iodine intake in those who do not use iodized salt and/or supplements, and excess iodine intake in over-the-counter supplements and/or seaweed users. In any case, a nutritional counselling is essential to guarantee an adequate iodine intake while planning a vegetarian/vegan diet, particularly among the most susceptible population groups.

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Conflict of interest

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Effects of metabolic and organ function factors on the efficacy of radioactive iodine therapy for hyperthyroidism

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Introduction: Recent studies suggest that metabolic and organ function indicators could influence radioactive iodine treatment (RAIT) efficacy in hyperthyroid patients, but their relationships with thyroid function remain unclear.

Objective: This study aims to explore the impact of these markers on RAIT efficacy and thyroid function.

Method: A total of 135 hyperthyroid patients undergoing RAIT were enrolled, with biomarkers collected at baseline and 1, 3, and 6 months posttreatment. These included thyrotropin receptor antibody (TRAb), free triiodothyronine (FT3), free thyroxine (FT4), thyroid-stimulating hormone (TSH), urinary iodine concentration (UIC), serum iodine concentration (SIC), 25-hydroxyvitamin D, alanine aminotransferase, aspartate aminotransferase, and serum creatinine (Scr). We mainly focus on the outcomes of the impact of metabolic and organ function markers on RAIT efficacy and their correlation with changes in thyroid function.

Results: Logistic regression identified age as a predictor of RAIT efficacy (OR = 0.957, $p = 0.025$), with older patients less likely to achieve clinical improvement. Scr showed borderline significance ($p = 0.049$). Early after treatment, SIC was positively correlated with TSH ($r = 0.204$, $p = 0.018$), whereas UIC and SIC were significantly negatively correlated with TSH at 6 months during treatment ($p < 0.05$). In addition, higher Scr levels were consistently and significantly associated with lower FT3 and FT4 and higher TSH at multiple time points ($p < 0.05$).

Conclusion: Age was a relative factor influencing RAIT efficacy, while iodine nutritional status exhibited dynamic, time-dependent effects on TRAb and TSH. Elevated Scr promotes reductions in FT3 and FT4 and an increase in TSH.

KEYWORDS

hyperthyroidism, radioactive iodine treatment, iodine nutritional status, vitamin D, renal function

Introduction

Thyrotoxicosis is a clinical syndrome characterized by an excess of circulating thyroid hormones within the body (1). The most prevalent cause of this condition is hyperthyroidism, an endocrine disorder arising from the overproduction and secretion of thyroid hormones (1, 2). The global prevalence of hyperthyroidism is estimated to range from 0.2% to 1.3% (3), with approximately 70% of these cases attributed to Graves' hyperthyroidism (GH) (3). Currently, antithyroid drugs (ATDs), radioactive iodine treatment (RAIT), and thyroidectomy are the primary treatment options for hyperthyroidism, particularly in cases of GH (3). The initial treatment strategy for hyperthyroidism frequently involves the use of ATDs. However, the long-term effectiveness of ATDs is limited, with recurrence rates reaching up to 50%, and their use may be accompanied by adverse effects, including hepatotoxicity (4, 5). While thyroidectomy offers a potentially effective solution, its irreversible nature often results in permanent hypothyroidism or complications such as weight gain (3, 6). Conversely, RAIT has emerged as the preferred treatment modality for certain patients, particularly in North America, due to its noninvasive nature, sustained efficacy, and comparatively low recurrence rate (7, 8).

Radioactive iodine (RAI) is transported to thyroid tissue via the sodium/iodide symporter (9). Once accumulated, it emits beta radiation that generates free radicals and induces deoxyribonucleic acid damage, inducing cytotoxicity that destroys thyroid tissue and reduces hormone synthesis (10). For patients with GH who have a poor response to ATDs or experience relapse, RAIT has shown significant advantages (11). However, despite the long-term efficacy achieved in most patients, a minority may still develop adverse effects, such as hypothyroidism (12, 13). Although RAIT primarily targets thyroid tissue, other cells can also accumulate RAI. For example, salivary gland uptake may result in glandular injury (14, 15), and RAI can also activate thyroid-specific autoimmune responses, evidenced by changes in thyrotropin receptor antibody (TRAb) and antithyroglobulin antibody levels and transient elevations of inflammatory cytokines, potentially leading to new-onset or worsened thyroid-associated ophthalmopathy (16). Moreover, the potential carcinogenic risk of RAIT cannot be completely excluded (16, 17). Therefore, close posttreatment monitoring and long-term follow-up are essential to ensure its safety and efficacy.

A growing body of research indicates that the effectiveness of RAIT may be modulated by multiple interacting factors, including RAI dose, thyroid volume, age, and sex (16). Furthermore, iodine nutritional status emerges as a pivotal determinant influencing RAIT outcome. To optimize thyroid uptake of RAI, patients are frequently recommended to follow a low-iodine diet (LID) before treatment (12, 18, 19). However, recent evidence suggests that there is no significant association between short-term LID and the effects of RAIT (20, 21). Although lower urinary iodine concentration (UIC) can increase RAI uptake, it does not significantly improve the treatment's success rate, thereby questioning the necessity of LID (21). Compared to UIC, serum iodine concentration (SIC) is a more

immediate indicator of thyroid iodine utilization and metabolic dynamics (22, 23). Studies have shown that both excessively low and high SIC may lead to thyroid dysfunction, thereby impacting the effectiveness of RAIT (23).

The immunomodulating effects of vitamin D (vit D) have been shown to improve treatment outcomes in patients with GH (24–26). Studies have indicated vit D deficiency is associated with a high prevalence of thyroid autoimmune disease, especially during RAIT. Supplementation with vit D has a potential positive impact on reducing hyperthyroidism recurrence (24), but further studies are necessary to confirm the specific role in RAIT (13).

The liver is integral to the metabolism and conversion of thyroid hormones, and impaired liver function may affect the restoration and regulation of thyroid hormone homeostasis (27). Likewise, the kidneys serve as the principal organs for the excretion of RAI (28). Renal dysfunction may prolong the retention time of RAI within the body, thereby consequently affecting the therapeutic efficacy (28). Since many patients may have pre-existing liver and kidney damage caused by prior medication treatments before undergoing RAIT, the functional status of these organs may indirectly impact the efficacy and safety of RAIT by altering the processes of RAI uptake, distribution, and clearance (27, 28).

While previous studies have explored the relationship between RAIT and metabolic as well as organ functional status, there remains a paucity of research directly examining how these factors influence the efficacy of RAIT. This study aims to systematically evaluate the relationship between iodine nutritional status, vit D levels, liver function, kidney function, and the effectiveness of RAIT in the treatment of hyperthyroidism. It seeks to analyze the potential factors that may affect the therapeutic outcomes of RAIT.

Materials and methods

Patients selection

This study was approved by the Medical Ethics Committee of the Second Hospital of Jilin University (Approval No.: 2023-231). All participants provided informed consent prior to their involvement in the study. We collected data from patients who received RAIT at the Department of Nuclear Medicine of the Second Hospital of Jilin University between 12 November 2023 and 1 May 2024. Eligible patients meeting the following criteria were included: (1) diagnosed with hyperthyroidism; (2) suitable for and receiving RAIT for the first time; and (3) followed up at 1, 3, or 6 months after treatment. Exclusion criteria were as follows: (1) a history of neoplasm; (2) patients with comorbid nonhyperthyroid thyroid disorders, psychiatric disorders, or pregnancy/lactation status (3) structural damage to vital organs such as the liver and kidney, or hepatic and renal dysfunction; and (4) incomplete follow-up information. All patients included in the study provided written informed consent for RAIT and the associated procedures.

Data collection

We collected biomarker data from patients at baseline (before RAIT) and at 1, 3, and 6 months after treatment. The biomarkers included thyroid function (TRAb, free triiodothyronine [FT3], free thyroxine [FT4], and thyroid-stimulating hormone [TSH]), as well as UIC, SIC, 25-hydroxyvitamin D (25[OH]D), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum creatinine (Scr). Thyroid function (TRAb, FT3, FT4, and TSH) was measured by electrochemiluminescence immunoassay. The detection ranges for these markers were as follows: TRAb, up to 40 IU/L; FT3, up to 50 pmol/L; FT4, up to 100 pmol/L; and TSH, from a minimum of 0.005 mIU/L to a maximum of 100 mIU/L. UIC and SIC were determined using the DAT-20SG and DAT-500 systems (Silky-Road Medical Technology, Changsha, China), respectively. Both systems employ arsenic-cerium catalytic spectrophotometry; additionally, the DAT-500 protocol incorporates a cold-digestion step based on international guidelines for serum iodine analysis, achieving an analytical accuracy error of $\leq 5\%$, an intra-assay coefficient of variation (CV) $\leq 2\%$, and an interassay CV $\leq 6\%$. 25 (OH) D was analyzed by chemiluminescence. ALT and AST were determined using the IFCC method without the addition of P5P, while Scr was assessed via the creatinine oxidase assay. Detailed information on data analysis methods and analytical instruments is provided in [Supplementary Table S1](#).

Statistical analysis

Normality tests were conducted on the collected data to assess whether they followed a normal distribution. Based on the results of the normality tests, we used either paired sample t-tests or Wilcoxon signed-rank tests, along with one-way analysis of variance (ANOVA) or Friedman tests, to evaluate significant

changes in thyroid function at different time points before and after RAI treatment. ANOVA or Kruskal–Wallis tests were used to assess the differences in biomarkers across groups with varying treatment outcomes. For biomarkers with significant differences, logistic regression analysis was applied to identify outcome-related predictors. To analyze the potential relationships between biomarkers and thyroid function at various time points, we used Pearson or Spearman bivariate correlation analyses, depending on data distribution. A linear mixed model (LMM) was used to further analyze the effects of time and biomarkers on thyroid function and its rate of change. The model incorporated fixed effects, and parameter estimation was performed using maximum likelihood estimation, with individual patients treated as random effects to control for inter-individual variability. Residual analysis was conducted to verify model assumptions. All statistical analyses were performed using SPSS 29.0 (IBM Corp, Armonk, NY, USA), with a two-sided $p < 0.05$ considered statistically significant.

Results

Patient clinical characteristics

As shown in [Figure 1](#), of the 256 patients with hyperthyroidism who underwent RAIT, 135 met our inclusion criteria by completing the required follow-up and were thus included in the study. The characteristics of the included patient cohort are summarized in [Table 1](#). Overall, the median age was 45 years (range: 21–72), and 61.48% (83 of 135) were women. Prior to RAIT, 77.04% (104 of 135) of patients had received drug therapy, with 38.46% (40 of 104) experiencing relapse after treatment. Palpitations (82.22%) and tremors (60.00%) were the most common symptoms observed before RAIT. Additionally, 65.19% (88 of 135) of the patients had

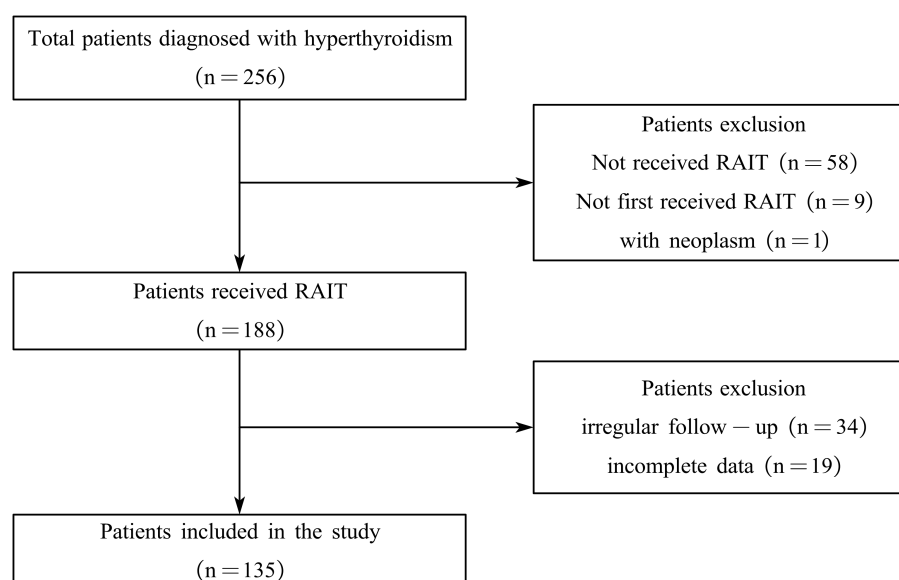


FIGURE 1
Flowchart of the enrollment and exclusion of study participants. RAIT, radioactive iodine treatment.

TABLE 1 Patient baseline characteristics.

Characteristic	Total (N = 135)
Median age (range; years)	45 (21–72)
Sex (n; %)	
Male	52 (38.52)
Female	83 (61.48)
Smoker (n; %)	88 (65.19)
Median disease duration (range; years)	3 (0–30)
Prior therapy (n; %)	
Untreated	31 (22.96)
Drug therapy	104 (77.04)
ATD/ATD and LT4	99/5 (95.19/4.81)
Surgery	1 (0.74)
Relapse after treatment (n; % of drug therapy)	40 (38.46)
Symptomatic (n; %)	
Palpitations	111 (82.22)
Tremor	81 (60.00)
Exophthalmos	65 (48.15)
Iodine-rich food consumption (n; %)	33 (24.44)
Thyroid median weight (range; g)	
Left lobe weight	19 (4–41.9)
Right lobe weight	19 (4.8–47.2)
Total weight	37.9 (10.2–88.9)
Median RAIU (%; range)	54.3 (22.3–99.0)

ATD, antithyroid drug; LT4, levothyroxine; RAIU, radioactive iodine uptake.

a history of smoking, and 24.44% (33 of 135) had a history of consuming iodine-rich foods. The median total thyroid weight was 37.9 g (range: 10.2–88.9), with median weights of the left and right lobes being 19 g (range: 4.4–41.9) and 19 g (range: 4.8–47.2), respectively. The median radioactive iodine uptake (RAIU) was 54.3% (range 22.3%–99.0%).

To systematically assess the dynamic effects of RAIT on biomarkers in patients, we collected and analyzed biomarkers at baseline and at 1, 3, and 6 months posttreatment (Table 2). Due to the lower detection limit of the laboratory assay, baseline TSH levels for most patients were recorded as 0.005 mIU/L (0.005, 0.005). TRAb levels increased from a baseline of 17.8 IU/L (8.7, 36.0) to 40.0 IU/L (25.8, 40.0) at 6 months. TSH also rose significantly to 5.160 mIU/L (0.005, 37.600) at 6 months. FT3 and FT4 levels exhibited a progressive decline (FT3: baseline, 27.4 pmol/L [15.1, 42.1], 6 months posttreatment, 5.1 pmol/L [3.0, 8.2]; FT4: baseline, 75.8 pmol/L [42.5, 100.0], 6 months posttreatment, 16.8 pmol/L [7.0, 25.2]). Iodine nutritional status showed an initial increase followed by a decrease (e.g., SIC: baseline, 183.1 µg/L [143.7, 241.0]; 1 month posttreatment, 200.9 µg/L [149.5, 253.4]; 6 months

posttreatment, 126.7 µg/L [98.4, 178.3]). In contrast, the interquartile ranges of 25(OH)D, ALT, AST, and Scr exhibited relatively minor changes across all time points before and after treatment.

Normality test results indicated that most biomarkers did not follow a normal distribution at various time points (Supplementary Table S2). Based on this result, nonparametric tests were applied. The results of the Wilcoxon signed-rank test and the Friedman test showed significant changes in TRAb, FT3, FT4, TSH, UIC, SIC, 25(OH)D, and Scr across different time points before and after treatment ($p < 0.05$), whereas no significant changes were observed for ALT and AST between time points (Supplementary Tables S3, S4).

Analysis of factors associated with the efficacy of RAIT

Efficacy was categorized according to the thyroid function and clinical manifestations of the patients after 6 months of treatment. Clinical cure was defined as the complete resolution of hyperthyroid symptoms and signs, with FT3, FT4, and TSH levels returning to normal, or the appearance of hypothyroid features (FT3 or FT4 below normal and TSH above normal). Improvement was defined as a reduction in hyperthyroid symptoms and partial resolution of signs, with significant reductions in FT3 and FT4, though not within normal limits. Ineffective was classified as the lack of improvement or worsening of hyperthyroid symptoms and signs following treatment. A significant change in thyroid function was defined as a posttreatment change of 20% or more relative to pretreatment levels over the 6-month period. Among the patients, 56 achieved clinical cure, 69 showed improvement, and 10 were ineffective (Supplementary Table S5). Patients in the ineffective group had lower FT3 and FT4 levels before treatment compared to the clinical cure and improvement groups, while their TRAb levels were higher than those in the other two groups.

Patient characteristics and baseline biomarkers between groups were included in nonparametric tests. Using the Kruskal–Wallis test to evaluate the significance of age and biomarkers among different groups, we identified significant differences in age, baseline FT3, and baseline Scr across efficacy (Figure 2; Supplementary Table S5; age, $p = 0.044$; FT3, $p = 0.022$; Scr, $p = 0.047$). These factors were subsequently incorporated into a multivariate logistic regression model to analyze their influence on efficacy in hyperthyroid patients at 6 months after RAIT. The results indicated that age was an independent predictor for clinical cure and improvement (odds ratio (OR) = 0.957, 95% confidence interval (CI) = 0.921–0.994, $p = 0.025$), suggesting a significant negative correlation between age and efficacy (Table 3). Specifically, older patients were less likely to achieve a clinical cure. Meanwhile, we observed that pretreatment Scr demonstrated statistical significance between clinical cure and improvement ($p = 0.049$), and this significance was borderline (Table 3). Furthermore, with an OR of 0.970 and the upper limit of the 95% CI approaching 1.0, these findings suggest that while Scr maintains a correlation with treatment outcome, its independent

TABLE 2 Baseline and posttreatment biomarker characteristics by follow-up period.

Category	Biomarker	Baseline (median [Q1, Q3])	1 Month posttreatment (median [Q1, Q3])	3 Months posttreatment (median [Q1, Q3])	6 Months posttreatment (median [Q1, Q3])
Thyroid function	TRAb (IU/L) ^a	17.8 [8.7, 36.0]	21.5 [12.8, 34.6]	36.3 [26.9, 40.0]	40.0 [25.8, 40.0]
	FT3 (pmol/L) ^b	27.4 [15.1, 42.1]	8.2 [5.5, 20.3]	5.0 [2.6, 8.5]	5.1 [3.0, 8.2]
	FT4 (pmol/L) ^c	75.8 [42.5, 100.0]	26.9 [18.6, 48.5]	15.8 [7.2, 24.6]	16.8 [7.0, 25.2]
	TSH (mIU/L) ^d	0.005 [0.005, 0.005]	0.005 [0.005, 0.005]	4.270 [0.005, 27.100]	5.160 [0.005, 37.600]
Iodine nutrition	UIC (μg/L)	103.8 [66.4, 187.6]	221.6 [142.6, 322.0]	152.4 [94.7, 194.6]	114.0 [76.4, 165.8]
	SIC (μg/L)	183.1 [143.7, 241.0]	200.9 [149.5, 253.4]	169.1 [132.2, 225.0]	126.7 [98.4, 178.3]
Vitamin D	25(OH)D (nmol/L)	42.30 [28.9, 57.8]	52.0 [36.7, 61.4]	47.2 [36.4, 56.0]	49.2 [42.6, 56.4]
Liver function	ALT (U/L)	26.0 [21.0, 42.0]	26.0 [19.0, 36.0]	24.0 [17.0, 31.0]	25.0 [18.0, 34.0]
	AST (U/L)	25.0 [19.0, 35.0]	25.0 [19.0, 32.0]	25.0 [18.0, 33.0]	25.0 [19.0, 34.0]
Kidney function	Scr (μmol/L)	44.0 [37.0, 58.0]	49.6 [40.0, 60.0]	56.0 [45.0, 67.0]	56.0 [47.0, 69.3]

Median [Q1, Q3]: values are presented as the median with the first quartile (Q1) and third quartile (Q3) in brackets.
TRAb, thyrotropin receptor antibody; *FT3*, free triiodothyronine; *FT4*, free thyroxine; *TSH*, thyroid-stimulating hormone; *UIC*, urinary iodine concentrations; *SIC*, serum iodine concentrations; *25(OH)D*, 25-hydroxyvitamin D; *ALT*, alanine aminotransferase; *AST*, aspartate aminotransferase; *Scr*, serum creatinine.
^aTRAb has an upper detection limit of 40 IU/L in the laboratory assay; values exceeding this threshold are reported as 40 IU/L.
^bFT3 has an upper detection limit of 50 pmol/L in the laboratory assay; values exceeding this threshold are reported as 50 pmol/L.
^cFT4 has an upper detection limit of 100 pmol/L in the laboratory assay; values exceeding this threshold are reported as 100 pmol/L.
^dTSH has a lower detection limit of 0.005 mIU/L in the laboratory assay; values below this threshold are reported as 0.005 mIU/L.

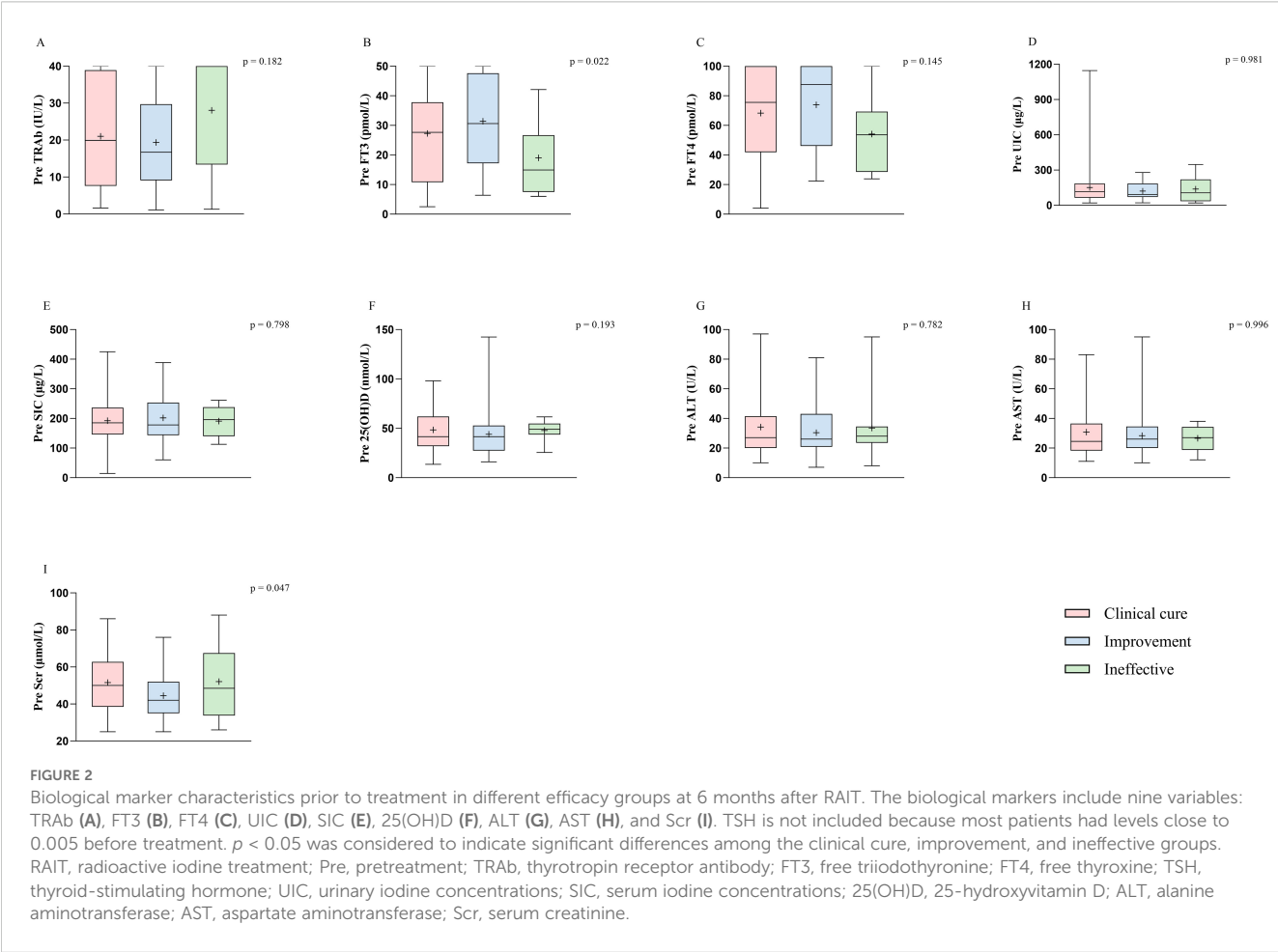


TABLE 3 Multivariate logistic regression analysis of influencing factors at 6 months after RAIT in patients with hyperthyroidism.

Influencing factors	Improvement vs. clinical cure		Ineffective vs. clinical cure	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age	0.957 (0.921, 0.994)	0.025	0.954 (0.888, 1.025)	0.198
Pre FT3	0.999 (0.971, 1.028)	0.953	0.947 (0.892, 1.004)	0.070
Pre Scr	0.970 (0.941, 1.000)	0.049	0.980 (0.930, 1.032)	0.446

RAIT, radioactive iodine treatment; 95% CI, 95% confidence interval; Pre, pretreatment; FT3, free triiodothyronine; Scr, serum creatinine. Bold values indicate statistical significance ($p < 0.05$).

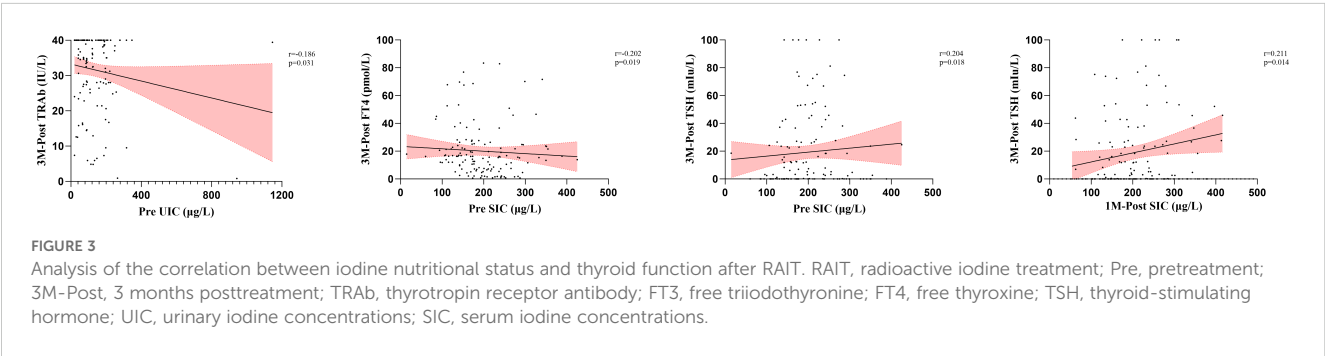
influence might be relatively weak. Pretreatment FT3 did not demonstrate a significant independent predictive role between clinical cure and improvement. Additionally, no significant independent predictors, including sex, age, and pretreatment FT3 and Scr, were identified between the clinical cure and ineffective treatment groups.

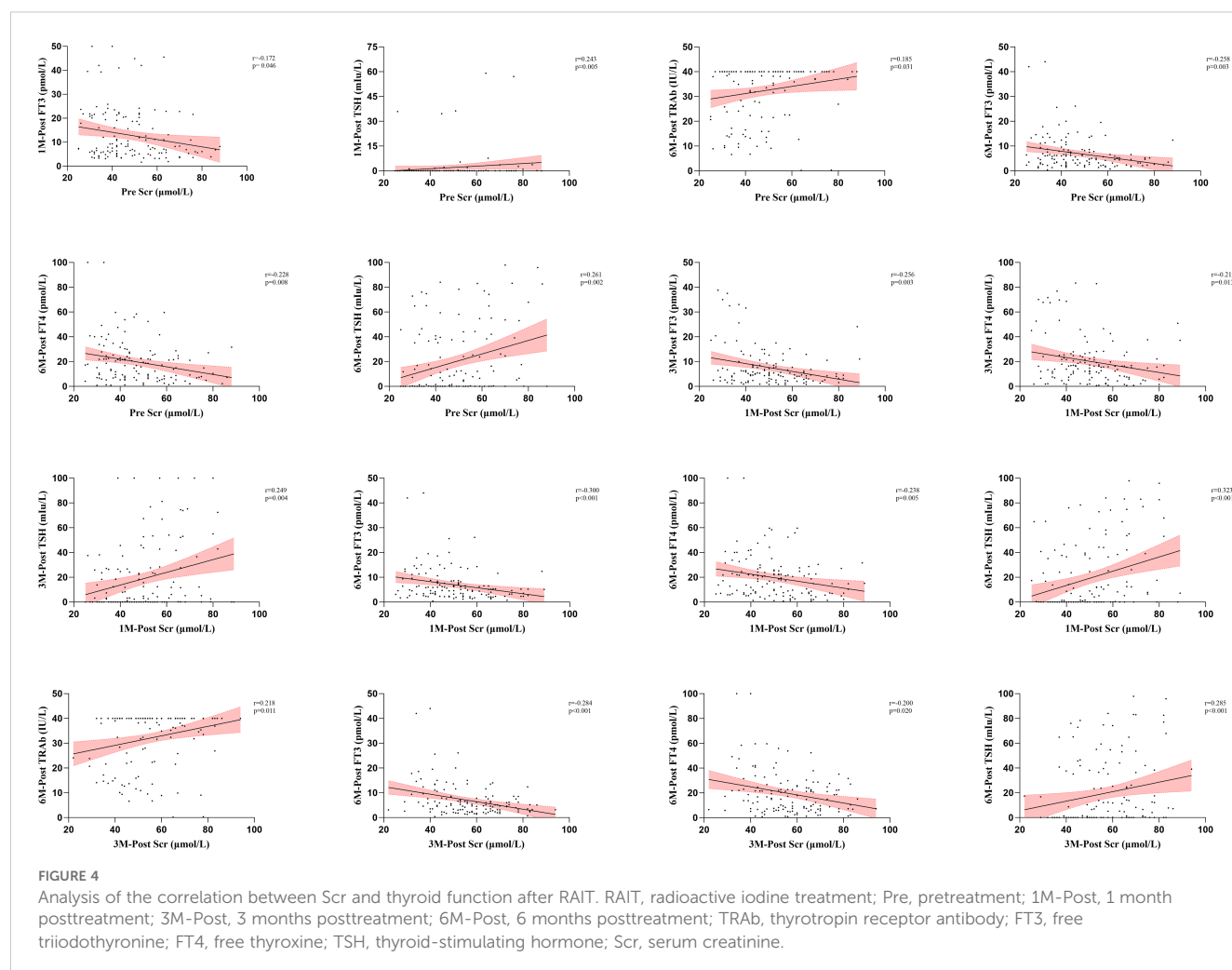
Correlation between metabolic and organ function markers and thyroid function

Spearman correlation analysis was used to evaluate the potential impacts of metabolic and organ function markers on thyroid function at different time points. In this analysis, we identified 28 significant correlations between metabolic and organ function indicators before and after treatment and thyroid function after treatment (Figures 3, 4, Supplementary Table S6). Specifically, there were 1, 3, 3, 5, and 16 correlations between UIC, SIC, ALT, AST, and Scr and thyroid functional status, respectively. The correlation between UIC and thyroid function was relatively weak. Pretreatment UIC exhibited a negative correlation with TRAb at 3 months posttreatment (Figure 3, $r = -0.186$, $p = 0.031$). In contrast, SIC demonstrated more complex associations. Pretreatment SIC showed a negative correlation with FT3 ($r = -0.202$, $p = 0.019$) and a positive correlation with TSH ($r = 0.204$, $p = 0.018$) at 3 months posttreatment (Figure 3). Additionally, at 1 month posttreatment, SIC remained positively correlated with TSH at 3 months posttreatment ($r = 0.211$, $p = 0.014$), suggesting a relatively strong

influence of SIC on TSH at 3 months posttreatment. The relationship between Scr and thyroid function was the most pronounced and exhibited consistent trends across multiple time points. Pretreatment Scr was strongly negatively correlated with FT3 ($r = -0.258$, $p = 0.003$) and FT4 ($r = -0.228$, $p = 0.008$) at 6 months posttreatment, whereas it was positively correlated with TSH ($r = 0.261$, $p = 0.002$). This trend persisted after treatment, particularly at 3 months posttreatment, where Scr remained negatively correlated with FT3 ($r = -0.284$, $p < 0.001$) and FT4 ($r = -0.200$, $p = 0.020$) and positively correlated with TSH ($r = 0.285$, $p < 0.001$). These correlations were consistent across several time points (Figure 4).

During the treatment process, no significant correlation was found between 25(OH)D and posttreatment thyroid function (Supplementary Table S6). However, ALT and AST exhibited some associations with thyroid function, mainly in the pre-treatment period and the early stage of treatment. For instance, pretreatment ALT showed a positive correlation with FT3 ($r = 0.174$, $p = 0.043$) and FT4 ($r = 0.195$, $p = 0.024$) at 1 month posttreatment (Supplementary Table S6). Additionally, pretreatment AST was positively correlated with TRAb at 3 months posttreatment (Supplementary Table S6, $r = 0.209$, $p = 0.015$). Notably, these associations did not persist at later time points. Results from nonparametric tests indicated that ALT and AST did not exhibit significant differences throughout the treatment process (Supplementary Tables S3, S4). While these indicators showed correlation with thyroid function at specific time points, they may not constitute the primary influencing factors for alterations in thyroid function.





Longitudinal impact of metabolic and organ function markers on treatment

In the LMM analysis, incorporating time as a repeated measure and individual patients as random effects, we observed that multiple metabolic and organ function markers were significantly associated with changes in thyroid function before and after treatment (Table 4). The analysis results showed that both UIC and SIC were significantly negatively correlated with TRAb and TSH. Specifically, the negative correlations between SIC and TRAb ($\beta = -0.041$, $p < 0.001$) as well as TSH ($\beta = -0.059$, $p < 0.001$) were stronger than those between UIC and TRAb ($\beta = -0.010$, $p = 0.002$) and TSH ($\beta = -0.020$, $p = 0.002$), and these negative associations were more pronounced. Additionally, SIC showed significant positive correlations with FT3 and FT4 (FT3: $\beta = 0.017$, $p = 0.022$; FT4: $\beta = 0.060$, $p = 0.003$), but no similar association was observed between UIC and FT3. These findings suggest that iodine nutritional status has a significant regulatory effect on thyroid function, particularly with regard to TRAb and TSH, following RAIT, and this effect may change with the progression of treatment. Through repeated measures analysis, Scr was found to be significantly negatively correlated with both FT3 ($\beta = -0.460$,

$p < 0.001$) and FT4 ($\beta = -1.029$, $p < 0.001$), while it was significantly positively correlated with TRAb ($\beta = 0.102$, $p = 0.018$) and TSH ($\beta = 0.651$, $p < 0.001$). This further indicated that renal function exerts a significant and persistent influence on thyroid function changes before and after RAIT.

Throughout the treatment course, no significant correlation was observed between 25(OH)D and thyroid function alterations. Although nonparametric tests revealed no significant intertemporal differences in ALT and AST, LMM analysis demonstrated potential associations between these parameters following RAIT. Notably, in the temporal dimension of multiple measurements, ALT and AST exhibited certain correlations with thyroid function fluctuations across different time points, suggesting possible time-dependent effects of thyroid function on ALT and AST.

During the residual analysis, we found that the residuals did not obey a normal distribution (Supplementary Table S7). This result may be related to the distribution characteristics of the data, potentially reflecting sample heterogeneity or the influence of some unaccounted variables. Nevertheless, considering that the LMM, after controlling for individual differences and time factors, still demonstrated significant associations between metabolic and

TABLE 4 Effects of iodine nutritional status, vitamin D, liver function, and renal function-related biomarkers on thyroid function assessed using LMM analysis.

Variables	Mean ± SE	UIC	SIC	25(OH)D	ALT	AST	Scr
TRAb	Pre = 20.68 ± 1.17	$\beta = -0.010$	$\beta = -0.041$	$\beta = 0.008$	$\beta = 0.014$	$\beta = 0.109$	$\beta = 0.102$
	1M = 22.80 ± 1.12	$p = 0.002$	$p < 0.001$	$p = 0.454$	$p = 0.688$	$p < 0.001$	$p = 0.018$
	3M = 31.62 ± 0.93						
	6M = 32.34 ± 0.97						
FT3	Pre = 28.75 ± 1.30	$\beta = -0.008$	$\beta = 0.017$	$\beta = -0.010$	$\beta = 0.126$	$\beta = 0.024$	$\beta = -0.460$
	1M = 12.91 ± 0.93	$p = 0.050$	$p = 0.022$	$p = 0.509$	$p = 0.002$	$p = 0.497$	$p < 0.001$
	3M = 7.49 ± 0.66						
	6M = 6.92 ± 0.57						
FT4	Pre = 70.09 ± 2.56	$\beta = -0.007$	$\beta = 0.060$	$\beta = -0.025$	$\beta = 0.298$	$\beta = 0.045$	$\beta = -1.029$
	1M = 36.37 ± 2.21	$p = 0.442$	$p = 0.003$	$p = 0.442$	$p < 0.001$	$p = 0.567$	$p < 0.001$
	3M = 20.02 ± 1.60						
	6M = 19.49 ± 1.47						
TSH	Pre = 0.04 ± 0.19	$\beta = -0.020$	$\beta = -0.059$	$\beta = 0.000$	$\beta = 0.032$	$\beta = 0.232$	$\beta = 0.651$
	1M = 1.89 ± 0.75	$p = 0.002$	$p < 0.001$	$p = 0.986$	$p = 0.606$	$p < 0.001$	$p < 0.001$
	3M = 19.21 ± 2.36						
	6M = 19.60 ± 2.32						

LMM, linear mixed model; SE, standard error; Pre, pretreatment; 1M, 1 Month posttreatment; 3M, 3 months posttreatment; 6M, 6 months posttreatment; TRAb, thyrotropin receptor antibody; FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid-stimulating hormone; UIC, urinary iodine concentrations; SIC, serum iodine concentrations; 25(OH)D, 25-hydroxyvitamin D; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Scr, serum creatinine. Bold values indicate statistical significance ($p < 0.05$).

organ function markers and thyroid function, we maintain that the analysis results are robust and reliable.

Discussion

In recent years, RAIT has demonstrated significant advantages in treating hyperthyroidism and has increasingly been considered the preferred treatment option for patients (7, 8). Emerging findings underscore the complexity of factors that can impact RAIT efficacy and highlight the need for personalized treatment approaches to optimize outcomes for patients with hyperthyroidism. Thus far, factors affecting RAIT efficacy still remain a focal point of current clinical research.

In this study, 135 patients undergoing RAIT were enrolled. Data on TRAb, FT3, FT4, TSH, UIC, SIC, 25(OH)D, ALT, AST, and Scr were collected and analyzed at baseline and at 1, 3, and 6 months posttreatment, identifying four key findings. First, approximately 41.49% of patients achieved clinical cure following RAIT, and approximately 51.11% of patients showed improvement. Age was identified as a relative influencing factor for the efficacy of RAIT (Table 3). Second, the study revealed that iodine nutritional status significantly affected thyroid function changes after RAIT, and the extent of the effect varied over time (Table 4, Supplementary Table S6). In the early posttreatment period, higher iodine nutrition levels were more conducive to the recovery of thyroid function, while in the later stages, lower iodine levels were more beneficial for

treatment efficacy. Third, within the normal range, relatively higher Scr levels were associated with a greater decrease in FT3 and FT4, as well as an increase in TSH (Table 4, Supplementary Table S6). Finally, data analysis revealed that changes in ALT and AST before and after RAIT were not significant (Supplementary Tables S3, S4), and 25(OH)D had little impact on the efficacy following RAIT (Tables 3, 4, Supplementary Table S6).

In recent years, the advent of RAIT has markedly transformed the therapeutic approach to hyperthyroidism. Nonetheless, the influence of patient age on the efficacy of RAIT remains a subject of debate within the academic community. Certain studies propose that age does not significantly affect the efficacy of RAIT in individuals with GH, suggesting that younger patients generally exhibit lower success rates, a pattern that endures even after controlling for confounding variables (29–31). In contrast, other studies show a negative correlation between age and cure rates, with younger patients more likely to develop hypothyroidism, suggesting higher sensitivity to RAIT (32–34). Bonnema et al. proposed that these discrepancies may be attributed to differences in smoking habits, disease severity, medication use, and thyroid enlargement in younger patients (16). The results presented in this study suggest that age may serve as an independent predictor for clinical cure and improvement ($OR = 0.957$, $p = 0.025$). This also supports the finding that younger patients are more sensitive to RAI and are more likely to benefit from the treatment. However, in the comparative analysis of clinical cure and ineffectiveness, the independent predictive role of age did not reach statistical

significance (OR = 0.954, $p = 0.198$). Therefore, although the conclusions from existing studies are inconsistent, our data suggest that age may serve as a relative predictor of treatment efficacy rather than an independent predictor. Additionally, our study also supports that younger patients are more likely to benefit from RAIT.

UIC is the predominant metric employed for evaluating the iodine nutritional status of populations; however, it is subject to variation due to several factors, including dietary iodine intake and fluid consumption (35, 36). In contrast, SIC is less affected by dietary factors and remains relatively stable (37). This aligns with the findings of our study, where SIC showed a more significant association with thyroid function compared to UIC (Supplementary Table S6) (23, 38). Although a LID is typically recommended prior to RAIT, recent studies on iodine nutrition status and RAIT efficacy indicate that a LID does not provide additional benefits for RAIT (20, 21). In our analysis, we observed a more complex relationship between iodine nutrition status and RAIT efficacy.

In the LMM with repeated measurements over 6 months after RAIT, both UIC and SIC showed significant negative correlations with TRAb and TSH. Notably, despite the expected therapeutic effects of RAIT to decrease TRAb and increase TSH, some studies have reported that TRAb may actually rise within 6 months of RAIT, which is associated with thyroid cell damage, antigen release, and autoimmune reactions (39–41). Based on this finding, we hypothesize that the dynamic regulation of iodine nutrition status may have a bidirectional impact on RAIT efficacy. Higher iodine nutritional status may help reduce TRAb; however, it could also lead to a further increase in thyroid hormone levels, thereby exacerbating the feedback inhibition on TSH. Lindgren et al. and Fang et al. also confirmed this viewpoint (39, 42).

Furthermore, the role of time factors in the relationship between iodine nutritional status and thyroid function parameters following RAIT deserves further exploration. The results indicate that a higher iodine nutritional status prior to treatment may contribute to a decrease in TRAb and an increase in TSH during the early phase posttreatment. In the LMM analysis, however, a lower iodine nutritional status throughout the follow-up period appears to be more favorable for the stabilization of thyroid function. This finding suggests that iodine nutritional status not only exhibits a bidirectional regulatory effect on thyroid function after RAIT but also has time-dependent characteristics. An elevated SIC at the onset of treatment may improve therapeutic outcomes by suppressing TRAb and increasing TSH levels. However, as treatment advances, the persistent feedback inhibition of TSH influenced by iodine nutritional status may become more significant. This also helps to explain the observed negative correlation between TSH and iodine nutritional status. Since our study is limited to cross-sectional data, further longitudinal studies are required to validate these findings.

In our analysis, we found that patients with relatively higher levels of Scr were more likely to benefit from RAIT. Published studies have also highlighted the importance of renal function in the metabolism of RAI and its retention time in the body (28, 43). Our analysis provides some evidence for a potential association between renal function and RAIT efficacy. Impaired renal function can slow

the excretion of RAI, thereby prolonging its retention time in the body, which may enhance the localized cytotoxic effects of β radiation in thyroid tissue (43). However, it is important to note that patients with higher Scr levels in this cohort may also be influenced by other metabolic factors, and the additional risks associated with radiation exposure should not be overlooked. Therefore, further prospective clinical trials are necessary to better define the impact of Scr on RAIT efficacy and to explore the potential underlying mechanisms. Interestingly, we observed that a relatively higher Scr level within the normal physiological range may be associated with more favorable recovery of thyroid hormone levels posttreatment. This raised a hypothesis that dietary interventions, such as a moderate increase in protein intake, might influence treatment outcomes by modulating Scr levels. However, it remains essential to ensure that Scr levels are maintained within the normal range to avoid renal burden or other adverse effects. This potential mechanism is still speculative and warrants clarification through further clinical studies.

In this analysis, 25(OH)D was found to have no impact on the efficacy of RAIT or thyroid function. This finding contrasts with several studies that propose a potential role for vit D in modulating thyroid function following RAIT. Płazińska et al. found that vit D deficiency, particularly in smokers, affects the recovery of thyroid function in GH patients undergoing RAIT, especially leading to an increase in TRAb (24). In CD4⁺ T-cell responses, vit D directly inhibits the production of Th1 cytokines (IL-2 and IFN- γ) and increases the production of Th2 cytokines (IL-4). The increase in Th2 cytokines helps reduce TRAb and alleviates the autoimmune response in patients with GH (24, 44, 45). In contrast, our study did not differentiate between smokers and nonsmokers, which may partly explain the lack of such an association. Furthermore, our study had a relatively short follow-up period after RAIT, and the potential immunomodulatory effects of vit D during long-term follow-up were not fully observed. The effects of 25(OH)D in RAIT still require further investigation.

Although the study found a partially significant correlation between liver function indicators (ALT and AST) and changes in thyroid function after RAIT, the changes in ALT and AST before and after RAIT were not significant. Therefore, even though liver function may play a role in other metabolic pathways, its specific impact on RAIT outcomes was not supported by the findings of this study (27).

In this study, we found no significant associations between thyroid weight, RAIU, smoking status, or iodine-rich food consumption and the efficacy of RAIT (Supplementary Table S5). These results are consistent with the study of Nishio et al., further underscoring the complexity and potential limitations of these variables in predicting RAIT efficacy (20).

Smoking has been repeatedly linked to higher relapse rates in GH, an increased risk of Graves ophthalmopathy (GO) following RAIT, and poorer GO treatment responses (46). Płazińska et al. also demonstrated that smokers had higher titers of thyroid-stimulating hormone receptor antibodies with or without concomitant vit D supplementation, suggesting that smoking may exacerbate the immunopathology and clinical course of GH (24). Nevertheless, we observed no significant relationship between smoking and RAIT efficacy in our cohort. This discrepancy may be attributed to

immune-mediated pathways or clinical characteristics through which smoking influences treatment response, but which were not fully measured in our study. In addition, heterogeneity in treatment regimens, patient demographics, or other unaccounted confounding factors may also have contributed to the lack of observed association.

Although dietary iodine intake is recognized as a modulator of thyroid function, we did not detect a significant impact of an iodine-rich diet on RAIT outcomes, in agreement with the findings of Nishio et al. (20). Interestingly, however, our data revealed that iodine nutritional status correlated differently with treatment efficacy at early versus late stages after RAIT. Whereas previous studies have treated iodine intake as a static variable that is considered to have a minimal impact on RAIT success, our observations suggest that the timing and dynamic adjustment of iodine consumption throughout the treatment course may be critical. We therefore hypothesize that modulating iodine intake in accordance with the RAIT phase could optimize therapeutic response. It should be noted that our analyses were confined to biomarker-level data. The utility of dynamically tailored dietary iodine interventions to enhance RAIT efficacy remains to be validated in prospective clinical trials.

This study has several inherent limitations. First, the follow-up period was confined to 6 months after the initial RAIT. As a subset of patients who remained unresponsive after 6 months may have undergone a second RAIT, a 6-month observation period may be insufficient to fully capture rates of relapse or the long-term risk of hypothyroidism. Therefore, a prospective study with extended follow-up (e.g., ≥ 12 months) is warranted in the future to confirm our findings and to more fully assess the efficacy and safety of RAIT. Second, owing to the limitations of our clinical follow-up protocol, thyroid function assessment was confined to four biomarkers (TRAb, FT3, FT4, and TSH), therefore unable to fully characterize thyroid autoimmunity after RAIT. Future work should incorporate a broader range of immune markers to clarify how nutritional status and organ functional status affect immune dynamics during RAIT. Third, the cohort included in this study is from Northeast Asia, a region with high iodine levels, where dietary habits are characterized by high salt and seasoning intake. This may somewhat limit the generalizability of the findings to other populations. Moreover, factors such as sunlight exposure and lifestyle may affect 25(OH)D, which could contribute to discrepancies when comparing with results from previous studies.

Conclusion

To the best of our knowledge, this is the first systematic analysis to investigate the potential impacts of iodine nutritional status, 25(OH)D, and liver and kidney function on therapeutic efficacy and thyroid function during RAIT in patients with hyperthyroid. The results indicated that age was a relatively influential factor affecting RAIT efficacy. Additionally, iodine nutritional status exhibited dynamic changes in its effect on TRAb and TSH at different time points, with SIC found to be more advantageous than UIC for monitoring

iodine nutritional status. Scr was significantly associated with changes in thyroid function following RAIT. Within the normal range, higher Scr levels were potentially associated to decreases in FT3 and FT4, as well as increases in TSH. In contrast, ALT and AST did not show significant changes before and after RAIT and exhibited a weak correlation with thyroid function.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#). Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Ethics Committee of The Second Hospital of Jilin University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

YH: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. SL: Conceptualization, Writing – review & editing. XX: Writing – review & editing. LW: Writing – review & editing. YZ: Conceptualization, Funding acquisition, Methodology, Writing – review & editing.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fendo.2025.1568699/full#supplementary-material>

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Case Report: Mild and complete thyroid peroxidase deficiency in a family with literature review

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Mild thyroid peroxidase (TPO) deficiency is an extremely rare autosomal recessive genetic disorder, with fewer than 10 cases reported globally. This condition is often misdiagnosed as primary hypothyroidism. We report a family with mild and complete TPO deficiency due to *TPO* gene mutations, including a novel mutation (p.R584W; c.1750 T > G). The literature was reviewed to provide references for early clinical diagnosis and treatment. The proband was a 27-year-old man with a 20-year history of goiter and abnormal thyroid function. Thyroid function tests showed decreased thyroxine and free thyroxine levels, increased triiodothyronine and free triiodothyronine levels, elevated FT3/FT4 ratios, normal thyroid stimulating hormone levels, and elevated thyroglobulin levels. Ultrasound highlighted goiter with multiple nodules. Previous treatment with levothyroxine (L-T4) showed no improvement in goiter nor thyroid function, leading to discontinuation. Genetic sequencing revealed a heterozygous *TPO* gene mutation (p.R584W; c.1750 T > G), predicted to be harmful by software including REVEL, PolyPhen2, and MutationTaster (REVEL score: 0.959). The proband's brother carried the same mutation albeit with different clinical manifestations, diagnosed as complete TPO deficiency. Moreover, the clinical characteristics and gene mutations of the nine previously reported cases of mild TPO deficiency were reviewed and summarized. Hence, this study reported a family with mild and complete TPO deficiency due to *TPO* gene mutations, and the literature was reviewed to enhance clinicians' understanding of the disease. Gene mutations aid in diagnosis. This is the first study to report the p.R584W; c.1750 T > G gene mutation, enriching the gene pool for this rare disease. The efficacy of L-T4 treatment for mild TPO deficiency requires further observation and research.

KEYWORDS

TPO gene mutation, mild TPO deficiency, goiter, elevated FT3/FT4 ratio, thyroglobulin

1 Introduction

Thyroid peroxidase (TPO) is a key enzyme in thyroid hormone synthesis. Its deficiency can lead to varying degrees of iodide organification defect (IOD) (1). Clinically, TPO deficiency is classified into two main categories, namely complete TPO deficiency and mild TPO deficiency, with the latter being extremely rare. Mild TPO deficiency, which causes a partial IOD (PIOD), is an autosomal recessive disorder characterized by late-onset goiter, elevated free triiodothyronine (FT3)/free thyroxine (FT4) ratio, normal or slightly elevated thyroid stimulating hormone (TSH), and increased thyroglobulin (Tg) (2). Due to partial loss of TPO enzymatic activity, patients can still produce some thyroid hormones during the first few years of life. Most cases are diagnosed around the age of 10 years due to progressive goiter.

To date, there have been nine reported cases of mild TPO deficiency at home and abroad. These cases are characterized by elevated FT3/FT4 ratios, with residual TPO enzymatic activity ranging from 30 to 65% (3–5). In contrast, classic TPO deficiency results in a total IOD (TIOD), manifested as severe congenital hypothyroidism (CH), elevated TSH, and reduced thyroxine (T4), often detected through newborn screening (6).

Here, we report clinical data and genetic testing results for two brothers who exhibited mild and complete TPO deficiency leading to CH due to *TPO* gene mutations. Additionally, the relevant literature was retrospectively analyzed to provide references for early clinical diagnosis and treatment.

2 Case description

2.1 Case 1

The proband was a 27-year-old man who presented to the authors' department in 2020 with a history of goiter and abnormal thyroid function for over 20 years (starting at 5 years of age). He had been evaluated at multiple hospitals in Beijing and Shanghai for goiter, where thyroid function tests showed decreased T4 and FT4 levels, increased triiodothyronine (T3) and FT3 levels, normal TSH levels, and no Tg measurement. Thyroid ultrasound indicated goiter. The proband was diagnosed with “nodular goiter” and recommended for thyroidectomy, which his family declined.

Between 5 and 20 years, his thyroid progressively enlarged. At 12 years, the proband was diagnosed with “hypothyroidism” and was administered levothyroxine (L-T4) 25 µg for treatment. After 1 month, there were no significant changes in thyroid function, and the thyroid did not shrink; hence, medication was discontinued. Annual TSH monitoring since then showed no increase; moreover, his neck swelling did not progress after 20 years of age. Over the past 10 years, the proband's weight remained stable, and he did not experience symptoms such as fatigue, edema, heat intolerance, excessive sweating, or palpitations. His growth, development, and intelligence were normal. Physical examination revealed a height of 188 cm and weight of 75 kg. The thyroid was grade 3 enlarged, soft, and had a 4 × 2 cm palpable nodule in the left and right lobes.

Thyroid function tests (Table 1) indicated decreased T4 and FT4 levels, increased T3 and FT3 levels, elevated FT3/FT4 ratios, normal TSH levels, and Tg more than 300 ng/mL (normal range: 0–20 ng/mL). Anti-TPO and anti-Tg antibodies were normal; TSH receptor antibody (TRAb) was 0.30 U/L (normal range: 0–1.75 U/L), immunoglobulin G was 40.649 g/L (normal range: 0.03–2.01 g/L), and calcitonin was 4.0 pg./mL (normal range: 0–18.2 pg./mL). Thyroid ultrasound (Table 1) suggested diffuse goiter with multiple mixed and solid nodules in both lobes, classified as ACR TIRADS 2–3. The dimensions of the largest nodule were 47 × 43 × 32 mm.

Peripheral blood was taken from the patient for next-generation sequencing (performed by Guangzhou Jiajian Medical Science Detection Co.). Genetic analysis revealed that the patient carried a

TABLE 1 Mild TPO deficiency patient data.

Changes in thyroid function, related antibodies, and thyroglobulin in Case 1.									
Date	T4 58.1–140.6 nmol/L	FT4 11.5–22.7 pmol/L	T3 0.92–2.79 nmol/L	FT3 3.5–6.5 pmol/L	TSH 0.55–4.78 mU/L	FT3/FT4	Anti-TPO 0–60 IU/mL	Anti-TG 0–60 IU/mL	Tg ng/mL
Feb 7, 2014	27.1↓	–	3.01↑	–	2.798	–	< 28	< 20	–
Dec 15, 2020	6.6↓	3.49↓	2.79	7.37↑	2.868	2.11	< 28	< 15	–
Nov 1, 2021	12.9↓	4.12↓	4.82↑	10.77↑	2.853	2.61	< 28	< 15	–
Nov 29, 2022	12.8↓	2.89↓	4.58↑	6.45	6.21↑	2.23	< 28	< 15	> 300

Changes in thyroid ultrasound							
Date	Dimensions of the left thyroid lobe	Dimensions of the right thyroid lobe	Isthmus anteroposterior diameter	Dimensions of the largest nodule in the left lobe	Dimensions of the largest nodule in the right lobe	Dimensions of the isthmus nodule	ACR TIARDS
Dec 14, 2020	40 + *23*37 mm	40 + *24*55 mm	15 mm	24*17 mm	46*27 mm	8*8 mm	Category 2–3
Mar 31, 2022	40 + *28*42 mm	40 + *37*53 mm	16 mm	34*26*18 mm	46*43*26 mm	19*7*5 mm	Category 2–3
Nov 29, 2022	40 + *33*42 mm	40 + *41*51 mm	15 mm	40*30*23 mm	47*43*32 mm	15*8*13 mm	Category 2–3

Patient, male, 26 years old. “–” denotes missing data.

Thyroid size measurement method (superoinferior diameter * anteroposterior diameter * transverse diameter); the maximum measurable superoinferior diameter by the ultrasound probe is 40 mm.

TABLE 2 Next-generation sequencing results.

Gene name	OMIM number	HG19 location	Transcript	Nucleotide and amino acid changes	Zygosity	ACMG variant classification
<i>TPO</i>	606765	chr2: 1491745	NM_000547.5	c.1750C > T (p.R584W)	Heterozygous	Class 3—variants of uncertain significance

1. ACMG: American College of Medical Genetics and Genomics.
2. Chromosome Location: Referencing the human genome version Human GRCh37/hg19.
3. According to the ACMG guidelines (2015 edition), this variant is classified as 3—variants of uncertain significance. The existing literature suggests that *TPO* protein mutations may be associated with type 2A hypothyroidism, an autosomal recessive condition characterized by significantly low thyroid hormone levels and goiter after birth (PMID:28648508).

heterozygous variant of the *TPO* gene on chromosome 2, namely NM_000547.5: c.1750C > T: p.R584W (heterozygous) (Table 2).

The diagnosis was as follows: 1. Mild *TPO* deficiency (c.1750C > T: p.R584W); 2. Multiple nodules in both thyroid lobes.

For the treatment strategy, regular thyroid function monitoring and thyroid ultrasound were recommended. If necessary, a fine-needle aspiration biopsy should be performed to determine the nature of the thyroid nodules. The patient was advised to seek genetic counseling and prenatal diagnosis when planning to have children. Thyroid function tests and mutation detection were also recommended for the family.

2.2 Case 2

The older brother of Case 1, aged 34 years, was diagnosed with CH accompanied by goiter at 1 year due to unstable walking and delayed growth and development. He subsequently started oral L-T4 treatment, the dosage of which was regularly adjusted based on routine thyroid function tests. At the time of the study, the patient was receiving 100 µg L-T4 daily. Physical examination showed a height of 178 cm, weight of 70 kg, and grade 3 goiter that was soft in texture, without palpable nodules. Genetic testing results were the same as those in Case 1. Case 2 had one son and one daughter, both of whom exhibited no gene mutations on genetic testing, with normal thyroid function and no goiter on ultrasound.

The diagnosis was CH (c.1750C > T: p.R584W).

For the treatment strategy, the patient was advised to continue regular oral administration of L-T4, with periodic monitoring of thyroid function and thyroid ultrasound.

Regarding family history, the parents were non-consanguineous. Previous thyroid function tests indicated normal results and no history of goiter. Nevertheless, both parents exhibited elevated levels of *TPO* antibodies. Genetic testing revealed that the mother had low-level mosaicism for the *TPO* gene mutation NM_000547.5: c.1750C > T: p.R584W (with a zygosity of 7.91%), whereas the father did not carry the *TPO* gene mutation.

3 Discussion

TPO deficiency is a group of autosomal recessive disorders (7). Based on the mutation, *TPO* deficiency is divided into two types: PIOD and TIOD (7). In patients with PIOD, radioactive iodine levels drop by 10–50% after a perchlorate discharge test, whereas in patients with TIOD, levels drop by >90%. In normal individuals, radioactive iodine levels drop to <10%. Complete *TPO* deficiency can lead to

TIOD, with a CH incidence rate of 1 in 2000–4000 (8). This is mainly due to thyroid dysgenesis and thyroid hormone synthesis disorders, with *TPO* deficiency being the most prevalent cause (2, 3). CH exhibits typical clinical manifestations, including significantly elevated TSH and Tg levels, decreased T4 and FT4 levels, reduced T3 and FT3 levels, and associated developmental delays, making it easy to diagnose through neonatal screening (6). PIOD, induced by mild *TPO* deficiency, is primarily characterized by delayed-onset goiter, elevated FT3/FT4 ratios, normal or mildly elevated TSH levels, and elevated Tg levels (2). These clinical manifestations are closely related to the degree of *TPO* enzyme activity reduction.

Here, we report two brothers with the same *TPO* gene mutation, resulting in mild *TPO* deficiency in the older brother and complete *TPO* deficiency in the proband. The older brother presented with CH, which was easy to recognize clinically. Therefore, we focused on the proband. Owing to residual *TPO* activity, the proband presented in childhood with goiter and abnormal thyroid function. Specifically, thyroid function tests indicated decreased T4 and FT4 levels, elevated T3 and FT3 levels, increased FT3/FT4 ratios, normal TSH levels, elevated Tg levels, and normal thyroid-related autoantibodies. Treatment with L-T4 was not effective. To date, nine cases of PIOD with an elevated FT3/FT4 ratio as a common feature of mild *TPO* deficiency have been reported (five abroad and four domestically). Including this case, 10 patients' clinical characteristics were reviewed. The median age of onset was 10 years, with four women and six men. Common features included pronounced goiter and abnormal thyroid function, indicated by decreased T4 and FT4 levels, increased T3 and FT3 levels, elevated FT3/FT4 ratios, normal or mildly elevated TSH levels (not exceeding 10 mIU/L), and significantly increased Tg levels. Meanwhile, the levels of anti-*TPO*, anti-Tg, and TRAb were all normal. All patients received L-T4 treatment (dose not reported), with three showing improvement in goiter, three showing no change, and one experiencing further enlargement of the thyroid. The responses in the remaining three cases were not reported. Details on thyroid function, thyroid size, response to L-T4 treatment, gene mutation sites, and nucleotide changes are summarized in Table 3. Among the cases, three siblings from Japan shared the same gene mutation site, G1687T/1808-13del. Another three cases (one from Japan, two from China) shared the same gene mutation site, C670-672del, while the remaining four patients had different gene mutations.

These 10 cases of mild *TPO* deficiency leading to PIOD revealed several clinical characteristics, including early childhood onset, goiter, elevated FT3/FT4 ratios, elevated Tg levels, and normal anti-*TPO* and anti-Tg levels. Four patients, including the proband, had normal TSH levels, whereas the remaining six exhibited mildly elevated TSH levels (all <10 mIU/L). This condition is often misdiagnosed as hypothyroidism due to decreased T4 and FT4 levels and is consequently

TABLE 3 Summary of gene results and clinical characteristics of 10 patients with mild TPO deficiency reported in literature.

Case	Mutation site		Exon	Zygosity	Origin	Goiter	L-T4 dose (ugqd)	L-T4 response	Thyroid function								Perchlorate discharge rate	Thyroid ultrasound
	Nucleotide change	Amino acid change							T4	FT4	T3	FT3	FT3/FT4	TSH	Tg	Anti-TPO anti-Tg		
Case 1 (8-year-old girl)	C670-672del	p.A224del	–	Compound Heterozygous	Mother	Yes	–	Improved	–	0.99	–	4.1	0.49↑ (0.33–0.41)	6.5↑	1057↑	Normal	36.5%↑	68 mL
Case 2 (8-year-old boy)	–	p.T527c	–	Homozygous		Yes	–	Worsened	3.5↓	–	2.2↑		T3/T4 0.075↑ (0.010–0.027)	5.9↑	250↑	Normal	–	–
Case 3 (12-year-old boy)	G1687 1808.13del	G533C A574/L575del	9	Compound Heterozygous	Father	Yes	–	–	–	11.6	–	5.5	0.47	4.0	123↑	Normal	83.7%↑	45*20 mm
Case 4 (10-year-old boy)	Same as Case 3	–	10	Same as Case 3	–	Yes	–	–	–	9.0↓	–	6.0	0.67	6.0↑	129↑	Normal	76.3%↑	50*30 mm
Case 5 (8-month-old girl)	Same as Case 3	–	10	Same as Case 3	–	Yes	–	–	–	16.8	–	7.9↑	0.47	9.2↑	577↑	–	–	32.9 mm (30.8 mm)
Case 6 (27-year-old man)	C1631C > T C1921del	p.Ala544Val p.Glu641Lys p.G641L	10 11	Heterozygous	–	Yes	50	No change	32.49↓	< 5.15↓	2.26↓	5.21	> 1	2.47	> 500↑	Normal	–	Right 39*50 mm Left 39*48 mm
Case 7 (18-year-old woman)	C1009G > A	p.Glu337Lys p.Ala544Val	8 10	Compound heterozygous	–	Yes	25	Reduced	43.59↓	8.17↓	2.15↓	4.55	0.557	3.35	> 500↑	Normal	37.3%↑	Right 47*63 mm Left 40*52 mm
Case 8 (15-year-old girl)	C.670-672del	p.Asp224del p.Arg665Gln	7 11	Heterozygous	–	Yes	25	No change	44.61↓	7.91↓	2.54↓	6.08↑	0.769	6.205↑	> 500↑	Normal	–	Right 25*37 mm Left 40*52 mm
Case 9 (5-year-old boy)	C.670-672delGAC	p.Asp224del p.Cys808Ala	7 14	Heterozygous	None	Yes	–	Reduced	74.09	10.44	3.45	7.05↑	0.675	7.706↑	> 500↑	Normal	–	Right 25*30 mm Left 24*32 mm
Case 10 (26-year-old man)	C1750C > T	p.R584W	2	Heterozygous	None	Yes	–	No change	8.2↓	3.65↓	2.79	7.37↑	2.01	2.868	> 300↑	Normal	–	Right 40*37*53 mm Left 40*26*42 mm

1. L-T4, levothyroxine; TPO, thyroid peroxidase; TSH, thyroid stimulating hormone; T3, triiodothyronine; T4, thyroxine; FT3, free triiodothyronine; FT4, free thyroxine; Tg, thyroglobulin.

2. Reference ranges for the thyroid function of Cases 1 and 2: TSH 0.5–5 mU/L, T4 6–13.8 ug/dL, FT4 0.9–1.8 pg/mL, T3 0.6–1.81 ng/mL, FT3 2.6–4.9 pg/mL. Tg < 30 ng/mL (4).

3. Reference ranges for the thyroid function of Cases 3–5: TSH 0.51–4.34 uIU/mL, FT4 10.6–21.0 pmol/L, FT3 3.1–7.5 pmol/L. Tg < 30 ng/mL (5).

4. Reference ranges for the thyroid function of Cases 6–9: TSH 0.35–4.94 uIU/mL, T4 62.67–150.84 nmol/L, FT4 9.01–19.04 pmol/L, T3 2.63–5.7 nmol/L, FT3 2.63–5.7 pmol/L. Tg 3.5–77 ng/mL (3).

5. Case 10 is the case reported in this study.

6. “–” denotes missing data.

treated with L-T4. However, the clinical efficacy is often limited. Notably, unlike hypothyroidism, which presents with elevated TSH levels, decreased T4 and FT4 levels, and normal or decreased T3 and FT3 levels, usually caused by autoimmune thyroiditis with positive TPO antibodies, this condition features negative TPO antibodies.

Patients with PIOD often present with significant goiter as the initial symptom; however, the degree of goiter is not directly correlated with TSH levels. Despite exhibiting normal TSH levels, proband and two cases reported by Ruijin Hospital (27-year-old man and 17-year-old girl) still had grade 3 goiters, which did not reduce in size after treatment. The extent of goiter depends not only on TSH levels but also on the duration of hyperthyrotropinemia (9). Additionally, the direct action of the mutant TPO protein contributes to the formation of goiter (4). As the body's demand for thyroid hormone increases with growth and development, the lack of TPO protein prevents the thyroid from synthesizing sufficient thyroid hormones, thereby leading to compensatory enlargement of the thyroid. Consequently, as the age of patients with PIOD increases, and the goiter often progressively enlarges. Meanwhile, the enlargement of the thyroid itself compensates for the reduced synthesis of thyroid hormones, which is why the clinical symptoms of hypothyroidism were not obvious in the patient in this study. This suggests that the degree of TPO deficiency in this patient was relatively mild, possibly due to the small degree of structural and enzymatic activity damage in the protein. This high retention of enzymatic activity resulted in less severe impairment of thyroid function.

Another common feature of patients with IOD is abnormal thyroid function, manifested as elevated T3 and FT3 levels, decreased T4 and FT4 levels, increased FT3/FT4 ratios, and normal or mildly elevated TSH levels (all <10 mIU/L). In normal individuals, T4 is directly secreted by the thyroid. Only about 20% of T3 is secreted by the thyroid, whereas the remaining 80% is produced by the peripheral conversion of T4 by removing a single 5' iodine atom. Narumi et al. reported that in patients with mild TPO deficiency, T3 was secreted by the thyroid, and the conversion of T4 to T3 in peripheral tissues was impaired (4). The thyroid of patients with mild Tg deficiency usually prioritizes T3 secretion, which reflects chronic overstimulation of the gland (10). Narumi et al. speculated that similar adaptive responses may occur in patients with mild TPO deficiency (4), and

elevated FT3/FT4 ratios may explain why their metabolism remained normal despite low T4 levels.

Elevated Tg levels is another common feature in patients with IOD. Exons 8, 9, and 10 encode the catalytic center of the TPO protein, and TPO enzyme activity depends on correct folding, membrane insertion, and intact catalysis (11). Abnormal TPO molecules can lead to defects such as TPO's inability to bind heme, inability to bind Tg as a substrate, and abnormal cellular localization of TPO (12). Because the denatured TPO protein cannot facilitate the binding of Tg and iodine atoms, serum Tg levels are significantly elevated in these patients.

According to our literature search, the *TPO* mutation (NM_000547.5: c.1750C > T: p.R584W) we observed has not been reported previously. This variant was listed in the gnomAD database as a rare mutation with an allele frequency of 0.000012. Further analysis using software predictors like REVEL, Polyphen2 (Figure 1), and MutationTaster predicted it to be a harmful variant (REVEL score: 0.959). This mutation is not listed in the ClinVar database, suggesting that it is a novel mutation. According to the ACMG guidelines (2015 edition), this mutation is classified as a variant of uncertain significance. The literature suggests that *TPO* mutations may be associated with type 2A hypothyroidism, an autosomal recessive condition characterized by significantly low thyroid hormone levels and goiter after birth (PMID:28648508). Conservation predictions indicated that the mutation site was highly conserved across different species (Figure 2), and the resultant amino acid change at position 584 in the TPO protein (from arginine to tryptophan) could impact the protein's structure and function (Figure 3). The mutation NM_000547.5: c.1750C > T: p.R584W resulted in a nucleotide change from cytosine to thymine at position 1750 in the coding region of the *TPO* gene, leading to an amino acid substitution at position 584 from arginine to tryptophan. Furthermore, the site was highly conserved across multiple species, suggesting that the amino acid change could alter the TPO protein structure, ultimately impairing or eliminating TPO enzyme activity. Nevertheless, further validation through *in vitro* functional tests is required. The milder symptoms in Case 1 suggest that although TPO activity was partially compromised by the mutation, some functionality was retained. In contrast, Case 2, with a complete TPO mutation, presented with CH and required lifelong L-T4 therapy.



FIGURE 1
Polyphen2 prediction of mutation harmfulness: predicted as harmful mutation.

tr H3BC80 H3BC80_LATCH	E	A	L	S	L	D	L	A	S	L	N	L	Q	R	G	R	D	H	G	L	P	S	Y	N	A	W	R	Q	F	C	N	L	P	E	A	K	552
tr A0A8D2IIV4 A0A8D2IIV4_VARKO	T	N	G	S	L	D	L	A	S	L	D	L	Q	R	G	R	D	H	G	L	P	G	Y	N	D	W	R	E	F	C	D	L	P	R	L	R	559
sp P07202 PERT_HUMAN	N	S	S	T	L	D	L	A	S	I	N	L	Q	R	G	R	D	H	G	L	P	G	Y	N	E	W	R	E	F	C	G	L	P	R	L	E	604
sp P14650 PERT_RAT	N	V	G	T	L	D	L	A	S	L	N	L	Q	R	G	R	D	H	G	L	P	G	Y	N	E	W	R	E	F	C	G	L	S	R	L	D	592
sp P35419 PERT_MOUSE	N	V	G	T	L	D	L	A	S	L	N	L	Q	R	G	R	D	H	G	L	P	D	Y	N	E	W	R	E	F	C	G	L	S	R	L	E	592
sp Q8HYB7 PERT_CANLF	S	S	G	S	L	D	L	A	S	I	N	L	Q	R	G	R	D	H	G	L	P	G	Y	N	A	W	R	E	F	C	G	L	G	R	L	H	612
tr A0A3Q1LK39 A0A3Q1LK39_BOVIN	D	A	G	T	L	D	L	A	S	I	N	L	Q	R	G	R	D	H	G	L	P	G	Y	N	E	W	R	Q	F	C	G	L	S	R	L	E	552
sp P09933 PERT_PIG	N	S	G	T	L	D	L	A	S	I	N	L	Q	R	G	R	D	H	G	L	P	G	Y	N	E	W	R	E	F	C	G	L	S	R	L	E	602

FIGURE 2

Conservation prediction indicates that the mutation site is highly conserved across different species.

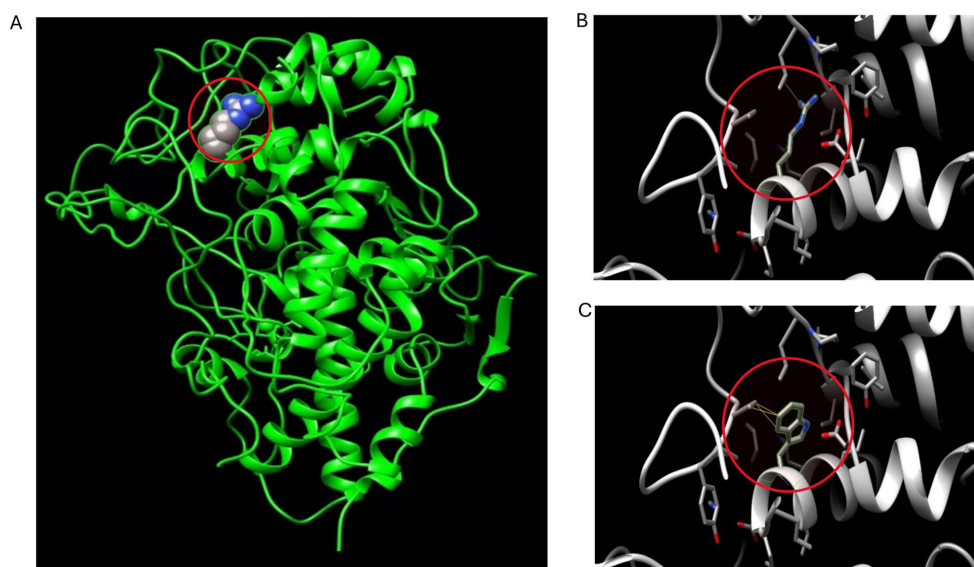


FIGURE 3

3D conformation prediction of wild-type and mutant TPO proteins. This patient's *TPO* gene mutation results in an amino acid change at position 584 of the TPO protein (from arginine to tryptophan). (A) 3D conformation of the amino acid at position 584 in the mutation site (arginine) of wild-type TPO protein, highlighted in the red circle; (B) Micrograph of the amino acid at position 584 (arginine) in the 3D conformation of wild-type TPO protein, highlighted in the red circle; (C) Micrograph of the amino acid at position 584 (tryptophan) in the 3D conformation of mutant TPO protein (p.R584W), highlighted in the red circle.

The efficacy of L-T4 in controlling goiter in patients with classic or mild TPO deficiency phenotypes is generally poor and may even exacerbate thyroid enlargement (13). In four patients with mild TPO deficiency reported by Ruijin Hospital, two patients did not show improvement in thyroid function or alleviation in goiter after L-T4 treatment, whereas the other two saw a reduction in goiter volume (3). In our study, the symptoms of the patient did not improve with L-T4 treatment, and the thyroid did not further enlarge after discontinuing the medication. Theoretically, oral L-T4 can reduce TSH levels, thereby decreasing goiter volume. However, long-term impaired thyroid hormone synthesis may lead to a relatively high set point for the TSH-thyroid hormone regulatory axis, potentially requiring lower TSH levels to suppress further goiter enlargement. Other factors related to the development of goiter may be involved in this process. For example, iodide can inhibit thyroid epithelial cell proliferation (3). Therefore, due to the lack of iodide, *TPO* mutations may increase the

risk of multinodular goiter and even thyroid cancer. Consequently, patients with mild TPO deficiency may respond poorly to L-T4 treatment, necessitating surgical intervention if the patient experiences tracheal compression due to significant goiter. To determine whether mild TPO deficiency requires treatment, further clinical observations are required, given the limited number of current clinical cases.

The incidence of thyroid cancer in patients with nodular goiter is approximately 3–5% (14). Reduced TPO expression and overexpression of shorter splice variants in thyroid tissue are associated with follicular thyroid tumors (15). Patients with CH due to *TPO* gene mutations are subject to a higher risk of developing thyroid cancer than other populations (16). Nevertheless, there have been no reports of thyroid cancer in patients with mild TPO deficiency. Patients with CH and nodular goiter can develop thyroid cancer [follicular thyroid cancer reported (17, 18)]. Therefore, such patients should be assessed for the malignancy of all suspected nodules, with particular attention to the risk

of developing follicular thyroid carcinoma. Since the relationship between mild TPO deficiency and thyroid cancer is not fully elucidated and due to the limited number of reported cases and the absence of thyroid cancer in the previously reported cases, long-term follow-up and regular neck ultrasounds are warranted for these patients. If necessary, fine-needle aspiration biopsy of the thyroid should be performed to confirm the presence of follicular thyroid carcinoma.

Clinically, identifying and screening high-risk individuals for mild TPO deficiency is essential. Patients with goiter and normal TPO antibodies should be carefully evaluated. Because T4 and FT4 levels are decreased, these patients are easily misdiagnosed with hypothyroidism and inappropriately treated with L-T4 replacement therapy. Clinicians should pay close attention to patients with abnormal thyroid function, with specific manifestations including elevated FT3/FT4 ratios, decreased T4 and FT4 levels, elevated T3 and FT3 levels, and normal or mildly elevated TSH levels, especially when TPO antibodies are negative. Even if TSH levels are normal, mild TPO deficiency should be considered. Additionally, Tg testing is recommended, while genetic testing and the perchlorate discharge test can help diagnose and differentiate mild TPO deficiency early. For patients with mild TPO deficiency, the decision to undergo thyroid hormone replacement therapy requires more clinical data due to the small sample size of the existing research. If there is no TSH elevation, L-T4 treatment usually provides no benefits, and follow-up is recommended. Conversely, when goiter is evident, surgical intervention or thermal ablation may be required to reduce the thyroid volume. After diagnosis, long-term follow-up is necessary to detect clinical hypothyroidism and follicular thyroid carcinoma early. For newborns with significant goiter, elevated TSH levels, increased FT3/FT4 ratios, elevated Tg levels, and normal anti-TPO and anti-Tg levels, genetic testing should be performed to confirm the diagnosis as it helps diagnose TPO deficiency and differentiate it from other types of CH.

In conclusion, mild TPO deficiency is an autosomal recessive disorder primarily characterized by goiter, increased FT3/FT4 ratios, elevated Tg levels, and normal anti-TPO and anti-Tg levels. Existing diagnosis is mainly based on elevated FT3/FT4 ratios and Tg levels, with genetic testing aiding confirmation. This c.1750 T > G (p.R584W) is a novel mutation in the population, expanding the mutation spectrum of mild TPO deficiency. Genetic testing of the proband and their parents may provide prenatal diagnosis for families planning to have children.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

The studies involving humans were approved by Medical Ethics Committee, Sun Yat-sen Memorial Hospital, Sun Yat-sen University. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from a by-product of routine care or industry. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements. Written informed consent was

obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

XW: Data curation, Formal analysis, Methodology, Project administration, Writing – original draft, Writing – review & editing, Investigation. YY: Formal analysis, Methodology, Software, Writing – review & editing. HW: Data curation, Methodology, Resources, Writing – review & editing. KW: Data curation, Methodology, Software, Visualization, Writing – review & editing. MW: Data curation, Methodology, Resources, Writing – review & editing. LY: Writing – review & editing, Supervision. YL: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. JZ: Conceptualization, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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