Multiple nutritional factors and the risk of Hashimoto's Thyroiditis

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ABSTRACT

Background

Hashimoto's Thyroiditis (HT) is considered to be the most common autoimmune disease. It is currently accepted that genetic susceptibility, environmental factors and immune disorders contribute to its development. Regarding nutritional factors, evidence implicates high iodine intake, deficiencies of selenium and iron with a potential relevance of vitamin D status as contributing factors. To elucidate the role of nutritional factors in the risk, pathogenesis and treatment of HT, PubMed and the Cochrane Library were searched for publications on iodine, iron, selenium and vitamin D and risk/treatment of HT.

Summary

Iodine: Chronic exposure to excess iodine intake induces autoimmune thyroiditis, partly because highly-iodinated thyroglobulin is more immunogenic. Recent introduction of universal salt iodization can have a similar, though transient, effect.

Selenium: Selenoproteins are essential to thyroid action. In particular, the glutathione peroxidases protect the thyroid by removing excessive hydrogen peroxide produced for thyroglobulin iodination. Genetic data implicate the anti-inflammatory selenoprotein S in HT risk. There is evidence from observational studies and randomized controlled trials that selenium/selenoproteins can reduce TPO-antibody titers, hypothyroidism and postpartum thyroiditis.

Iron: Iron deficiency impairs thyroid metabolism. Thyroid peroxidase (TPO), the enzyme responsible for the production of thyroid hormones is a heme (iron-containing) enzyme; it becomes active at the apical surface of thyrocytes only after binding heme. HT patients are frequently iron-deficient since autoimmune gastritis, which impairs iron absorption, is a common co-morbidity. Treatment of anemic women with impaired thyroid function with iron improves thyroid-hormone concentrations while thyroxine and iron together are more effective in improving iron status.

Vitamin D: Lower vitamin D status has been found in HT patients than in controls and inverse relationships of serum vitamin D with TPO/thyroglobulin antibodies have been reported. However, other data and the lack of trial evidence suggest that low vitamin-D status

is more likely the result of autoimmune disease processes that include vitamin D-receptor dysfunction.

Conclusions

Clinicians should check patients' iron- (particularly in menstruating women) and vitamin-D status to correct any deficiency. Adequate selenium intake is vital in areas of iodine-deficiency/excess and in regions of low selenium intake a supplement of 50 to 100 µg/day selenium may be appropriate.

INTRODUCTION

Hashimoto's Thyroiditis (HT), also known as chronic lymphocytic thyroiditis and chronic autoimmune thyroiditis, is now considered to be the most common autoimmune disease (1) and is the most common cause of primary hypothyroidism in iodine-sufficient areas (2). It includes a spectrum of histopathological and clinical entities that are collectively characterized by intrathyroidal lymphocytic infiltration (1). Currently, HT is mainly diagnosed by positive titers of serum autoantibodies against thyroid peroxidase (TPOAb) and thyroglobulin (TgAb) as well as diffuse hypoechogenicity on thyroid ultrasonography (1). Patients may present with various thyroid function states but most of them eventually evolve into hypothyroidism (1). HT patients can suffer from a variety of local and systemic manifestations accompanied by other autoimmune disorders (1). Moreover, HT has been found to be associated with an increased risk of primary thyroid lymphoma and papillary thyroid cancer (3).

Although discovered a century ago, the pathogenesis of HT remains unclear. It is currently accepted that the complex interactions of genetic susceptibility, environmental factors and immune disorders contribute to its development (4). Family and twin studies have confirmed a significant genetic influence on HT susceptibility (4). However, the concordance rate for overt Hashimoto's hypothyroidism found in Danish monozygotic twins was 55%, indicating an almost equally important role of environmental factors in the disease pathogenesis (5). A probable mechanistic model is that in genetically susceptible individuals, several environmental factors may trigger thyroid autoimmunity by increasing the immunogenicity of thyroid autoantigens, enhancing antigen presentation in the thyroid and reducing self-tolerance (4). Consequently, various pro-inflammatory cytokines are produced by immune and thyroid cells, resulting in predominantly Th1 and Th17 responses with an increased Th1/Th2 ratio (4). Meanwhile, increased production of pro-apoptotic cytokines leads to thyrocyte apoptosis and finally, thyroid destruction (4). In addition, a decreased number or impaired function of regulatory T cells (Tregs), which are pivotal for maintaining peripheral tolerance and suppressing excessive immune response, has been recognized to play an

important role in the pathogenesis of HT (6).

Regarding environmental factors, evidence implicates high iodine intake, selenium deficiency, infection, certain drugs and chemicals in risk, while smoking and moderate alcohol consumption have a protective effect (7, 8). Studies have also suggested a role for other common micronutrients, most notably iron and vitamin D, on HT risk (8). Although there is some evidence of an effect of vitamin A and zinc on thyroid metabolism, only sparse data are available on their relationship with HT. Hence in this review, we will concentrate on iodine, selenium, iron and vitamin D.

METHODS

PubMed and the Cochrane Library were searched for publications up to October 2016 using the search terms “Hashimoto’s thyroiditis” OR “autoimmune thyroiditis” OR “autoimmune thyroid disease” in combination with “iodine”, “selenium”, “iron”, “vitamin D” and “nutrition OR diet”. Articles were filtered by relevance of title, abstract and finally the full text. Relevant conclusions or results were extracted from each article.

REVIEW

Iodine

The micronutrient, iodine, is an essential constituent of the thyroid hormones which play a pivotal role in growth, development and metabolism (9). The biologically active form, tri-iodothyronine (T3), stimulates oxygen consumption, controls basal metabolic rate and thermogenesis, regulates the expression of numerous target genes that affect protein synthesis either positively or negatively, regulates cell activity and growth, and is essential for brain development and function, particularly in fetal life (9).

Role of iodine in the thyroid

Iodide is taken up from blood by thyroid epithelial cells. At the apical surface of the thyrocyte in the follicular lumen, in the presence of hydrogen peroxide (H₂O₂), the enzyme

thyroid peroxidase (TPO) iodinates tyrosine molecules on the surface of thyroglobulin, a large glycoprotein synthesized by thyroid epithelial cells (9). The products are mono- and di-iodotyrosines (9). Coupling of these products, still attached to thyroglobulin, again catalyzed by TPO in the presence of H₂O₂, forms the thyroid hormones, T₃ and thyroxine (T₄) (9). The iodinated thyroglobulin molecule then enters the thyrocyte and is digested, releasing T₄ and T₃ into the circulation (9).

Evidence for a relationship between iodine intake/status and HT risk

Iodine intake has a key influence on the spectrum of thyroid disorders in populations (2, 10). Severe iodine deficiency causes goiter and hypothyroidism due to reduced production of thyroid hormone whereas chronic mild-to-moderate iodine deficiency may increase the prevalence of toxic nodular goiter and hyperthyroidism (2, 10). Excess iodine intake or a rise in intake after initiating iodine fortification of an iodine-deficient population are associated with an increased risk of subclinical hypothyroidism and thyroid autoimmunity (2, 10).

Iodine deficiency has long been an important public health issue worldwide, causing multiple detrimental consequences (11). Since the implementation of iodine fortification, particularly universal salt iodization, substantial progress has been made to prevent and control iodine-deficiency disorders. However, excessive iodine intake, which can also result from the implementation of universal salt iodization programs at too high a level of supplementation, increases the risk of several thyroid disorders, including autoimmune thyroiditis (12).

Numerous epidemiological studies have associated high iodine intake with increased prevalence of autoimmune thyroiditis in populations (12-16). However, the optimization of nutritional iodine intake ultimately results in decreased prevalence of autoimmune thyroiditis (17). The association between high iodine exposure and increased incidence of autoimmune thyroiditis has also been demonstrated in a variety of animal models with genetic susceptibility (18-20). The ingestion of excess iodide through drinking water by the autoimmune thyroiditis-prone NOD.H2^{h4} mouse significantly enhances and accelerates the incidence and severity of autoimmune thyroiditis in a dose-dependent manner (21).

The mechanisms by which increased chronic intake of dietary iodine induces autoimmune thyroiditis are unclear. It is thought that in genetically susceptible individuals, chronic high iodine exposure may: (i) increase the immunogenicity of thyroglobulin, (ii) induce auto-antigen presentation activity of thyrocytes and dendritic cells, (iii) impair peripheral tolerance by inhibiting Tregs, (iv) cause oxidative stress leading to thyroid tissue injury, (v) activate auto-reactive T cells which increases cytokine secretion and eventually triggers apoptosis-signaling pathways, leading to thyroid destruction (12, 21, 22).

Recommendations on iodine intake

The level of dietary iodine intake has a very significant effect on the pattern of thyroid disorders in populations. While iodine deficiency is recognized to have multiple adverse effects on the thyroid, with regard to autoimmune thyroiditis/HT, there is more evidence for an association with iodine excess, especially in genetically susceptible individuals (12, 16, 22). To avoid an increased risk of HT, it is therefore important to ensure, as far as possible, that iodine intake falls within a relatively narrow range of the recommended levels (23) [see Table 1 (24-26)]. On a population basis, this would be represented by a median urinary iodine concentration in adults of 100-200 µg/l (26). Authorities introducing iodine fortification of the food supply in a country (e.g. universal salt iodization) need to ensure that such fortification is introduced very cautiously; Denmark provides an excellent example of how this can be done (27).

Selenium

Selenium is a trace mineral essential for human health (28). As selenocysteine (an analogue of cysteine), it is incorporated into 25 human selenoproteins that have a wide range of functions ranging from antioxidant and anti-inflammatory agents to the production of active thyroid hormone (28, 29). An indication of the importance of selenium to the thyroid is the fact that it contains the highest concentration of selenium in the human body and is able to retain that selenium under conditions of severe deficiency that cause its loss from many other

tissues (30).

Role of selenium in the thyroid: selenoproteins

A number of selenoproteins are expressed in thyrocytes, e.g. the deiodinase isozymes (Dio1, Dio2, though not Dio3), members of the glutathione peroxidase family (GPx1, GPx3, GPx4), the thioredoxin reductases (TxnRd1 and TxnRd2), selenoprotein 15, selenoprotein P (SELENOP), and selenoproteins M and S (SELENOM, SELENOS) (31). Those below play particularly important roles.

The deiodinases (DIO): DIO1 and DIO2 can activate T4 by transforming it into T3 by removal of the 5'-iodine, while DIO1 and DIO3 can prevent T4 from being activated by converting it to the inactive reverse T3 (32). DIO3 can also inactivate T3 by 5-deiodination to T2. Outside the thyroid, DIO2 is largely responsible for local conversion of T4 to T3 in target tissues (29). DIO3 is found in fetal tissue, the placenta and central nervous system where it protects sensitive cells from thyrotoxic concentrations of active T3 (29, 33).

The glutathione peroxidases (GPx): GPx3, normally found in the plasma is also secreted at the apical side of the thyrocyte membrane where it degrades excess H₂O₂ that has not been used by TPO for the iodination of tyrosyl residues of thyroglobulin or for iodotyrosine coupling (34) (see Figure 1). GPx-1 protects the intracellular compartment from excessive H₂O₂ that may diffuse into the thyrocytes while GPx4 can remove excessive lipid hydroperoxides in the mitochondria (33, 34).

Selenoprotein S (SELENOS): SELENOS is involved in the control of the inflammatory response in the endoplasmic reticulum (ER) (35). In a Portuguese study, the *SELENOS* -105G/A promoter polymorphism (rs28665122) was strongly associated with circulating levels of cytokines such as IL-1 β , IL-6 and TNF- α (36), that are known to be involved in HT pathogenesis. A-allele carriers of this polymorphism were more than twice as likely as GG-homozygotes to have HT; in male carriers, the risk was four-fold higher (36).

Evidence for a relationship between selenium intake/status and HT risk/treatment

Deficiency of selenium has been associated with a number of adverse thyroid conditions,

including hypothyroidism, subclinical hypothyroidism and autoimmune thyroiditis/HT (37), an enlarged thyroid (37-39); thyroid cancer (31, 40, 41); and Graves' disease (42).

A recent study, very relevant to the effect of selenium status on HT risk, was carried out in two counties of Shaanxi Province, China, that had high genetic, environmental and lifestyle similarities, and comparable iodine status, but very different selenium status – adequate and low (37). Participants (n=6152) completed demographic and dietary questionnaires, underwent physical and thyroid-ultrasound examinations and had serum samples analyzed for thyroid-function parameters and selenium concentration. Median (IQR) selenium concentrations differed almost two-fold [103.6 (79.7, 135.9) vs. 57.4 (39.4, 82.1) µg/L; P=0.001] between participants from the two counties. After adjustment for potential confounders, the prevalence of pathological thyroid conditions was significantly lower in the adequate-selenium than in the low-selenium county (18.0% vs. 30.5%; P<0.001). Higher serum selenium was associated with lower odds [OR (95% CI)] of autoimmune thyroiditis [0.47 (0.35, 0.65)], hypothyroidism [0.75 (0.63, 0.90)], subclinical hypothyroidism [0.68 (0.58, 0.93)], and enlarged thyroid [0.75 (0.59, 0.97)] (37). Both these counties had an iodine intake that was more-than-adequate (26, 37, 43) which may have accounted to some extent for the high level of thyroid disease prevalence (15, 44). This study suggests that in such a situation, having an adequate selenium status may be protective.

Several trials of selenium supplementation have been carried out in both autoimmune thyroiditis (HT) and mild Graves' orbitopathy. In a large, multicenter, randomized, controlled trial (RCT) with selenium, patients with mild Graves' orbitopathy significantly improved (45). There have been a number of systematic reviews/meta-analyses of controlled trials of selenium treatment in patients with autoimmune thyroiditis/HT (33, 46-48). The most recent included 16 trials in a meta-analysis that found that selenium supplementation reduced serum TPO-Ab levels after 3, 6 and 12 months in a population with chronic autoimmune thyroiditis treated with levothyroxine, and after three months in an untreated population (46). Some of these studies also saw a reduction in Tg-Ab titers at 12 months, an improvement in thyroid

echogenicity, and an increase in subjective well-being. However, the methodology of many of the studies was flawed; e.g., they were underpowered, not double-blinded, not placebo-controlled, and disparities in iodine intake were not considered (46, 47, 49). The beneficial effect in some studies and not in others cannot easily be explained on the basis of baseline selenium status, stage of disease, baseline TPO-Ab titers, form or dose of selenium used (8). Hence, we still need well designed, properly powered, RCTs of selenium in the treatment of autoimmune thyroiditis/HT before we can confidently recommend selenium supplementation in HT patients.

Despite these caveats, there is a rationale for a beneficial effect of selenium – probably through its role within selenoproteins – on autoimmune thyroid disease/HT: (i) selenium, as the glutathione peroxidases and the thioredoxin reductases has an antioxidant, protective function (28, 50); (ii) selenium can up-regulate regulatory T-cells resulting in increased immune tolerance (in an autoimmune thyroiditis model system) (51); (iii) selenium has anti-inflammatory effects (35, 52, 53); and (iv) selenium may suppress the expression of HLA-DR molecules on thyrocytes, reducing the development of thyroid autoimmunity (50, 54).

Evidence for a relationship between intake/status of selenium and autoimmune thyroid disease in pregnancy and the postpartum period

Several clinico-pathologic variants are now thought to be included under the term HT, including postpartum thyroiditis (1). Pregnant women positive for TPO-Abs are likely to develop hypothyroxinemia during pregnancy and postpartum thyroiditis in the year after delivery (55). Up to 50% of TPO-Ab-positive pregnant women develop postpartum thyroiditis of whom 20-40% subsequently become hypothyroid (55).

An RCT in TPO-Ab-positive women in Italy found that selenium supplementation reduced thyroid inflammatory activity and the risk of postpartum thyroid disease (56). During pregnancy and the postpartum period, 151 TPO-Ab-positive women were randomized to selenium (200 µg/d as selenomethionine) or placebo. TPO-Abs fell significantly during

gestation but the reduction was significantly greater in the selenium-supplemented group (P=0.01) and remained so in the postpartum period (P=0.01) (see Figure 2). Importantly, there was a significant reduction in the incidence of postpartum thyroid disease and hypothyroidism in the selenium-supplemented group (28.6% vs. 48.6%, P<0.01 and 11.7% vs. 20.3%, P<0.01, respectively) (56). Furthermore, during treatment, women on selenium maintained the same level of ultra-sound echogenicity whereas in those on placebo, echogenicity significantly worsened. At the end of the postpartum period, grade 2-3 thyroiditis had developed in 44.3% of women on placebo but only in 27.3% of women on selenium (P<0.01).

The only other RCT that investigated the effect of selenium supplementation on autoimmune thyroid disease in pregnancy found no difference in the magnitude of decrease between selenium and placebo groups (57). However the trial was underpowered, the median baseline TPO-Ab concentrations in the women were much lower than in the study by Negro et al. (56), and the selenium dose given was considerably less, i.e. 60 vs. 200 µg/d. There is clearly a need for a further, high-quality, adequately powered RCT in the TPO-Ab-positive pregnant population to see if the results of Negro and colleagues can be replicated (56).

Is selenium intake/status adequate?

The intake of selenium shows tremendous variability from one part of the world to another, ranging from deficient (7 µg/d) to toxic (4990 µg/d) levels (28). Figure 3 shows the variability and gives an indication of the level of intake believed to be needed to optimize the activity of GPx3 (58), the main selenoenzyme responsible for removing excess H₂O₂ from the thyroid. This geographical variability in intake (and hence, status) relates not only to the selenium content of the soil on which crops and fodder are grown, but to many other factors that determine the availability of selenium to the food chain such as selenium speciation, soil pH and organic-matter content. Mean intake is some 40 µg/d in Europe and 93 (females) to 134 (males) µg/d in the USA (28). Recommended selenium intake varies by authority and averages 60 µg/d for men and 53 µg/d for women (59). Supplements of selenium contribute

to intake and are quite commonly consumed, particularly in the US, where some 50% of the population takes dietary supplements (60).

Food sources of Selenium

Brazil nuts are the richest food source of selenium though they are generally not a commonly eaten food, and in any case cannot be recommended as a main selenium source as the content is very variable, ranging from 0.03 to 512 mg/kg fresh weight, and they are high in barium (59). Organ meats and seafoods are good sources, followed by muscle meats, cereals and grains, though the selenium content of the latter varies widely (see Figure 4) (28, 59). Thus, in the USA, grains such as wheat are excellent selenium sources and provide some 37% of dietary Se (61) whereas in the UK, they only provide 26% of Se intake (62).

Recommendations for selenium intake

Though we lack evidence that selenium supplementation results in clinical improvement in autoimmune thyroiditis (other than in mild Graves' orbitopathy), it still makes sense to ensure that selenium intake is adequate, given the roles played by selenium/selenoproteins in human health (28) and particularly in the thyroid. Regions of deficient, more-than-adequate or high iodine intake may have more need for selenium owing to the capacity of selenoproteins to protect the thyroid from damage from H₂O₂, reactive oxygen species and inflammation and to increase immune tolerance (see above). Hence, under these circumstances, clinicians need to be especially vigilant to ensure that selenium intake/status is adequate. Women are at greater risk of thyroid disorders and may thus have a higher requirement for additional selenium, particularly in pregnancy. In addition, geographical location will give a good indication of selenium adequacy or otherwise (see Figure 3). It is also important to enquire into the dietary habits of a given patient and see if he/she eats foods that supply selenium (see above) (28). In China, for instance, selenium-enriched tea is an excellent selenium source (37) and is available in many areas.

If there appear to be few, or no, selenium-rich sources in a patient's diet, low-dose

supplementation (50-100 µg selenium/d) is suggested. Multi-vitamin/mineral tablets may contain 50 µg selenium/d, an amount that will generally be adequate for women. A dose of 100 µg selenium/d (as selenium-yeast) given to someone in the UK will raise plasma selenium to around 140 µg/L which is more than enough to optimize the synthesis of all the selenoproteins (63). Either selenium-yeast (which behaves in the body like wheat-selenium) or sodium selenite (the latter is not non-specifically incorporated into body proteins in place of methionine) is adequate (59).

Even if patient with HT is being treated with levothyroxine, one needs to be aware that some studies found that giving Se as well as levothyroxine resulted in a greater reduction in TPO-Abs, inflammatory cytokines and C-reactive protein (53, 64).

It is also of importance to bear in mind that though selenium is essential, excessive intake of selenium is toxic and supplements of selenium of 200 µg/d, generally considered to be quite safe, have been associated with toxic effects (alopecia, dermatitis, squamous cell carcinoma, type-2 diabetes mellitus) in North American men (65-67), though these men had higher selenium status than European men. As for many nutrients, there is a U-shaped relationship between selenium status and disease risk; therefore, it is recommended to aim for an adequate intake that does not stray into levels associated with potential toxicity (28).

Iron

Iron is an essential major mineral; through its presence in hemoglobin, myoglobin and many iron-containing enzymes, it is involved in a great number of metabolic processes in the body. These include oxygen transport and storage, DNA synthesis, ATP generation, oxidation-reduction reactions, electron transfer and regulation of the cell cycle (68-70). In healthy adults, iron metabolism is strictly regulated to maintain body iron content within a restricted range. This is because iron deficiency leads to decreased oxygen transport and impaired activity of iron-containing enzymes, and because iron excess may predispose to iron-overload diseases and cancer (68, 69).

Role of iron in the thyroid

TPO, the enzyme required for the organification and coupling reactions in thyroid hormone synthesis (71, 72) becomes active at the apical surface of thyrocytes only after it binds a prosthetic heme I group (73). Hence an adequate iron status is essential for the production of the thyroid hormones, T3 and T4.

Evidence for a relationship between iron intake/status and HT risk/treatment

Hashimoto's Thyroiditis and iron deficiency

Studies have revealed that HT patients with subclinical hypothyroidism have lower serum iron concentrations and a higher prevalence of iron deficiency than healthy controls (74, 75). As an organ-specific autoimmune disease, HT is frequently associated with other autoimmune disorders. Indeed, a considerable proportion of HT patients have celiac disease (76-78) or autoimmune gastritis co-morbidity (79-82) and these co-morbid conditions are regarded as the major cause of iron deficiency in HT patients. Iron-deficiency anemia is the most common extra-intestinal manifestation of celiac disease, which impairs iron absorption and leads to iron deficiency (83). Autoimmune gastritis is characterized by serum anti-parietal cell antibodies and anti-intrinsic factor antibodies; it can finally evolve to severe atrophic gastritis with subsequent hypochlorhydria and chronic iron deficiency (84). Because of the abnormal gastric secretion and low acidity, dietary non-heme iron cannot effectively be solubilized leading to iron malabsorption (85).

It appears likely, however, that hypothyroidism *per se*, which is common in HT patients, impairs gastrointestinal iron absorption. Early experiments in hypothyroid rats showed diminished gastrointestinal iron absorption that was restored to normal on supplementation with T3 (86). In two studies in patients with coexisting iron-deficiency anemia and subclinical hypothyroidism, treatment with iron and T4 together was considerably more effective in improving iron status than treatment with iron alone (87, 88).

Iron deficiency affects thyroid metabolism

Not only do HT patients have a higher prevalence of iron deficiency, but iron deficiency impairs thyroid metabolism. It reduces thyroid hormone production by decreasing the activity of the iron-dependent enzyme, TPO (71-73). In rodent studies, iron deficiency, with or without anemia, decreased serum T4 and T3 concentrations, lowered 5'-deiodinase activity, and reduced the ability to thermoregulate in response to a cold environment (72, 89-91). Apart from the observed effect of iron deficiency on thyroid hormone production (72, 73), it has been suggested that it may lead to functional hypothyroidism by altering the central regulation of the thyroid axis (90) and hampering the binding of T3 to hepatic nuclear receptors (92).

Human studies have provided equivocal outcomes. While a few studies found no significant association between serum thyroid hormone concentrations and iron status (93, 94), others had different results. Lower serum T4 and/or T3 and higher TSH levels were reported in women with iron-deficiency anemia than in non-anemic controls, and iron supplementation partially normalized plasma thyroid hormone concentrations (95, 96). A small Finnish study illustrates that low iron stores may contribute to symptom persistence in patients treated for hypothyroidism (97). Twenty-five women with persistent symptoms of hypothyroidism despite appropriate levothyroxine therapy became symptom-free when treated with oral iron supplements for 6-12 months. None of the women had anemia or red-cell indices outside the reference range though all had serum ferritin < 60 mg/L(97). A study conducted in 4392 women of childbearing age indicated that iron deficiency was independently correlated with isolated hypothyroxinemia in both pregnant and non-pregnant women (98). Two cross-sectional studies showed significantly higher risks of goiter in children with low serum iron (99, 100).

Recommendations for iron intake

As explained above, HT patients have a high prevalence of iron deficiency or low iron stores that may impair thyroid metabolism. Hence, we recommend routine screening of HT patients

for iron deficiency. If either deficiency or a serum ferritin $< 70 \mu\text{g/L}$ is found (97), co-morbid celiac disease or autoimmune gastritis should be suspected as a potential cause and treated if necessary. If celiac disease is diagnosed, it should be formally documented. Hematological tests can be used to distinguish between iron-deficiency anemia that will respond to iron supplementation and the anemia of chronic disease that will not. Assuming the latter is not involved, supplementation to restore iron sufficiency should be instituted and will help prevent the deleterious effects of iron deficiency on thyroid function (95, 96). There are alternative supplements to ferrous sulfate (e.g. ferrous bisglycinate) that may be better tolerated by the gastro-intestinal tract (101, 102).

Vitamin D

Vitamin D is a steroid hormone precursor, pivotal for bone and mineral homeostasis that balances serum levels of calcium and phosphorus (103). It has two major forms of which vitamin D₂ comes exclusively from diet and vitamin D₃ is largely synthesized in the human skin through sunlight exposure. Despite the difference in side-chain structure, both are hydroxylated in the liver by 25-hydroxylase to 25-hydroxyvitamin D [25(OH)D, calcidiol], which is carried by vitamin D binding protein (VDBP) and is used as the circulating indicator of vitamin D status (104). In the classic pathway, 25(OH)D is then converted to 1 α ,25-dihydroxyvitamin D [1 α ,25(OH)₂D, calcitriol] by a cytochrome P450 enzyme, 1- α -hydroxylase (CYP27B1) in the kidney; this is the hormonally active form and exerts its endocrine effects by binding to the vitamin D receptor (VDR) and regulating VDR-responsive genes (105). However, numerous recent studies have shown that many tissues have local 1- α -hydroxylase that can produce 1 α -25(OH)₂D that has both autocrine and paracrine effects (103, 106). Moreover, the discovery of VDRs in more than 35 tissues unrelated to bone metabolism demonstrates the pleiotropic effects of vitamin D (107). In fact, many observational studies have demonstrated inverse correlations between circulating 25(OH)D concentrations and the risk of extra-skeletal diseases (108-110).

Role of vitamin D in the thyroid

If vitamin D has a role in the thyroid, it is likely to be *via* its effect on the immune system and its role in dealing with infection. The potential for chronic infectious agents to be a causal factor for autoimmune disease has long been recognized (111). For instance, Epstein-Barr virus (EBV) is a ubiquitous herpes virus that is suspected to be involved in the pathogenesis of many autoimmune diseases (112). There is also some serological evidence of bacterial infection in patients with HT (113-115).

Multiple *in vitro* studies have provided compelling evidence that $1\alpha,25(\text{OH})_2\text{D}$, acting through the VDR, induces innate antimicrobial activity by regulating the expression of antimicrobial peptides (cathelicidin hCAP18 and defensin beta 4) that are responsible for extensive antimicrobial action (116-119) and the activation of antibacterial autophagy (120, 121). While $1\alpha,25(\text{OH})_2\text{D}$ is a promoter of innate immunity, it suppresses the adaptive immune response (116) by inhibiting the pro-inflammatory effects of Th1 and Th17 cells and enhancing the anti-inflammatory activities of Th2 and Treg cells (122-124). $1\alpha,25(\text{OH})_2\text{D}$ is believed to play a protective role against autoimmunity; on the one hand, it exerts special immunoregulatory and tolerogenic effects by hampering the maturation and autoantigen presentation of many dendritic-cell subsets (125), on the other, it increases the count of CD8⁺ T cells that are capable of controlling EBV infection and clearing EBV-infected autoreactive B cells (112).

Evidence for a relationship between intake/status of vitamin D and HT risk/treatment

Since excessive activation of Th1 and Th17 cells, as well as impaired function of Treg cells (6) and deficiency of CD8⁺ T cells (112) are implicated in the pathogenesis of HT, it is conceivable that vitamin D status may affect the development of this disorder.

Evidence from animal studies

In vivo studies showed that a low-dose combination of $1\alpha,25(\text{OH})_2\text{D}_3$ and cyclosporine could effectively prevent the induction of experimental autoimmune thyroiditis (EAT) in an mouse model of thyroiditis similar to human Hashimoto's thyroiditis (126). Injection of high-dose $1\alpha,25(\text{OH})_2\text{D}_3$ showed a therapeutic effect in established EAT rat models by improving

thyroid-gland structure and restoring Th1/Th2 cytokine equilibrium (127).

Evidence from human studies

Evidence from human studies is not strong; almost all studies were cross-sectional in nature and there are no randomized, controlled trials of vitamin D and thyroid autoimmunity. A number of case-control studies (128-137) reported lower mean levels of 25(OH)D as well as higher rates of vitamin D deficiency or insufficiency in HT patients than in healthy controls (see Table 2). Furthermore, subjects with vitamin D deficiency had a higher risk of developing HT than those with normal levels, e.g. for every 5 nmol/L increase in plasma 25(OH)D concentration, a 1.62-times decrease in HT risk was found (129). Moreover, inverse relationships of serum 25(OH)D concentrations with TPO-Ab and Tg-Ab titers in HT patients have been seen in a number of studies (130, 132, 133, 135, 136). With regard to thyroid function, data from a case-control study demonstrated that serum 25(OH)D status correlated inversely with thyroid-stimulating hormone (TSH) levels and positively with T3 levels in hypothyroid patients (134) while hypothyroidism at diagnosis was more prevalent in HT patients with serum 25(OH)D concentrations below 10 ng/ml (25 nmol/L) than in those within the normal range (138). In a comparison of chronic and new-onset HT patients and healthy controls, a clear association was found between the severity of vitamin D deficiency and disease duration, as well as a positive correlation between serum 25(OH)D levels and thyroid-gland volume in the patients (133).

However, two studies had different findings. In a case-control study, serum 25(OH)D concentration was found to be no lower in HT patients than in controls (139). More significantly, a longitudinal study failed to show lower vitamin D levels in women who developed TPO-Abs during follow-up than in those who remained thyroid-antibody negative, indicating a lack of relationship between early-stage thyroid autoimmunity and vitamin D insufficiency (140). Some of the divergence between study results might be attributable to differences in latitude, season, sunlight exposure, ethnicity, body-mass index, assay methods as well as inadequate matching between cases and controls with respect to confounding

factors that can affect vitamin D levels (139, 141, 142).

Could the association between low vitamin D status and HT be a result of ill health?

However, a more likely scenario may be that low concentrations of 25(OH)D in HT patients are simply a result of disease; for instance, in HT, increased body-fat mass due to hypothyroidism as well as other co-morbid autoimmune diseases may predispose to vitamin D deficiency (143). Low serum concentrations of 25(OH)D have been observed in many extra-skeletal diseases and may simply be a sign of ill-health (144). The validity of this hypothesis is strengthened by the fact that none of the numerous randomized trials carried out with vitamin D (though none was in HT) confirmed the health benefits of increased 25(OH)D, even when high doses of supplementation were given to participants with low vitamin D status prior to randomization (144, 145).

Could the association between low vitamin D status and HT be a result of vitamin D receptor (VDR) dysfunction?

Another explanation for the low serum 25(OH)D observed in autoimmune diseases, including HT, is VDR dysfunction in phagocytes resulting from chronic infection with intracellular bacteria that dysregulate vitamin D metabolism (111). Because the VDR controls expression of the cathelicidin and beta-defensin antimicrobial peptides, dysregulation of the receptor greatly compromises the innate immune response (113). Bacterial-induced VDR dysfunction can explain the low concentrations of 25(OH)D and high concentrations of 1,25(OH)₂D (111). Thus, in inflammatory conditions, unregulated extra-renal production of 1,25(OH)₂D occurs and escapes breakdown by binding to the pregnane X nuclear receptor (PXR), thus inhibiting the activity of the deactivating enzyme, 24-hydroxylase (CYP24A1), which would normally degrade it to a mono-hydroxy vitamin D [e.g. 25(OH)D] (111, 114). The end effect of this VDR dysfunction is lowered 25(OH)D, implying low vitamin D status as usually measured, and elevated 1,25(OH)₂D (though this is seldom measured). In other words, the low level of 25(OH)D observed in autoimmune disease is the result of the autoimmune disease process rather than its cause (113). In support of this explanation, there is evidence

that some autoimmune diseases can be reversed by gradually restoring VDR function with the administration of a VDR agonist, olmesartan, in conjunction with bacteriostatic antibiotics (111). Eight of nine genetic studies have implicated SNPs of both the VDR or 1-alpha-hydroxylase genes in HT risk, though results are not wholly consistent [(partly reviewed in (146) and see Supplemental Table 1].

Is vitamin D status adequate?

A review of six different geographical regions has demonstrated that vitamin D deficiency is widespread across the world (147). Although both the Institute of Medicine (148) and the European Food Safety Authority (EFSA) (149) consider that a dietary intake that achieves a serum 25(OH)D concentration of 50 nmol/L is sufficient, a number of organizations prefer to define sufficiency as the higher value of 75 nmol/L, values between 75 and 50 nmol/L as insufficient and those below 50 nmol/L as deficient (141). In most of the studies that have investigated the relationship between vitamin D and autoimmune thyroid disease, vitamin D deficiency has been defined as a serum 25(OH)D concentration less than 50 nmol/L (20 ng/ml). There are no available data on the optimal vitamin D concentration that can support diverse tissue responses, though it appears likely that local tissue levels need to be higher than typical serum levels (139). Locally synthesized $1\alpha,25(\text{OH})_2\text{D}_3$ has been shown to be degraded immediately after its autocrine action without entering the circulation, hence even measurements of serum $1\alpha,25(\text{OH})_2\text{D}_3$ may not be meaningful (139).

Recommendations for vitamin D

It appears highly questionable that the low serum/plasma 25(OH)D concentration in HT patients is a true reflection of a deficient vitamin D status, let alone that vitamin D deficiency is a cause of HT. Studies need to measure serum/plasma concentrations of not only 25(OH)D but of $1,25(\text{OH})_2\text{D}$ and indeed of $24,25(\text{OH})_2\text{D}$, to get a clearer picture. Even then, the concentrations in thyroid and immune cells will not necessarily be revealed. Trials are needed to elucidate the association between vitamin D and HT so that clear evidence-based suggestions can be made. In the meantime, however, it would be wise to ensure that patients

avoid overt vitamin D deficiency.

However, be aware that at high levels (e.g. achieved from supplementation), 1,25(OH)₂D may have the potential to displace the natural ligands from nuclear receptors such as the α - and β -thyroid hormone receptors (111, 113). If T3 is indeed displaced from the thyroid hormone receptors, there may be adverse effects on the endocrine system. New data also show that the greater the increase in 25(OH)D on supplementation, the greater the conversion to the inactive 24,25(OH)₂D, resulting in a null effect (150).

Conclusions

HT affects more people than any other autoimmune condition. Hence, awareness of the nutritional factors discussed above that can interact to alter the risk, progression or development of HT or associated conditions can provide an additional strategy in the hands of concerned clinicians to the benefit of a large number of patients.

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Table 1. Iodine intake requirements by life stage according to various authorities

Age	EFSA AI (µg/d) (24)	USA RDA (µg/d) (25)	ICCIDD/UNICEF/WHO RNI (µg/d) (26)
0-6 mth	-	110 (AI)	90
7-12 mth	70	130 (AI)	90
1-6 yr	90	90	90
7-10 yr	90	90-120	120
11-14 yr	120	120-150	120-150
15-17 yr	130	-	-
15-50 yr	-	150	150
≥ 18 yr	150	-	-
Pregnancy	200	220	250
Lactation	200	290	250

Abbreviations: AI, Adequate Intake; RDA, Recommended Dietary Allowance; RNI
Recommended Nutrient Intake.

Table 2. Case-control studies of the association between vitamin D status and HT risk

Author & reference	Year	Country	Participants	Time of Sampling	Main Indices	Significance
Evliyaoglu et al. (128)	2015	Turkey	90 HT patients and 79 healthy controls	Not specified	Vit D deficiency rate 25(OH)D concentration	P = 0.025 P = 0.001
Ma et al. (129)	2015	China	70 newly diagnosed HT patients and 70 controls	Winter	25(OH)D concentration	P < 0.001
Shin et al. (130)	2014	Korea	111 AITD patients and 193 non-AITD patients	Throughout the year	25(OH)D concentration	P < 0.001
Mansournia et al. (131)	2014	Iran	41 hypothyroid HT patients and 45 euthyroid controls	Autumn	25(OH)D concentration	P = 0.008
Unal et al. (132)	2014	Turkey	254 newly diagnosed HT patients and 124 healthy controls	Not specified	25(OH)D concentration	P < 0.001
Bozkurt et al. (133)	2013	Turkey	180 chronic HT patients, 180 newly onset HT patients, and 180 healthy controls	Not specified	25(OH)D concentration Severe vit D deficiency rate (<10 ng/mL)	P = 0.002 P < 0.001
Mackawy et al. (134)	2013	KSA	30 hypothyroid patients and 30 healthy controls	Autumn, Winter and Spring	25(OH)D concentration	P = 0.000
Camurdan et al. (135)	2012	Turkey	78 recently diagnosed children HT patients and 74 controls	Not specified	25(OH)D concentration Vit D deficiency rate	P < 0.001 P < 0.0001
Kivity et al. (136)	2011	Hungary	50 AITD patients (28 HT, 22 GD), 42 non-AITD patients and 98 healthy controls	Spring	Vit D deficiency rate TPOAb-positive rate	P < 0.001 P < 0.001
Tamer et al. (137)	2011	Turkey	161 HT patients and 162 healthy controls	Not specified	Vit D insufficiency rate	P < 0.0001

Abbreviations: AITD, Autoimmune Thyroid Disease; GD, Graves' Disease; HT, Hashimoto's Thyroiditis.

Figure legends

Figure 1. GPXs catalyse the removal of H₂O₂ (and lipid hydroperoxides) converting it to harmless water thus protecting the thyroid from excessive exposure to H₂O₂

Figure 2. Selenium protects against post-partum autoimmune thyroid disease [adapted from (56) with permission]

Figure 3. Mean selenium intake levels (µg/d) in different countries and the range of Se intake (55-75 µg/d) believed to be required for optimal activity of plasma GPX (GPX3) [adapted from (58)]

Figure 4. Typical selenium content of food sources, adapted from WHO. Selenium. A report of the International Programme on Chemical Safety. Environmental Health Criteria number 58. Geneva: WHO, 1987 (reproduced from 28).

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Supplemental Table 1. Case-control studies of the association between polymorphisms of the *VDR* and *CYP27B1* genes and HT risk

Author	Year	Country	Participants	Gene	SNP	Significance
Guleryuz et al.	2016	Turkey	136 HT patients and 50 healthy controls	VDR	TaqI FokI	Significant NS
Giovinazzo et al.	2016	Italy	100 newly diagnosed HT patients and 100 healthy controls	VDR	Bsml, Apal, TaqI	NS
Djurovic et al.	2015	Serbia	44 female HT patients and 32 healthy controls	VDR	FokI	P = 0.009
Inoue et al.	2014	Japan	116 HT patients and 76 controls	VDR	FokI	P = 0.0174
Yazici et al.	2013	Turkey	111 HT patients and 159 healthy controls	VDR	TaqI, FokI	Significant
Stefanic et al.	2008	Croatia	145 HT patients and 145 euthyroid controls	VDR	Bsml BB Bsml-TaqI BT haplotype Bsml-TaqI bT haplotype Bsml-Apal-TaqI baT haplotypes Bsml-Apal-TaqI BaT variants	Pc = 0.0052 Pc = 0.0008 Pc = 0.0004 Pc = 0.012 Pc = 0.0012
Lin et al.	2006	China	109 HT patients and 90 healthy controls	VDR	FokI	P = 0.0458
Yang & Xiong	2008	China	171 HT patients and 172 healthy controls	CYP27B1	CYP27B1 promoter (-1260) C/A	P < 0.05
Lopez et al.	2004	Germany	139 HT Patients and 320 healthy controls	CYP27B1	CYP27B1 intron 6 (+2838) C/T CYP27B1 promoter (-1260) C/A	P = 0.0058 P = 0.0173

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985 Abbreviations: VDR, vitamin D receptor; CYP27B1, vitamin D activating enzyme, 1- α -hydroxylase; HT, Hashimoto's Thyroiditis; NS, Not Significant;

986 Pc=Permutation-corrected P-value (corrected for multiple testing); SNP=Single Nucleotide Polymorphism; VDR, Vitamin D Receptor.

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