The role of iodine in the evolution of thyroid disease in Greece: from endemic goiter to thyroid autoimmunity

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ABSTRACT
The thyroid gland is dependent on dietary iodine for the production of thyroid hormones, normal iodine requirement being about 150-200μg/day. Long-term deficiency in iodine intake is associated with the development of goiter. When the prevalence of goiter in a population rises above 5-10%, the problem is considered endemic. Greece is a country with a recent history of moderate iodine deficiency, endemic goiter being prevalent in the 1960s in inhabitants of mountainous regions. Despite recognition of the problem, an iodine prophylaxis program was never officially implemented. Instead, “silent iodine prophylaxis” took place during the 1980s and 1990s with Greece’s improvement in socioeconomic conditions. This resulted in the elimination of iodine deficiency and a parallel decrease in the prevalence of goiter among schoolchildren in formerly iodine deficient areas. However, the transition from iodine deficiency to iodine sufficiency or excess was followed by the emergence of autoimmune thyroiditis, especially among young girls, indicating that exposure to excess iodine may trigger thyroid autoimmunity. Thus, the modification of an environmental factor, ie dietary iodine, over the last 40 years in Greece has been associated with changes in the phenotypic expression of thyroid disease from endemic goiter to goiter associated with autoimmune thyroiditis.

Key words: Autoimmune thyroiditis, Goiter, Iodine deficiency, Thyroid.

INTRODUCTION
Thyroid hormones play an important role via their impact on the growth and maturation of a variety of tissues as well as their metabolism. Thyroid function is controlled by the thyroid stimulating hormone (TSH) whose main role is the stimulation of the biosynthesis and secretion of the thyroid hormones. TSH binding to its receptor on the basolateral membrane of thyroid follicular cells activates intracellular signaling pathways that regulate thyroid cell function and growth. The synthesis of thyroid hormones requires the entry of adequate quantities of iodine into the thyroid. Iodine enters the thyroid in the inorganic form as iodide that is derived either from food, water and drugs, or from the deiodination of thyroid hormones. A daily dietary iodine intake of approximately 150-200μg is considered normal and sufficient to sustain a plasma iodide concentration of
0.5 μg/dl and a urinary excretion greater than 100 μg/g creatinine (Cr).\textsuperscript{1,2}

Iodine is actively co-transported, together with two sodium ions, into the thyroid cell via an intrinsic plasma membrane protein termed the Na\textsuperscript{+}/I\textsuperscript{−} symporter (NIS).\textsuperscript{3} Energy for this process is provided by oxidative metabolism in the gland. Subsequently, iodine is translocated across the apical membrane of the thyroid follicular cell where, following oxidation by thyroid peroxidase (TPO), it becomes capable of binding to tyrosyl residues in thyroglobulin (Tg). This results in the formation of the Tg-bound monoiodotyrosine (MIT) and diiodotyrosine (DIT). The coupling reaction of the iodotyrosines, which occurs within the Tg molecule, leads to the formation of triiodothyronine (T\textsubscript{3}) and thyroxine (T\textsubscript{4}). Thyroid hormones on Tg are then stored in the colloid until the complex is taken up by thyrocytes, hydrolyzed by proteases, and free hormones are released into the circulation.\textsuperscript{4}

Insufficient intake of dietary iodine over a long period of time is associated with the development of goiter. This is due to the dependence of the thyroid gland on dietary iodine intake in order to maintain plasma inorganic iodine within normal limits, since there are no renal iodine homeostatic mechanisms.\textsuperscript{5}

The term “goiter” refers to a non-neoplastic enlargement of the thyroid gland, sometimes visible as an anterior neck swelling. A person is described as having a goiter if on clinical examination the size of the thyroid unilaterally exceeds the size of his/her distal thumb phalange. However, more precise assessment of the size and morphology of the thyroid gland is done by ultrasonography. Upper limits have been set by the World Health Organization (WHO) in order to evaluate thyroid volume, depending on the sex, age and body surface area (BSA).\textsuperscript{5} Increase in body weight and height during growth is followed by increase in the dimensions of the thyroid gland, at least until adolescence is completed. Furthermore, the size of the thyroid gland is also related to various racial characteristics and nutritional habits. The use of BSA was introduced in order to diminish these differences among diverse populations. Thyroid volume greater than 18 ml in adult males and 14 ml in adult females is considered abnormal. These numbers should not, however, be considered as stringent, but rather as an estimate of the actual normal size of the thyroid gland.\textsuperscript{7}

In this review we discuss the role of an enviromental factor, dietary iodine, in the evolution of dominant thyroid disease in Greece over the last 40 years as a paradigm of transition from an iodine deficiency to an iodine sufficiency era. We present epidemiological evidence for the transition from a moderate iodine deficiency state causing endemic goiter to iodine sufficiency,\textsuperscript{8} associated with thyroid autoimmunity. This is preceeded by some general information on the classification and etiology of goiter and is followed by analysis of the possible mechanisms by which iodine may induce thyroid autoimmunity.

**GOITER CLASSIFICATION**

The classification of goiter is based on its endemic or sporadic nature, the morphology (diffuse or nodular goiter) of the thyroid parenchyma and its functional status (non-toxic or toxic goiter).

Goiter is primarily classified into endemic and sporadic form. The characterization of a region as endemic for the development of goiter is based on the prevalence of goiter in its population. When the prevalence of goiter in a population rises above 5-10%, then the term endemic goiter is used.\textsuperscript{2,9} Iodine deficiency is widely known to be the main cause of endemic goiter. Sporadic goiter is defined as a goiter occurring in an iodine-replete area. It is rare (up to 4%) and endogenous factors are implicated in its pathogenesis.\textsuperscript{9}

Nodular goiter covers a spectrum from uninodular to multinodular goiter. Multinodular goiter is characterized by the presence of multiple polyclonal thyroid nodules that are structurally and functionally heterogeneous.\textsuperscript{5} In diffuse goiter, homogenous hyperplasia and hypertrophy of the thyroid cells takes place, leading to a diffusely enlarged thyroid gland. With time, however, diffuse goiters also tend to form nodules.\textsuperscript{10} The natural history of nodular goiter is towards a gradual increase in size and functional autonomy. Thus, euthyroidism (non-toxic goiter) may gradually change to subclinical hyperthyroid-
ism and eventually to overt hyperthyroidism (toxic nodular goiter).11,12

Finally, when thyroid enlargement is accompanied by the presence of thyroid autoantibodies, the goiter is part of autoimmune thyroid disease. This can manifest as Graves’ disease with diffuse hyperplasia plus hyperthyroidism or Hashimoto’s thyroiditis with lymphocytic infiltration of the gland and hypothyroidism.10

**ETIOLOGY OF GOITER**

Both environmental and genetic factors may contribute to the development of goiter. Dietary iodine deficiency is the main cause of endemic goiter worldwide. On the other hand, enzymatic defects impairing thyroid hormone synthesis or other endogenous factors may play a role in the pathogenesis of sporadic goiter.13 Even though susceptibility genes have not yet been identified, cases of familial aggregation provide evidence of a genetic component.14 Genetic studies have excluded Tg, TPO and NIS gene mutations as a major cause of euthyroid goiter but have implicated activating mutations in the TSH receptor (TSHR) and Gs-alpha (Gsα) genes and have identified a locus for familial multinodular nontoxic goiter (MNG-1) on chromosome 14q.15-17 All these factors acting on thyroid cells, characterized by a constitutive heterogeneity in responding to various stimuli, may lead to focal thyroid cell hyperplasia and eventually to nodular thyroid disease.

**Environmental Factors**

**Iodine deficiency**

Iodine deficiency is the main pathogenetic factor contributing to endemic goiter. The observation that dietary iodine shortage is directly associated with the development of goiter has a history of many decades. Lack of iodine acts indirectly, through increased TSH secretion induced by decreased thyroid hormone production, or directly, through local feedback mechanisms within the thyroid gland. This may provide the stimulus for a subpopulation of rapidly responding thyroid cells to undergo proliferation leading to focal thyroid cell hyperplasia.13

The incidence of goiter increases in line with the increase in distance from the sea and is inversely related with the iodine content in soil. Long lasting periods of intense glaciation are known to leach iodine out of the soil. Similarly, the points at which continental tectonic plates collide are sites of major mountain system formation and intense geologic activity, where the newly formed soils are relatively poor in iodine. The major source of soil iodine in such areas is iodine-containing rain derived from seawater evaporation. Inhabitants of such regions predominantly consume local food and water, inadequate in providing the daily requirement of iodine.18,19 Furthermore, lack of easy road transportation and poor economic conditions prevent these inhabitants from obtaining imported goods. On the other hand, iodine deficiency is less likely to occur in larger cities where foodstuffs are imported from a much wider geographic area. For the same reason, upper socioeconomic classes are less likely to develop goiter because of their more varied diet. Thus, the areas most severely affected are remote mountainous regions away from rural centers.20,21

**Other environmental goitrogens**

Apart from iodine, the impact of less common environmental factors cannot be excluded. The thyroid is a gland considered highly vulnerable to exogenous agents. Chemical contamination may therefore play a modulatory role in the development of goiter. Any substance, synthetic or natural, that interferes with the biosynthesis of thyroid hormones is considered as a potential goitrogen. A variety of synthetic chemical compounds possesses thyroid disruptor properties and may influence thyroid function. Such chemical agents include polychlorinated biphenyls (PCBs), organophosphate and organochlorine pesticides, dioxin-byproducts of organochlorine chemicals and thiocyanate. The combined effect of multiple toxic exposure during the perinatal period can permanently alter the pituitary-thyroid axis, thereby affecting thyroid function.22-25

Thyroid disruptor properties have also been attributed to several plant derived substances. Flavonoids for example interfere with the synthesis of thyroid hormones by inhibiting TPO.26,27 Millet is rich in C-glycosylflavones and in areas where it is a major dietary component, its ingestion may contribute to the genesis of endemic goiter.28,29 The high rate of
Mutations in the TSH signal transduction pathway

The molecular mechanisms that generate thyroid nodules are still poorly understood, though activating mutations in the TSH signal transduction pathway have been established as a possible and most common pathogenetic mechanism underlying thyroid autonomy. Activating mutations in the TSHR and Gsa gene have been found in a large number of autonomously functioning nodules, while exposure of the population to iodine deficiency has been suspected of favoring the occurrence of these mutations.

Activating somatic mutations in the TSH signaling pathway are frequent in autonomous nodules in patients with endemic goiter. These mutations result in uncontrolled signaling through the TSH receptor that is likely to cause hyperfunction and proliferation. This supports the evidence that toxic thyroid nodules seem to originate from small autonomous areas in iodine-deficient euthyroid goiters containing a TSHR mutation.

Gsa protein is the product of a gene of the cAMP regulatory cascade which mediates the activation of adenylate cyclase after TSH binding to its receptor and thus increases intracellular cAMP. Activating mutations of Gsa leading to constitutive activation of this cascade have also been detected in many autonomous nodules in patients with nodular goiter.

Activating Gsa and TSHR mutations have been found in many subtypes of autonomous nodules but not in non-functioning nodules. Certainly, the recent detection of activating mutations in the TSHR and the Gsa in nodules may explain their hyperfunction and transformation from non-toxic to toxic goiter, but these mutations are unlikely to be the primary cause of goiter formation.

TSH stimulating immunoglobulins

Other factors implicated in the pathogenesis of goiter are TSH stimulating immunoglobulins (TSI) which cause hyperplasia of the thyroid follicular cells through activation of the TSHR. Such im-

endogenous and genetic factors

natural heterogeneity of thyroid cells

Another aspect of goitrogenesis is that of the heterogeneity among the individual thyrocytes themselves. This is based on evidence that the process of goitrogenesis may also operate through mechanisms involving the heterogeneity of growth and function of a variable, genetically predetermined fraction of thyrocytes. Individual thyrocytes are characterized by heritable metabolic and functional differences. Some of these cells also display high intrinsic growth potential. In this respect, the concept of naturally occurring heterogeneity of thyroid cells provides a plausible explanation for the early stages of nodular formation. If a thyrocyte is affected by overexpression of a growth factor or hit by other molecular events, the cell may start operating autonomously. Furthermore, acquisition of new heritable features during cell proliferation as well as disorders in cell to cell communication within the follicle could result in the dysfunction of the follicle as a well adjusted unit. It could be assumed that goitrogenesis ensues from the natural heterogeneity which, when amplified by certain stimuli, results in the development of nodules.
munoglobulins are the TSHR stimulating autoantibodies found in Graves’ disease.\textsuperscript{46,47} Recently, the production of such human monoclonal autoantibodies with powerful TSHR stimulating activity and all the characteristics of serum TSHR autoantibodies was achieved.\textsuperscript{48,49}

It is likely that when a mild stimulus (e.g. mild iodine deficiency, slight TSH increase) acts on this heterogeneous group of cells, only the most sensitive of the thyrocytes with the highest growth potential will respond, resulting in focal hyperplasia. If, on the other hand, the goitrogenic stimulus is strong and persistent (TSI), then the whole population of cells undergo replication leading to diffuse thyroid hyperplasia.

The role of growth factors

Increased expression of local growth factors (IGF-1, IGF-2, FGF and EGF) can promote cell hyperplasia through their autocrine and paracrine action. There is also compelling evidence that an imbalance in the interactions between the various growth factor axes exists in multinodular non-toxic goiter, which may favor cell replication.\textsuperscript{50}

Studies on goitrous mice showed that increased expression of growth factors and their receptors occurs and that complete inhibition of goiter requires combined gene therapy modification of angiopoietin, vascular endothelial growth factor and fibroblast growth factor signaling.\textsuperscript{51,52} By reducing the action of some of these growth factors, alone and in combination, goitrogenesis in mice might also be reduced.\textsuperscript{53}

EVOLUTION OF THYROID DISEASE: THE ROLE OF IODINE

From endemic goiter to thyroid autoimmunity

The studies on endemic goiter in Greece were first reported in the 1960s.\textsuperscript{54-56} Attention was drawn to the areas more severely affected, which were scattered all over the country but shared a common characteristic. They were all remote, mountainous regions, distant from the sea and cities, and depended on locally produced food.

The poor economic and social conditions, in conjunction with lack of road infrastructure that typified Greece during this era, prevented the inhabitants from buying food products enriched in iodine. Thus, the iodine content of various food items was low in endemic regions.\textsuperscript{59} These areas included the mountainous territories of Northwestern Greece, Thessaly and Crete.

Metabolic studies, first conducted 40 years ago, showed that plasma inorganic iodine and the urinary iodine excretion in endemic areas were low, thereby establishing iodine deficiency as the main cause of endemic goiter. The prevalence of goiter among schoolchildren was estimated to be as high as 40-60% in certain mountain villages of Northwestern Greece and the median urinary iodine concentration in schoolchildren was 17.1μg/g Cr. Even in Athens in 1964, the median urinary iodine concentration in schoolchildren was low (45.5μg/g Cr).\textsuperscript{55,56} Following these surveys, and according to WHO criteria, Greece was characterized as a country with moderate iodine deficiency. However, a national iodine prophylaxis program was never officially implemented.

In the 1980s the situation was re-evaluated. New data showed that even though no official measures had been taken to implement an obligatory program of salt iodination, dietary iodine intake had improved. The median urinary iodine concentration in Athens was found to be 94μg/g Cr.\textsuperscript{57} This improvement should be attributed to the so-called “silent iodine prophylaxis” resulting from improvement in socioeconomic conditions. This allowed easier access to imported food products richer in iodine. In the meantime, iodized salt became available on the market and, more importantly, the public became aware of the endemic goiter problem and the necessity of iodine supplementation.

Studies that followed confirmed that iodine supplementation resulted in a notable decrease of goitrous children. Median urinary iodine excretion in Athens was estimated at 208μg/g Cr in 1992 and 204μg/g Cr in 1999. The mean prevalence of goiter in schoolchildren from 17 different regions in Greece reached 12.5% with a urinary iodine excretion fluctuating between 43-167μg/g Cr in previously endemic areas, while in areas without endemic history the numbers were 1.7% and 95-191μg/g Cr, respectively.\textsuperscript{58}
Studies which took place in the Epirus region of Northwestern Greece confirmed the decrease in goiter prevalence and the rise in urinary iodine excretion. In 1994, the median iodine urinary concentration was 84μg/g Cr in goitrous schoolchildren, while the overall goiter prevalence was 21%. By the year 2001, the mean iodine urinary concentration increased to 202μg/g Cr and the goiter prevalence had fallen dramatically to 5%. These findings indicate that, even in formerly endemic areas, iodine deficiency is no longer a problem in Greece.

Although iodine deficiency was eliminated through “silent iodine prophylaxis”, it was followed by the emergence of goitrous autoimmune thyroiditis, especially in young people. Thus, Doufas et al. performed FNA biopsies looking for signs of lymphocytic infiltration, indicative of thyroid autoimmunity, and measured thyroid autoantibodies (anti-Tg, anti-TPO). Their results showed that the percentage of non-toxic goiter patients with markers for thyroid autoimmunity increased from 5.9% during the period 1985-1986 to 13.9% in the years 1994-1995. The prevalence of positive thyroid autoantibodies in non-toxic goiter patients was found to be 61.6% in Athens and 58.5% in Crete.

Similar observations were made in schoolchildren in Northwestern Greece. The criteria used for the diagnosis of autoimmune thyroiditis were the thyroid ultrasonographic pattern plus the titer of thyroid autoantibodies. The overall prevalence of autoimmune thyroiditis among schoolchildren increased to 9.6% in 2001 from 3.3% in 1994. Furthermore, the children with positive thyroid antibodies had higher levels of urinary iodine concentration compared to those with negative antibodies.

These findings led to the assumption that iodine is implicated in triggering or enhancing thyroid autoimmune, at least in the formerly iodine deficient areas. This phenomenon has also been observed in other formerly iodine deficient countries where the transition to sufficient or excessive iodine intake was followed by an increase in the incidence of thyroid autoimmunity. It should, however, be mentioned that other studies found no supporting evidence of autoimmunity induction after iodine administration to correct iodine deficiency. In the case of Morocco, salt iodination and administration to iodine-deficient schoolchildren resulted in a transient increase in the prevalence of detectable antibodies but levels returned to baseline after 1 year and no child developed clinical or ultrasonographic evidence of thyroid autoimmune disease.

Furthermore, thyroid autoimmunity may also evolve during the course of iodine prophylaxis as was elaborated by two consecutive studies among schoolchildren in Sri Lanka. The authors reported the appearance of thyroid autoantibodies at high frequency among schoolchildren within 5 years of the introduction of a universal salt iodination program in Sri Lanka. The predominant antibodies were anti-Tg, whereas anti-TPO antibodies were present in less than 10% of the children. Intrestingly, 3 years later there was a significant reduction in the prevalence of thyroid antibodies and the predominant antibodies were now anti-TPO. Subclinical hypothyroidism was more prevalent in antibody-positive subjects.

On the basis of these observations, the hypothesis of evolving thyroid autoimmunity during iodine prophylaxis was put forward. This includes an early phase during which anti-Tg antibodies appear at high frequency. In this phase, the highly iodinated Tg may become immunogenic, triggering off an autoimmune reaction against the thyroid gland. This phase appears to be reversible, with anti-Tg antibodies disappearing in half of the cases. The remainder manifest anti-TPO antibodies and may go on to develop autoimmune thyroiditis. During the second phase, the continuing exposure to iodine may play an immunomodulatory role propagating thyroid autoimmunity. The presence of anti-TPO antibodies may predict the future development of thyroid dysfunction. In this context, the finding of our studies in Northwestern Greece may reflect the second phase in the natural course of thyroid autoimmunity.

**Role of iodine in the induction of thyroid autoimmunity**

Iodine is considered an important environmental agent in the induction of thyroid autoimmunity. In 1983, Boukis et al reported the emergence of thyroid autoantibodies, both against thyroglobulin and the microsomal antigen, in 42.8% of 58 goitrous patients from a mildly iodine-deficient area in Greece who...
had received iodized oil im. Clinical studies have also linked iodine intake with the appearance of thyroid autoantibodies and the initiation and promotion of thyroid autoimmunity. In addition, experimental studies in autoimmune-prone animal models, fed with iodine-rich diets and tested for signs of thyroiditis and thyroid autoantibodies, have confirmed that intake of excessive quantities of iodine can accelerate autoimmune thyroiditis. Human studies regarding the effect of iodine administration in subjects with endemic goiter showed development of thyroid autoantibodies in approximately 8-20% of the patients, depending on the dose, as well as an increased intrathyroidal lymphocytic infiltration in a number of patients. Nevertheless, these findings were transient and after discontinuation of iodine, antibody titers and lymphocytic infiltration decreased significantly.

Iodine is found in a number of dietary products including sea-food and food additives (kelp and seaweed, iodinated salt, iodine additives in bread, flour, preservatives and red coloring). Therapeutic use of iodine rich compounds such as amiodarone, vitamins, local antiseptics and iodinated radiographic contrast dyes can also lead to the release of large amounts of iodine. Amiodarone contains 37% iodine. This commonly used antiarrythmic drug can impair thyroid function and cause clinical hyperthyroidism or hypothyroidism in 2-10% of patients.

The mechanisms by which excessive iodine is related to the development of thyroid autoimmunity are as yet unknown but several hypotheses have been put forward. Intake of large iodine quantities results in its increased incorporation in the Tg molecule. This highly iodinated Tg is characterized by alterations in its stereochemical conformation. The modifications that occur in Tg structure can change its properties, leading to loss of antigenic epitopes and creation of novel, iodine containing ones. New antigenic determinants may be created by tyrosine iodination at critical points within the Tg molecule. When presented to T and/or B lymphocytes, these new determinants exhibit an increased affinity for the T cell receptor or the MHC-presenting molecule on antigen-presenting cells (APCs). This may consequently enhance the Tg presentation by APCs and lead to specific T lymphocyte activation, thereby initiating the autoimmune process. Excessive iodination of Tg can thus heighten its immunogenic potential compared with Tg containing fewer iodine atoms.

Another suggested mechanism is direct iodine toxicity to thyrocytes, possibly through induction of oxidative stress. Excessive amounts of iodine may comprise a direct threat for thyrocytes. TPO rapidly oxidizes excessive amounts of iodine in the hyperplastic thyrocytes and generates oxidative intermediates of iodine. These oxidative elements are highly reactive and able to bind to proteins, nucleic acids and membrane lipids, forming iodoconjugates which damage thyroid cell and mitochondrial membrane integrity. Oxidative stress caused by the generation of free radicals can also lead to thyroid cell necrosis, while autoantigens may be released. Excessive iodine intake is also related to the induction of thyrocyte apoptosis and the development of thyroid autoimmunity.

Iodine may also exert a direct stimulatory effect on cells of the immune system, in particular APCs such as macrophages and dendritic cells. There is increasing evidence that changes in the microenvironment within the thyroid by iodine excess may affect the function of APCs which in turn influences the class of T-cell development and the type of immune (T or B cell) responses. However, more studies are required to clarify the role of iodine excess in triggering thyroid autoimmunity.

**SYNOPSIS AND CONCLUSIONS**

The present review has discussed the evolution of thyroid disease in Greece in relation to changes in dietary iodine intake, as a paradigm of how changes in an environmental factor may influence the phenotypic expression of thyroid disease. Epidemiological surveys in the early 1960s revealed a high prevalence of goiter among inhabitants in the mountainous areas of Greece. The prevalence of goiter in schoolchildren was as high as 40-60% and the median urinary iodine concentration was low (17.1 μg/gCr), indicating that iodine deficiency was the main cause of endemic goiter in Greece. Despite this information, an iodine prophylaxis program was never implemented by the State. Instead, dietary iodine intake improved gradually in parallel with the improvement in so-
cioeconomic conditions during the following three decades. The elimination of iodine deficiency by the late 1990s, through “silent iodine prophylaxis”, was followed by the disappearance of endemic goiter. However, the transition from iodine deficiency to sufficient or excess iodine intake was associated with the emergence of thyroid autoimmunity, especially in young women.

In conclusion, over the last 40 years we have witnessed a change in the phenotypic expression of thyroid disease in Greece that runs parallel with the changes in the availability of dietary iodine (Figure 1). Although iodine in excess may be the main reason for the current rise in thyroid autoimmunity, other as yet unknown environmental factors may have contributed. These remain to be identified.

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