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Review

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Global view on the pathogenesis of benign thyroid disease based on historical, experimental, biochemical and genetic data, identifying the role of magnesium, selenium, coenzyme Q10 and iron in the context of the unfolded protein response and protein quality control of thyroglobulin

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Abstract

We conducted a global review on the characteristics of benign thyroid disease beginning with the original clinical descriptions by Riedel, de Quervain and Hashimoto. A special section describes the attempts to fit human thyroid disease into animal models. The main emphasis is set on thyroglobulin including descriptions of isolation procedures and identification of molecular variations. The next element in this review describes the genetic knowledge about thyroglobulin synthesis and processing, showing that several mutations can result in a misfolded thyroglobulin molecule. This fact identifies the endoplasmic reticulum as a key element. Up to this point, thyroid disease still appears to be invincible. We proceeded to describe our own clinical experiences empirically aimed at improving mitochondrial function, i.e., ATP generation. We demonstrate several mechanisms, e.g., heat, stress and pregnancy, that can lead to an inflammatory thyroid condition. These changes can be reversed by supplementation with magnesium, selenium and coenzyme Q10. Finally, we reveal a functional relation between magnesium and anti-thyroglobulin antibody levels such that the antibodies disappear when magnesium levels normalize. We conclude that benign thyroid disease has the characteristics of an acquired mitochondrial dysfunction, which

consequently compromises the function of the endoplasmic reticulum because of limited ATP supply. Altered thyroglobulin has the potential to change thyroid function on the basis of its autoregulatory thyroidal property. At the same time, it can be a stimulus for the production of anti-thyroglobulin antibodies. We propose a general applicability of this concept to beta-cell and ovarian function where compromised function of the endoplasmic reticulum can be expected.

Keywords: Thyroid diseases, thyroiditis, magnesium, selenium, coenzyme Q10, power Doppler sonography, thyroglobulin

INTRODUCTION

Our commitment to medicine and medical research has always been inspired by the teachings and writings of many colleagues which we would like to present at the beginning. These research hints have paved our professional work through decades of clinical research. We have always looked back into historical accounts and have tried to bring them into the future. In a detailed narrative fashion, the reader will be confronted with historical data and valuable clinical descriptions as well as with advanced genetic investigations in the age of Precision Medicine.

Gregorio Marañón y Posadillo[1] - Spanish endocrinologist, born 1887, died 1960.

«Vivir no es solo existir, sino existir y crear, saber gozar y sufrir, y no dormir, sino soñar. Descansar es empezar a morir»

«Living is not just existing, but existing and creating, knowing how to enjoy and suffer, and not sleeping, but dreaming. To rest is to start to die»

Albert Szent-Györgyi (Bioenergetics, page 57 in[2]), Hungarian biochemist, born 1893, died 1986.
«Research is to see what everybody has seen and think what nobody has thought»

Christian Albert Theodor Billroth, German surgeon, born 1829, died 1894.

«Only the man who is familiar with the art and science of the past is competent to aid in its progress in the future»

A practical synthesis will consolidate the fundamental biochemical pieces into a general model of disease that is centered on the function of the endoplasmic reticulum and mitochondria. This concept will be demonstrated for thyroid diseases taking thyroglobulin as the main indicator. It will also be applied to diabetes and to reproductive physiology.

THE MAIN PLAYERS: GENETICS IN THE TIME OF PRECISION MEDICINE AND THE WOMED MODEL OF BENIGN THYROID DISEASE

With the introduction of precision medicine in 2015[3], research actions of the medical community have turned towards genetic methods to advance the understanding of mechanisms of disease, hoping to improve therapy options.

Long time before genetic details could be envisioned, medical research on thyroid disease had evolved according to the existing techniques since the 1890s. Early researchers investigating goiter concentrated on the clinical description of cases including their evolution and surgical therapy. Since the 1950s, experimental researchers have attempted to develop animal models that could resemble human disease, e.g., thyroiditis. In 1956, Danziger and Elmergreen presented a model of a homeostatic mechanism of the

thyroid-pituitary relation based on a mathematical negative-feedback model[4], thus paving the way for the use of laboratory methods.

Over the past decades, developments of diagnostic methods have significantly improved and expanded the scope of clinical work. This has been achieved through accurate laboratory tests and imaging procedures. In spite of these advancements, subclinical thyroid disease was still being simply described as a biochemical condition in 2019[5]. Clinicians concerned about the recommendations launched by Bekkering[5] commented: "Thus, urgent search action is needed, not clinical inaction"[6], a comment that can be applied to the numerous clinical practice guidelines.

Our clinical work on benign thyroid diseases over the past 15 years has avoided inaction by introducing an advanced ultrasound methodology with 3D power Doppler imaging[7] and has gone through a step-by-step inclusion of additional laboratory parameters related to the new aspects found in sonography[8]. Power Doppler examination has allowed us to characterize an inflammatory condition common to subacute thyroiditis, hypothyroidism or hyperthyroidism related to a compound deficiency state affecting magnesium, coenzyme Q_{10} (CoQ_{10}) and selenium levels. Our observational research results have allowed us to propose a model of acquired mitochondrial dysfunction as the central event in thyroid disease. Consequently, we designed a therapy concept aimed at clinical improvement of mitochondrial function[8,9]. This therapy can reverse the hypo-echogenic changes of the thyroid and also correct the inflammatory hyperperfusion of the gland[10]. Full therapy success needs at least 18 to 24 months of supplementation. Unfortunately, numerous clinical practice guidelines concentrate on hormone determinations, leaving no place for ultrasound or power Doppler.

The aim of this review was to look back in time and to analyze key findings in the field of benign thyroid diseases. By reading and extracting the original publications, we summarized historical, clinical, experimental, genetic and biochemical data. The synthesis of these findings will be used to further develop a new pathogenesis concept of thyroid disease based on the relation between magnesium levels and anti-thyroglobulin antibodies. This will bring us to a description of thyroglobulin synthesis including both specific and general aspects of the unfolded protein response as well as of the heat shock response.

Basic concepts of thyroid disease from Bernhard Riedel to Fritz de Quervain to Robert McCarrison and to Hakaru Hashimoto

In 1896, Riedel[11] presented his report on a special type of thyroid affection which was later called after him, Riedel thyroiditis. The publication started with the mention of 2 previous cases of chronic pancreatitis which presented as a large tumor surrounding the bile duct. Histological examination revealed chronic inflammation. With this background information, he then described 2 special cases of goiter which had been identified after having conducted thyroid surgery in 300 cases over 12 years. The first patient was a 42-year-old man who had noticed an enlargement of the thyroid over the past 6 months. The enlargement of the thyroid resulted in symptoms of local compression. The goiter firmly embraced both carotid arteries and the internal jugular veins. Histological examination revealed signs of inflammation with round cells. The second patient was a 23-year-old woman who presented with a fast-growing goiter over the past 10 weeks. This goiter showed a similar infiltration of the neck vessels as the previous case. Goiter growth stopped after surgery even though only one fifth of the gland was resected. In the discussion there is a mention of a similar case which had been observed in a 12-year-old girl.

In 1902, de Quervain[12] described a process of thyroid inflammation, which was later named after him, de Quervain thyroiditis. He differentiated the etiology of thyroiditis as being either purulent or non-purulent. Case 1 corresponded to a 16-year-old girl who had noticed a painful goiter which developed some 8 days after having had an angina. An emergency operation was necessary because of local compression problems. Histology showed no inflammatory changes, and the final diagnosis was that

of a simple goiter. Neither the connective tissue nor vessels showed any changes. Case 2 described a 38-year-old woman who had also had an angina and who presented with a painful goiter. Case 3 described a 38-year-old woman who presented with a painful goiter which developed some 4½ months post-partum. Case 4 corresponded to a 40-year-old woman who presented with sudden swelling of the thyroid. A hemithyroidectomy of the left lobe was carried out. Histology revealed polynuclear leukocytes. The final diagnosis was parenchymal thyroiditis. A short phrase - lost amid these descriptions - stated that the pain symptoms of cases 2, 3 and 4 were successfully treated with salicylic acid, i.e., aspirin.

In 1908, McCarrison[13] described the histological appearance of the thyroid in a case of endemic cretinism demonstrating large fibrous strains together with a diminution of functional thyroid tissue (figure 5 in[13]).

In 1912, the classical description of lymphadenoid goiter was published by Hashimoto[14]. His report was based on the histological findings from 4 women who were thyroidectomized because of hypertrophic goiter. The histological analysis revealed no signs of acute inflammation. Speculating about the nature of the disease, Hashimoto proposed a process of chronic inflammation.

In 1931, Graham and McCullagh[15] described the association of lymphoid tissue with atrophy and fibrosis of the thyroid. The authors referred to the lack of publications related to the topic described by Hashimoto since 1912. They added the description of 4 cases of women with goiter who were seen at the Department of Surgical Pathology and Surgery of the Cleveland Clinic. The individual cases can be summarized as follows. Case 1, a 75-year-old woman with thyroid enlargement who was treated with radiotherapy 2 times. On surgery, both lobes were found to be enlarged. The gland was hard and fixed around the trachea, and no acute inflammation was seen. Case 2, a 44-year-old woman with diffuse enlargement of the gland. The gland was fibrotic and had no normal thyroid tissue. Case 3, a 53-year-old woman with long-standing goiter, which increased in size over the last 4 years. The thyroid expanded behind the trachea. The gland was fibrotic and contained no normal thyroid tissue. Case 4, a 75-year-old woman with a large goiter, which had been taken as malignant. The gland was fibrotic and had no normal thyroid tissue. In summary, the main characteristics were atrophy of the parenchyma, replacement fibrosis and lymphoid infiltration. Besides the presentation of these cases, the authors included an informative table that summarized the data taken from Hashimoto together with the data of Graham's patients (page 561 in[15]). A pathogenic explanation, however, was not found. In the same year Graham published a comparison between Riedel's struma and struma lymphomatosa[16]. The author collected the literature available at that time finding data from 104 cases (table 1 on page 64 in[16]). In the conclusions the author proposed that with time the changes of the thyroid become degenerative and sclerosing and that the lymphoid tissue "is non-specific for Hashimoto's struma".

The relationship between Riedel's struma and struma lymphomatosa (Hashimoto) was described by Eisen in 1934[17]. He also collected the relevant literature of that time (references 4 to 12) making special reference to Ewing (1922) who considered Hashimoto's thyroiditis to be an early stage of Riedel's thyroiditis. Eisen's conclusion was similar to that of Ewing when he stated: "Riedel's struma and Hashimoto's struma are different morphological manifestations of the same disease process".

In 1935, a clinically oriented publication on Hashimoto's disease by Clute, Eckerson and Warren appeared in a surgical journal[18]. They described the findings in 9 women with a mean age of 55.5 years. The main complaint was pressure on the midline structures, i.e., trachea. A common feature was extensive fibrosis, which was limited by the capsule, while inflammation was not described.

In 1937, McClintock and Wright[19] carried out a comparative study dealing with the overlap in the diagnosis of Riedel's struma and Hashimoto's disease. They reviewed the literature available at that

time and were able to identify 60 patients with Riedel's struma and 50 with Hashimoto's disease (table 1 on page 12 in[19]). An additional group was that of patients who presented with atrophy and fibrosis. Tables 2 and 3 in[19] summarized the relations of each entity to sex, age, duration of symptoms, type of thyroid involvement and thyroid function. In Hashimoto's thyroiditis, 95% of the cases were women. The duration of symptoms was much longer in Hashimoto's thyroiditis. Thyroid involvement was unilateral in 30% of cases with Riedel's thyroiditis, while it was bilateral in Hashimoto's thyroiditis. The cases with atrophy had inflammatory fibrosis. Recovery after surgery for Riedel's thyroiditis was rather rapid, while patients with Hashimoto's thyroiditis needed a longer time to return to normal.

In 1941, Goodman[20] added a report on 2 cases of Riedel's thyroiditis. The author emphasized that Hashimoto's thyroiditis and Riedel's thyroiditis must be distinguished and not taken as being similar. The wedge resection in cases of Riedel's struma was recommended.

In 1942, Lundbæk reported the case of a woman, 41 years old, presenting with a moderate enlargement of the thyroid. The gland felt firm and hard but was not adherent. The histological diagnosis was Riedel's struma. Treatment was given in the form of X-ray radiation administered 6 times at a dose of 100 r. The effect of this treatment was a diminution of goiter size[21]. This publication made mention of earlier studies on chronic thyroiditis[22], but unfortunately, we could find no details.

The clinical aspects of 24 cases presenting with chronic thyroiditis were described by Patterson and Starkey in 1948[23]. The authors stressed that a correct preoperative diagnosis was not always possible. After surgery, the final diagnosis revealed 11 cases of the Hashimoto type, 11 of the Riedel type, and 2 of the giant-cell type of de Quervain.

In 1951, Statland, Wasserman and Vickery, working at the Thyroid Clinic and Pathology Department of Massachusetts General Hospital, reported on 51 cases with Hashimoto's thyroiditis described as struma lymphomatosa[24]. In the introduction, the authors made a clear differentiation between Riedel's thyroiditis and Hashimoto's struma, which led them to exclude cases with Riedel's thyroiditis from their report. The frequency of struma lymphomatosa was 51 out of 3,676 thyroid operations. Forty-four of the cases were described as being typical for the disease, while 11 were not. The mean weight of the thyroid was 66.8 g compared to 20 g as seen in normal subjects in the fifth decade of life. The main complaint was enlargement in the neck. Thyroid tissue was described as being yellowish pink to tan and separated into lobulations separated by fibrous septa. The most outstanding feature was the diffuse distribution of lymphoid tissue and associated parenchymal degeneration. In their conclusions, they recognized that the cause for the diminished functional capacity of the altered thyroid cells was unknown.

The proposed "autoimmune" pathogenesis of human thyroid disease made a start with an extremely limited number of histological observations from surgical material (n = 3) in 1957[25]. Applying the medical knowledge available then and reviewed above, the true number of cases with chronic thyroiditis should have been reduced to one since one patient had thyroid carcinoma and the other patient had Riedel's thyroiditis. Therefore, it turned out that a disease concept was launched as "a disease in one". In spite of this severe medical and scientific limitation, this loose idea has been followed and investigated further by other researchers[26-28]. Despite research efforts, this concept has not produced any therapy approach in humans.

In 1959, Graham and Gilliland presented a case of a woman presenting with Riedel's thyroiditis, where a hemithyroidectomy of the left lobe was required. In a follow-up examination, the right lobe appeared functional, showing ^{131}iodine uptake[29].

Katz and Vickery[30] looked at Hashimoto's thyroiditis from a pathologist's point of view in 1974. They

reviewed the material from the Massachusetts General Hospital available from 1931 until 1969. The sources of the material were: surgery (n = 16), needle biopsy (n = 40), open biopsy (n = 7), and autopsy (n = 2). The authors found fibrosis of the gland seen as broad bands of connective tissue which separated islands of thyroid parenchyma. Thirty percent of thyroid parenchyma was replaced by fibrous tissue (figures 2 to 4 in[30]). Working with the tanned red cell method for antibody detection they found anti thyroglobulin antibodies in many patients. These antibodies showed a correlation to situations of recent development of goiter as well as to fibrosis.

Experimental models of thyroid disease: lymphadenoid goiter, "autoimmunization" and the obese/obese strain of chickens

McCarrison[31] described in 1929 a model of experimental lymphadenoid goiter in rats. The animals received a dietary mixture consisting of white flour, meat residue, olive oil, and a salt mixture containing 0.45% potassium iodide. The resulting alterations in the thyroid were described as showing fibrosis and atrophy, i.e., regressive changes. The author concluded that dietary deficiencies, e.g., vitamins, could be considered to have caused the goiter.

The publication from Witebsky *et al.*[25] which proposed an "autoimmune" pathogenesis of human thyroid disease also presented experimental data to describe a so-called "autoimmunization" condition. Careful reading of the text reveals that this experiment was the product of a manipulated intervention, i.e., immunization, using organ extracts from rabbit thyroids together with Freund's complete adjuvant. This - non physiological mixture - was applied to the footpads of the experimental animals. Freund's adjuvant[32] is a toxic substance containing a mineral oil, Arlacel (mixture of mannide mono-oleate), together with killed Mycobacterium butyricum or Mycobacterium smegmatis. Our 2020 look at these data is critical since the words chosen for the title of the original publication suggested a natural connection between chronic thyroiditis in humans and autoimmunization in experimental animals. In the same publication, the authors referred to this situation as being used "under certain experimental conditions" (Page 1445 in[25]).

One important molecular issue identified by Schulman, Rose and Witebsky in 1955 was the alteration of thyroglobulin structure found after biochemical extraction[33]. The authors attributed this change to a shift in equilibrium between different aggregation states. This biochemical feature of thyroglobulin has not been followed in the literature.

In 1964, Weigle[34] described an experimental approach aimed at the induction of an immune reaction in rabbits. This publication also referred to "autoimmunity" even though it was clearly stated that the study was based on the use of - externally - altered tissue components, i.e., thyroglobulin. The alterations were produced by heating and coupling to diazonium derivatives. By using heat, 65 °C for 15 min, dissociated thyroglobulin was produced. Two slower sedimenting components, i.e., of lower molecular weight, were found. The diazonium derivatives were produced using either sulfanilic acid or arsanilic acid. This chemical intervention produced a breakdown of thyroglobulin into 4 components including 3 with a slower sedimentation. PubChem describes sulfanilic acid (4-aminobenzenesulfonic acid) as an irritant and arsanilic acid (4-aminophenyl arsonic acid) as being toxic and an environmental hazard. PubMed has no additional entries relating these substances to any "autoimmunization".

In 1962, Van Tienhoven and Cole[35] described a complex disease including thyroiditis, which was observed in obese chickens. Many researchers have used this strain of chickens in further experiments. In 1994, Rose[36] summarized the topic of autoimmunity in relation to lessons from the birds. Careful reading of the publication discloses a conceptual pathogenesis paradox in the first lines when it is stated that autoimmune disease can be achieved by immunization. It could also be called a dedicated semantic mistake, i.e., dedicated to promoting the idea of autoimmunity. The self or auto intervention is physically

Table 1. Selected publications in the development of the pathogenesis concept of thyroiditis and goiter together with modern concepts

Authors	Year	Topic
Riedel[11]	1896	Riedel's thyroiditis
de Quervain[12]	1902	Description of acute thyroiditis and treatment with salicylic acid
McCarrison[13]	1908	Fibrotic thyroid in endemic cretinism, loss of functional thyroid tissue
Hashimoto[14]	1912	Original description of struma lymphomatosa
Rose[39] and Witebsky[40]	1955	Serological reactions with thyroid extracts, calling thyroglobulin a major player
Roitt[26,27,41]	1958	The idea of auto-antibodies
Van Tienhoven and Cole[35]	1962	Description of endocrine disturbances in obese chickens
Ritossa[42]	1962	Description of the heat shock response
Wartenberg *et al.*[43]	1973	Mitochondria in leukocyte migration inhibition test
Katz and Vickery[30]	1974	The fibrotic variant of Hashimoto thyroiditis
White and Walmsley[44]	1984	Plasma enzyme activities in 3 cases of hypothyroidism
Rose[36]	1994	Avian models of autoimmune disease
Medeiros-Neto *et al.*[45]	1996	Congenital hypothyroidism as an endoplasmic reticulum storage disease
Leo *et al.*[37]	2011	Fundamentals of immunization
Caturegli *et al.*[38]	2014	Therapeutic options for HT are either substitution or surgery
Jancic and Stosic[46]	2014	Cadmium toxicity in the thyroid via mitochondrial function
Zimmermann *et al.*[47]	2016	Alteration of the mitochondrial respiratory complex I in HT
Grootjans *et al.*[48]	2016	The unfolded protein response and immunization
Barić *et al.*[49]	2019	Relation between symptom burden and thyroglobulin antibodies
Wang *et al.*[50]	2018	Relation between magnesium levels and thyroglobulin antibodies
Hu *et al.*[51]	2020	Generalized concept of mitochondrial systems biology

and philosophically impossible since the experimental animals were actually vaccinated against foreign material. Rose coincides with our opinion when he declares and admits that "Autoimmune diseases can be produced in chickens, as they are in mammals, by experimental immunization" (page 984 in[36]). For these semantic reasons, we consider that the term autoimmune, referring to experimental immunization, is rather misleading just as fake news is today in 2020. Immunization is a process that involves external elements not found in the self[37].

Even though numerous publications have explored thyroid disease on the basis of the concept of autoimmunity as proposed by Rose, there has been no practical evolution into a therapy that would control the disease. Caturegli, a co-worker of Rose, provided a modern view on Hashimoto's thyroiditis and included the following statement on therapy: "The treatment remains symptomatic and based on the administration of synthetic thyroid hormones to correct the hypothyroidism as needed"[38].

The following Table 1 summarizes some of the milestones of thyroid research including some hints as to how the views can change. In the following sections, we will look at the therapy attempts based on concepts of oxidative damage to the thyroid in relation to selenium deficiency. We will demonstrate some causes leading to thyroid inflammation and how this can be approached by prescribing a combined supplementation with magnesium, selenium and CoQ_{10}[9,10].

Oxidative damage to the thyroid and the potential use of selenium

On the basis of simple models of selenium involvement in protective mechanisms in the body as an anti-oxidant[52], some researchers have investigated the potential benefit of giving selenium as a single substance to treat thyroid disease, specially Hashimoto's thyroiditis. A publication of the Cochrane Library explored the utility of selenium administration on Hashimoto's disease in 2013[53], analyzing the data originating from 4 studies published between 2006 and 2011[54-57].

One aspect that was missed in the proposal of the evaluation, was that they did not select studies that had measured selenium. Keeping selenium levels as an unknown variable invalidates any conclusion.

Invalidated analyses would not make Archie Cochrane happy since they cannot fulfill his ambitious postulate of finding a successful therapy in the sense of effectiveness and efficiency[58].

The citation in the Box 1 illustrates the inconclusive conclusions of the Cochrane analysis[53].

Box 1. Conclusions from the Cochrane Study on Selenium Supplementation and Hashimoto's Thyroiditis

"Results of these four studies show that evidence to support or refute the efficacy of selenium supplementation in people with Hashimoto's thyroiditis is incomplete. The current level of evidence for the efficacy of selenium supplementation in the management of people with Hashimoto's thyroiditis is based on four randomised controlled trials assessed at unclear to high risk of bias; this does not at present allow confident decision making about the use of selenium supplementation for Hashimoto's thyroiditis."

In the following paragraphs, we will refer to recent publications that have evaluated selenium supplementation in relation to thyroid disease. Between 2015 and 2017, 3 studies coming from Danish researchers appeared in the literature. In 2015, they reported the results of a double-blinded selenium supplementation trial[59]. The outcome measure was defined as changes of thyroid function, i.e., fT3, fT4 and TSH, as well as of thyroid peroxidase antibodies. The study used a patented selenium product SelenoPrecise (https://www.pharmanord.eu/) which is described by the producer as containing selenomethionine and more than 20 other organic selenium compounds. Selenium levels were determined at the beginning and during the supplementation period, 6 months and 5 years later. The doses administered were, 0, 100, 200 and 300 μg daily. Thyroid function parameters showed no change. A peculiar aspect of the study was the high total weight of the placebo preparation which adds up to 400 mg! (Page 660 in[59]). In 2016, a publication with a provocative title appeared. Referring to selenium, they posed the following question: "An element in search of the relevant indications?"[60]. The authors presented an analysis of a web-based survey done by the Italian Associazione Medici Endocrinologi in 2016[61]. The authors concluded that selenium use is inappropriate in several thyroid-related conditions. In 2017, Winther *et al.*[62] published a meta-analysis that looked at the efficacy of selenium supplementation in chronic autoimmune thyroiditis. The authors started by declaring that this disease has no cure but fail to add any validated reference for this statement. The notion of low selenium being associated with thyroid disease was taken from a previous Chinese publication[63]. Wu *et al.*[63] previously looked at thyroid function parameters, thyroid peroxidase antibodies, selenium, and thyroid volume determined by ultrasound examination using a 7.5-MHz probe. The authors found an association between an enlarged thyroid and low selenium levels. Unfortunately, this key clinical element was not considered in the analysis by Winther *et al.*[62]. In their opinion, selenium supplementation in patients with thyroid disease should be discouraged. They proposed carrying out well-powered randomised clinical trials (RCTs) evaluating disease progression or health-related quality of life. The evaluation of disease progression was not defined.

Mao *et al.*[64] studied the effect of low-dose selenium supplementation in pregnant women. They looked at parameters of thyroid function as well as at the levels of thyroid peroxidase antibodies. The study did not contain any data on selenium levels or on thyroid ultrasonography. The study failed to show an effect on antibody levels.

In 2017, Esposito *et al.*[65] studied the effects of short-term selenium supplementation including euthyroid subjects with Hashimoto's thyroiditis. Thyroid parameters included were TSH, fT3, fT4, thyroid peroxidase antibodies, and thyroid echogenicity evaluated with a 10-MHz ultrasound probe. CXCL10 or interferon-gamma inducible protein 10 kD[66], originally considered to be involved in inflammatory processes[67], was included as a secondary outcome parameter. The authors concluded that short-time selenium supplementation was ineffective as no end-point parameter changed.

In 2020, a study on selenium supplementation in patients with subclinical hypothyroidism was published by Pirola *et al.*[68]. The study included the analysis of thyroid hormones and thyroid peroxidase antibodies, selenium and iodine. This study also included determinations of CXCL9, CXCL10 and CXCL11, taken as

being representative for interferon-γ- inducible chemokines. The only change reported by the authors was normalization of TSH levels.

These recent publications coincide with the outcome of the Cochrane evaluation[53], i.e., the sole supplementation of selenium is not effective in modifying or treating thyroid disease.

Prior to the Cochrane analysis and in strong contrast to the results described above, Rani and Lalitha[69] in 1996 paved the road for selenium research in connection with mitochondrial function. They showed that electron transport function was impaired at the same time as mitochondrial structure was affected when selenium deficiency was present. We personally consider a mitochondrial involvement to be more important than any antioxidative presumption.

An interlude: updating the contribution of genetics

In 2011, Panicker[70] published an article on the genetics of thyroid disease. Some key statements are quoted here. "Although the advancement of genetic technology has led to many significant findings in the last decade or two, it is clear that we are only just beginning to understand the role of genetics in thyroid function and disease". A second statement reads: "Early optimistic expectations that all genes responsible would be rapidly discovered have had to be reined in, and researchers across many fields are searching for the reasons this has not happened. The need for larger sample sizes and collaboration between groups with access to large cohorts has now been understood, and these studies will undoubtedly discover further genes. Furthermore, whole genome sequencing may provide more information as other types of genetic variation, such as copy number variants may also be found to play a role. In addition, influences on accessibility of genes to transcription may be at work. However, what we have already discovered has increased our understanding of normal thyroid hormone action and physiology, and we are beginning to understand the complex origins of autoimmune thyroid disease. Important for future work is the need to replicate the early findings presented above and perform functional studies to identify the true associations and the mechanisms behind them. These mechanisms will increase our understanding of thyroid physiology and identify therapeutic targets". Also in 2011, Davies declared that there are no really significant genes for autoimmune thyroid disease[71].

Looking specifically at causes of Grave's disease, Marinò *et al.*[72] concluded that the ultimate cause of the disease remains obscure. At the same time they hoped that further genetic studies would contribute to the clarification of the situation. Using the advanced technique of metabolomics, Struja *et al.*[73] found no distinct pattern that could relate to disease relapse in cases of Grave's disease. The same researchers tried to find markers for the early diagnosis of thyroid disease on the basis of metabolomics. The authors could not find any metabolomics parameter that was able to fulfill this expectation[74].

At the time Precision Medicine arrived, Galofre and Ladenson added some clinical features and described some expectations for thyroid diseases in 2016. Galofre wrote: "The aim of the initiative is to eradicate imprecision in estimating the probability of a correct diagnosis, to be as sure as possible of the most effective treatment, and to maximize the chances of a successful outcome"[75]. Ladenson commented: "The broad spectrum of thyroid disease severity - from subclinical hypothyroidism to myxedema coma, subclinical thyrotoxicosis to thyroid storm, and microscopic papillary to anaplastic cancers - has always demanded that clinicians individualize their management of thyroid patients. Deepening knowledge of thyroid pathophysiology along with advances in diagnostic, prognostic, and therapeutic technologies applicable to thyroid diseases position this field to ride the wave of precision medicine in the decade ahead"[76].

Another source of general information on genetics and thyroid disease is the "Genetics Home Reference: your guide to understanding genetic conditions"(https://ghr.nlm.nih.gov/). The interested reader will find the following description of the inheritance of Hashimoto's disease: "The inheritance pattern of Hashimoto

thyroiditis is unclear because many genetic and environmental factors appear to be involved"(https://ghr.nlm.nih.gov/condition/hashimoto-thyroiditis#inheritance). For Graves' disease, they state the following: "The inheritance pattern of Graves' disease is unclear because many genetic and environmental factors appear to be involved. However, the condition can cluster in families" (https://ghr.nlm.nih.gov/condition/graves-disease).

Going through the literature, we got the impression that one can consider genetic studies done in relation to thyroiditis to fall into 2 groups: descriptive and interpretative. The first group tells the reader that there are defects somewhere, while the second group goes beyond to describe cellular mechanisms.

Among studies that we consider to be descriptive, one can find mutations in patients with thyroid disease. Genetic changes in Hashimoto's thyroiditis involving thyroglobulin gene polymorphisms were described by Caputo *et al.*[77], showing that the TGrl29 microsatellite was significantly associated with the disease. Lo *et al.*[78] published the results of a study conducted in a family that presented early onset of Hashimoto's thyroiditis. They found a splice site variant in all family members who were affected, but also in one child without the disease. The change they found was TG c.1076-1G > C. In a setting of genetic polymorphism, i.e., TG rs2076740, the anti-thyroglobulin antibody titers were described as being higher than in controls[79]. In a study that looked at a group of Chinese Han subjects, two independent SNPs (rs2294025 and rs7005834) for Graves' disease susceptibility were described. Unfortunately, they showed no correlation with clinical phenotypes[80].

Analyses of naturally occurring genetic defects in congenital goiter have concluded that allelic alterations of the thyroglobulin gene lead to a defect in secretion in the sense of an endoplasmic reticulum storage disease[81]. Retention of thyroglobulin, or in general of any protein, will lead to a response via the endoplasmic reticulum in the sense of the unfolded protein response[82,83]. This process requires sufficient ATP supply[84]. In 3 of their publications, Barishev *et al.*[85,86] Sargsyan *et al.*[87] described the mechanisms involved in congenital hypothyroidism. Pardo *et al.*[88] reported that genetic changes in Tg, i.e., the p.A2215D thyroglobulin gene mutation, can produce molecule retention in cells, thus involving the endoplasmic reticulum. Using a bioinformatics analysis approach, Zheng *et al.*[89] recently identified key genes associated with Hashimoto's disease. Another player in Hashimoto's thyroiditis involves polymorphisms of the STAT3 gene[90,91].

A new publication on genetics has shown that the apoptosis antagonizing transcription factor (AATF)[92] is involved in thyroid disease in determining the volume of the gland in situations of thyroiditis[93]. AATF appears to regulate the anti-apoptotic effect of the unfolded protein response through transcriptional regulation of AKT1[94].

We can summarize this section by declaring that on the basis of the data presented on autoimmunity, selenium supplementation and genetics, it is not possible to develop any therapy concept that can improve or cure benign thyroid disease. The following Box 2 is Bob Dylan quotation from his 1967 song "All Along the Watchtower" describes the situation precisely.

Box 2. I can't get no relief… from my thyroid disease

There must be some kind of way outta here
Said the joker to the thief
There's too much confusion
I can't get no relief
Bob Dylan, 1967, All Along the Watchtower

To provide relief, we will present our approach to diagnosing and treating thyroid disease based on our WOMED model of benign thyroid disease[8,9].

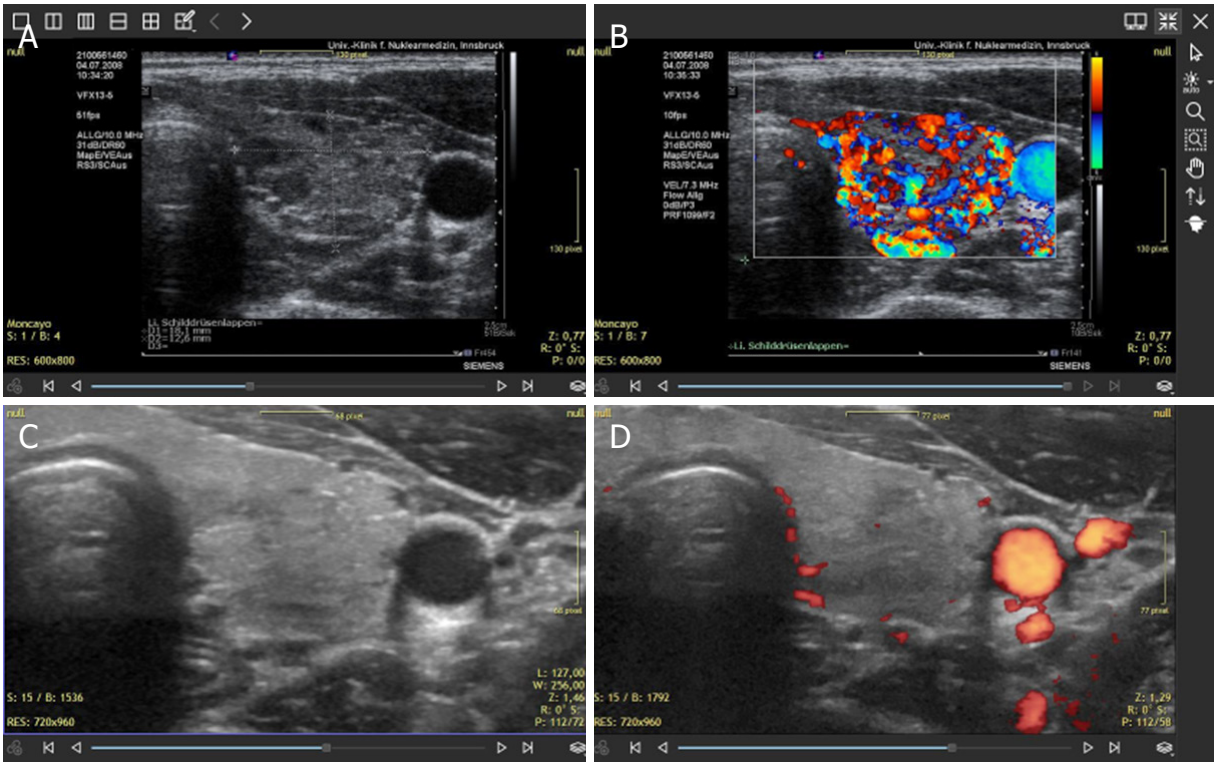


Figure 1. The Index Case showing combined deficiencies of magnesium, selenium, and CoQ_{10}. Thyroid sonography was done using 2 different systems: SIEMENS Antares in 2008 (A, B) and GE LOGIQ E9 in 2019 (C, D). In 2009, the thyroid showed diminished echogenicity and hyperperfusion as seen in color mode. The images C and D demonstrate the improvement of thyroid morphology together with a normalization of perfusion. These changes disappeared after combined supplementation was started. Currently the patient does not require any thyroid medication

Finding relief for thyroid disease: correlative observations of thyroid perfusion changes

(1) The Index Case: combined deficiencies of magnesium, selenium and CoQ_{10}

The Index Case of a complex biochemical and clinical situation involved a young woman who was examined between 2008 and 2020. The patient presented with a series of sequential medical conditions including hyperthyroidism, cerebral ischemia, and myocardial infarction. The biochemical determinations detected low levels of magnesium and CoQ_{10}. The first thyroid sonography was done in May 2008 and showed a hypo-echogenic gland with massive hyperperfusion as seen in color mode. Supplementation with magnesium and CoQ_{10} normalized thyroid morphology and function. In 2018, a postpartum thyroiditis with hyperthyroidism and marked magnesium deficiency was diagnosed. On ultrasound, the thyroid showed a pronounced diminution of echogenicity together with hyperperfusion suggestive of CoQ_{10} deficiency. Since the patient was breastfeeding, a combined supplementation was started again using a higher daily dose of magnesium, i.e., 11.2 mmol of elemental magnesium (~3.2 g magnesium citrate). CoQ_{10} was given at a dose of 30 mg/d. Within 3 months, thyroid function, thyroid morphology and perfusion returned to normal. During her first pregnancy, hyperperfusion with the pattern of CoQ_{10} deficiency reappeared. Currently, in the second pregnancy, the changes were prevented by continuing supplementation [Figure 1].

(2) Thyroid perfusion changes during pregnancy

One pregnant woman seen between April and August 2019 showed sequentially appearing patterns of altered thyroid perfusion: (1) magnesium deficiency; and (2) CoQ_{10} deficiency [Figure 2].

(3) Hyperthyroidism developing after combined heat and exertional stress

In the past 2 years, we observed a small number of hyperthyroid patients who initially presented

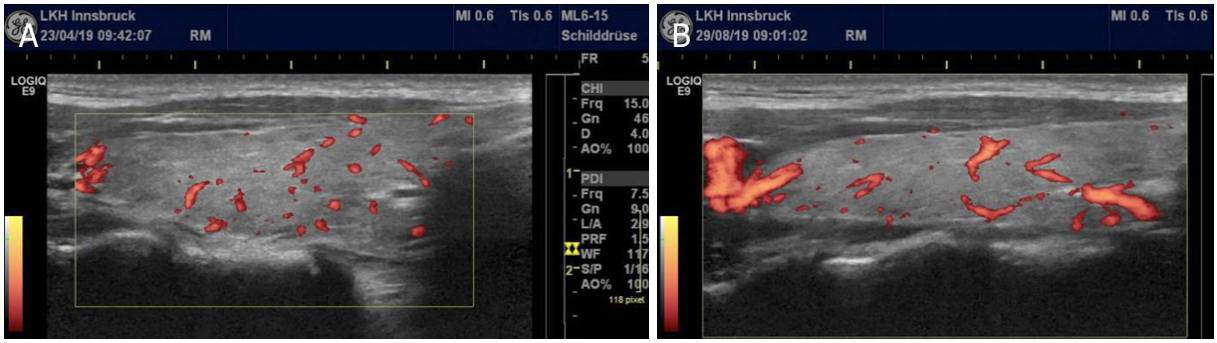


Figure 2. Changes in thyroid perfusion during pregnancy in the same patient: the characteristic spot-like hyperperfusion due to magnesium deficiency on the 25th week of pregnancy (A); the situation 10 weeks later with the typical vessel form seen in CoQ_{10} deficiency, i.e., vessels with a wide appearance (B). Thyroid function was normal at both examinations

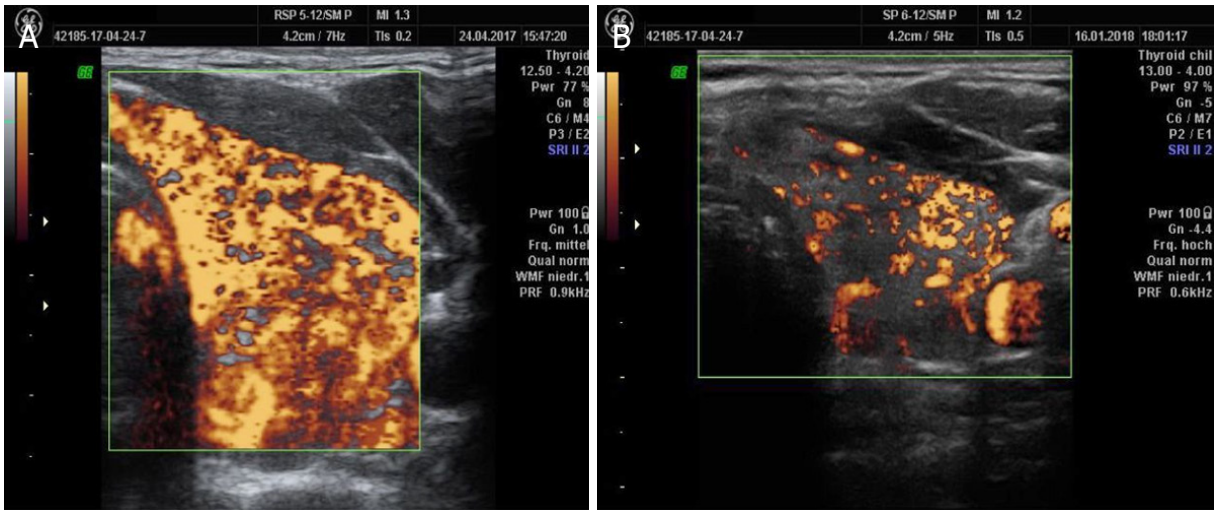


Figure 3. Hyperthyroidism after exertional stress under conditions of elevated ambient temperature. Initial examination showing diffusely increased vascularization (A); in the follow-up examination after 9 months of combined supplementation, hyperperfusion is less pronounced while the thyroid still shows decreased echogenicity (B). The patient is currently euthyroid after 2 years

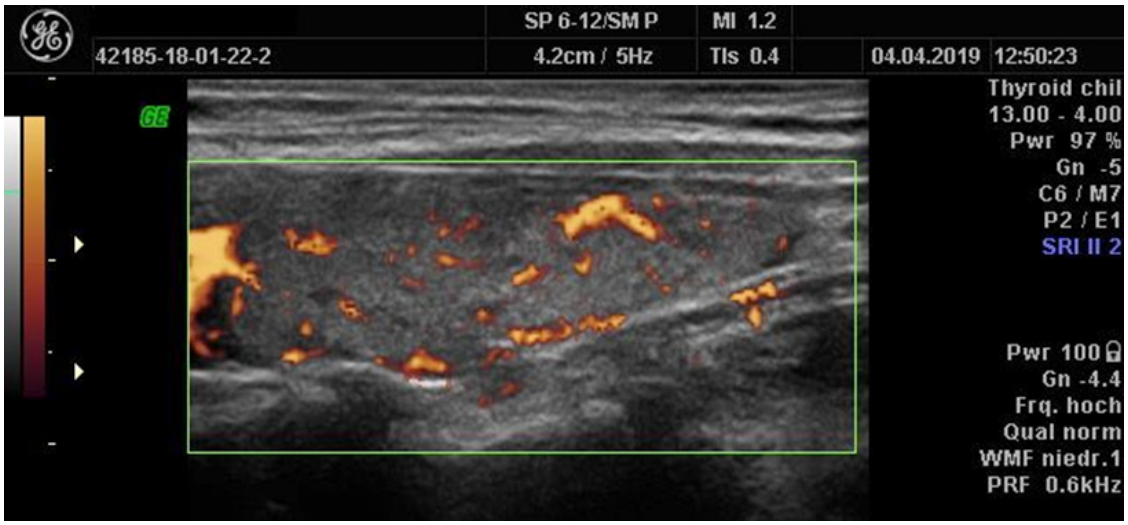


Figure 4. Postpartum thyroiditis with hyperthyroidism. The thyroid is hypo-echogenic. The perfusion pattern corresponds to a combined deficiency of magnesium (small spots) and CoQ_{10} (wide vessels)

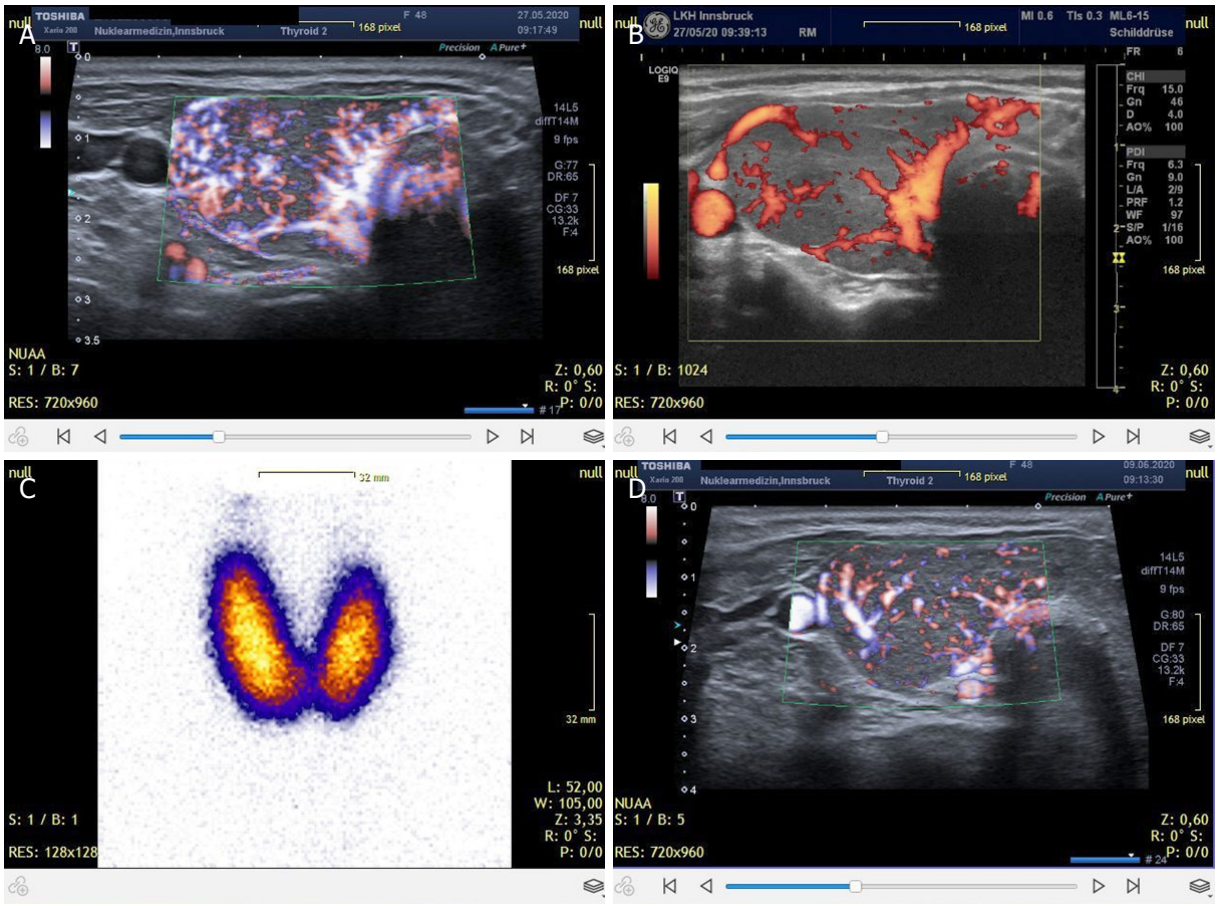


Figure 5. Recently diagnosed case of hyperthyroidism showing hyperperfusion corresponding to CoQ_{10} deficiency. Sonography images are compared for 2 different machines, i.e., Toshiba (now Canon) (A, B) and Loqiq Q9, G.E (C, D). The scintigraphy shows an intense tracer uptake. The lower right panel shows the response to 3 weeks of combined supplementation

with signs and symptoms suggestive of cardiac disease. Laboratory tests showed an elevation of NT-pro-BNP and creatine kinase. Echocardiography did not reveal cardiomyopathy. In one male patient, heat stress together with intense physical activity (active sports vacation) was the precipitating event for hyperthyroidism [Figure 3]. The patient complained of reduced physical capacity almost to the level of physical exhaustion, while the time of physical recovery was prolonged. This situation corresponds to the picture seen in heatstroke when there has been vigorous muscle exertion[95].

(4) Postpartum thyroiditis and hyperthyroidism

The sibling of the previous patient [Figure 4] presented with a condition of postpartum thyroiditis with hyperthyroidism, showing less pronounced changes in thyroid morphology. Signs of CoQ_{10}-associated hyperperfusion were present. She responded well to supplementation within 3 months. Thyroid morphology and function returned to normal in 2020.

(5) Hyperthyroidism and elevated proBNP

A recent case presented with symptoms of decreased muscular function, i.e., fatigability. Laboratory tests demonstrated a T3-predominant hyperthyroidism with > 30.8 pmol/L fT3, 64.2 pmol/L fT4 together with elevated proBNP of 811 pg/ml. Combined supplementation with magnesium citrate (4 g daily), 60 mg CoQ_{10}, and 200 µg selenomethione was commenced together with thyreostatic treatment. A follow-up 3 weeks later showed significant decrease of thyroid hormone levels and normalization of proBNP. Thyroid hyperperfusion was also diminished [Figure 5].

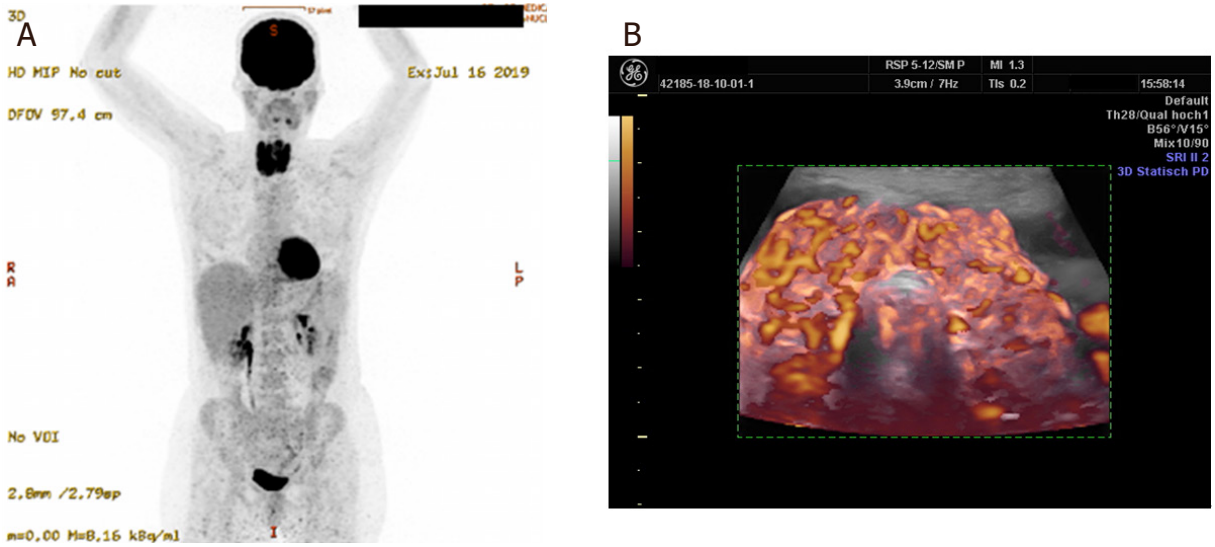


Figure 6. Woman with chronic relapsing thyroiditis, showing increased uptake of ^{18}F-FDG in the thyroid and the heart in a PET examination (A). This tracer uptake pattern indicates glycolytic metabolism of the organs. On 3D power Doppler sonography (B), one can recognize the marked perfusion changes that correspond to CoQ_{10} deficiency, showing enlarged vessels

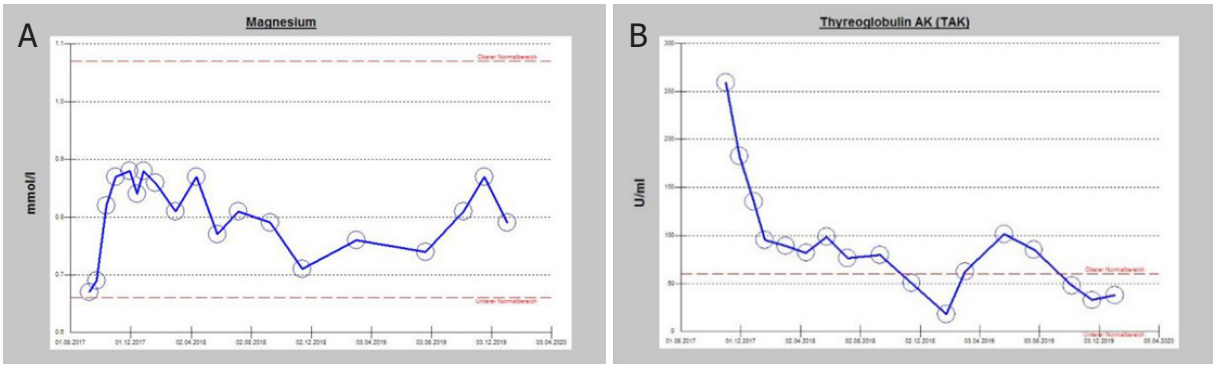


Figure 7. The graphs show the sequential determinations of magnesium levels (A) and anti-thyroglobulin antibody levels (B) documented between 2017 and 2020

(6) Chronic relapsing thyroiditis: 3D power Doppler and ^{18}F-FDG PET investigation

The first case of thyroiditis that could be investigated some 15 years ago and showed a course of repeated relapses of the thyroid affection. Every time a relapse occurred, hyperperfusion of the thyroid with the pattern of CoQ_{10} deficiency reappeared. In July 2019 a whole body ^{18}F-FDG PET examination was requested by the patient's general practitioner. The study revealed that the metabolism of the thyroid and the heart had switched to glycolysis. The patient had no signs of heart disease [Figure 6].

Correlative observations on blood levels of magnesium and anti-thyroglobulin antibodies

The following images are an example of the effects of combined supplementation on the levels of anti-thyroglobulin antibodies and magnesium in a female patient with hyperthyroidism. At the same time as magnesium levels tended to normalize, i.e., > 0.80 mmol/L, anti-thyroglobulin antibodies decreased into the normal range [Figure 7]. Fluctuations in magnesium levels were due to compliance failures.

The corresponding ultrasound images of this patient are shown in Figures 8 and 9. Thyroid volume decreases and morphology improves, while perfusion normalizes.

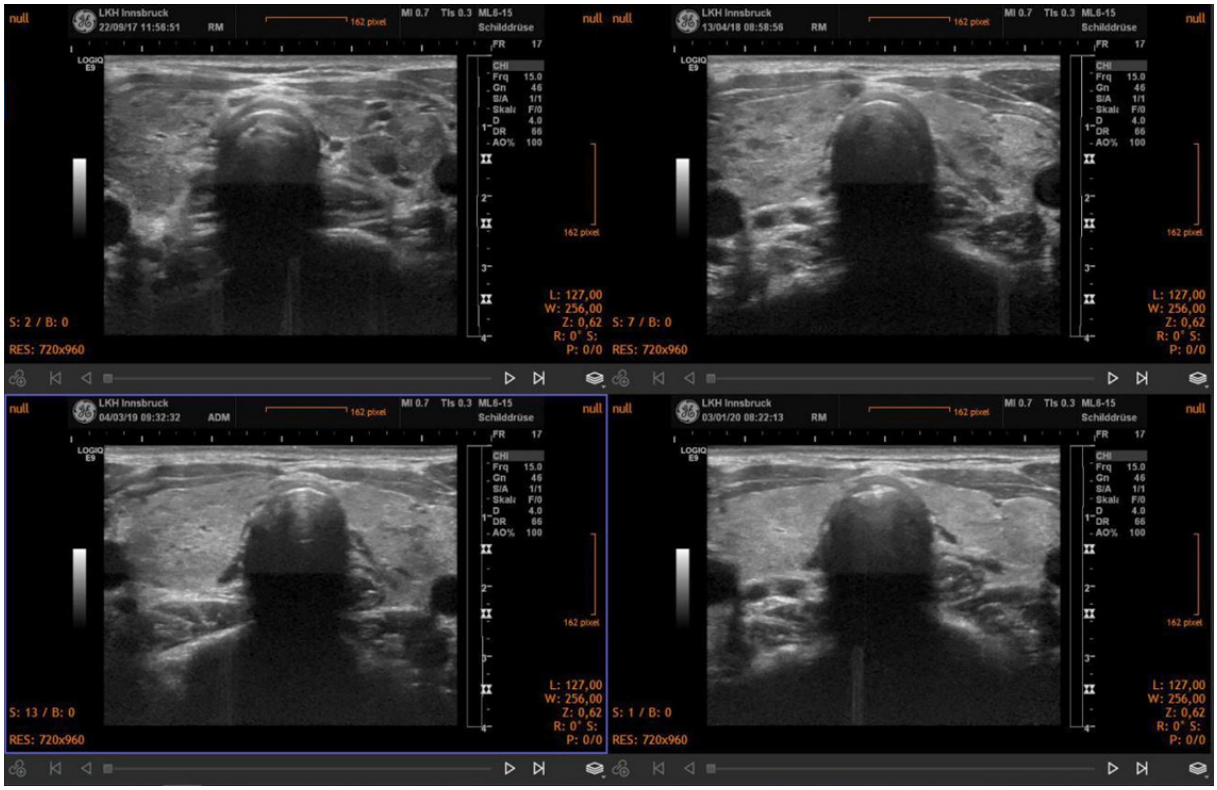


Figure 8. B-mode sonography showing improved morphology

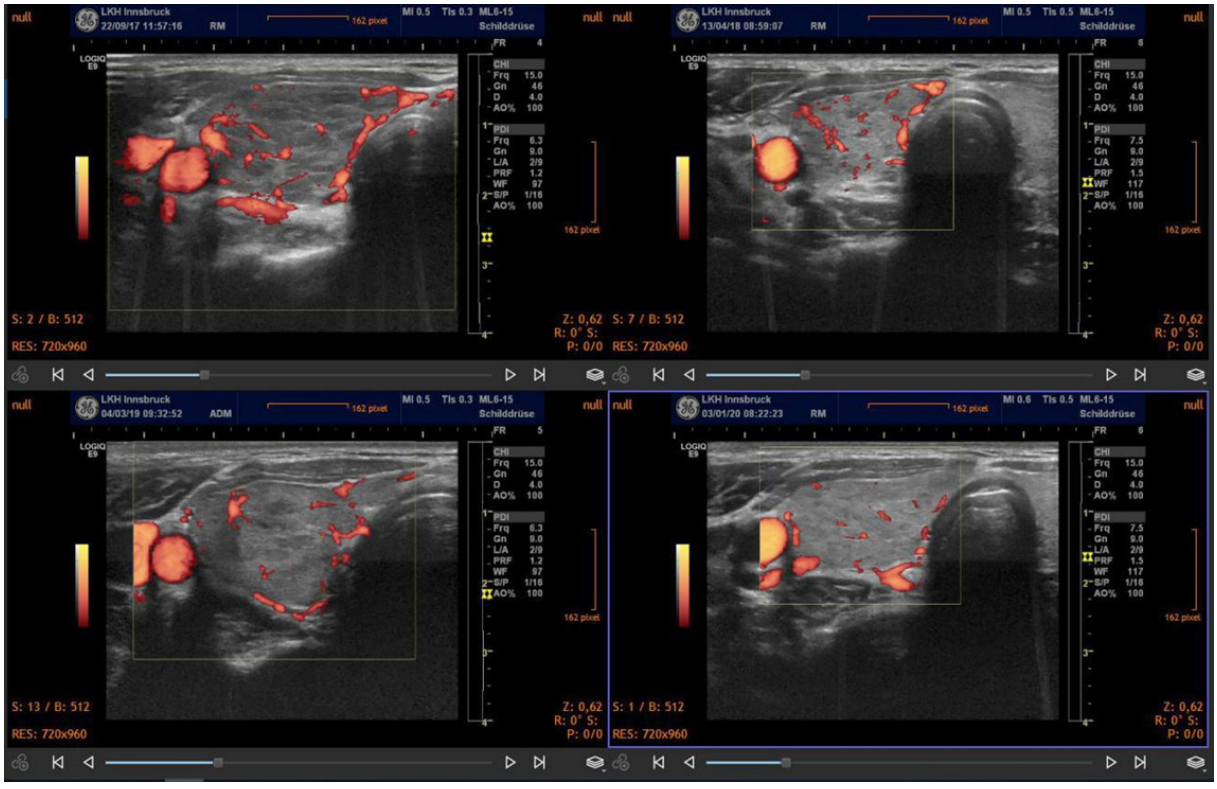


Figure 9. Power Doppler sonography showing a diminution of perfusion September 2017 to January 2020. Thyroid size decreases, morphology improves and perfusion returns to normal

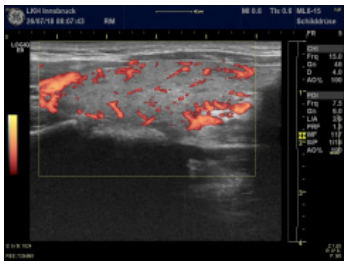
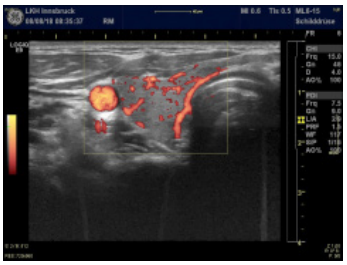
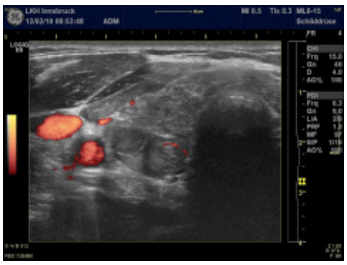
	"eigenthümliche Art von chronischer Entzündung" Chronic inflammation	**"Wucherung des Gefässendothels" Enlarged endothel**	**"Fällt selbst dem Schwunde anheim" Destruction**
TSH	No	No	Elevated in hypothyroidism
Thyroid -Abs	Partial correlation	No	No
Ferritin	No	No	No
Magnesium	Yes	Yes	Partly
Coenzyme Q_{10}	Yes	Yes - relates to the dynamics of perfusion	No
Selenium	Yes	No	Yes in fibrosis
Sonography	Yes	Yes	Yes
Power Doppler Images			
Condition	Magnesium def. pattern	CoQ_{10} def. pattern	Chronic fibrosis

Figure 10. Summary of the WOMED clinical approach to thyroiditis in relation to the 3 main findings described by Hashimoto (in the original German description). The clinical validity of diagnostic procedures including power Doppler sonography is explained for every stage

We (Moncayo R and Reisenzahn J) are currently analyzing the relationships between anti-thyroglobulin antibodies and magnesium. A rough estimate shows a good response rate of about 80%, partial improvement in 10%, and no improvement in the remaining 10% of cases. The non-responding cases fall into the category of fibrosis, where only replacement therapy with thyroid hormone can be offered. Some biochemical and genetic features of thyroglobulin will be discussed below.

Modern ultrasound and biochemistry parameters depict the finding made by Hakaru Hashimoto

On the basis of the data presented above it is possible to put together a functional model of thyroid disease pathogenesis centered on thyroiditis, where discrete biochemical players assume a specific role in the process of thyroid damage. The tool needed to recognize these changes is power Doppler sonography done with a modern probe of at least 15 MHz frequency[7]. In each patient, it is possible to differentiate distinct phases of thyroid inflammation and consequently to prescribe a tailored supplementation. Follow-up controls rely on 3 elements: (1) clinical history; (2) ultrasound examination with power Doppler; and (3) laboratory determinations of thyroid hormones, thyroid antibodies and magnesium. In some cases, ferritin and vitamin D are also evaluated. Within a time period of 1-2 years, normalization of parenchymal and perfusion changes can be seen[9,10]. The following Figure 10 shows our practical clinical approach[7-10] as compared to the classical description made by Hashimoto[14].

The findings of the sonography examinations can be translated into clinical action, i.e., into a therapeutic approach [Table 2]. Since sonography is regularly excluded from clinical practice recommendations, we cannot compare our approach with any previous publications.

Clinical practice has led us to the following prescription recommendations. Magnesium deficiency should be treated with pure magnesium citrate as a magistral formulation at a dose of 3.5 to 4.0 g per day. This applies to hypothyroidism as well as to hyperthyroidism. The main advantage of this formulation lies in the ability of citrate to localize inflammation[96]. For the correction of selenium and CoQ_{10} deficiencies, we have relied on products from Pure Encapsulations for 15 years and have obtained satisfactory results [Figures 1-9, and Figure 11]. The preparations used include selenomethionine (200 µg capsule) and CoQ_{10} (60 mg

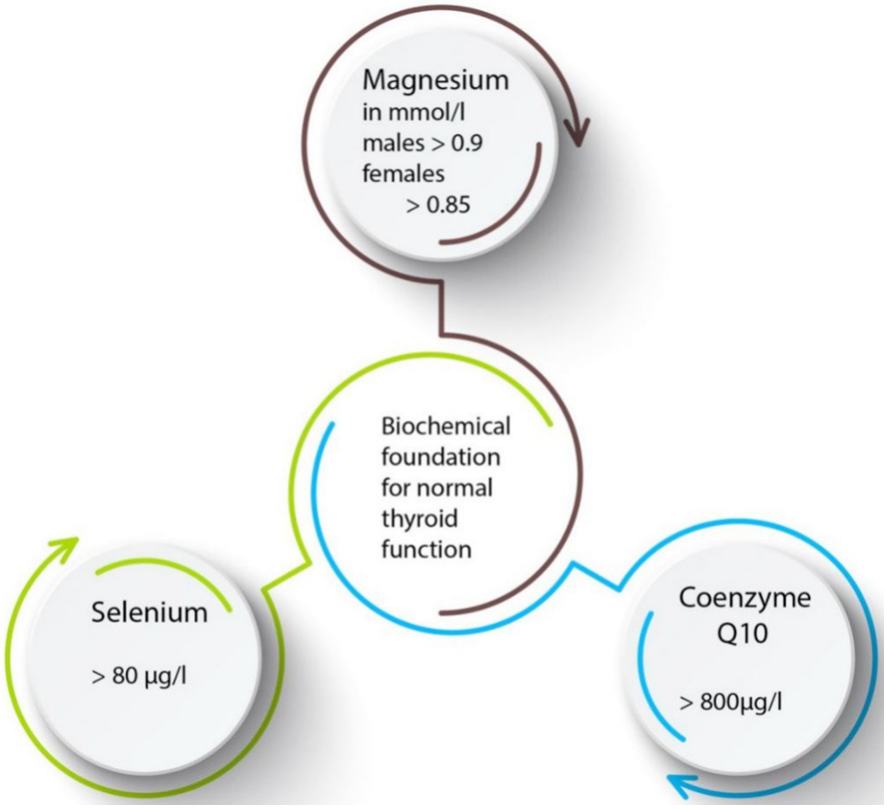


Figure 11. The target concentrations to be reached following supplementation. The graphic represents the interlaced function of the individual biochemical elements. Sequential ultrasound controls showing morphology (upper panel) and power Doppler perfusion (lower panel) September 2017 to January 2020. Thyroid size decreases, morphology improves and perfusion returns to normal

capsule). Supplementation begins with 1 capsule of selenomethionine and CoQ_{10} daily taken together at night during the first 2 weeks. Beginning on the 3rd week the dose is reduced to only 3 capsules per week of each preparation taken on alternate days. Treatment costs amount to €220 per year or €0.66 per day. The target values to be reached through supplementation are shown in Figure 11. The figure suggests an interlacing of the substances in human physiology. The essence of such an approach can be taken from the abstract of the publication by Kitano on Systems biology: "To understand biology at the system level, we must examine the structure and dynamics of cellular and organismal function, rather than the characteristics of isolated parts of a cell or organism"[97]. This interlacing is shown in Figure 12.

We have observed that heat stress can affect levels of CoQ_{10} and initiate or complicate the course of thyroid disease. Israeli authors recognized 40 years ago that intermittent thyroid dysfunction corresponded to a heat stress syndrome[98]. Under experimental conditions, CoQ_{10} can ameliorate the consequences of heat stress in chickens[99]. For thyroid disease patients planning a holiday where they will be exposed to elevated temperatures, we recommend CoQ_{10} daily[100]. Theoretically the same applies to UV-radiation[101,102], which also affects CoQ_{10}. Physical and psychological stressors can have a negative effect on magnesium, selenium and CoQ_{10}. Loss of CoQ_{10} could affect oxidative phosphorylation in muscle in such a manner that energy production will rely on glycolysis. This has been described in brown adipose tissue[103]. The inverse situation can be seen in the regulation of muscle oxidative function away from glycolysis achieved by CoQ_{10}[104].

The role of thyroglobulin conformation: isolation methods and biochemistry to genetics related to the endoplasmic reticulum

The biochemical methods for the isolation of thyroglobulin have been developed over the past 120 years, contributing with information about molecular variability in the thyroid. An early description of the

Table 2. Interpretation matrix of 3D power Doppler thyroid sonography

Morphology	Perfusion	Magnesium	Coenzyme Q_{10}	Selenium
Normal	Normal			
Hypo-echogenic homogeneous	Fine granular	Deficiency		
Hypo-echogenic inhomogeneous	Thickened vessels	Deficiency	Deficiency	
Hypo-echogenic inhomogeneous with fibrosis signs	Moderately increased	Probable	Probable	Deficiency
Very low echogenic	Increased	Deficiency	Deficiency	Deficiency
Very low echogenic with fibrosis signs	Not increased = final stage	-	-	-

isolation of thyroglobulin was provided by Oswald in 1898[105] while working at the Chemical Laboratory of the Medical Clinic in Zürich. The method was based on the extraction of the thyroid glands with 0.9% NaCl, followed by precipitation by either half saturation with ammonium sulfate or with acetic acid. In 1933, Heidelberg and Palmer commented that Oswald's method was to be considered as a reference work even though it produced denaturation of the molecule and it also contained some contaminants[106]. In the following years, Heidelberger and Svedberg determined the molecular weight of thyroglobulin[107], and Heidelberger and Pedersen determined the isoelectric point of the molecule[108].

In 1948, Derrien, Michel and Roche published their research work dealing with the isolation of thyroglobulin based on a salting-out procedure[109]. A short discussion on the role of thyroglobulin in thyroid disease was published by Witebsky in 1958[110]. In that study, thyroglobulin was extracted by sedimentation with 1.6 and 1.7 M ammonium sulfate. The authors suggested that thyroglobulin was the agent leading to a serological reaction. In 1968, Cheng *et al.*[111] described an extraction method that combined sequential salting-out with ammonium sulfate, starch-gel electrophoresis, and gel filtration. They concluded that this procedure allowed them to purify thyroglobulin, eliminating contaminants.

In 1998, Druetta[112] compared the molecular form of thyroglobulin in patients with Graves' disease (n = 21) and compared these results with the findings of anti-thyroglobulin antibodies. Ten patients were positive for anti-thyroglobulin antibodies. Thyroglobulin was extracted using an 8 to 25% sucrose gradient, followed by centrifugation at 100,000 g for 18 h at 4 °C. In patients with Graves' disease and positive for anti-thyroglobulin antibodies, the main form of thyroglobulin was found to be a heavy sedimentation peak, i.e., faster than the normal 19S form. Split forms of thyroglobulin were not associated with the presence of antibodies. In subacute thyroiditis, they described small oligomeric forms.

Besides the traditional thought of thyroglobulin being only related to thyroid hormone synthesis, a new perspective of thyroglobulin in the regulation of thyroid function has been proposed by Sellitti and Suzuki[113]. In this model, thyroglobulin is responsible for heterogeneity of follicular cells in which colloidal concentration of thyroglobulin adapts to improve hormone production. They consider the thyroid follicle to be the morphological function unit of the gland having a basket-like surrounding capillary network. Differences in the content of thyroglobulin can be found in adjacent follicles. Gérard *et al.*[114] showed a process where thyrocytes metabolize thyroglobulin globules by step-wise fragmentation changing the thyroglobulin form from being insoluble compact into soluble molecules. Morphological studies of the thyroid done by Smeds and Anderberg showed that administration of propylthiouracil was associated with a decrease of thyroglobulin aggregates[115]. Smeds[116] also noted that thyroglobulin aggregates localized preferentially to the periphery of the colloid in close association with the apical portion of the thyroid cell membrane.

During the process of thyroglobulin synthesis, a transient aggregation of nascent thyroglobulin in the endoplasmic reticulum can be observed[117]. Thyroglobulin production is regulated by TSH, and at the same time, TSH produces a regulated increase in folding capacity[118]. The 1996 study by Medeiros-Neto *et al.*[45]

dealing with congenital hypothyroidism, demonstrated that these patients suffer from an endoplasmic reticulum storage disease. Electron microscopy clearly showed an abnormal distension of the endoplasmic reticulum. Using biochemical methods they found a strong induction of chaperones such as binding immunoglobulin protein (BiP), a hsp70 homolog, and glucose-related protein 94 (GRP94) in an animal model of congenital goiter, the cog/cog mouse[119,120]. These results indicate an activation of the endoplasmic reticulum[121]. The GRP were originally described in 1977 by Shiu, Pouyssegur, and Pastan who worked with Rous sarcoma virus-transformed chick embryo fibroblasts[122]. They elucidated that the stimulus for GRP induction was glucose depletion in the growth medium. In 1981, Lee[123] added a functional relationship of thermal stress to the induction of these proteins. Thermal stress is related to the heat shock reaction, originally described by Ritossa[42,124].

In some patients with congenital goiter, anti-thyroglobulin antibodies can be found in the blood. Ordookhani *et al.*[125] found an association between neonatal hypothyroidism and elevated levels of anti-thyroglobulin antibodies. Cho *et al.*[126] described high titers of anti-thyroglobulin antibodies in cases of non-transient congenital hypothyroidism. They proposed that the antigenicity of thyroid proteins could be related to the underlying pathogenesis of congenital goiter. Peteiro-Gonzalez *et al.*[127] characterized changes in thyroglobulin genes in a family with congenital goiter, thus confirming their relation to altered function of the endoplasmic reticulum. A general investigation on genetic changes found in cases of congenital goiter was presented by Nicholas *et al.*[128] in 2016. A specific mutation affecting the linker domain of thyroglobulin, i.e., p.L571P, has been recently described in relation to the conformational maturation and intracellular handling of rat thyroglobulin leading to intracellular retention[129]. An extensive description of the genetics and biochemistry of thyroglobulin was recently published by Citterio *et al.*[130].

Cell repair, programmed cell death, the unfolded protein response and the heat shock response, and ROS

Cell repair mechanisms have seen an evolution in the terminology used to describe them. There has been the Milieu Intérieur of Claude Bernard[131], homeostasis from Cannon[132], and proteostasis from Balch[133], just to name a few. Beyond internal equilibrium, these processes are now related to chronic modern day diseases, where homeostasis has failed[134]. Susan Elmore published a review on the topic of programmed cell death in 2007[135]. Referring to apoptosis, she reminds the readers that this process is energy-dependent, while on the other hand, necrosis is not. It follows that it is conceivable that transition from apoptosis to necrosis could occur when energy supply is limited, i.e., conservation of ATP production is essential for life. Prior to cell death, other cellular repair mechanisms are activated. Altered molecular structures, i.e., unfolded proteins, can induce a repair mechanism[83].

Looking at thyroid disease, the conformational changes of thyroglobulin, either due to a genetic cause or found in extraction procedures, present a challenge for the endoplasmic reticulum. The endoplasmic reticulum must clear misfolded proteins by activating repair mechanisms[136]. The endoplasmic reticulum relies on ATP translocation to carry out its functions[137]. Furthermore, the activation of the unfolded protein response and the degradation of misfolded proteins involves the ubiquitin system[138] and the 26S proteasome. Stabilization of the 26S proteasome is both ATP- and NADH-dependent[139]. ATP can add a further contribution to structural maintenance since it has important functions as a hydrophore, maintaining protein solubility[140,141].

On the basis of these modern concepts, it is also possible to add an explanation to some experimental observations. Referring to the obese chicken model of thyroid disease, the authors mentioned two mechanisms that seemed to increase disease severity when the animals were kept in crowded cages: temperature and stress. The authors called this condition the “cage effect”, which, however, was not further characterized. Current studies about a similar situation of heat exposure have demonstrated that

the process of heat shock reaction is taking place[142] resulting in genomic changes that are associated with outcome survival conditions. Another relevant situation in "the cage" is that of stress since physical stressors can lead to a deficiency condition for magnesium[143] and CoQ_{10} (oral presentation by Gvozdjáková A. in 2000 cited by Boreková[144]). Low magnesium levels are related to decreased thyroid function[145]. In Figure 3, we demonstrated the effect of heat exposure on CoQ_{10} levels in relation to thyroid disease as well as the positive corrective action. Similar observations on the protective action of administration of CoQ_{10} to chickens under heat stress are known in the literature[146,147]. The addition of acetyl salicylic acid to CoQ_{10} has an enhancing effect on the expression of hsp70 in the chicken heat stress model[148]. Modern studies have demonstrated that exogenous aspirin can activate the signaling pathways of the unfolded protein response[149]. Further information on aspirin and the endoplasmic reticulum can be found in publications dealing with plant physiology[150,151]. We can now say that the empiric use of aspirin to treat patients with thyroiditis as described in 1902 by de Quervain[12] was on the right track and simply ahead of its time.

Besides heat shock and unfolded protein situations, oxidative damage caused by radicals has been investigated in relation to aging and disease for many decades. Early studies on this topic were presented by Gerschman in 1953 (published in 1954[152]). She discussed common mechanisms found in oxygen poisoning and x-irradiation. In 1956, Harman presented his theory of aging, where he considered the deleterious effect arising from cellular metabolism[153]. He compared these effects to those resulting from irradiation in relation to OH and HO_2 radicals. In addition to external sources of reactive oxygen species, disruption of complex I of the oxidative phosphorylation chain can turn to be noxious as has been described by Adam-Vizi and Chinopoulos[154]. Another peculiarity of CoQ_{10} to keep in mind is its tendency towards lower levels with increasing age (table 3, page 582 in[155]).

In a previous section, we discussed the lack of effect of selenium supplementation in thyroid disease when only putative antioxidant effects are sought. Our working model has a different philosophy. Our decision to consider selenium and CoQ_{10} in our supplementation prescription is based on observations made by Green *et al.*[156] in 1961 and by Hidiroglou *et al.*[157] in 1967. Both groups revealed a functional relation between both substances such that administration of selenium improved the tissue levels of CoQ_{10} in the experimental animals. This important physiological aspect has not been considered in many studies that have chosen to study the effects selenium or CoQ_{10} given as single agents to overcome oxidative changes. A similar observation on this relation was published by Vadhanavikit and Ganther[158]. In a further study, they speculated that the lowering of CoQ_{10} levels depended on the depressed GSH-Px activity that resulted from selenium deficiency[159]. In situations of selenium deficiency, mitochondrial structure and also electron transport function were found to be altered[69]. A study on the distribution of selenium in mitochondria revealed that the highest concentration was found in the inner membrane space, the inner membrane and the matrix[160]. Finally, we would like to mention that selenium and the endoplasmic reticulum are a matter of current research related to resident selenoproteins. Details can be found in the references[161-169].

A concept of shared resources as a general basis of health and disease

The medical literature contains a considerable number of publications that suggest that thyroid function has effects on other body systems, e.g., fertility and reproduction. We would like to address this topic adding a mechanism that we refer to as the WOMED Concept of Shared Resources. The basic interaction of magnesium, selenium, CoQ_{10} and iron in relation to thyroid function is shown in Figure 13. What we initially conceived for thyroid function has applicability in the whole body. Iron has a high relevance since it is a constituent in countless iron-sulfur proteins[170-178].

The WOMED Concept of Shared Resources is shown in Figure 12. The central condition is defined by sufficient supply of magnesium, iron, selenium, and CoQ_{10}. B. This common source will allow normal mitochondrial function and consequently ensure the function of the endoplasmic reticulum, i.e., protein

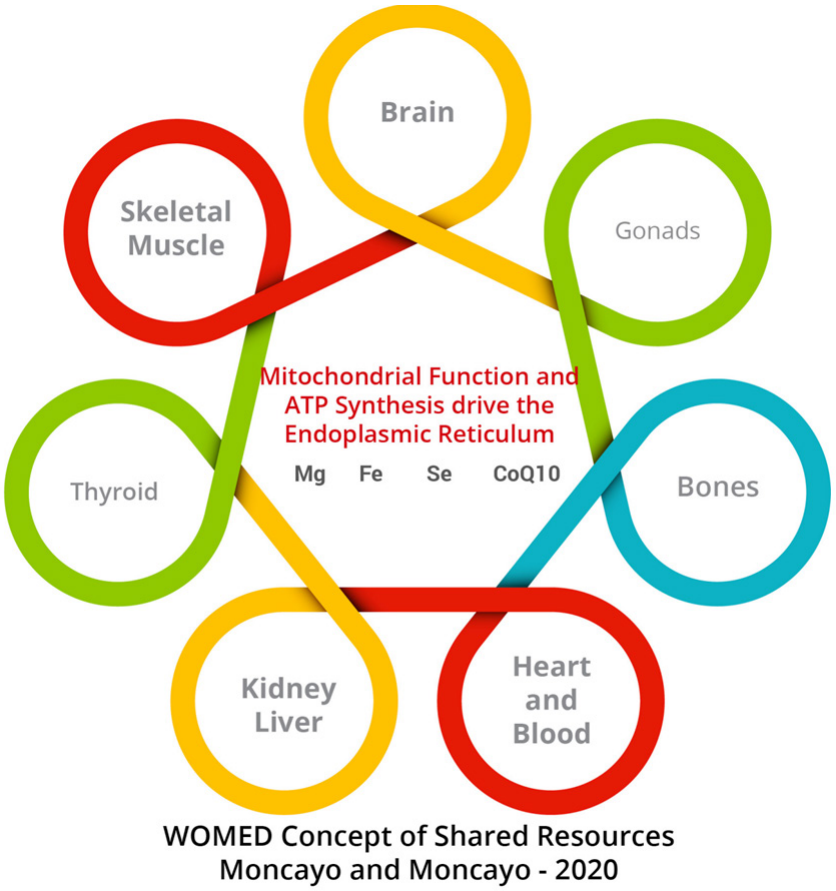


Figure 12. The WOMED concept of Shared Resources. The central element is defined by sufficient supply of magnesium, iron, selenium, and $CoQ_{10.}$ This common source will allow normal mitochondrial function. Sufficient ATP production will have positive effects on the function of the endoplasmic reticulum

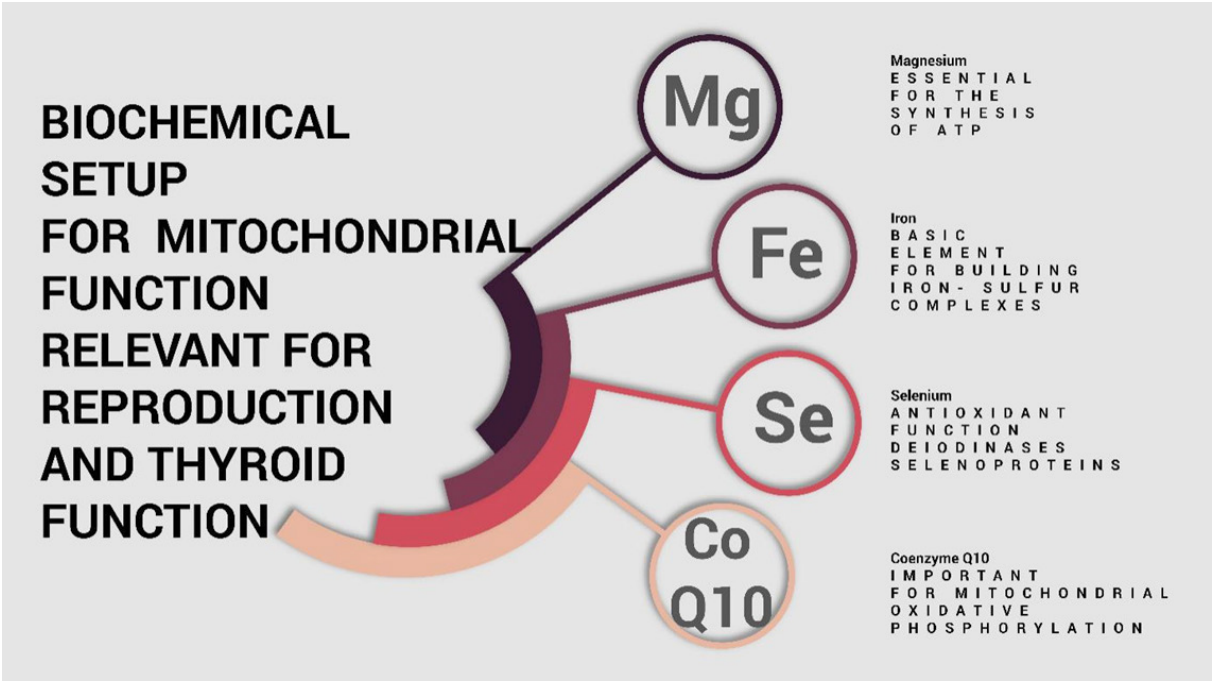


Figure 13. The combined availability of magnesium, iron, selenium, and CoQ_{10} will maintain key functions in the body, i.e., mitochondria

repair. Lack of CoQ_{10} affects primarily energy generation and is related to multi-organ involvement[179]. The graphic represents Resource Distribution as a flow continuum inspired by the movement of Qi in Chinese Medicine[180-191] and taking into consideration the idea of metabolic flexibility[192].

Looking back and beyond, we now consider thyroid disease as a model of acquired unfolded protein disease, thus proposing that the concepts of thyroid physiology should be modified. In the final phases of writing this manuscript, we found a recent contribution from the research group of Peter Arvan describing the role of endoplasmic reticulum stress on the survival and adaptation of thyrocytes[193]. We believe that magnesium plays an important role in this process of adaptation by being a key element in the generation of ATP, which in turn results in the recovery of the function of the endoplasmic reticulum. In other words, low levels of magnesium are associated with misfolded thyroglobulin molecules, which induce the production of anti-thyroglobulin antibodies. The risk of developing anti-thyroglobulin antibodies in situations of magnesium deficiency was described by Wang in 2018[50]. Our data confirm and expand these previous findings. In 2019, Ana Barić described an association between symptom burden and anti-thyroglobulin antibodies in patients with Hashimoto's thyroiditis[49]. These findings coincide with our previous description of the psychosomatic aspects of thyroid disease, where we found an association between magnesium deficiency and symptoms[194]. In 2020, Luka Brčić together with Ana Barić and others reported that the expression of AATF is diminished in cases of Hashimoto's thyroiditis and is associated with increased thyroid volume[93]. In the historical and experimental sections of this paper, we mentioned that increased thyroid volume has been a characteristic known for decades.

The important role of genetics in this context can be recognized in recent studies by Santos *et al.*[195], demonstrating a relation between the SELENOS promoter genotype and NFE2L2 promoter genotype, which together modulate the risk of Hashimoto's thyroiditis. SELENOS is of importance since it constitutes a basic element of the endoplasmic reticulum, especially under conditions of glycolytic metabolism[196]. In Figure 6, we indeed demonstrated that chronic thyroiditis in an active phase can be associated with glycolytic metabolism of the thyroid.

Since the elements leading to this acquired situation are amenable to correction, the fears related to the artificial concept of autoimmunity should disappear from the minds of patients and medical practitioners. More emphasis should be put on maintaining a balanced state of the nutritional elements that the body must share - magnesium, selenium, iron and CoQ_{10}.

DECLARATIONS

Acknowledgments

The principal author (Moncayo R) was introduced to thyroid research by Rodrigo Fierro-Benítez (Quito, Ecuador, 1976-1979). At this time, the teachings of Gregorio Marañon[197,198] transmitted by Fierro-Benítez were also absorbed. This period was followed by valuable scientific exchange with John B. Stanbury (Boston), John Dunn (Charlottesville) and Georg Riccabona (Innsbruck) in the context of the iodine deficiency network[199-202]. Many important and contemporary concepts and lessons have been found in the publications from Gerardo Medeiros-Neto (São Paulo), Peter Arvan (Ann Arbor), and Carina M. Rivolta, Cintia E. Citterio, and Héctor M. Targovnik (Buenos Aires).

Authors' contributions

Main concept for the manuscript, literature search, preparing the figures, writing the manuscript: Moncayo R

Developed the method of power Doppler sonography for thyroid patients, financial support: Moncayo H

Extracted clinical data from patients shown in the Figures, literature search on selenium and thyroid, reading the publications and extracting the content: Reisenzahn J

Availability of data and materials

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This paper was written in the form of a case report, retrospectively describing the course of the diagnostics and therapy. The concept was considered and approved by the Medical University of Innsbruck (Studienabteilung).

Consent for publication

Informed consent was obtained from the patient for the publication of this report.

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