



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

ARCHIVIO ISTITUZIONALE
DELLA RICERCA

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Oxidative stress as a key feature of autoimmune thyroiditis: an update.

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Oxidative stress as a key feature of autoimmune thyroiditis: an update / Ruggeri RM, Campennì A, Giuffrida G, Casciaro M, Barbalace MC, Hrelia S, Trimarchi F, Cannavò S, Gangemi S.. - In: MINERVA ENDOCRINOLOGICA. - ISSN 0391-1977. - ELETTRONICO. - 45:4(2020), pp. 326-344. [10.23736/S0391-1977.20.03268-X]

Availability:

This version is available at: <https://hdl.handle.net/11585/800904> since: 2021-02-18

Published:

DOI: <http://doi.org/10.23736/S0391-1977.20.03268-X>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).
When citing, please refer to the published version.

(Article begins on next page)

This is the final peer-reviewed accepted manuscript of:

Ruggeri RM, Campennì A, Giuffrida G, Casciaro M, Barbalace MC, Hrelia S, et al. Oxidative stress as a key feature of autoimmune thyroiditis: An update. *Minerva Endocrinologica*. 2021;45(4):326–43.

The final published version is available online at: <https://dx.doi.org/10.23736/S0391-1977.20.03268-X>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

When citing, please refer to the published version.

OXIDATIVE STRESS AS A KEY FEATURE OF AUTOIMMUNE THYROIDITIS: AN UPDATE.

Journal: Minerva Endocrinologica

Paper code: Minerva Endocrinol-3268

Submission date: June 8, 2020

Article type: Review Article

Files:

1. Reply letter to comments on the manuscript
Version: 3
Description: response to referees with highlighted modified text
File format: application/vnd.openxmlformats-officedocument.wordprocessingml.document
2. Manuscript
Version: 6
Description: revised manuscript as suggested by the referees
File format: application/vnd.openxmlformats-officedocument.wordprocessingml.document
3. Figures 1
Version: 1
Description: Figure 1
File format: image/jpeg
4. Figures 2
Version: 1
Description: Figure 2 A) and B)
File format: image/jpeg

1 We thank the Editor and the reviewers for the interest in our work and for their constructive comments.
2 We have accepted the suggestions and comments and have modified the manuscript accordingly. To
3 facilitate the reviewing process, the sentences added and/or changed are highlighted in yellow in the text. We
4 provide a point-by-point response to Referees' comments.
5

6 Both the Abstract and the Reference list were modified according to journal guidelines, as per Editorial
7 Office's request. The Tables were added at the end of the manuscript file.
8

9 We hope that the manuscript is now in good order for publication.
10

11 **Response to the Editor**

12 We thank the Editor for the interest and the overall favorable evaluation given to our work. We
13 really appreciate his pertinent suggestion to add a short description of the interplay between inflammation,
14 the aging of the thyroid gland (including the anti-oxidative system) and the development of thyroid diseases.
15 The suggested references were quoted.
16

17 **Response to Referee 1.**

18 We thank referee 1 for the overall favorable evaluation and his/her comments and suggestions, that we
19 carefully met. We modified the manuscript accordingly.
20

21 *MINOR CORRECTIONS*

22 1. *I suggest authors to discuss about the relationship between HT and thyroid cancer, particularly the*
23 *papillary histotype. Specifically, authors should report some data and reflect about the interplay between*
24 *AT, thyroid cancer and oxidative stress. Indeed, an amount of data are available about the relationship*
25 *between oxidative damage and thyroid carcinogenesis. This may further enrich the paper.*
26

27 We thank the Referee for her/his suggestion. We added a paragraph concerning the interplay between
28 autoimmune thyroiditis and thyroid cancer and oxidative stress (in yellow), to further improve the review.
29 Pertinent references were quoted.
30

31 2. *In the paragraph Possible Therapeutic Role of Antioxidants, I suggest to insert some subparagraphs*
32 *dedicated to clarify how specific supplements impact on the thyroid oxidative status and the current*
33 *indications about clinical use. Particularly, authors should refer not only to selenium or LT4 treatment, but*
34 *also to other possible interventions with antioxidant effect such as VITD supplementation.*
35

36 Once again, we thank the Referee for this very pertinent comment. We added some subparagraphs to
37 specify the potential role of each specific supplement on the thyroid oxidative status, also discussing the
38 possible antioxidant effects of Vitamin D. Pertinent references were quoted.
39

40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

1 3. Please add, if possible basing on journal guidelines, an expert opinion paragraph where authors
2 provide their own considerations and indications about current therapeutic use of antioxidants and suggest
3 strategies of future research and development.
4

5
6 We thank the Referee for his suggestion. We have had our considerations about current and future
7 antioxidant therapeutic strategies in the conclusions.
8

9 10 11 12 **Responses to Reviewer 2**

13 We thank the Referee for his/her overall favorable and constructive comments.
14

15 16 **MINOR CORRECTIONS**

17
18 The sentence "Therefore, as already demonstrated in vitiligo, also in AT oxidative stress may represent
19 a pathogenetic link between environmental agents and autoimmunity, playing a role both in initiation (novel
20 autoantigens) and progression (autoimmune-related inflammation, cell apoptosis and parenchymal
21 destruction) of the disorder" should be rephrased. In particular, what do the Authors refer to with the term
22 "novel autoantigens". Using this term might lead to confusion, I would suggest to make the sentence more
23 clear.
24
25
26
27

28 We thank the Referee for this suggestion. The pertinent sentence was rephrased to make it clearer.
29

30 31 **REVISED VERSION OF THE MANUSCRIPT (TEXT ONLY)**

32 **Highlighted= added; colored text= modified.**
33

34 **ABSTRACT**

35
36
37
38 Introduction. Oxidative stress has been proposed as one of the factors concurring in the pathophysiology of
39 autoimmune thyroid diseases. Reactive oxygen species are the main expression of oxidative stress in
40 biological systems, and their production can overcome antioxidant defenses ultimately leading to cell
41 damage, apoptosis, and death. The present review was aimed at describing the state of the art of the
42 relationships between oxidative stress and autoimmune thyroiditis. The most used biomarkers of oxidative
43 stress and their correlation with thyroid function are reported.
44
45
46

47
48 Evidence Acquisition. We conducted a search of the literature in the English language starting from 2000,
49 using the following search terms: "Hashimoto thyroiditis", "autoimmune thyroiditis", "hypothyroidism",
50 "hyperthyroidism", "oxidative stress", "oxidants", "antioxidant", "advanced glycation end products". Both
51 clinical studies and animal models were evaluated.
52
53
54
55

1 Evidence Synthesis. Data from clinical studies clearly indicate that the balance between oxidants and
2 antioxidants is shifted towards the oxidative side in patients with autoimmune thyroiditis, suggesting that
3 oxidative stress may be a key event in the pathophysiology of the disease, irrespective of thyroid function.
4 Studies in animal models, such as the NOD.H2h4 mouse, confirm that thyroidal accumulation of ROS plays
5 a role in the initiation and progression of autoimmune thyroiditis.
6
7

8
9 Conclusions. Oxidant/antioxidant imbalance represent a key feature of thyroid autoimmunity. Oxidative
10 stress parameters could be used as biochemical markers of chronic inflammation, to better predict the disease
11 evolution along its natural history. Dietary habits and antioxidant supplements may provide protection from
12 autoimmunity, opening new perspectives in the development of more tailored therapies.
13
14

15 16 17 18 INTRODUCTION.

19
20 Autoimmune thyroiditis (AT), also referred to as Hashimoto's thyroiditis (HT), is the most common
21 autoimmune endocrine disorder and the main cause of hypothyroidism in iodine-sufficient areas 1,2. AT
22 covers a wide spectrum of phenotypes, encompassing different clinico-pathological entities: the classic form,
23 which features goiter with or without hypothyroidism; the fibrous variant with glandular fibrosis and rapid
24 progression towards hypothyroidism; the IgG4-related variant; the juvenile form and the painless (or silent)
25 thyroiditis, occurring either sporadically or in the post-partum 1,2. Overall, AT has an estimated prevalence
26 of around 3-5% of the general population, with peaks during adolescence and middle adulthood 3, 4. In
27 addition, the prevalence of thyroid autoantibodies increases in people over the age of seventy 5. At any age,
28 females are more affected than males with a female to male ratio around 5-7:1 1,2. Incidence has been
29 raising in last decades, mostly in developed countries 3. AT often occurs in association with other endocrine
30 and non-endocrine autoimmune diseases in the same patient (autoimmune comorbidity) and/or in in other
31 members of the same family (familial clustering), facilitated by a predisposing polygenic background. The
32 clinical presentation of AT can widely vary, from the rapid development of severe hypothyroidism to an
33 initial but transient thyrotoxicosis (Hashitoxicosis). In many cases, AT generally proceeds from an
34 asymptomatic autoimmune condition, featured by circulating anti-thyroid autoantibodies and normal thyroid
35 function, towards subclinical and then overt hypothyroidism 1,2,3,. Even if return to normal thyroid function
36 has been reported, the final outcome of AT is permanent hypothyroidism due to progressive destruction of
37 thyroid follicular cells 1,2.
38
39

40
41 AT is a chronic inflammation of the thyroid that results from an inappropriate immune reaction against the
42 gland. Failure of immunological self-tolerance leads to activation and expansion of autoreactive T cells,
43 release of pro-inflammatory cytokines and differentiation of self-reactive B cells with production of organ-
44 specific autoantibodies 6. Together, these events are responsible for chronic inflammation with infiltration of
45 hematopoietic mononuclear cells (T and B lymphocytes, plasma cells and macrophages), interstitial fibrosis
46 and follicular cells damage by means of both cell- and antibody-mediated cytotoxic and apoptotic
47
48
49
50
51
52
53
54
55

1 pathways⁶. In this cascade of events, a crucial role is played by pro-inflammatory cytokines, such as IL-1 β ,
2 IL-6, IFN- γ , TNF- α , IL-22 and IL-23, that promote and amplify inflammation, contribute to tissue damage
3 and modulate the metabolic and immune function of thyroid follicular cells (Figure 1) ^{6,7,8,9}.

4
5
6 Underlying this process there is a complex interplay between genetic and environmental factors: exogenous
7 and existential factors trigger the development of the immune response against thyroid autoantigens in
8 genetically susceptible individuals ^{2,6,10}. The genetic background of the disease is still not fully understood
9 and includes a wide number of genes, encompassing the HLA and immune-regulatory genes, that confer
10 generalized susceptibility to autoimmunity on one hand, and several different tissue-specific genes, which
11 exert either predisposing or protective effects for particular types of disease in a tissue-specific fashion on
12 the other ². In face of the constancy of the genetic basis, the number of potential environmental triggers has
13 been enormously expanding over the last decades. They include changes in lifestyle (modified infectious
14 habitat and ameliorated personal hygiene, stress, dietary habits, sedentary life), increased exposure to
15 pollutants and toxics, radiations, novel (i.e. tyrosine-kinase inhibitors and immune check-point inhibitors)
16 and old (i.e. lithium, interferons, amiodarone) drugs, gut microbiome alterations and nutrients (notably,
17 vitamin D deficiency, iodine and selenium intake) ^{2,6,10}. These environmental factors may affect the thyroid
18 gland and trigger/favor the development of autoimmunity through a wide range of mechanisms, including
19 increased free radicals accumulation and enhanced oxidative stress ^{11,12}.

20
21
22
23
24
25
26
27
28 Oxidative stress is the result of an imbalance between oxidants production and antioxidant defense
29 mechanisms. This condition concerns all the alterations that may occur at tissue, cellular and biological
30 macromolecular level. In biological systems oxidants are represented by free radicals, i.e. partially reduced
31 forms of oxygen and nitrogen, the so-called reactive oxygen (ROS) and nitrogen (RNS) species. From a
32 chemical point of view, a free radical is a molecular entity having one or more unpaired electrons on one
33 atomic or molecular orbital. They present an extremely high reactivity and instability and tend to catch the
34 missing electron from other molecules. Free radicals start chain reactions leading to shutdown of initial
35 radical and/or to the generation of new radicals. These molecules are products of normal cell metabolism and
36 are essential for several biochemical processes inside the cell when at low levels. On the contrary, the
37 alteration of the normal redox state due to their excessive production and/or accumulation into the cells
38 causes oxidation of all macromolecules (membrane lipids, proteins and nucleic acids), leading to cell
39 damage, apoptosis and death ¹³. Indeed, as postulated in the 'redox window' hypothesis, adequate ROS
40 levels help physiological cellular functions, but excessive ROS production is involved in the development of
41 several pathologies ¹⁴. The main types of ROS that can be generated in the cell are superoxide (O₂^{•-}) anion,
42 hydroxyl (OH[•]) radical, and hydrogen peroxide (H₂O₂) which is a non-free radical. There are several
43 enzymes involved in ROS production. At mitochondrial level, the complex I and III are implicated in the
44 production of large amounts of superoxide, and their activity is slowed during increased ROS production
45 which, in turn, promotes further ROS release ¹⁵. Moreover, mito-ROS can subsequently activate other ROS
46 sources¹⁶. NAD(P)H (nicotinamide adenine dinucleotide phosphate) oxidase catalyzes the release of
47
48
49
50
51
52
53
54
55

1 superoxide anion or hydrogen peroxide through the reduction of molecular oxygen, using as electron donor
2 NADPH, in various intracellular and extracellular compartments 17. Nitric oxide synthase (NOS) enzyme is
3 responsible for NO production but excessive superoxide levels, in condition of oxidative stress, depletes
4 (6R)5,6,7,8-tetrahydrobiopterin (BH₄), the essential NOS cofactor 18, causing NOS impairment, which
5 becomes itself a source of superoxide. Xanthine oxidase (XO), released during pathophysiological processes,
6 donates electrons to molecular oxygen producing superoxide and hydrogen peroxide (Figure 2).
7
8
9

10 Multiple enzymatic and non-enzymatic systems, the so-called antioxidants, are present in both the
11 bloodstream and peripheral tissues, to prevent/counteract excess free radicals' production and/or
12 accumulation. The enzymatic defenses can remove radicals with a catalytic mechanism, while the non-
13 enzymatic defenses have heterogeneous working mechanisms, as they can sequestrate pro-oxidant
14 molecules, or act as radical scavenger. Non-enzymatic antioxidants may be either endogenous, like the
15 mitochondrial uncoupling proteins, reduced glutathione (GSH) and transport proteins synthesized in the liver
16 (ceruloplasmin, transferrin, albumin etc...) or exogenous products, including uric acid, vitamin E and C,
17 which are mainly derived from diet 13. The enzymatic antioxidants include the glutathione peroxidase
18 (GPx)/glutathione reductase (GR) system and the glutathione-S-transferases (GSTs), which represent the
19 first-line defense against oxidants in almost every cell types, with a tissue-specific distribution (Figure 2).
20 GPx/GR system reduces H₂O₂ or hydroperoxides reduction, using GSH as electron donor and NADPH as a
21 co-factor, meanwhile GSTs are a class of enzymes which catalyzes the conjugation of GSH to electrophilic
22 compounds 19. Thioredoxin (TRx)/thioredoxin reductase (TRxR) constitutes another important system for
23 H₂O₂ detoxification. TRxR acts using NADPH to restore the oxidized thioredoxin in the reduced form. Both
24 GPx and TRxR are selenoproteins, with a Se atom incorporated in their catalytic domain in the form of
25 selenocysteine, and require an appropriate supply of Se to be active 20. At the highest levels of oxidants, also
26 catalases (CAT) and superoxide dismutase (SOD) contribute to enzymatic degradation of free radicals 21.
27
28
29
30
31
32
33
34
35

36 Under physiological conditions, there is an equilibrium between the production and detoxification of free
37 radicals, the so-called redox homeostasis, which is essential for every kind of aerobic life 20,22. When free
38 radicals are produced in excess or are not adequately degraded by the cells or both, then a condition of
39 oxidative stress occurs and causes cell damage and death, and tissue inflammation, also worsened by the
40 release of pro-inflammatory cytokines in damaged tissues 20,22. Almost every cell type and tissue are prone
41 to oxidative damage, with tissue-specific differences, and oxidative stress is present at the site of
42 inflammation²³.
43
44
45
46

47 For these reasons, oxidative stress has been thought to represent a key feature of several inflammatory and
48 immune-related disorders, including autoimmune thyroid diseases (AITDs) 24,25,26. Increased ROS
49 production due to environmental agents (i.e. iodine excess, radiations, toxics and drugs, pollutants) could
50 induce a modification of tissue proteins, or might dysregulate the immune system, influencing the
51 appearance of the autoimmune disorder 24.
52
53
54
55

1 The present review was aimed at summarizing the evidence of the recent literature concerning the
2 bidirectional association between oxidative stress and AT, since the relevance of oxidative stress and the
3 beneficial effects of antioxidant supplementation in ATs are intensely debated at present. We also revised the
4 different biomarkers that have been measured to evaluate the impact of ROS in the setting of AT, in an effort
5 to identify useful and reliable markers of oxidative stress.
6
7

8 EVIDENCE ACQUISITION.

9
10 For this purpose, we extensively examined both in vivo and in vitro data on changes in oxidative balance and
11 oxidative stress markers/indices, published in the last two decades in the field of AITDs. The review of the
12 pertinent literature has been conducted employing MEDLINE database. On this website, we searched for
13 articles using key terms related to Hashimoto's thyroiditis and oxidative stress. A MeSH search has been
14 performed using "Hashimoto thyroiditis"/"autoimmune thyroiditis" AND "oxidative stress", followed by a
15 simple search using "Hashimoto thyroiditis", "autoimmune thyroiditis" AND "oxidative stress" OR
16 "oxidants" OR "antioxidant" OR "advanced glycation end products" and a search using "oxidative stress"
17 AND autoimmune thyroiditis" OR "hypothyroidism" OR "hyperthyroidism" as key terms. We obtained 196
18 results; we included in the present paper only the articles matching the following inclusion criteria: English
19 language, publication in peer-reviewed journals starting from 2000, research papers. Articles considered
20 relevant and cited in the references of the selected papers were included too. We excluded articles for
21 irrelevance to the topic in question, duplicates, papers written in other languages apart from English and
22 articles published before 2000.
23
24

25 EVIDENCE SYNTHESIS.

26 Animal Models.

27 The NOD-H2(h4) mouse represents an animal model of autoimmune lymphocytic thyroiditis that mimics
28 human Hashimoto's thyroiditis. The NOD-H2h4 mice spontaneously develop an autoimmune thyroiditis,
29 whose incidence dramatically increases when adding iodine to the drinking water 27,28,29,30. Excess iodine
30 triggers autoimmunity by multiple mechanisms, including changing the immunogenicity of the thyroglobulin
31 molecule, upregulating intracellular adhesion molecule-1 (ICAM-1) expression on thyrocytes and increasing
32 ROS production by the thyrocytes themselves 26. Burek and co-workers demonstrated that thyrocytes
33 isolated from NOD-H2h4 mice produced significantly more H₂O₂ than control thyrocytes when exposed to
34 iodine²⁶. ROS accumulation also contributes to upregulation of ICAM-1 expression on the surface of
35 thyrocytes, enhancing immune cells infiltration of the thyroid gland and pro-inflammatory cytokines
36 production 31,32. Incubation with the antioxidant diphenyleneiodium, an inhibitor of NADPH oxidase,
37 reduced both ROS production and ICAM-1 expression in cultured NOD-H2h4 thyrocytes 32. Kolypetri and
38 Carayanniotis showed that impaired control of oxidative stress mechanisms is associated with high
39 susceptibility to apoptosis in NOD.H2(h4) thyrocytes exposed to iodine 33 . Finally, the antioxidant N-
40 acetylcysteine (NAC) reduced ROS and the immune infiltration, thereby leading to a restoration of thyroid
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

1 morphology, in NOD.H2h4 thyroid glands 34. Likely NAC exert its protective effects by acting on
2 infiltrating inflammatory cells rather than directly on thyrocytes. In the same model, increased thyroid
3 content of 4-HNE, a toxic product from lipid peroxidation used as a marker of oxidative stress, was reported
4 34. Overall, studies in the NOD.H2h4 model suggest that thyroidal accumulation of ROS plays a role in the
5 initiation and progression of autoimmune thyroiditis.
6
7

8 CLINICAL STUDIES.

9 Thyroid Function and Oxidative Stress.

10
11 There is a close and bidirectional relationship between the thyroid gland and oxidative stress since it
12 concerns both the effects of thyroid function on oxidants/antioxidants balance in peripheral tissues and the
13 effects of oxidative stress on thyroid gland itself.
14
15

16
17 Firstly, thyroid hormones play a crucial role in regulating redox homeostasis. On the one hand, they
18 accelerate the basal metabolic rate and cell oxidative metabolism by inducing mitochondrial respiration, and
19 enhance free radical production; on the other, they regulate the synthesis of enzymatic and non-enzymatic
20 antioxidants. As a consequence, both hyperthyroidism and hypothyroidism have been associated with
21 oxidative stress, since the former has been shown to increase oxidants production as a result of increase of
22 metabolic processes into cells and to cause consequent exhaustion of antioxidants 35,36,37,38,39,40 and the
23 latter to reduce the antioxidant defense systems 25,37,39,41,42,43 .
24
25

26
27 Moreover, the thyroid itself is exposed to lifelong oxidative stress, induced by the continuous generation of
28 H₂O₂, fundamental for the iodine oxidation during the process of thyroid hormone synthesis. For this
29 reason, thyroid cells display efficient detoxification systems, mainly represented by the cytoplasmic GPx1
30 and by the secretive GPx3 in the colloid lumen 44,45. Inadequate Se supplementation may impair both the
31 expression and the enzymatic activity of the antioxidant selenoproteins GPx1 and GPx3, altering the natural
32 oxidant/antioxidant thyroid cycle and resulting in a reduced antioxidant activity in thyrocytes 21. In animal
33 models, Se deficiency is associated to oxidative cell damage, defective tissue repair and thyroid fibrosis,
34 whilst Se supplementation is protective against experimentally induced autoimmune thyroiditis 46,47.
35
36 Similarly, in in vitro studies Se supplementation prevents cell damage from oxidative injury and protects
37 against apoptosis 48,49,50. Also, iodine deficiency may increase oxidative stress, by uncoupling H₂O₂
38 generation, which is stimulated by TSH in response to iodine deficiency, from iodine oxidation, which is
39 reduced because of iodine deficiency. As a result, excess H₂O₂ accumulates in the colloid lumen. When
40 combined selenium deficiency occurs, it results in decreased expression of the selenoprotein GPx3 by
41 thyrocytes. As a result, inadequate GPx activity does not remove excess H₂O₂, which cannot be consumed
42 by thyroperoxidase (TPO) for tyrosine iodination and iodothyronine coupling under conditions of iodine
43 deficiency, leading to H₂O₂-induced tissue necrosis and fibrosis 21. Hypothyroidism enhances such an
44 effect because H₂O₂ production, which is increased under continuous and elevated stimulation of the thyroid
45 gland by TSH, is not adequately counteract because of the reduced synthesis of antioxidants, as above
46
47
48
49
50
51
52
53
54
55

1 reported 51. A high iodine intake might cause an excessive H₂O₂ generation too, as clearly demonstrated in
2 animal models 52,32

3
4
5 Finally, oxidative stress seems to have a relevant role in the aging of thyroid gland and in the pathogenesis of
6 age-related thyroid dysfunction. Aging is associated with a decrease in thyroid volume and hormone
7 secretion, as well as with a reset of the hypothalamic–pituitary–thyroid axis 53. The production of free
8 radicals and ROS gradually increases with aging, whereas the activity of antioxidant defenses decreases,
9 leading to ROS accumulation in cells and tissues 54. The oxidative stress caused by this imbalance might
10 contribute to the progressive age-related dysfunction of the thyroid gland directly, through cellular oxidative
11 damage, and indirectly, through alterations in protein synthesis and function. Prolonged age-dependent ROS
12 exposure also causes genomic damage and telomere shortening, contributing to accumulation of senescent
13 cells in the thyroid with age. Moreover, ROS excess favors inflammation by increasing immune cells
14 recruitment into tissues (potentially damaging), by stimulating pro-inflammatory cytokines synthesis/release
15 and by modulating other processes such as mitochondrial function and microRNA production. A chronic,
16 low-level inflammation is a key feature of aging process, the so-called “inflammaging”, and a major
17 contributor to both thyroid senescence and age-related thyroid disorders 53,54. Indeed, aging is associated
18 with an increase in the prevalence of several thyroid diseases, and solid evidence indicates that a condition of
19 oxidative stress is relevant for their development and/or progression.

27 Thyroid Autoimmunity and Oxidative Stress.

28
29 A close relationship exists between oxidative stress and thyroid autoimmunity irrespective of thyroid
30 dysfunction 55,56,57,58. In autoimmune disorders such as AT, the infiltrating immune cells develop a
31 chronic inflammatory milieu in which ROS accumulate and exert a toxic effect on surrounding cells and
32 tissues. In these conditions, oxidative stress may play a role in both the induction of autoimmune response
33 against self-antigens, and the amplification of tissue inflammation and damage, once the autoimmune
34 process has been initiated 24,26. First, oxidative imbalance may play a role in the onset of the autoimmune
35 response. ROS excess causes oxidative modifications of proteins, lipids and DNA, which become highly
36 immunogenic and may act as neo-antigens, leading to loss of self-tolerance in genetically predisposed
37 individuals 56,57. Thyroidal accumulation of ROS has been shown to promote cleavage of thyroglobulin
38 into several fragments, likely exposing the immune system to novel epitopes and thus enhancing the
39 autoimmune response 59. Once the autoimmune reaction has been triggered, the related inflammation may
40 promote excess ROS production and enhanced oxidative stress in thyroid tissue via activation of T and B
41 lymphocytes infiltrating the gland. In fact, it has been demonstrated that Th1 cytokines released by activated
42 lymphocytes induce ROS production by thyrocytes 60,61. Activated lymphocytes themselves produce excess
43 ROS 62,63. Whatever the source is, ROS accumulation causes oxidative damage of the cells, leading to
44 apoptosis, necrosis, and parenchymal destruction, as it occurs in other autoimmune diseases 11,64,65.
45 Moreover, the antioxidant system is not sufficient to counteract ROS overproduction, since the antioxidant
46 potential is reduced in HT patients, even in euthyroidism 25,55,56,57,58. Therefore, as already demonstrated

1 in vitiligo, also in AT oxidative stress may play a role both in initiation (modified proteins acting as neo-
2 antigens) and progression (autoimmune-related inflammation, cell apoptosis and parenchymal destruction) of
3 the disorder 25,26. Studies in euthyroid patients with AT are more limited than those in hypo- and
4 hyperthyroid patients, but they all agree on demonstrating higher oxidative stress in AT cases than in
5 controls, due to increased oxidants or decreased antioxidants or both 25,55,56,57,58 . In each study a
6 significant correlation with thyroid autoimmunity was found. Ates et al reported a negative correlation
7 between serum total antioxidant activity and anti-thyroperoxidase antibodies (TPO-Ab), while Baser et al.
8 reported a positive correlation between serum oxidants and anti- thyroglobulin (Tg-Ab) antibodies 55, and
9 Ruggeri et al. confirmed the TPO-Ab were independent predictors of the oxidative status in euthyroid HT
10 patients 56. Overall, human studies report an increased oxidative status in AT, even in euthyroidism, but do
11 not clarify whether it is the cause or the consequence of thyroid autoimmunity. Maybe autoimmunity and
12 oxidative stress coexist and act in synergism in initiating and/or perpetrating the progressive damage of
13 thyrocytes.
14
15
16
17
18
19

20 Autoimmunity, cancer and oxidative stress.

21
22
23 The relationship between AT and thyroid cancer, especially papillary thyroid cancer (PTC), is a well-known
24 fact 66,67 and several research groups have studied the role played by oxidative stress in thyroid
25 carcinogenesis, reporting an increase in levels of oxidants and/or a decrease in antioxidant activity in patients
26 with thyroid cancer 68,69. The accumulation of excess ROS in the thyroid gland can cause DNA damage,
27 resulting in mutagenic genetic alterations and promoting tumour initiation and development 70,71. Thus, it is
28 conceivable that inflammation and oxidative stress, that are closely related processes, may contribute to the
29 increased risk of thyroid cancer that has been reported in AT 67,71, whilst antioxidant may exert protective
30 effects 72. However, scanty data are available concerning the interplay between AT, thyroid cancer and
31 oxidative stress. Lassoued et al evaluated the presence of OS markers in patients suffering from AITDs
32 (Graves' disease and Hashimoto's Thyroiditis) and patients with PTC, before and after thyroidectomy and
33 radioiodine therapy 73. Comparing their oxidative stress profile with that of HT patients, malondialdehyde
34 (MDA), SOD and CAT activities were high, with reduced levels of GPx, in both groups. However, the
35 absolute values were higher (and lower regarding GPx), in the PTC patients, thus suggesting a higher grade
36 of oxidative stress in this population deriving from a more sustained production of free radicals and/or a
37 damaged antioxidant system 73. Moreover, these alterations did not change after thyroidectomy and
38 radioiodine therapy, thus confirming a previous evidence of an intrinsic oxidative imbalance in PTC 74
39 .Such data could be a starting point to further analyze the potential diagnostic/prognostic role of OS
40 parameters in PTC, also considering the use of antioxidant compounds to ameliorate patients' recovery 73.
41
42
43
44
45
46
47
48
49

50 Biomarkers of Oxidative Stress.

51 Hashimoto's thyroiditis (HT)

1 Starting from 2000s, growing evidence emerged concerning several peripheral/circulating markers of
2 oxidative stress in HT patients (Table 1). In 2006 Taddei et al. demonstrated that patients with HT and
3 subclinical hypothyroidism presented with higher C-reactive protein and IL-6 values. In these subjects, the
4 antioxidant vitamin C did not improve endothelial dysfunction and nitric oxide (NO) availability after the
5 administration of indomethacin, that unselectively blocked a COX2-dependent pathway 75. In 2008 Erdamar
6 et al. observed an increase in MDA, nitrite, vitamin E, and myeloperoxidase (MPO) activity in hypothyroid
7 HT patients, as well as high levels of MDA and MPO activity in hyperthyroid subjects with Graves' disease
8 (GD). Treatments for both conditions revealed a reduction in nitrite and vitamin E in HT patients and a
9 decrease of the raised parameters in GD ones versus a homogenous group of healthy controls. In particular,
10 levothyroxine (L-T4) therapy took two months to lead markers back to normal values, while a faster
11 response (one month) was observed with propylthiouracil (PTU) treatment for hyperthyroidism, thus
12 confirming a role of thyroid hormones oscillations into influencing the redox homeostasis of thyroid gland
13 37. A decrease in plasmatic levels of transforming growth factor-beta 1 (TGF- β 1) and vascular endothelial
14 growth factor (VEGF), and an increase in nitrite/nitrate (NO_x, metabolites deriving from NO), was observed
15 in a group of HT patients versus controls 76. In the study by Torun and co-workers, MDA was elevated in
16 both hypothyroid and subclinical hypothyroid patients compared with controls and showed a correlation with
17 altered lipid metabolism in hypothyroidism states. On the contrary, total antioxidant status TAS levels show
18 no significant differences between groups, suggesting an insufficient increase in the antioxidant status in
19 hypothyroid patients 42. In the study by Lassoued et al. oxidative stress in patients with untreated HT and
20 GD resulted higher than in healthy controls, especially concerning SOD activities and MDA. Besides, the
21 same evidence with more elevated values, was observed in patients with surgically treated PTC, thus
22 demonstrating a disturbed oxidative profile as in autoimmune diseases 73. As stated before, oxidative stress
23 could also be influenced by TSH levels, as demonstrated by Ozturk and colleagues who evaluated oxidative
24 stress parameters in a cohort of HT patients, differently affected by subclinical (SHypo) or overt
25 hypothyroidism (OHypo). Several serum parameters (MDA, diene conjugate – DC, protein carbonyl – PC,
26 nitrotyrosine – NT and ferric reducing antioxidant power - FRAP) were altered in comparison with healthy
27 controls, but while MDA and DC levels were normal in SHypo, all these analytes were increased in the
28 OHypo group, and Dc and copper-induced MDA were also measurable in low-density lipoprotein (LDL)
29 fraction in OHypo patients only 77. Moreover, even GSH status has been investigated in HT. As
30 demonstrated by Rostami et al., serum glutathione was significantly reduced in affected subjects versus
31 controls, and it correlated with TPO-Ab values, thus suggesting that this decrease could be a hallmark of
32 oxidative stress activation and immunological intolerance development 78. In the study by Reddy and
33 coworkers, MDA and GPx values were elevated, while GSH, TAC as FRAP, SOD, and SOD/GPx ratio were
34 decreased in hypothyroid HT patient compared to controls, the observed decrease being more relevant in
35 overt than in SHypo HT patients. Thus, hypothyroid subjects displayed deficient antioxidant defenses in
36 relation to the degree of hormonal dysfunction and lipid peroxidation 43. Oxidative stress can act at
37 thyroxisome level, that is impairing the homeostasis of the thyroid hormone-producing unit in the follicle
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

1 apical membrane, composed by TPO, Caveolin-1 (Cav-1) and dual oxidase (DUOX). It has been observed
2 that all these components were reduced in HT, in which the Th1 immune response could down-regulate Cav-
3 1 expression, leading to a mislocalization of TPO and DUOX and a decrease of T4 synthesis in the colloid,
4 with consequent oxidative stress and cell apoptosis as main features of HT pathogenesis 61. However,
5 system perturbations involve the whole redox balance, being not only limited to an increased production of
6 reactive species. For example, Ates et al. demonstrated raised total oxidant status (TOS) and oxidative stress
7 index (OSI), as well a reduction in total antioxidant status (TAS), total thiol and ARE levels in HT patients in
8 comparison with healthy volunteers. Indeed, these alterations were progressively more marked passing from
9 euthyroid to subclinical or overt hypothyroid subjects, with a negative correlation between TAS and TPO-Ab
10 25. The same group showed how TSH, FT4 and OSI ratio could have an independent predictive role of
11 progression from euthyroidism to subclinical, and finally overt, hypothyroidism in HT 79. A similar
12 evidence was found by Ruggeri et al., who analyzed the redox status of a group of euthyroid HT patients in
13 comparison with healthy controls. In the first group, a significant decrease in biological antioxidant potential
14 (BAP), as well as increased levels of derived reactive oxygen metabolites (dROMs) and advanced glycation
15 end products (AGEs), that were both inversely correlated to the former. Moreover, TPO-Ab were the main
16 predictors for all the aforementioned parameters 56. Also, a common polymorphism of AGEs receptor
17 (RAGE) related to HT, namely -429T>C, has been associated with the risk of progression from euthyroidism
18 to hypothyroidism, since patients under L-T4 treatment presented with higher oxidative stress levels 80.
19 AGEs are well known to be increased in conditions of oxidative stress and to promote inflammation by
20 interacting with their receptor RAGE on cell membrane. By contrast, the soluble receptor sRAGE exerts
21 protective effects by competing with RAGE for ligand binding. More recently, reduced levels of the soluble
22 form of RAGE (sRAGE) have been reported in euthyroid HT patients compared to controls, along with
23 increased serum AGEs levels, and the two parameters were inversely correlated 58. Accordingly, the
24 AGEs/sRAGEs ratio was threefold higher in HT patients than controls, suggesting a dysregulation of
25 AGE/sRAGEs-related oxidative homeostasis in HT patients even when in euthyroid status. In regression
26 analysis models, serum TPO-Ab were the main predictors for AGEs and sRAGEs levels and AGEs/sRAGEs
27 ratio, irrespective of TSH and/or FT4 values 58. In other experiences, patients with AITDs presented with
28 reduced levels of paraoxonase-1 (PON1) and total free sulfhydryl (-SH) levels – both compounds having a
29 well-known antioxidant function – while lipid oxidation expressed as lipid hydroperoxide (LOOH) values
30 was significantly higher versus healthy controls 81. The same reduction of PON, and arylesterase (ARE) was
31 observed in a group of female adolescents with euthyroid HT, and it was paired with significantly higher
32 levels of anti-Mullerian hormone 82 . Finally, an increase in serum interleukin-37 (IL-37) has been recently
33 observed in HT patients vs controls, directly correlating with anti-thyroid antibodies titre and AGEs levels.
34 This evidence could lead to hypothesize a protective role of IL-37 against oxidative stress in HT 57.

51
52 Graves' disease
53
54
55

1 Most of the above reported biomarkers have been investigated also in Graves' disease patients, since
2 autoimmune hyperthyroidism and the related orbitopathy are well known to be oxidative stress-related
3 disorders 83. For the sake of completeness and comparison, we briefly report the more recent data on
4 oxidative stress biomarkers in GD patients (Table 2).
5
6

7
8 As stated before, in 2010 Lassoued et al. demonstrated high levels of SOD activity and MDA in patients with
9 GD 73. Also, metalloprotease (MMP) expression is stimulated by a high-oxidative stress environment, as
10 observed by Korkmaz et al. in a cohort of GD patients without Graves' orbitopathy (GO). They presented
11 high levels of the MMP prolidase, which positively correlated with TOS/OSI indexes, while -SH groups
12 were significantly reduced 84. A reduction in native and total thiol levels in GD patients was also observed
13 by Agan et al, with a positive correlation between free triiodothyronine (FT3) and FT4 levels and thiol
14 homeostasis impairment/oxidative stress parameters 85. High levels of MDA bound to proteins or carbonyl
15 groups, as well as a hyper-reactivity towards hydrogen peroxide (H₂O₂)-oxidized thyroid antigens, has been
16 observed in patients affected by AITD as expression of oxidative stress presence/increase 86. Indeed, the
17 high production of H₂O₂ during hormone synthesis could enhance the antigen reactivity through the creation
18 of new epitopes. In GD patients this phenomenon had a positive correlation with FT3 levels 86. Gargouri et
19 al also demonstrated a positive correlation between T3 levels and the immunoreactivity towards MDA-
20 modified catalase in GD patients vs controls 87. In a study by Choi et al, MDA and 8-hydroxy-2'-
21 deoxyquanosine (8-OHdG), H₂O₂ and intracellular superoxide anion levels were measured in the tear fluid
22 of GD patients 88. These markers resulted increased in comparison to healthy controls, and progressively
23 higher in affected subjects without and with GO, respectively. There was also a positive correlation between
24 markers and clinical activity score (CAS) in GO, while increased levels of extracellular ROS were
25 demonstrated in fibroadipose tissue, blood, orbital fibroblasts, and urine from these subjects 88. Marique et
26 al. detected an increased expression of oxidative stress parameters in both adipose and muscular orbital cells
27 in patients with GO. On the other hand, an upregulation of some antioxidants (peroxiredoxin 5, catalase) was
28 also observed, while the overexpression of adiponectin (ApN) and proliferator-activated receptor gamma
29 (PPAR γ) exerted direct and indirect protective effects. These data confirmed the role of antioxidant
30 supplementation in active and chronic GO 89. In another study, increased serum concentrations of
31 nicotinamide adenine dinucleotide phosphate oxidase, isoform 2 (NOX2) were measured in untreated
32 hyperthyroid GD patients, being significantly higher than in GD euthyroid treated patients, subjects with
33 multinodular toxic goiter and healthy controls. Moreover, higher oxidative stress parameters were also
34 detected in urine samples of untreated GD patients, as well as an increased respiratory burst of leukocytes in
35 whole blood 90. In the same study by Diana et al, the Authors measured superoxide production in human
36 embryonic kidney (HEK)-293 cells with overexpressed TSH receptor (TSHR), and lipid peroxidation in
37 these cells and in human primary thyrocytes. Monoclonal M22 TSABs, bovine TSH and sera from
38 hyperthyroid GD patients stimulated cAMP in HEK cells, significantly increasing superoxide levels vs
39 controls. However, in this case there was no correlation between T3 levels and ROS production. A similar
40 result was obtained in primary thyrocytes, with higher oxidative stress parameters in GD patients vs controls,
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

1 and in untreated GD patients vs controls 90. Recently, Ko et al demonstrated a significant increase in ROS
2 production and decrease in antioxidant enzymes in cultured orbital fibroblasts from GD patients with GO,
3 induced by H₂O₂ or cigarette smoke extract. Of note, treatment with caffeine determined a dose-dependent
4 decrease in intracellular ROS and antioxidant enzymes levels, while PPAR γ , C/EBP α , and C/EBP β protein
5 expression levels were inhibited during adipocyte differentiation 91.
6
7

8 Possible Therapeutic Role of Antioxidants.

9
10 Several antioxidant compounds have been evaluated to counteract oxidative stress in thyroid disorders and
11 their role in clinical practice is under debate.
12
13

14 Selenium.

15
16 Selenium, a well-known element involved in thyroid homeostasis, has been demonstrated to exert some
17 antioxidant effects in AITDs, although its practical applications are still a matter of debate especially
18 concerning AT. In fact, thyrocytes express many selenoproteins, some of which, like type 1 and 2
19 iodothyronine deiodinases (DIO1 and 2) and GPX (isoforms 1, 3 and 4), are linked to thyroid hormone
20 metabolism and participate into controlling oxidative stress levels in the gland 92. Others, like selenoprotein
21 S (SELENOS), modulate the transcription of genes encoding proinflammatory cytokines involved in AT
22 pathogenesis 93. Selenium intake is widely variable in the world due to environmental reasons and dietary
23 habits, ranging from deficiency to toxicity doses (7-4990 μ g/day), and in Europe it is in general under the
24 levels recommended by the US Institute of Medicine or the European Food Safety Authority (EFSA), that is
25 55 and 70 μ g/day respectively 94.
26
27
28
29
30
31
32

33 In vitro studies demonstrated that incubation of thyrocytes with Se supplements is able to prevent oxidative
34 cell necrosis and apoptosis and enhances cell viability by preventing H₂O₂-induced degradation of DNA and
35 by reducing caspase-3 activity and BAX mRNA levels and increasing BCL-2 mRNA levels 46–50. An
36 increase in MDA levels, which was prevented by the pretreatment with both selenomethionine and selenite,
37 was also reported after exposure to H₂O₂ 50. Both selenocompounds induced an increase in GPx activity,
38 suggesting that these protective effects may be, almost in part, mediated by these selenoproteins 50.
39
40
41

42 A number of studies evaluating selenium supplementation in HT have demonstrated a reduction in TPO-Ab
43 concentration, but there are scarce evidences about its effects on disease progression and remission,
44 influences on L-T₄ replacement dosage and patients' quality of life 95,94. For example, the addition of
45 selenomethionine 200 μ g/day to levothyroxine treatment in a cohort of 170 Polish women with HT vs 41
46 controls demonstrated a reduced release of proinflammatory cytokines comprehending IL-2, IFN γ and TNF,
47 and decreased levels of C-reactive protein 96. On the converse, another Austrian study in women with HT
48 treated with levothyroxine plus sodium selenite 200 μ g/day for three months did not record significant
49 modifications in cytokine profile 97. While some ongoing trials are evaluating this association, the
50 paradoxical aspect to be clarified is that, in a recent survey performed by the European Thyroid Association
51
52
53
54
55

(ETA), around 65% of European endocrinologists occasionally or frequently prescribe selenium despite its use in HT is not routinely recommended 94,98.

The use of selenium in mild and active GO is an established tool in clinical practice, but the same consensus has not been extended to the treatment of Graves' disease without GO 40,99,100. In fact, even if one study investigating the effects of selenium added to methimazole (MMI) treatment for GD reported a good biochemical control of hyperthyroidism, two randomized clinical trials (RCTs) did not demonstrated a short-term improvement of hyperthyroidism or an amelioration of response/recurrence rates 101,102,103. However, new evidences could emerge from more recent studies thoroughly analysing clinical parameters, like the work by Xu et al, in which the comparison between MMI alone and MMI plus selenium groups revealed significantly lower levels of FT3, FT4, TPO-Ab, Tg-Ab and TRAb, and a marked increase in TSH after 6 months of treatment 104.

Vitamin antioxidants.

Vitamin antioxidant compounds, such as vitamin C, vitamin E, β -carotene, have been employed in Graves' disease patients under MMI or carbimazole therapy with positive results, providing evidence in favour of the use of these antioxidant supplements in the early phases of anti-thyroid treatment 105. For example, Guerra et al tested a combination between antithyroid drugs and an antioxidant mixture (vitamin E, β -carotene, vitamin C, Cu, Zn, Mn, and selenium) vs anti-thyroid drugs alone in hyperthyroid GD patients: in the first group, a significant reduction in MDA levels was recorded, with a shorter time required to normalize thyroid hormones and the clinical score, thus suggesting a synergistic mechanism at thyroid hormone synthesis level 106. Larger cohort of patients are needed to confirm these findings.

In recent years, a growing importance of the abovementioned vitamin antioxidants, namely alpha-tocopherol and ascorbate, has been linked to a better efficiency of the antioxidant systems and the endogenous antioxidants like SOD and CAT, as observed for example in benign prostate hyperplasia (BPH), in which ROS production could contribute to carcinogenesis 107. A similar evidence has been observed in a group of thyroid cancer patients, treated with radioiodine and supplemented with 2000 mg vitamin C, 1000 mg vitamin E, and 400 μ g selenium for 21 days before therapy. They presented with significantly lower plasma levels of 8-epi-PGF_{2a}, a marker of lipid peroxidation, thus allowing to extend the hypothesis of a beneficial effects of these substances in AITDs 108.

Vitamin D.

Vitamin D is a steroid hormone, which has been demonstrated to be involved in several processes in the human body, including the regulation of immune response. In fact, its receptor (vitamin D receptor, VDR) is present in monocytes and activated T cells, influencing both the innate and adaptive immunity 109,110. Several observational studies showed a relationship between vitamin D deficiency and the risk of developing AITDs, especially HT, and a strong negative correlation has been observed between reduced vitamin D

1 levels and TPO-Ab, whereas the association of HT with VDR polymorphisms was variable 111–113.
2 Moreover, this connection could also be confirmed by the fact that, conversely, vitamin D can downregulate
3 the expression of proinflammatory cytokines, such as Interleukin 6 (IL-6) and tumor necrosis factor α (TNF-
4 α), which are responsible for T and B cells migration and proliferation and for an increase in oxidative stress
5 levels 114. In the majority of randomized controlled trials (6 out of 7) conducted up to now in HT patients,
6 the main effect of cholecalciferol supplementation is a significant reduction of TPO-Ab titer, but several
7 questions remain unanswered i.e. regarding the best dosage, the best detection method, etc 110. Considering
8 these data, an empirical approach could be to consider measuring vitamin D levels in patients affected by
9 HT, especially when they are middle aged-women (the most affected part of population) and/or presenting
10 with slightly elevated TSH levels: this passage could increase the number of patients treated with vitamin D,
11 not only for the prevention of bone metabolism alterations, but also in order to slow down the progression of
12 HT towards overt hypothyroidism and/or thyroid atrophy 115. Similar considerations could be applied in GD
13 patients, but further studies with larger cohorts of patients and more stringent selection criteria are required.
14
15
16
17
18
19

20 Dietary intake of plant-derived antioxidants.

21
22
23 Plant-based foods are rich sources of vitamins, oligoelements and metabolites (such as phenols,
24 polyphenols, phytoestrogens, flavonoids, flavones, proanthocyanins, catechins) with documented antioxidant
25 activity and may exert protective effects against oxidative stress-related disorders, including autoimmune
26 processes. In isolated rural populations a vegetarian diet has been associated with a low prevalence of
27 autoimmune diseases, such a protective influence being attributed to the effects of plant foods, to the
28 exclusion of animal products, or both. On the contrary, a Western-type diet, rich in calories, fats, and
29 proteins, high in salt and refined sugars, and low in fibers, is thought to favor the development of
30 autoimmunity through enhanced oxidative stress and inflammation. Indeed, consumption of large amounts of
31 meat, fats and refined sugar in the long run results in gut microbiota disruption and inflammation with ROS
32 overproduction, while the low intake of fruits and vegetables causes lack of exogenous antioxidants 116. In
33 the last decade, Tonstad et al., using data from the Adventist Health Study-2, reported a reduced prevalence
34 and incidence of both hypothyroidism and hyperthyroidism among subjects following vegan/vegetarian diets
35 compared to omnivorous diets, providing congruent, though not always statistically significant, data in
36 favour of a protective role of diet excluding meat against thyroid dysfunction 117,118. More recently,
37 Ruggeri and co-workers provided evidence that low dietary intake of animal foods has a potentially
38 protective effect towards thyroid autoimmunity as a result of the positive influence of this dietary habit on
39 redox balance¹¹⁹. According to this survey, the nutritional pattern of HT subjects was characterized by
40 increased consumption of animal proteins, higher intake of saturated fats and refined sugars, and lower
41 intake of fibers and antioxidants compared with healthy control subjects. In other words, nutritional patterns
42 of HT subjects resembled the Western-type diet, while controls displayed a higher level of adherence to the
43 Mediterranean diet. The study points to meat in omnivorous diets as the main nutritional factor associated
44
45
46
47
48
49
50
51
52
53
54
55

1 with redox dysregulation and increased risk of thyroid autoimmunity, whilst plant-based foods and
2 Mediterranean diet traits are protective¹¹⁹.

3
4 L-T4 treatment.

5
6 Another interesting topic is the role of thyroid hormone replacement therapy towards oxidative stress, with
7 many evidences emerging in the literature. In a study by Marchiori et al, in a cohort of 17 hypothyroid HT
8 patients treated with L-T4, oxidative stress parameters were measured at 6 and 12 months, respectively. A
9 significant reduction in non-protein thiol (NP-SH) was observed, together with significant modifications in
10 interleukin levels (increase in IL-10 and decreases in IL-1, IL-6, INF- γ and TNF- α), thus suggesting that
11 hormone replacement therapy could condition the inflammatory response in thyroid autoimmunity¹²⁰. A
12 similar effectiveness of L-T4 was reported in a paper by Ates et al, in which a group of treated HT patients
13 presented with increased levels of TAS, total thiol, ARE, and PON1 and decreased TOS and OSI levels in a
14 6-month period, compared to healthy controls. Moreover, pre-treatment TOS and OSI levels positively
15 correlated with TSH values, TPO- and Tg-Ab titer¹²¹. More recently, another group recorded an increase in
16 antioxidant CAT levels, and a significant decrease in thiobarbituric acid reactive substances (TBARS) in a
17 group of 25 female patients with primary hypothyroidism treated with L-T4¹²². On the other hand,
18 Chakrabarti et al. described the effects of the association of L-T4 replacement therapy with selenium
19 supplementation (100 mcg twice a day) in hypothyroid HT patients, vs L-T4 alone: although in both groups a
20 reduction in MDA levels was recorded after 6 months, there was no statistical significance in the
21 measurements obtained in the combination group¹²³.

30 CONCLUSION.

31
32 In the context of a growing amount of data about the role of oxidative stress and antioxidants in the
33 pathophysiology of ATDs, and especially in AT progression towards hypothyroidism, oxidative stress
34 parameters could be used as biochemical markers of chronic inflammation, to better predict the disease
35 evolution along its natural history. Besides, the well-known link between oxidative alterations and thyroid
36 hormone replacement treatment could be the theoretical basis to use these parameters for a finer patients'
37 monitoring. A larger knowledge of the substances which locally counteract the oxidative stress imbalance
38 typical of chronic inflammatory disorders could open new perspectives in the development of more tailored
39 medical therapies for these autoimmune conditions that significantly impair patients' quality of life.
40 Currently, natural sources of antioxidants in the form of a plant-based foods may represent the best option
41 for protecting against chronic oxidative stress-related disorders. Reducing the intake of animal proteins and
42 fats and increasing that of fruits and vegetables has proven to be a useful lifestyle strategy for contrasting
43 oxidative stress and reducing the risk for autoimmune diseases, including AT. In particular, a predominantly
44 plant-based Mediterranean diet, high in naturally occurring antioxidants, low in saturated fat and cholesterol,
45 and good source of vitamins and minerals, may represent a healthy food model for people suffering from AT.
46 Conversely, there is still a debate as to whether assuming antioxidants in supplement form can actually
47
48
49
50
51
52
53
54
55

1 reduce the risk of AT development/progression, by preventing or slowing down thyroid damage. In spite of
2 the experimental evidence for a protective effect of antioxidant molecules against oxidative damage to cells
3 in animal models and in vitro studies, clinical studies of antioxidant supplements in AT patients reached
4 disappointing and largely inconclusive results and have not demonstrate them to provide substantial benefits
5 in preventing/slowing down AT development and progression towards thyroid dysfunction. On this basis, a
6 routine use of vitamins and antioxidants supplementation in the treatment of AT patients should be
7 discouraged, but correction of nutrient deficiencies (for instance, vitamin D or selenium deficit) is advisable
8 to avoid negative health effects due to the lack of these elements essential to proper thyroid and immune
9 function. Moreover, antioxidant supplements cannot be routinely given for a long enough time to prevent
10 chronic diseases, such as HT and hypothyroidism, which develop over decades, but short courses of
11 antioxidants supplementation can be considered as an option and justified in selected conditions and/or
12 populations. A 6-month trial of selenium supplements has indication in the treatment of patients with
13 Graves' disease and associated mild orbitopathy since selenium may enhance the effectiveness of anti-
14 thyroid drugs, improves clinical manifestations and quality of life and prevents progression of the disease.
15 Importantly, current guidelines and scientific societies do not recommend Se supplementation for other
16 indications. Future antioxidant therapeutic strategies should include the design of protocols for the inhibition
17 of oxidative stress damage through administration of synthetic and natural antioxidants and enhancement of
18 the antioxidant defenses by increasing the production of endogenous antioxidants and activation of
19 antioxidant mechanisms.

PEER REVIEW COPY
Minerva Endocrinologica

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

OXIDATIVE STRESS AS A KEY FEATURE OF AUTOIMMUNE THYROIDITIS: AN UPDATE.

Rosaria Maddalena Ruggeri^{1,2}, Alfredo Campenni³, Giuseppe Giuffrida^{1,2}, Marco Casciaro⁴, Maria Cristina Barbalace⁵, Silvana Hrelia⁵, Francesco Trimarchi⁶, Salvatore Cannavò^{2,7}, Sebastiano Gangemi⁴.

Affiliations:

¹Department of Clinical and Experimental Medicine, University of Messina; ²Endocrinology Unit, University Hospital of Messina; ³Department of Biomedical Sciences and Morpho-Functional Imaging, University of Messina; ⁴School and Unit of Allergy and Clinical Immunology, Department of Clinical and Experimental Medicine, University of Messina; ⁵Department for Life Quality Studies, Alma Mater Studiorum, University of Bologna, Bologna, Italy; ⁶Accademia Peloritana dei Pericolanti, University of Messina; ⁷Department of Human Pathology DETEV, University of Messina, Messina, Italy.

Running title: oxidative stress and autoimmune thyroiditis

CORRESPONDING AUTHOR:

Rosaria Maddalena Ruggeri, MD, PhD.
Endocrine Unit, Department of Clinical and Experimental Medicine
“Gaetano Martino” University Hospital,
98125 Messina, ITALY
Telephone +39-090-221.3840; fax: +39-090-221.3518
e-mail: rmruggeri@unime.it

Key words: Hashimoto’s thyroiditis, oxidative stress, ROS, antioxidants, thyroid autoimmunity.

ABSTRACT

1
2 **Introduction.** Oxidative stress has been proposed as one of the factors concurring in the pathophysiology of
3 autoimmune thyroid diseases. Reactive oxygen species are the main expression of oxidative stress in
4 biological systems, and their production can overcome antioxidant defenses ultimately leading to cell
5 damage, apoptosis, and death. The present review was aimed at describing the state of the art of the
6 relationships between oxidative stress and autoimmune thyroiditis. The most used biomarkers of oxidative
7 stress and their correlation with thyroid function are reported.

8
9
10 **Evidence Acquisition.** We conducted a search of the literature in the English language starting from 2000,
11 using the following search terms: "Hashimoto thyroiditis", "autoimmune thyroiditis", "hypothyroidism",
12 "hyperthyroidism", "oxidative stress", "oxidants", "antioxidant", "advanced glycation end products". Both
13 clinical studies and animal models were evaluated.

14
15
16 **Evidence Synthesis.** Data from clinical studies clearly indicate that the balance between oxidants and
17 antioxidants is shifted towards the oxidative side in patients with autoimmune thyroiditis, suggesting that
18 oxidative stress may be a key event in the pathophysiology of the disease, irrespective of thyroid function.
19 Studies in animal models, such as the NOD.H2h4 mouse, confirm that thyroidal accumulation of ROS plays
20 a role in the initiation and progression of autoimmune thyroiditis.

21
22 **Conclusions.** Oxidant/antioxidant imbalance represent a key feature of thyroid autoimmunity. Oxidative
23 stress parameters could be used as biochemical markers of chronic inflammation, to better predict the disease
24 evolution along its natural history. Dietary habits and antioxidant supplements may provide protection from
25 autoimmunity, opening new perspectives in the development of more tailored therapies.

26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

INTRODUCTION.

1
2 Autoimmune thyroiditis (AT), also referred to as Hashimoto's thyroiditis (HT), is the most common
3
4 autoimmune endocrine disorder and the main cause of hypothyroidism in iodine-sufficient areas ^{1,2}. AT
5
6 covers a wide spectrum of phenotypes, encompassing different clinico-pathological entities: the classic form,
7
8 which features goiter with or without hypothyroidism; the fibrous variant with glandular fibrosis and rapid
9
10 progression towards hypothyroidism; the IgG4-related variant; the juvenile form and the painless (or silent)
11
12 thyroiditis, occurring either sporadically or in the post-partum ^{1,2}. Overall, AT has an estimated prevalence of
13
14 around 3-5% of the general population, with peaks during adolescence and middle adulthood ^{3,4}. In addition,
15
16 the prevalence of thyroid autoantibodies increases in people over the age of seventy ⁵. At any age, females
17
18 are more affected than males with a female to male ratio around 5-7:1 ^{1,2}. Incidence has been raising in last
19
20 decades, mostly in developed countries ³. AT often occurs in association with other endocrine and non-
21
22 endocrine autoimmune diseases in the same patient (autoimmune comorbidity) and/or in in other members of
23
24 the same family (familial clustering), facilitated by a predisposing polygenic background. The clinical
25
26 presentation of AT can widely vary, from the rapid development of severe hypothyroidism to an initial but
27
28 transient thyrotoxicosis (Hashitoxicosis). In many cases, AT generally proceeds from an asymptomatic
29
30 autoimmune condition, featured by circulating anti-thyroid autoantibodies and normal thyroid function,
31
32 towards subclinical and then overt hypothyroidism ^{1,2,3}. Even if return to normal thyroid function has been
33
34 reported, the final outcome of AT is permanent hypothyroidism due to progressive destruction of thyroid
35
36 follicular cells ^{1,2}.

37
38 AT is a chronic inflammation of the thyroid that results from an inappropriate immune reaction against the
39
40 gland. Failure of immunological self-tolerance leads to activation and expansion of autoreactive T cells,
41
42 release of pro-inflammatory cytokines and differentiation of self-reactive B cells with production of organ-
43
44 specific autoantibodies ⁶. Together, these events are responsible for chronic inflammation with infiltration of
45
46 hematopoietic mononuclear cells (T and B lymphocytes, plasma cells and macrophages), interstitial fibrosis
47
48 and follicular cells damage by means of both cell- and antibody-mediated cytotoxic and apoptotic pathways⁶.
49
50 In this cascade of events, a crucial role is played by pro-inflammatory cytokines, such as IL-1 β , IL-6, IFN- γ ,
51
52 TNF- α , IL-22 and IL-23, that promote and amplify inflammation, contribute to tissue damage and modulate
53
54 the metabolic and immune function of thyroid follicular cells (**Figure 1**) ^{6,7,8,9}.
55

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

Underlying this process there is a complex interplay between genetic and environmental factors: exogenous and existential factors trigger the development of the immune response against thyroid autoantigens in genetically susceptible individuals ^{2,6,10}. The genetic background of the disease is still not fully understood and includes a wide number of genes, encompassing the HLA and immune-regulatory genes, that confer generalized susceptibility to autoimmunity on one hand, and several different tissue-specific genes, which exert either predisposing or protective effects for particular types of disease in a tissue-specific fashion on the other ². In face of the constancy of the genetic basis, the number of potential environmental triggers has been enormously expanding over the last decades. They include changes in lifestyle (modified infectious habitat and ameliorated personal hygiene, stress, dietary habits, sedentary life), increased exposure to pollutants and toxics, radiations, novel (i.e. tyrosine-kinase inhibitors and immune check-point inhibitors) and old (i.e. lithium, interferons, amiodarone) drugs, gut microbiome alterations and nutrients (notably, vitamin D deficiency, iodine and selenium intake) ^{2,6,10}. These environmental factors may affect the thyroid gland and trigger/favor the development of autoimmunity through a wide range of mechanisms, including increased free radicals accumulation and enhanced oxidative stress ^{11,12}.

Oxidative stress is the result of an imbalance between oxidants production and antioxidant defense mechanisms. This condition concerns all the alterations that may occur at tissue, cellular and biological macromolecular level. In biological systems oxidants are represented by free radicals, i.e. partially reduced forms of oxygen and nitrogen, the so-called reactive oxygen (ROS) and nitrogen (RNS) species. From a chemical point of view, a free radical is a molecular entity having one or more unpaired electrons on one atomic or molecular orbital. They present an extremely high reactivity and instability and tend to catch the missing electron from other molecules. Free radicals start chain reactions leading to shutdown of initial radical and/or to the generation of new radicals. These molecules are products of normal cell metabolism and are essential for several biochemical processes inside the cell when at low levels. On the contrary, the alteration of the normal redox state due to their excessive production and/or accumulation into the cells causes oxidation of all macromolecules (membrane lipids, proteins and nucleic acids), leading to cell damage, apoptosis and death ¹³. Indeed, as postulated in the 'redox window' hypothesis, adequate ROS levels help physiological cellular functions, but excessive ROS production is involved in the development of several pathologies ¹⁴. The main types of ROS that can be generated in the cell are superoxide ($O_2^{\bullet-}$) anion, hydroxyl (OH^{\bullet}) radical, and hydrogen peroxide (H_2O_2) which is a non-free radical. There are several

1 enzymes involved in ROS production. At mitochondrial level, the complex I and III are implicated in the
2 production of large amounts of superoxide, and their activity is slowed during increased ROS production
3 which, in turn, promotes further ROS release ¹⁵. Moreover, mito-ROS can subsequently activate other ROS
4 sources¹⁶. NAD(P)H (nicotinamide adenine dinucleotide phosphate) oxidase catalyzes the release of
5 superoxide anion or hydrogen peroxide through the reduction of molecular oxygen, using as electron donor
6 NADPH, in various intracellular and extracellular compartments ¹⁷. Nitric oxide synthase (NOS) enzyme is
7 responsible for NO production but excessive superoxide levels, in condition of oxidative stress, depletes
8 (6R)5,6,7,8-tetrahydrobiopterin (BH4), the essential NOS cofactor ¹⁸, causing NOS impairment, which
9 becomes itself a source of superoxide. Xanthine oxidase (XO), released during pathophysiological processes,
10 donates electrons to molecular oxygen producing superoxide and hydrogen peroxide (**Figure 2**).

11 Multiple enzymatic and non-enzymatic systems, the so-called antioxidants, are present in both the
12 bloodstream and peripheral tissues, to prevent/counteract excess free radicals' production and/or
13 accumulation. The enzymatic defenses can remove radicals with a catalytic mechanism, while the non-
14 enzymatic defenses have heterogeneous working mechanisms, as they can sequester pro-oxidant
15 molecules, or act as radical scavenger. Non-enzymatic antioxidants may be either endogenous, like the
16 mitochondrial uncoupling proteins, reduced glutathione (GSH) and transport proteins synthesized in the liver
17 (ceruloplasmin, transferrin, albumin etc...) or exogenous products, including uric acid, vitamin E and C,
18 which are mainly derived from diet ¹³. The enzymatic antioxidants include the glutathione peroxidase
19 (GPx)/glutathione reductase (GR) system and the glutathione-S-transferases (GSTs), which represent the
20 first-line defense against oxidants in almost every cell types, with a tissue-specific distribution (**Figure 2**).
21 GPx/GR system reduces H₂O₂ or hydroperoxides reduction, using GSH as electron donor and NADPH as a
22 co-factor, meanwhile GSTs are a class of enzymes which catalyzes the conjugation of GSH to electrophilic
23 compounds ¹⁹. Thioredoxin (TRx)/thioredoxin reductase (TRxR) constitutes another important system for
24 H₂O₂ detoxification. TRxR acts using NADPH to restore the oxidized thioredoxin in the reduced form. Both
25 GPx and TRxR are selenoproteins, with a Se atom incorporated in their catalytic domain in the form of
26 selenocysteine, and require an appropriate supply of Se to be active ²⁰. At the highest levels of oxidants, also
27 catalases (CAT) and superoxide dismutase (SOD) contribute to enzymatic degradation of free radicals ²¹.
28 Under physiological conditions, there is an equilibrium between the production and detoxification of free
29 radicals, the so-called redox homeostasis, which is essential for every kind of aerobic life ^{20,22}. When free
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

1 radicals are produced in excess or are not adequately degraded by the cells or both, then a condition of
2 oxidative stress occurs and causes cell damage and death, and tissue inflammation, also worsened by the
3 release of pro-inflammatory cytokines in damaged tissues^{20,22}. Almost every cell type and tissue are prone to
4 oxidative damage, with tissue-specific differences, and oxidative stress is present at the site of
5 inflammation²³.
6
7

8
9 For these reasons, oxidative stress has been thought to represent a key feature of several inflammatory and
10 immune-related disorders, including autoimmune thyroid diseases (AITDs)^{24,25,26}. Increased ROS production
11 due to environmental agents (i.e. iodine excess, radiations, toxics and drugs, pollutants) could induce a
12 modification of tissue proteins, or might dysregulate the immune system, influencing the appearance of the
13 autoimmune disorder²⁴.
14
15
16
17
18

19 The present review was aimed at summarizing the evidence of the recent literature concerning the
20 bidirectional association between oxidative stress and AT, since the relevance of oxidative stress and the
21 beneficial effects of antioxidant supplementation in ATs are intensely debated at present. We also revised the
22 different biomarkers that have been measured to evaluate the impact of ROS in the setting of AT, in an effort
23 to identify useful and reliable markers of oxidative stress.
24
25
26
27
28

29 **EVIDENCE ACQUISITION.**

30 For this purpose, we extensively examined both *in vivo* and *in vitro* data on changes in oxidative balance and
31 oxidative stress markers/indices, published in the last two decades in the field of AITDs. The review of the
32 pertinent literature has been conducted employing MEDLINE database. On this website, we searched for
33 articles using key terms related to Hashimoto's thyroiditis and oxidative stress. A MeSH search has been
34 performed using "Hashimoto thyroiditis"/"autoimmune thyroiditis" AND "oxidative stress", followed by a
35 simple search using "Hashimoto thyroiditis", "autoimmune thyroiditis" AND "oxidative stress" OR
36 "oxidants" OR "antioxidant" OR "advanced glycation end products" and a search using "oxidative stress"
37 AND "autoimmune thyroiditis" OR "hypothyroidism" OR "hyperthyroidism" as key terms. We obtained 196
38 results; we included in the present paper only the articles matching the following inclusion criteria: English
39 language, publication in peer-reviewed journals starting from 2000, research papers. Articles considered
40 relevant and cited in the references of the selected papers were included too. We excluded articles for
41 irrelevance to the topic in question, duplicates, papers written in other languages apart from English and
42 articles published before 2000.
43
44
45
46
47
48
49
50
51
52
53
54
55

EVIDENCE SYNTHESIS.

Animal Models.

The NOD-H2(h4) mouse represents an animal model of autoimmune lymphocytic thyroiditis that mimics human Hashimoto's thyroiditis. The NOD-H2h4 mice spontaneously develop an autoimmune thyroiditis, whose incidence dramatically increases when adding iodine to the drinking water^{27,28,29,30}. Excess iodine triggers autoimmunity by multiple mechanisms, including changing the immunogenicity of the thyroglobulin molecule, upregulating intracellular adhesion molecule-1 (ICAM-1) expression on thyrocytes and increasing ROS production by the thyrocytes themselves²⁶. Burek and co-workers demonstrated that thyrocytes isolated from NOD-H2h4 mice produced significantly more H₂O₂ than control thyrocytes when exposed to iodine²⁶. ROS accumulation also contributes to upregulation of ICAM-1 expression on the surface of thyrocytes, enhancing immune cells infiltration of the thyroid gland and pro-inflammatory cytokines production^{31,32}. Incubation with the antioxidant diphenyleneiodium, an inhibitor of NADPH oxidase, reduced both ROS production and ICAM-1 expression in cultured NOD-H2h4 thyrocytes³². Kolypetri and Carayanniotis showed that impaired control of oxidative stress mechanisms is associated with high susceptibility to apoptosis in NOD.H2(h4) thyrocytes exposed to iodine³³. Finally, the antioxidant N-acetylcysteine (NAC) reduced ROS and the immune infiltration, thereby leading to a restoration of thyroid morphology, in NOD.H2h4 thyroid glands³⁴. Likely NAC exert its protective effects by acting on infiltrating inflammatory cells rather than directly on thyrocytes. In the same model, increased thyroid content of 4-HNE, a toxic product from lipid peroxidation used as a marker of oxidative stress, was reported³⁴. Overall, studies in the NOD.H2h4 model suggest that thyroidal accumulation of ROS plays a role in the initiation and progression of autoimmune thyroiditis.

CLINICAL STUDIES.

Thyroid Function and Oxidative Stress.

There is a close and bidirectional relationship between the thyroid gland and oxidative stress since it concerns both the effects of thyroid function on oxidants/antioxidants balance in peripheral tissues and the effects of oxidative stress on thyroid gland itself.

Firstly, thyroid hormones play a crucial role in regulating redox homeostasis. On the one hand, they accelerate the basal metabolic rate and cell oxidative metabolism by inducing mitochondrial respiration, and enhance free radical production; on the other, they regulate the synthesis of enzymatic and non-enzymatic

1 antioxidants. As a consequence, both hyperthyroidism and hypothyroidism have been associated with
2 oxidative stress, since the former has been shown to increase oxidants production as a result of increase of
3 metabolic processes into cells and to cause consequent exhaustion of antioxidants^{35,36,37,38,39,40} and the latter
4 to reduce the antioxidant defense systems^{25,37,39,41,42,43}.

5
6
7 Moreover, the thyroid itself is exposed to lifelong oxidative stress, induced by the continuous generation of
8 H_2O_2 , fundamental for the iodine oxidation during the process of thyroid hormone synthesis. For this reason,
9 thyroid cells display efficient detoxification systems, mainly represented by the cytoplasmic GPx1 and by the
10 secretive GPx3 in the colloid lumen^{44,45}. Inadequate Se supplementation may impair both the expression and
11 the enzymatic activity of the antioxidant selenoproteins GPx1 and GPx3, altering the natural
12 oxidant/antioxidant thyroid cycle and resulting in a reduced antioxidant activity in thyrocytes²¹. In animal
13 models, Se deficiency is associated to oxidative cell damage, defective tissue repair and thyroid fibrosis,
14 whilst Se supplementation is protective against experimentally induced autoimmune thyroiditis^{46,47}.
15 Similarly, in *in vitro* studies Se supplementation prevents cell damage from oxidative injury and protects
16 against apoptosis^{48,49,50}. Also, iodine deficiency may increase oxidative stress, by uncoupling H_2O_2
17 generation, which is stimulated by TSH in response to iodine deficiency, from iodine oxidation, which is
18 reduced because of iodine deficiency. As a result, excess H_2O_2 accumulates in the colloid lumen. When
19 combined selenium deficiency occurs, it results in decreased expression of the selenoprotein GPx3 by
20 thyrocytes. As a result, inadequate GPx activity does not remove excess H_2O_2 , which cannot be consumed by
21 thyroperoxidase (TPO) for tyrosine iodination and iodothyronine coupling under conditions of iodine
22 deficiency, leading to H_2O_2 -induced tissue necrosis and fibrosis²¹. Hypothyroidism enhances such an effect
23 because H_2O_2 production, which is increased under continuous and elevated stimulation of the thyroid gland
24 by TSH, is not adequately counteract because of the reduced synthesis of antioxidants, as above reported⁵¹.

25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
A high iodine intake might cause an excessive H_2O_2 generation too, as clearly demonstrated in animal
models^{52,32}

Finally, oxidative stress seems to have a relevant role in the aging of thyroid gland and in the pathogenesis of
age-related thyroid dysfunction. Aging is associated with a decrease in thyroid volume and hormone
secretion, as well as with a reset of the hypothalamic–pituitary–thyroid axis⁵³. The production of free
radicals and ROS gradually increases with aging, whereas the activity of antioxidant defenses decreases,
leading to ROS accumulation in cells and tissues⁵⁴. The oxidative stress caused by this imbalance might

1 contribute to the progressive age-related dysfunction of the thyroid gland directly, through cellular oxidative
2 damage, and indirectly, through alterations in protein synthesis and function. Prolonged age-dependent ROS
3 exposure also causes genomic damage and telomere shortening, contributing to accumulation of senescent
4 cells in the thyroid with age. Moreover, ROS excess favors inflammation by increasing immune cells
5 recruitment into tissues (potentially damaging), by stimulating pro-inflammatory cytokines synthesis/release
6 and by modulating other processes such as mitochondrial function and microRNA production. A chronic,
7 low-level inflammation is a key feature of aging process, the so-called “inflammaging”, and a major
8 contributor to both thyroid senescence and age-related thyroid disorders^{53,54}. Indeed, aging is associated
9 with an increase in the prevalence of several thyroid diseases, and solid evidence indicates that a condition of
10 oxidative stress is relevant for their development and/or progression.

11 ***Thyroid Autoimmunity and Oxidative Stress.***

12 A close relationship exists between oxidative stress and thyroid autoimmunity irrespective of thyroid
13 dysfunction^{55,56,57,58}. In autoimmune disorders such as AT, the infiltrating immune cells develop a chronic
14 inflammatory milieu in which ROS accumulate and exert a toxic effect on surrounding cells and tissues. In
15 these conditions, oxidative stress may play a role in both the induction of autoimmune response against self-
16 antigens, and the amplification of tissue inflammation and damage, once the autoimmune process has been
17 initiated^{24,26}. First, oxidative imbalance may play a role in the onset of the autoimmune response. ROS
18 excess causes oxidative modifications of proteins, lipids and DNA, which become highly immunogenic and
19 may act as neo-antigens, leading to loss of self-tolerance in genetically predisposed individuals^{56,57}.
20 Thyroidal accumulation of ROS has been shown to promote cleavage of thyroglobulin into several
21 fragments, likely exposing the immune system to novel epitopes and thus enhancing the autoimmune
22 response⁵⁹. Once the autoimmune reaction has been triggered, the related inflammation may promote excess
23 ROS production and enhanced oxidative stress in thyroid tissue via activation of T and B lymphocytes
24 infiltrating the gland. In fact, it has been demonstrated that Th1 cytokines released by activated lymphocytes
25 induce ROS production by thyrocytes^{60,61}. Activated lymphocytes themselves produce excess ROS^{62,63}.
26 Whatever the source is, ROS accumulation causes oxidative damage of the cells, leading to apoptosis,
27 necrosis, and parenchymal destruction, as it occurs in other autoimmune diseases^{11,64,65}. Moreover, the
28 antioxidant system is not sufficient to counteract ROS overproduction, since the antioxidant potential is
29 reduced in HT patients, even in euthyroidism^{25,55,56,57,58}. Therefore, as already demonstrated in vitiligo, also

1 in AT oxidative stress may play a role both in initiation (modified proteins acting as neo-antigens) and
2 progression (autoimmune-related inflammation, cell apoptosis and parenchymal destruction) of the disorder
3
4^{25,26}. Studies in euthyroid patients with AT are more limited than those in hypo- and hyperthyroid patients,
5
6 but they all agree on demonstrating higher oxidative stress in AT cases than in controls, due to increased
7
8 oxidants or decreased antioxidants or both^{25,55,56,57,58}. In each study a significant correlation with thyroid
9
10 autoimmunity was found. Ates et al reported a negative correlation between serum total antioxidant activity
11
12 and anti-thyroperoxidase antibodies (TPO-Ab), while Baser et al. reported a positive correlation between
13
14 serum oxidants and anti- thyroglobulin (Tg-Ab) antibodies⁵⁵, and Ruggeri et al. confirmed the TPO-Ab were
15
16 independent predictors of the oxidative status in euthyroid HT patients⁵⁶. Overall, human studies report an
17
18 increased oxidative status in AT, even in euthyroidism, but do not clarify whether it is the cause or the
19
20 consequence of thyroid autoimmunity. Maybe autoimmunity and oxidative stress coexist and act in
21
22 synergism in initiating and/or perpetrating the progressive damage of thyrocytes.

23 ***Autoimmunity, cancer and oxidative stress.***

24
25 The relationship between AT and thyroid cancer, especially papillary thyroid cancer (PTC), is a well-known
26
27 fact^{66,67} and several research groups have studied the role played by oxidative stress in thyroid
28
29 carcinogenesis, reporting an increase in levels of oxidants and/or a decrease in antioxidant activity in patients
30
31 with thyroid cancer^{68,69}. The accumulation of excess ROS in the thyroid gland can cause DNA damage,
32
33 resulting in mutagenic genetic alterations and promoting tumour initiation and development^{70,71}. Thus, it is
34
35 conceivable that inflammation and oxidative stress, that are closely related processes, may contribute to the
36
37 increased risk of thyroid cancer that has been reported in AT^{67,71}, whilst antioxidant may exert protective
38
39 effects⁷². However, scanty data are available concerning the interplay between AT, thyroid cancer and
40
41 oxidative stress. Lassoued et al evaluated the presence of OS markers in patients suffering from AITDs
42
43 (Graves' disease and Hashimoto's Thyroiditis) and patients with PTC, before and after thyroidectomy and
44
45 radioiodine therapy⁷³. Comparing their oxidative stress profile with that of HT patients, malondialdehyde
46
47 (MDA), SOD and CAT activities were high, with reduced levels of GPx, in both groups. However, the
48
49 absolute values were higher (and lower regarding GPx), in the PTC patients, thus suggesting a higher grade
50
51 of oxidative stress in this population deriving from a more sustained production of free radicals and/or a
52
53 damaged antioxidant system⁷³. Moreover, these alterations did not change after thyroidectomy and
54
55 radioiodine therapy, thus confirming a previous evidence of an intrinsic oxidative imbalance in PTC⁷⁴. Such

1 data could be a starting point to further analyze the potential diagnostic/prognostic role of OS parameters in
2 PTC, also considering the use of antioxidant compounds to ameliorate patients' recovery⁷³.

3 ***Biomarkers of Oxidative Stress.***

4 *Hashimoto's thyroiditis (HT)*

5
6 Starting from 2000s, growing evidence emerged concerning several peripheral/circulating markers of
7 oxidative stress in HT patients (**Table 1**). In 2006 Taddei et al. demonstrated that patients with HT and
8 subclinical hypothyroidism presented with higher C-reactive protein and IL-6 values. In these subjects, the
9 antioxidant vitamin C did not improve endothelial dysfunction and nitric oxide (NO) availability after the
10 administration of indomethacin, that unselectively blocked a COX2-dependent pathway⁷⁵. In 2008 Erdamar
11 et al. observed an increase in MDA, nitrite, vitamin E, and myeloperoxidase (MPO) activity in hypothyroid
12 HT patients, as well as high levels of MDA and MPO activity in hyperthyroid subjects with Graves' disease
13 (GD). Treatments for both conditions revealed a reduction in nitrite and vitamin E in HT patients and a
14 decrease of the raised parameters in GD ones *versus* a homogenous group of healthy controls. In particular,
15 levothyroxine (L-T4) therapy took two months to lead markers back to normal values, while a faster
16 response (one month) was observed with propylthiouracil (PTU) treatment for hyperthyroidism, thus
17 confirming a role of thyroid hormones oscillations into influencing the redox homeostasis of thyroid gland³⁷.
18 A decrease in plasmatic levels of transforming growth factor-beta 1 (TGF- β 1) and vascular endothelial
19 growth factor (VEGF), and an increase in nitrite/nitrate (NOx, metabolites deriving from NO), was observed
20 in a group of HT patients *versus* controls⁷⁶. In the study by Torun and co-workers, MDA was elevated in
21 both hypothyroid and subclinical hypothyroid patients compared with controls and showed a correlation with
22 altered lipid metabolism in hypothyroidism states. On the contrary, total antioxidant status TAS levels show
23 no significant differences between groups, suggesting an insufficient increase in the antioxidant status in
24 hypothyroid patients⁴². In the study by Lassoued et al. oxidative stress in patients with untreated HT and GD
25 resulted higher than in healthy controls, especially concerning SOD activities and MDA. Besides, the same
26 evidence with more elevated values, was observed in patients with surgically treated PTC, thus
27 demonstrating a disturbed oxidative profile as in autoimmune diseases⁷³. As stated before, oxidative stress
28 could also be influenced by TSH levels, as demonstrated by Ozturk and colleagues who evaluated oxidative
29 stress parameters in a cohort of HT patients, differently affected by subclinical (SHypo) or overt
30 hypothyroidism (OHypo). Several serum parameters (MDA, diene conjugate – DC, protein carbonyl – PC,

1 nitrotyrosine – NT and ferric reducing antioxidant power - FRAP) were altered in comparison with healthy
2 controls, but while MDA and DC levels were normal in SHypo, all these analytes were increased in the
3 OHypo group, and Dc and copper-induced MDA were also measurable in low-density lipoprotein (LDL)
4 fraction in OHypo patients only ⁷⁷. Moreover, even GSH status has been investigated in HT. As
5 demonstrated by Rostami et al., serum glutathione was significantly reduced in affected subjects *versus*
6 controls, and it correlated with TPO-Ab values, thus suggesting that this decrease could be a hallmark of
7 oxidative stress activation and immunological intolerance development ⁷⁸. In the study by Reddy and
8 coworkers, MDA and GPx values were elevated, while GSH, TAC as FRAP, SOD, and SOD/GPx ratio were
9 decreased in hypothyroid HT patient compared to controls, the observed decrease being more relevant in
10 overt than in SHypo HT patients. Thus, hypothyroid subjects displayed deficient antioxidant defenses in
11 relation to the degree of hormonal dysfunction and lipid peroxidation ⁴³. Oxidative stress can act at
12 thyroxisome level, that is impairing the homeostasis of the thyroid hormone-producing unit in the follicle
13 apical membrane, composed by TPO, Caveolin-1 (Cav-1) and dual oxidase (DUOX). It has been observed
14 that all these components were reduced in HT, in which the Th1 immune response could down-regulate Cav-
15 1 expression, leading to a mislocalization of TPO and DUOX and a decrease of T4 synthesis in the colloid,
16 with consequent oxidative stress and cell apoptosis as main features of HT pathogenesis ⁶¹. However, system
17 perturbations involve the whole redox balance, being not only limited to an increased production of reactive
18 species. For example, Ates et al. demonstrated raised total oxidant status (TOS) and oxidative stress index
19 (OSI), as well a reduction in total antioxidant status (TAS), total thiol and ARE levels in HT patients in
20 comparison with healthy volunteers. Indeed, these alterations were progressively more marked passing from
21 euthyroid to subclinical or overt hypothyroid subjects, with a negative correlation between TAS and TPO-Ab
22 ²⁵. The same group showed how TSH, FT4 and OSI ratio could have an independent predictive role of
23 progression from euthyroidism to subclinical, and finally overt, hypothyroidism in HT ⁷⁹. A similar evidence
24 was found by Ruggeri et al., who analyzed the redox status of a group of euthyroid HT patients in
25 comparison with healthy controls. In the first group, a significant decrease in biological antioxidant potential
26 (BAP), as well as increased levels of derived reactive oxygen metabolites (dROMs) and advanced glycation
27 end products (AGEs), that were both inversely correlated to the former. Moreover, TPO-Ab were the main
28 predictors for all the aforementioned parameters ⁵⁶. Also, a common polymorphism of AGEs receptor
29 (RAGE) related to HT, namely -429T>C, has been associated with the risk of progression from euthyroidism
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

1 to hypothyroidism, since patients under L-T4 treatment presented with higher oxidative stress levels ⁸⁰.
2 AGEs are well known to be increased in conditions of oxidative stress and to promote inflammation by
3 interacting with their receptor RAGE on cell membrane. By contrast, the soluble receptor sRAGE exerts
4 protective effects by competing with RAGE for ligand binding. More recently, reduced levels of the soluble
5 form of RAGE (sRAGE) have been reported in euthyroid HT patients compared to controls, along with
6 increased serum AGEs levels, and the two parameters were inversely correlated ⁵⁸. Accordingly, the
7 AGEs/sRAGEs ratio was threefold higher in HT patients than controls, suggesting a dysregulation of
8 AGE/sRAGEs-related oxidative homeostasis in HT patients even when in euthyroid status. In regression
9 analysis models, serum TPO-Ab were the main predictors for AGEs and sRAGEs levels and AGEs/sRAGEs
10 ratio, irrespective of TSH and/or FT4 values ⁵⁸. In other experiences, patients with AITDs presented with
11 reduced levels of paraoxonase-1 (PON1) and total free sulfhydryl (-SH) levels – both compounds having a
12 well-known antioxidant function – while lipid oxidation expressed as lipid hydroperoxide (LOOH) values
13 was significantly higher *versus* healthy controls ⁸¹. The same reduction of PON, and arylesterase (ARE) was
14 observed in a group of female adolescents with euthyroid HT, and it was paired with significantly higher
15 levels of anti-Mullerian hormone ⁸². Finally, an increase in serum interleukin-37 (IL-37) has been recently
16 observed in HT patients *vs* controls, directly correlating with anti-thyroid antibodies titre and AGEs levels.
17 This evidence could lead to hypothesize a protective role of IL-37 against oxidative stress in HT ⁵⁷.

33 Graves' disease

34 Most of the above reported biomarkers have been investigated also in Graves' disease patients, since
35 autoimmune hyperthyroidism and the related orbitopathy are well known to be oxidative stress-related
36 disorders ⁸³. For the sake of completeness and comparison, we briefly report the more recent data on
37 oxidative stress biomarkers in GD patients (**Table 2**).

38 As stated before, in 2010 Lassoued et al. demonstrated high levels of SOD activity and MDA in patients with
39 GD ⁷³. Also, metalloprotease (MMP) expression is stimulated by a high-oxidative stress environment, as
40 observed by Korkmaz et al. in a cohort of GD patients without Graves' orbitopathy (GO). They presented
41 high levels of the MMP prolidase, which positively correlated with TOS/OSI indexes, while -SH groups
42 were significantly reduced ⁸⁴. A reduction in native and total thiol levels in GD patients was also observed by
43 Agan et al, with a positive correlation between free triiodothyronine (FT3) and FT4 levels and thiol
44 homeostasis impairment/oxidative stress parameters ⁸⁵. High levels of MDA bound to proteins or carbonyl

1 groups, as well as a hyper-reactivity towards hydrogen peroxide (H₂O₂)-oxidized thyroid antigens, has been
2 observed in patients affected by AITD as expression of oxidative stress presence/increase ⁸⁶. Indeed, the
3 high production of H₂O₂ during hormone synthesis could enhance the antigen reactivity through the creation
4 of new epitopes. In GD patients this phenomenon had a positive correlation with FT3 levels ⁸⁶. Gargouri et al
5 also demonstrated a positive correlation between T3 levels and the immunoreactivity towards MDA-
6 modified catalase in GD patients vs controls ⁸⁷. In a study by Choi et al, MDA and 8-hydroxy-2'-
7 deoxyquanosine (8-OHdG), H₂O₂ and intracellular superoxide anion levels were measured in the tear fluid of
8 GD patients ⁸⁸. These markers resulted increased in comparison to healthy controls, and progressively higher
9 in affected subjects without and with GO, respectively. There was also a positive correlation between
10 markers and clinical activity score (CAS) in GO, while increased levels of extracellular ROS were
11 demonstrated in fibroadipose tissue, blood, orbital fibroblasts, and urine from these subjects ⁸⁸. Marique et al.
12 detected an increased expression of oxidative stress parameters in both adipose and muscular orbital cells in
13 patients with GO. On the other hand, an upregulation of some antioxidants (peroxiredoxin 5, catalase) was
14 also observed, while the overexpression of adiponectin (ApN) and proliferator-activated receptor gamma
15 (PPAR γ) exerted direct and indirect protective effects. These data confirmed the role of antioxidant
16 supplementation in active and chronic GO ⁸⁹. In another study, increased serum concentrations of
17 nicotinamide adenine dinucleotide phosphate oxidase, isoform 2 (NOX2) were measured in untreated
18 hyperthyroid GD patients, being significantly higher than in GD euthyroid treated patients, subjects with
19 multinodular toxic goiter and healthy controls. Moreover, higher oxidative stress parameters were also
20 detected in urine samples of untreated GD patients, as well as an increased respiratory burst of leukocytes in
21 whole blood ⁹⁰. In the same study by Diana et al, the Authors measured superoxide production in human
22 embryonic kidney (HEK)-293 cells with overexpressed TSH receptor (TSHR), and lipid peroxidation in
23 these cells and in human primary thyrocytes. Monoclonal M22 TSABs, bovine TSH and sera from
24 hyperthyroid GD patients stimulated cAMP in HEK cells, significantly increasing superoxide levels vs
25 controls. However, in this case there was no correlation between T3 levels and ROS production. A similar
26 result was obtained in primary thyrocytes, with higher oxidative stress parameters in GD patients vs controls,
27 and in untreated GD patients vs controls ⁹⁰. Recently, Ko et al demonstrated a significant increase in ROS
28 production and decrease in antioxidant enzymes in cultured orbital fibroblasts from GD patients with GO,
29 induced by H₂O₂ or cigarette smoke extract. Of note, treatment with caffeine determined a dose-dependent
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

1 decrease in intracellular ROS and antioxidant enzymes levels, while PPAR γ , C/EBP α , and C/EBP β protein
2 expression levels were inhibited during adipocyte differentiation ⁹¹.

3 **Possible Therapeutic Role of Antioxidants.**

4 Several antioxidant compounds have been evaluated to counteract oxidative stress in thyroid disorders and
5 their role in clinical practice is under debate.
6

7 ***Selenium.***

8 Selenium, a well-known element involved in thyroid homeostasis, has been demonstrated to exert some
9 antioxidant effects in AITDs, although its practical applications are still a matter of debate especially
10 concerning AT. In fact, thyrocytes express many selenoproteins, some of which, like type 1 and 2
11 iodothyronine deiodinases (DIO1 and 2) and GPX (isoforms 1, 3 and 4), are linked to thyroid hormone
12 metabolism and participate into controlling oxidative stress levels in the gland ⁹². Others, like selenoprotein S
13 (SELENOS), modulate the transcription of genes encoding proinflammatory cytokines involved in AT
14 pathogenesis ⁹³. Selenium intake is widely variable in the world due to environmental reasons and dietary
15 habits, ranging from deficiency to toxicity doses (7-4990 μ g/day), and in Europe it is in general under the
16 levels recommended by the US Institute of Medicine or the European Food Safety Authority (EFSA), that is
17 55 and 70 μ g/day respectively ⁹⁴.

18 *In vitro* studies demonstrated that incubation of thyrocytes with Se supplements is able to prevent oxidative
19 cell necrosis and apoptosis and enhances cell viability by preventing H₂O₂-induced degradation of DNA and
20 by reducing caspase-3 activity and BAX mRNA levels and increasing BCL-2 mRNA levels ⁴⁶⁻⁵⁰. An increase
21 in MDA levels, which was prevented by the pretreatment with both selenomethionine and selenite, was also
22 reported after exposure to H₂O₂ ⁵⁰. Both selenocompounds induced an increase in GPx activity, suggesting
23 that these protective effects may be, almost in part, mediated by these selenoproteins ⁵⁰.

24 A number of studies evaluating selenium supplementation in HT have demonstrated a reduction in TPO-Ab
25 concentration, but there are scarce evidences about its effects on disease progression and remission,
26 influences on L-T4 replacement dosage and patients' quality of life ^{95,94}. For example, the addition of
27 selenomethionine 200 μ g/day to levothyroxine treatment in a cohort of 170 Polish women with HT vs 41
28 controls demonstrated a reduced release of proinflammatory cytokines comprehending IL-2, IFN γ and TNF,
29 and decreased levels of C-reactive protein ⁹⁶. On the converse, another Austrian study in women with HT
30 treated with levothyroxine plus sodium selenite 200 μ g/day for three months did not record significant
31

1 modifications in cytokine profile ⁹⁷. While some ongoing trials are evaluating this association, the
2 paradoxical aspect to be clarified is that, in a recent survey performed by the European Thyroid Association
3 (ETA), around 65% of European endocrinologists occasionally or frequently prescribe selenium despite its
4 use in HT is not routinely recommended ^{94,98}.
5

6
7 The use of selenium in mild and active GO is an established tool in clinical practice, but the same consensus
8 has not been extended to the treatment of Graves' disease without GO ^{40,99,100}. In fact, even if one study
9 investigating the effects of selenium added to methimazole (MMI) treatment for GD reported a good
10 biochemical control of hyperthyroidism, two randomized clinical trials (RCTs) did not demonstrated a short-
11 term improvement of hyperthyroidism or an amelioration of response/recurrence rates ^{101,102,103}. However,
12 new evidences could emerge from more recent studies thoroughly analysing clinical parameters, like the
13 work by Xu et al, in which the comparison between MMI alone and MMI plus selenium groups revealed
14 significantly lower levels of FT3, FT4, TPO-Ab, Tg-Ab and TRAb, and a marked increase in TSH after 6
15 months of treatment ¹⁰⁴.
16
17

18 ***Vitamin antioxidants.***

19 Vitamin antioxidant compounds, such as vitamin C, vitamin E, β -carotene, have been employed in Graves'
20 disease patients under MMI or carbimazole therapy with positive results, providing evidence in favour of the
21 use of these antioxidant supplements in the early phases of anti-thyroid treatment ¹⁰⁵. For example, Guerra et
22 al tested a combination between antithyroid drugs and an antioxidant mixture (vitamin E, β -carotene, vitamin
23 C, Cu, Zn, Mn, and selenium) vs anti-thyroid drugs alone in hyperthyroid GD patients: in the first group, a
24 significant reduction in MDA levels was recorded, with a shorter time required to normalize thyroid
25 hormones and the clinical score, thus suggesting a synergistic mechanism at thyroid hormone synthesis level
26 ¹⁰⁶. Larger cohort of patients are needed to confirm these findings.
27

28 In recent years, a growing importance of the abovementioned vitamin antioxidants, namely alpha-tocopherol
29 and ascorbate, has been linked to a better efficiency of the antioxidant systems and the endogenous
30 antioxidants like SOD and CAT, as observed for example in benign prostate hyperplasia (BPH), in which
31 ROS production could contribute to carcinogenesis ¹⁰⁷. A similar evidence has been observed in a group of
32 thyroid cancer patients, treated with radioiodine and supplemented with 2000 mg vitamin C, 1000 mg
33 vitamin E, and 400 μ g selenium for 21 days before therapy. They presented with significantly lower plasma
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

1 levels of 8-epi-PGF_{2a}, a marker of lipid peroxidation, thus allowing to extend the hypothesis of a beneficial
2 effects of these substances in AITDs ¹⁰⁸.

3 ***Vitamin D.***

4
5 Vitamin D is a steroid hormone, which has been demonstrated to be involved in several processes in the
6 human body, including the regulation of immune response. In fact, its receptor (vitamin D receptor, VDR) is
7 present in monocytes and activated T cells, influencing both the innate and adaptive immunity ^{109,110}. Several
8 observational studies showed a relationship between vitamin D deficiency and the risk of developing AITDs,
9 especially HT, and a strong negative correlation has been observed between reduced vitamin D levels and
10 TPO-Ab, whereas the association of HT with VDR polymorphisms was variable ^{111–113}. Moreover, this
11 connection could also be confirmed by the fact that, conversely, vitamin D can downregulate the expression
12 of proinflammatory cytokines, such as Interleukin 6 (IL-6) and tumor necrosis factor α (TNF- α), which are
13 responsible for T and B cells migration and proliferation and for an increase in oxidative stress levels ¹¹⁴. In
14 the majority of randomized controlled trials (6 out of 7) conducted up to now in HT patients, the main effect
15 of cholecalciferol supplementation is a significant reduction of TPO-Ab titer, but several questions remain
16 unanswered i.e. regarding the best dosage, the best detection method, etc ¹¹⁰. Considering these data, an
17 empirical approach could be to consider measuring vitamin D levels in patients affected by HT, especially
18 when they are middle aged-women (the most affected part of population) and/or presenting with slightly
19 elevated TSH levels: this passage could increase the number of patients treated with vitamin D, not only for
20 the prevention of bone metabolism alterations, but also in order to slow down the progression of HT towards
21 overt hypothyroidism and/or thyroid atrophy ¹¹⁵. Similar considerations could be applied in GD patients, but
22 further studies with larger cohorts of patients and more stringent selection criteria are required.
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40

41 ***Dietary intake of plant-derived antioxidants.***

42
43 Plant-based foods are rich sources of vitamins, oligoelements and metabolites (such as phenols,
44 polyphenols, phytoestrogens, flavonoids, flavones, proanthocyanins, catechins) with documented antioxidant
45 activity and may exert protective effects against oxidative stress-related disorders, including autoimmune
46 processes. In isolated rural populations a vegetarian diet has been associated with a low prevalence of
47 autoimmune diseases, such a protective influence being attributed to the effects of plant foods, to the
48 exclusion of animal products, or both. On the contrary, a Western-type diet, rich in calories, fats, and
49 proteins, high in salt and refined sugars, and low in fibers, is thought to favor the development of
50
51
52
53
54
55

1 autoimmunity through enhanced oxidative stress and inflammation. Indeed, consumption of large amounts of
2 meat, fats and refined sugar in the long run results in gut microbiota disruption and inflammation with ROS
3 overproduction, while the low intake of fruits and vegetables causes lack of exogenous antioxidants ¹¹⁶. In
4 the last decade, Tonstad et al., using data from the Adventist Health Study-2, reported a reduced prevalence
5 and incidence of both hypothyroidism and hyperthyroidism among subjects following vegan/vegetarian diets
6 compared to omnivorous diets, providing congruent, though not always statistically significant, data in
7 favour of a protective role of diet excluding meat against thyroid dysfunction ^{117,118}. More recently, Ruggeri
8 and co-workers provided evidence that low dietary intake of animal foods has a potentially protective effect
9 towards thyroid autoimmunity as a result of the positive influence of this dietary habit on redox balance¹¹⁹.
10 According to this survey, the nutritional pattern of HT subjects was characterized by increased consumption
11 of animal proteins, higher intake of saturated fats and refined sugars, and lower intake of fibers and
12 antioxidants compared with healthy control subjects. In other words, nutritional patterns of HT subjects
13 resembled the Western-type diet, while controls displayed a higher level of adherence to the Mediterranean
14 diet. The study points to meat in omnivorous diets as the main nutritional factor associated with redox
15 dysregulation and increased risk of thyroid autoimmunity, whilst plant-based foods and Mediterranean diet
16 traits are protective¹¹⁹.

34 ***L-T4 treatment.***

35 Another interesting topic is the role of thyroid hormone replacement therapy towards oxidative stress, with
36 many evidences emerging in the literature. In a study by Marchiori et al, in a cohort of 17 hypothyroid HT
37 patients treated with L-T4, oxidative stress parameters were measured at 6 and 12 months, respectively. A
38 significant reduction in non-protein thiol (NP-SH) was observed, together with significant modifications in
39 interleukin levels (increase in IL-10 and decreases in IL-1, IL-6, INF- γ and TNF- α), thus suggesting that
40 hormone replacement therapy could condition the inflammatory response in thyroid autoimmunity ¹²⁰. A
41 similar effectiveness of L-T4 was reported in a paper by Ates et al, in which a group of treated HT patients
42 presented with increased levels of TAS, total thiol, ARE, and PON1 and decreased TOS and OSI levels in a
43 6-month period, compared to healthy controls. Moreover, pre-treatment TOS and OSI levels positively
44 correlated with TSH values, TPO- and Tg-Ab titer ¹²¹. More recently, another group recorded an increase in
45 antioxidant CAT levels, and a significant decrease in thiobarbituric acid reactive substances (TBARS) in a
46
47
48
49
50
51
52
53
54
55

1 group of 25 female patients with primary hypothyroidism treated with L-T4¹²². On the other hand,
2 Chakrabarti et al. described the effects of the association of L-T4 replacement therapy with selenium
3 supplementation (100 mcg twice a day) in hypothyroid HT patients, vs L-T4 alone: although in both groups a
4 reduction in MDA levels was recorded after 6 months, there was no statistical significance in the
5 measurements obtained in the combination group¹²³.
6
7
8

9 **CONCLUSION.**

10 In the context of a growing amount of data about the role of oxidative stress and antioxidants in the
11 pathophysiology of AITDs, and especially in AT progression towards hypothyroidism, oxidative stress
12 parameters could be used as biochemical markers of chronic inflammation, to better predict the disease
13 evolution along its natural history. Besides, the well-known link between oxidative alterations and thyroid
14 hormone replacement treatment could be the theoretical basis to use these parameters for a finer patients'
15 monitoring. A larger knowledge of the substances which locally counteract the oxidative stress imbalance
16 typical of chronic inflammatory disorders could open new perspectives in the development of more tailored
17 medical therapies for these autoimmune conditions that significantly impair patients' quality of life.
18 Currently, natural sources of antioxidants in the form of a plant-based foods may represent the best option
19 for protecting against chronic oxidative stress-related disorders. Reducing the intake of animal proteins and
20 fats and increasing that of fruits and vegetables has proven to be a useful lifestyle strategy for contrasting
21 oxidative stress and reducing the risk for autoimmune diseases, including AT. In particular, a predominantly
22 plant-based Mediterranean diet, high in naturally occurring antioxidants, low in saturated fat and cholesterol,
23 and good source of vitamins and minerals, may represent a healthy food model for people suffering from AT.
24 Conversely, there is still a debate as to whether assuming antioxidants in supplement form can actually
25 reduce the risk of AT development/progression, by preventing or slowing down thyroid damage. In spite of
26 the experimental evidence for a protective effect of antioxidant molecules against oxidative damage to cells
27 in animal models and *in vitro* studies, clinical studies of antioxidant supplements in AT patients reached
28 disappointing and largely inconclusive results and have not demonstrate them to provide substantial benefits
29 in preventing/slowing down AT development and progression towards thyroid dysfunction. On this basis, a
30 routine use of vitamins and antioxidants supplementation in the treatment of AT patients should be
31 discouraged, but correction of nutrient deficiencies (for instance, vitamin D or selenium deficit) is advisable
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

1 to avoid negative health effects due to the lack of these elements essential to proper thyroid and immune
2 function. Moreover, antioxidant supplements cannot be routinely given for a long enough time to prevent
3 chronic diseases, such as HT and hypothyroidism, which develop over decades, but short courses of
4 antioxidants supplementation can be considered as an option and justified in selected conditions and/or
5 populations. A 6-month trial of selenium supplements has indication in the treatment of patients with
6 Graves' disease and associated mild orbitopathy since selenium may enhance the effectiveness of anti-
7 thyroid drugs, improves clinical manifestations and quality of life and prevents progression of the disease.
8 Importantly, current guidelines and scientific societies do not recommend Se supplementation for other
9 indications. Future antioxidant therapeutic strategies should include the design of protocols for the inhibition
10 of oxidative stress damage through administration of synthetic and natural antioxidants and enhancement of
11 the antioxidant defenses by increasing the production of endogenous antioxidants and activation of
12 antioxidant mechanisms.
13
14
15
16
17
18
19
20
21
22

23 24 25 26 **ACKNOWLEDGEMENT AND DISCLOSURES**

27
28 **Conflict of interests:** none of the authors has any conflict of interests.

29
30 **Funding:** this research did not receive any specific grant from funding agencies in the public, commercial,
31 or not-for-profit sectors.
32

33
34 **Authors' contribution:** each Author equally contributed to this paper. All Authors read and approved the
35 final version of the manuscript.
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

References:

1. Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: Clinical and diagnostic criteria. *Autoimmun Rev.* 2014;13(4):391-397.
2. Ruggeri RM, Giuffrida G, Campenni A. Autoimmune endocrine diseases. *Minerva Endocrinol.* 2018;43(3):305-322.
3. McLeod DSA, Cooper DS. The incidence and prevalence of thyroid autoimmunity. *Endocrine.* 2012;42(2):252-265. doi:10.1007/s12020-012-9703-2
4. Ruggeri RM, Trimarchi F, Giuffrida G, et al. Autoimmune comorbidities in Hashimoto's thyroiditis: different patterns of association in adulthood and childhood/adolescence. *Eur J Endocrinol.* 2017;176(2):133-141.
5. Ruggeri RM, Trimarchi F, Biondi B. MANAGEMENT OF ENDOCRINE DISEASE: 1-Thyroxine replacement therapy in the frail elderly: a challenge in clinical practice. *Eur J Endocrinol.* 2017;177(4):R199-R217.
6. Ajjan R, Weetman A. The Pathogenesis of Hashimoto's Thyroiditis: Further Developments in our Understanding. *Horm Metab Res.* 2015;47(10):702-710.
7. Figueroa-Vega N, Alfonso-Pérez M, Benedicto I, Sánchez-Madrid F, González-Amaro R, Marazuela M. Increased Circulating Pro-Inflammatory Cytokines and Th17 Lymphocytes in Hashimoto's Thyroiditis. *J Clin Endocrinol Metab.* 2010;95(2):953-962.
8. Rebuffat SA, Kammoun-Krichen M, Charfeddine I, Ayadi H, Bougacha-Elleuch N, Peraldi-Roux S. IL-1 β and TSH disturb thyroid epithelium integrity in autoimmune thyroid diseases. *Immunobiology.* 2013;218(3):285-291.
9. Ruggeri RM, Saitta S, Cristani M, et al. Serum interleukin-23 (IL-23) is increased in Hashimoto's thyroiditis. *Endocr J.* 2014;61(4):359-363.
10. Effraimidis G, Wiersinga WM. MECHANISMS IN ENDOCRINOLOGY: Autoimmune thyroid disease: old and new players. *Eur J Endocrinol.* 2014;170(6):R241-R252.
11. Kurien BT, Scofield RH. Autoimmunity and oxidatively modified autoantigens. *Autoimmun Rev.* 2008;7(7):567-573.
12. Srivastava S, Singh D, Patel S, Singh MR. Role of enzymatic free radical scavengers in management of oxidative stress in autoimmune disorders. *Int J Biol Macromol.* 2017;101:502-517.
13. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol.* 2007;39(1):44-84.
14. Devasagayam TPA, Tilak JC, Bloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and antioxidants in human health: current status and future prospects. *J Assoc Physicians India.* 2004;52:794-804.
15. Davidson SM, Duchon MR. Endothelial Mitochondria. *Circ Res.* 2007;100(8):1128-1141.

16. Schulz E, Wenzel P, Münzel T, Daiber A. Mitochondrial Redox Signaling: Interaction of Mitochondrial Reactive Oxygen Species with Other Sources of Oxidative Stress. *Antioxid Redox Signal*. 2014;20(2):308-324.
17. Drummond GR, Selemidis S, Griendling KK, Sobey CG. Combating oxidative stress in vascular disease: NADPH oxidases as therapeutic targets. *Nat Rev Drug Discov*. 2011;10(6):453-471.
18. Laursen JB, Somers M, Kurz S, et al. Endothelial Regulation of Vasomotion in ApoE-Deficient Mice. *Circulation*. 2001;103(9):1282-1288.
19. Gandhi S, Abramov AY. Mechanism of Oxidative Stress in Neurodegeneration. *Oxid Med Cell Longev*. 2012;2012:1-11.
20. Steinbrenner H, Speckmann B, Klotz L-O. Selenoproteins: Antioxidant selenoenzymes and beyond. *Arch Biochem Biophys*. 2016;595:113-119.
21. Kohrle J, Jakob F, Contempre B, Dumont JE. Selenium, the thyroid, and the endocrine system. *Endocr Rev*. 2005;26(7):944-984.
22. Sies H. Oxidative stress: a concept in redox biology and medicine. *Redox Biol*. 2015;4:180-183.
23. Saito Y, Nishio K, Ogawa Y, et al. Turning point in apoptosis/necrosis induced by hydrogen peroxide. *Free Radic Res*. 2006;40(6):619-630.
24. Di Dalmazi G, Hirshberg J, Lyle D, Freij JB, Caturegli P. Reactive oxygen species in organ-specific autoimmunity. *Autoimmun Highlights*. 2016;7(1).
25. Ates I, Yilmaz FM, Altay M, Yilmaz N, Berker D, Güler S. The relationship between oxidative stress and autoimmunity in Hashimoto's thyroiditis. *Eur J Endocrinol*. 2015;173(6):791-799.
26. Burek CL, Rose NR. Autoimmune thyroiditis and ROS. *Autoimmun Rev*. 2008;7(7):530-537.
27. Rasooly L, Burek CL, Rose NR. Iodine-induced autoimmune thyroiditis in NOD-H-2h4 mice. *Clin Immunol Immunopathol*. 1996;81(3):287-292.
28. Braley-Mullen H, Sharp GC, Medling B, Tang H. Spontaneous Autoimmune Thyroiditis in NOD.H-2h4 Mice. *J Autoimmun*. 1999;12(3):157-165.
29. Kolypetri P, King J, Larijani M, Carayanniotis G. Genes and environment as predisposing factors in autoimmunity: acceleration of spontaneous thyroiditis by dietary iodide in NOD.H2(h4) mice. *Int Rev Immunol*. 2015;34(6):542-556.
30. Braley-Mullen H, Yu S. NOD.H-2h4 mice: an important and underutilized animal model of autoimmune thyroiditis and Sjogren's syndrome. *Adv Immunol*. 2015;126:1-43.
31. Sharma RB, Alegria JD, Talor MV, Rose NR, Caturegli P, Burek CL. Iodine and IFN- γ Synergistically Enhance Intercellular Adhesion Molecule 1 Expression on NOD.H2h4 Mouse Thyrocytes. *J Immunol*. 2005;174(12):7740-7745.

- 1 32. Sharma R, Traore K, Trush MA, Rose NR, Burek CL. Intracellular adhesion molecule-1 up-
2 regulation on thyrocytes by iodine of non-obese diabetic.H2(h4) mice is reactive oxygen
3 species-dependent. *Clin Exp Immunol.* 2008;152(1):13-20.
- 4 33. Kolypetri P, Carayanniotis G. Apoptosis of NOD.H2h4 Thyrocytes by Low Concentrations
5 of Iodide is Associated with Impaired Control of Oxidative Stress. *Thyroid.* 2014;24(7):1170-
6 1178.
- 7 34. Poncin S, Colin IM, Decallonne B, et al. N-Acetylcysteine and 15 Deoxy- Δ 12,14-
8 Prostaglandin J2 Exert a Protective Effect Against Autoimmune Thyroid Destruction in Vivo
9 but Not Against Interleukin-1 α /Interferon γ -Induced Inhibitory Effects in Thyrocytes in
10 Vitro. *Am J Pathol.* 2010;177(1):219-228.
- 11 35. Abalovich M, Llesuy S, Gutierrez S, Repetto M. Peripheral parameters of oxidative stress in
12 Graves' disease: the effects of methimazole and 131 iodine treatments. *Clin Endocrinol*
13 *(Oxf).* 2003;59(3):321-327.
- 14 36. Bednarek J, Wysocki H, Sowiński J. Oxidative stress peripheral parameters in Graves'
15 disease: the effect of methimazole treatment in patients with and without infiltrative
16 ophthalmopathy. *Clin Biochem.* 2005;38(1):13-18.
- 17 37. Erdamar H, Demirci H, Yaman H, et al. The effect of hypothyroidism, hyperthyroidism, and
18 their treatment on parameters of oxidative stress and antioxidant status. *Clin Chem Lab Med.*
19 2008;46(7).
- 20 38. Aslan M, Cosar N, Celik H, et al. Evaluation of oxidative status in patients with
21 hyperthyroidism. *Endocrine.* 2011;40(2):285-289.
- 22 39. Resch U, Helsel G, Tatzber F, Sinzinger H. Antioxidant Status in Thyroid Dysfunction. *Clin*
23 *Chem Lab Med.* 2002;40(11).
- 24 40. Marcocci C, Bartalena L. Role of oxidative stress and selenium in Graves' hyperthyroidism
25 and orbitopathy. *J Endocrinol Invest.* 2013;36(10 Suppl):15-20.
- 26 41. Baskol G, Atmaca H, Tanrıverdi F, Baskol M, Kocer D, Bayram F. Oxidative Stress and
27 Enzymatic Antioxidant Status in Patients with Hypothyroidism before and after Treatment.
28 *Exp Clin Endocrinol Diabetes.* 2007;115(08):522-526.
- 29 42. Torun AN, Kulaksizoglu S, Kulaksizoglu M, Pamuk BO, Isbilen E, Tutuncu NB. Serum total
30 antioxidant status and lipid peroxidation marker malondialdehyde levels in overt and
31 subclinical hypothyroidism. *Clin Endocrinol (Oxf).* 2009;70(3):469-474.
- 32 43. Reddy V, Gouroju S, Suchitra M, et al. Antioxidant Defense in Overt and Subclinical
33 Hypothyroidism. *Horm Metab Res.* 2013;45(10):754-758.
- 34 44. Song Y, Driessens N, Costa M, et al. Roles of Hydrogen Peroxide in Thyroid Physiology and
35 Disease. *J Clin Endocrinol Metab.* 2007;92(10):3764-3773.
- 36 45. Ghaddhab C, Kyrilli A, Driessens N, et al. Factors contributing to the resistance of the
37 thyrocyte to hydrogen peroxide. *Mol Cell Endocrinol.* 2019;481:62-70.
- 38 46. Contempre B, Le Moine O, Dumont JE, Denef J-F, Many MC. Selenium deficiency and
39 thyroid fibrosis. A key role for macrophages and transforming growth factor β (TGF- β). *Mol*
40 *Cell Endocrinol.* 1996;124(1-2):7-15.
- 41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

- 1 47. Wang W, Xue H, Li Y, et al. Effects of Selenium Supplementation on Spontaneous
2 Autoimmune Thyroiditis in NOD.H-2h4 Mice. *Thyroid Off J Am Thyroid Assoc.*
3 2015;25(10):1137-1144.
- 4 48. Demelash A, Karlsson J, Nilsson M, Bjorkman U. Selenium has a protective role in caspase-
5 3-dependent apoptosis induced by H₂O₂ in primary cultured pig thyrocytes. *Eur J*
6 *Endocrinol.* Published online June 1, 2004:841-849.
- 7 49. Nettore IC, De Nisco E, Desiderio S, et al. Selenium supplementation modulates apoptotic
8 processes in thyroid follicular cells. *BioFactors.* 2017;43(3):415-423.
- 9 50. Ruggeri RM, D'Ascola A, Vicchio TM, et al. Selenium exerts protective effects against
10 oxidative stress and cell damage in human thyrocytes and fibroblasts. *Endocrine.*
11 2019;68(1):151-162.
- 12 51. Guastamacchia E, Giagulli V, Licchelli B, Triggiani V. Selenium and Iodine in Autoimmune
13 Thyroiditis. *Endocr Metab Immune Disord-Drug Targets.* 2015;15(4):288-292.
- 14 52. Teng X, Shan Z, Teng W, Fan C, Wang H, Guo R. Experimental study on the effects of
15 chronic iodine excess on thyroid function, structure, and autoimmunity in autoimmune-prone
16 NOD.H-2h4 mice. *Clin Exp Med.* 2008;9(1):51-59.
- 17 53. Franceschi C, Ostan R, Mariotti S, Monti D, Vitale G. The aging thyroid: a reappraisal within
18 the geroscience integrated perspective. *Endocr Rev.* 2019;40(5):1250–1270.
- 19 54. Vitale G, Salvioli S, Franceschi C. Oxidative stress and the ageing endocrine system. *Nat Rev*
20 *Endocrinol.* 2013;9(4):228–240.
- 21 55. Baser H, Can U, Baser S, Yerlikaya FH, Aslan U, Hidayetoglu BT. Assesment of oxidative
22 status and its association with thyroid autoantibodies in patients with euthyroid autoimmune
23 thyroiditis. *Endocrine.* 2014;48(3):916-923.
- 24 56. Ruggeri RM, Vicchio TM, Cristani M, et al. Oxidative Stress and Advanced Glycation End
25 Products in Hashimoto's Thyroiditis. *Thyroid.* 2016;26(4):504-511.
- 26 57. Ruggeri RM, Cristani M, Vicchio TM, et al. Increased serum interleukin-37 (IL-37) levels
27 correlate with oxidative stress parameters in Hashimoto's thyroiditis. *J Endocrinol Invest.*
28 2018;42(2):199-205.
- 29 58. Ruggeri RM, Barbalace MC, Cristani MT, et al. Serum levels of advanced glycation end
30 products (AGEs) are increased and their soluble receptor (sRAGE) reduced in Hashimoto's
31 thyroiditis. *J Endocrinol Invest.* Published online March 30, 2020.
- 32 59. DUTHOIT C, ESTIENNE V, GIRAUD A, et al. Hydrogen peroxide-induced production of a
33 40 kDa immunoreactive thyroglobulin fragment in human thyroid cells: the onset of thyroid
34 autoimmunity? *Biochem J.* 2001;360(3):557-562.
- 35 60. Colin IM, Poncin S, Levêque P, Gallez B, Gérard A-C. Differential Regulation of the
36 Production of Reactive Oxygen Species in Th1 Cytokine-Treated Thyroid Cells. *Thyroid.*
37 2014;24(3):441-452.
- 38 61. Marique L, Van Regemorter V, Gérard A-C, et al. The Expression of Dual Oxidase, Thyroid
39 Peroxidase, and Caveolin-1 Differs According to the Type of Immune Response (TH1/TH2)
- 40
- 41
- 42
- 43
- 44
- 45
- 46
- 47
- 48
- 49
- 50
- 51
- 52
- 53
- 54
- 55

Involved in Thyroid Autoimmune Disorders. *J Clin Endocrinol Metab.* 2014;99(5):1722-1732.

62. Jackson SH, Devadas S, Kwon J, Pinto LA, Williams MS. T cells express a phagocyte-type NADPH oxidase that is activated after T cell receptor stimulation. *Nat Immunol.* 2004;5(8):818-827.
63. Williams MS, Kwon J. T Cell Receptor Stimulation, Reactive Oxygen Species, and Cell Signaling. *Free Radic Biol Med.* 2004;37(8):1144-1151.
64. Davies CM, Guilak F, Weinberg JB, Fermor B. Reactive nitrogen and oxygen species in interleukin-1-mediated DNA damage associated with osteoarthritis. *Osteoarthritis Cartilage.* 2008;16(5):624-630.
65. Rabinovitch A, Suarez-Pinzon WL, Strynadka K, Lakey JR, Rajotte RV. Human pancreatic islet beta-cell destruction by cytokines involves oxygen free radicals and aldehyde production. *J Clin Endocrinol Metab.* 1996;81(9):3197-3202.
66. Chen YK, Lin CL, Cheng FT, Sung FC, Kao CH. Cancer risk in patients with Hashimoto's thyroiditis: a nationwide cohort study. *Br J Cancer.* 2013;109(9):2496-2501.
67. Guarino V, Castellone MD, Avilla E, Melillo RM. Thyroid cancer and inflammation. *Mol Cell Endocrinol.* 2010;321(1):94-102.
68. Wang D, Feng J-F, Zeng P, Yang Y-H, Luo J, Yang Y-W. Total oxidant/antioxidant status in sera of patients with thyroid cancers. *Endocr Relat Cancer.* 2011;18(6):773-782.
69. Erdamar H, Çimen B, Gülcemal H, Saraymen R, Yerer B, Demirci H. Increased lipid peroxidation and impaired enzymatic antioxidant defense mechanism in thyroid tissue with multinodular goiter and papillary carcinoma. *Clin Biochem.* 2010;43(7-8):650-654.
70. Karger S, Krause K, Engelhardt C, et al. Distinct pattern of oxidative DNA damage and DNA repair in follicular thyroid tumours. *J Mol Endocrinol.* 2012;48(3):193.
71. Xing M. Oxidative stress: a new risk factor for thyroid cancer. *Endocr Relat Cancer.* 2012;19(1):C7.
72. Habza-Kowalska E, Gawlik-Dziki U, Dziki D. Mechanism of Action and Interactions between Thyroid Peroxidase and Lipoxxygenase Inhibitors Derived from Plant Sources. *Biomolecules.* 2019;9(11):663.
73. Lassoued S, Mseddi M, Mnif F, et al. A Comparative Study of the Oxidative Profile in Graves' Disease, Hashimoto's Thyroiditis, and Papillary Thyroid Cancer. *Biol Trace Elem Res.* 2010;138(1-3):107-115.
74. Akinci M, Kosova F, Çetin B, et al. Oxidant/antioxidant balance in patients with thyroid cancer. *Acta Cir Bras.* 2008;23(6):551-554.
75. Taddei S, Caraccio N, Virdis A, et al. Low-Grade Systemic Inflammation Causes Endothelial Dysfunction in Patients with Hashimoto's Thyroiditis. *J Clin Endocrinol Metab.* 2006;91(12):5076-5082.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
76. Vural P, Degirmencioglu S, Erden S, Gelincik A. The relationship between transforming growth factor- β 1, vascular endothelial growth factor, nitric oxide and Hashimoto's thyroiditis. *Int Immunopharmacol*. 2009;9(2):212-215.
 77. Öztürk Ü, Vural P, Özdeyri A, Karadağ B, Doğru-Abbasoğlu S, Uysal M. Oxidative stress parameters in serum and low density lipoproteins of Hashimoto's thyroiditis patients with subclinical and overt hypothyroidism. *Int Immunopharmacol*. 2012;14(4):349-352.
 78. Rostami R, Aghasi MR, Mohammadi A, Nourooz-Zadeh J. Enhanced oxidative stress in Hashimoto's thyroiditis: Inter-relationships to biomarkers of thyroid function. *Clin Biochem*. 2013;46(4-5):308-312.
 79. Ates I, Arikan MF, Altay M, et al. The effect of oxidative stress on the progression of Hashimoto's thyroiditis. *Arch Physiol Biochem*. 2017;124(4):351-356.
 80. Giannakou M, Saltiki K, Mantzou E, et al. RAGE polymorphisms and oxidative stress levels in Hashimoto's thyroiditis. *Eur J Clin Invest*. 2017;47(5):341-347.
 81. Korkmaz H, Tabur S, Ozkaya M, et al. Paraoxonase and arylesterase levels in autoimmune thyroid diseases. *Redox Rep*. 2016;21(5):227-231.
 82. Erol O, Parlak M, Ellidağ HY, et al. Serum anti-Müllerian hormone levels in euthyroid adolescent girls with Hashimoto's thyroiditis: relationship to antioxidant status. *Eur J Obstet Gynecol Reprod Biol*. 2016;203:204-209.
 83. Bartalena L, Tanda ML, Piantanida E, Lai A. Oxidative stress and Graves' ophthalmopathy: In vitro studies and therapeutic implications. *BioFactors*. 2003;19(3-4):155-163.
 84. Korkmaz H, Tabur S, Ozkaya M, Oguz E, Aksoy N, Akarsu E. Serum prolidase levels in Graves' disease without ophthalmopathy and its association with oxidative status. *J Endocrinol Invest*. 2015;38(11):1167-1173.
 85. Agan V, Celik H, Eren MA, et al. An Investigation of Oxidative Stress and Thiol/Disulphide Homeostasis in Graves' Disease. *Medicina (Mex)*. 2019;55(6):275.
 86. Mseddi M, Ben Mansour R, Gargouri B, et al. Proteins oxidation and autoantibodies' reactivity against hydrogen peroxide and malondialdehyde -oxidized thyroid antigens in patients' plasmas with Graves' disease and Hashimoto Thyroiditis. *Chem Biol Interact*. 2017;272:145-152.
 87. Gargouri B, Mseddi M, Mnif F, Abid M, Attia H, Lassoued S. Oxidative stress enhances the immune response to oxidatively modified catalase enzyme in patients with Graves' disease. *J Clin Lab Anal*. 2019;34(2).
 88. Choi W, Li Y, Ji YS, Yoon KC. Oxidative stress markers in tears of patients with Graves' orbitopathy and their correlation with clinical activity score. *BMC Ophthalmol*. 2018;18(1).
 89. Marique L, Senou M, Craps J, et al. Oxidative Stress and Upregulation of Antioxidant Proteins, Including Adiponectin, in Extraocular Muscular Cells, Orbital Adipocytes, and Thyrocytes in Graves' Disease Associated with Orbitopathy. *Thyroid*. 2015;25(9):1033-1042.
 90. Diana T, Daiber A, Oelze M, et al. Stimulatory TSH-Receptor Antibodies and Oxidative Stress in Graves Disease. *J Clin Endocrinol Metab*. 2018;103(10):3668-3677.

- 1 91. Ko J, Kim J-Y, Kim J, Yoon JS. Anti-oxidative and anti-adipogenic effects of caffeine in an
2 in vitro model of Graves' orbitopathy. *Endocr J.* 2020;67(4):439-447.
- 3 92. Schmutzler C, Mentrup B, Schomburg L, Hoang-Vu C, Herzog V, Köhrle J. Selenoproteins
4 of the thyroid gland: expression, localization and possible function of glutathione peroxidase
5 3. *Biol Chem.* 2007;388(10).
- 6 93. Santos LR, Durães C, Mendes A, et al. A Polymorphism in the Promoter Region of the
7 Selenoprotein S Gene (SEPS1) Contributes to Hashimoto's Thyroiditis Susceptibility. *J Clin*
8 *Endocrinol Metab.* 2014;99(4):E719-E723.
- 9 94. Winther KH, Rayman MP, Bonnema SJ, Hegedüs L. Selenium in thyroid disorders
10 essential knowledge for clinicians. *Nat Rev Endocrinol.* 2020;16(3):165-176.
- 11 95. Schomburg L. Selenium, selenoproteins and the thyroid gland: interactions in health and
12 disease. *Nat Rev Endocrinol.* 2011;8(3):160-171.
- 13 96. Krysiak R, Okopien B. The Effect of Levothyroxine and Selenomethionine on Lymphocyte
14 and Monocyte Cytokine Release in Women with Hashimoto's Thyroiditis. *J Clin Endocrinol*
15 *Metab.* 2011;96(7):2206-2215.
- 16 97. Karanikas G, Dudczak R, Willheim M. Author's Response to Letters to the Editor
17 Concerning "No Immunological Benefit of Selenium in Consecutive Patients with
18 Autoimmune Thyroiditis." *Thyroid.* 2008;18(6):673-674.
- 19 98. Winther KH, Wichman JEM, Bonnema SJ, Hegedüs L. Insufficient documentation for
20 clinical efficacy of selenium supplementation in chronic autoimmune thyroiditis, based on a
21 systematic review and meta-analysis. *Endocrine.* 2017;55(2):376-385.
- 22 99. Kahaly GJ, Bartalena L, Hegedüs L, Leenhardt L, Poppe K, Pearce SH. 2018 European
23 Thyroid Association Guideline for the Management of Graves' Hyperthyroidism. *Eur*
24 *Thyroid J.* 2018;7(4):167-186.
- 25 100. Kotwal A, Stan M. Current and Future Treatments for Graves' Disease and Graves'
26 Ophthalmopathy. *Horm Metab Res Horm Stoffwechselforschung Horm Metab.*
27 2018;50(12):871-886.
- 28 101. Calissendorff J, Mikulski E, Larsen EH, Möller M. A Prospective Investigation of Graves'
29 Disease and Selenium: Thyroid Hormones, Auto-Antibodies and Self-Rated Symptoms. *Eur*
30 *Thyroid J.* 2015;4(2):93-98.
- 31 102. Leo M, Bartalena L, Rotondo Dottore G, et al. Effects of selenium on short-term control of
32 hyperthyroidism due to Graves' disease treated with methimazole: results of a randomized
33 clinical trial. *J Endocrinol Invest.* 2017;40(3):281-287.
- 34 103. Kahaly GJ, Riedl M, König J, Diana T, Schomburg L. Double-Blind, Placebo-Controlled,
35 Randomized Trial of Selenium in Graves Hyperthyroidism. *J Clin Endocrinol Metab.*
36 2017;102(11):4333-4341.
- 37 104. XU B, WU D, YING H, ZHANG Y. A Pilot Study on the Beneficial Effects of the
38 Additional Selenium Supplementation to Methimazole for Treating Patients with Graves'
39 disease. *Turk J Med Sci.* Published online January 24, 2019.
- 40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

105. Marcocci C, Leo M, Altea MA. Oxidative Stress in Graves' Disease. *Eur Thyroid J*. 2012;1(2):80-87.
106. Guerra LN, Moiguer S, Karner M, de Molina M del CR, Sreider CM, Burdman JA. Antioxidants in the Treatment of Graves Disease. *IUBMB Life*. 2001;51(2):105-109.
107. Minciullo PL, Inferrera A, Navarra M, Calapai G, Magno C, Gangemi S. Oxidative stress in benign prostatic hyperplasia: a systematic review. *Urol Int*. 2015;94(3):249-254.
108. Rosário PW, Batista KCS, Calsolari MR. Radioiodine-induced oxidative stress in patients with differentiated thyroid carcinoma and effect of supplementation with vitamins C and E and selenium (antioxidants). *Arch Endocrinol Metab*. 2016;60(4):328-332.
109. Muscogiuri G, Mitri J, Mathieu C, et al. Vitamin D as a potential contributor in endocrine health and disease. *Eur J Endocrinol*. 2014;171(3):R101–R110.
110. Gallo D, Mortara L, Gariboldi MB, et al. Immunomodulatory effect of vitamin D and its potential role in the prevention and treatment of thyroid autoimmunity: A narrative review. *J Endocrinol Invest*. Published online 2019:1–17.
111. Kivity S, Agmon-Levin N, Zisappl M, et al. Vitamin D and autoimmune thyroid diseases. *Cell Mol Immunol*. 2011;8(3):243–247.
112. Bozkurt N, Karbek B, Ucan B, et al. The association between severity of vitamin D deficiency and Hashimoto's thyroiditis. *Endocr Pract*. 2013;19(3):479–484.
113. Giovinazzo S, Vicchio TM, Certo R, et al. Vitamin D receptor gene polymorphisms/haplotypes and serum 25(OH)D₃ levels in Hashimoto's thyroiditis. *Endocrine*. 2016;55(2):599-606.
114. Zhang Y, Leung DY, Richers BN, et al. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. *J Immunol*. 2012;188(5):2127–2135.
115. Mirhosseini N, Brunel L, Muscogiuri G, Kimball S. Physiological serum 25-hydroxyvitamin D concentrations are associated with improved thyroid function—observations from a community-based program. *Endocrine*. 2017;58(3):563–573.
116. Manzel A, Muller DN, Hafler DA, Erdman SE, Linker RA, Kleinewietfeld M. Role of “Western Diet” in Inflammatory Autoimmune Diseases. *Curr Allergy Asthma Rep*. 2013;14(1).
117. Tonstad S, Nathan E, Oda K, Fraser G. Vegan diets and hypothyroidism. *Nutrients*. 2013;5(11):4642–4652.
118. Tonstad S, Nathan E, Oda K, Fraser GE. Prevalence of hyperthyroidism according to type of vegetarian diet. *Public Health Nutr*. 2015;18(8):1482–1487.
119. Ruggeri RM, Giovinazzo S, Barbalace MC, et al. Influence Of Dietary Habits On Oxidative Stress Markers In Hashimoto's Thyroiditis. *Thyroid*. 2020;(ja).
120. Marchiori RC, Pereira LAF, Naujorks AA, et al. Improvement of blood inflammatory marker levels in patients with hypothyroidism under levothyroxine treatment. *BMC Endocr Disord*. 2015;15(1).

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
121. Ates I, Altay M, Yilmaz FM, et al. The impact of levothyroxine sodium treatment on oxidative stress in Hashimoto's thyroiditis. *Eur J Endocrinol.* 2016;174(6):727-734.
 122. Masullo LF, Magalhães RA, Lemes RPG, et al. Levothyroxine Replacement Improves Oxidative Status in Primary Hypothyroidism. *Front Endocrinol.* 2018;9.
 123. Chakrabarti S, Ghosh S, Banerjee S, Mukherjee S, Chowdhury S. Oxidative stress in hypothyroid patients and the role of antioxidant supplementation. *Indian J Endocrinol Metab.* 2016;20(5):674.

PEER REVIEW COPY
Minerva Endocrinologica

LEGEND FOR FIGURES.

1
2 **Figure 1.** Schematic representation of ROS involvement in the pathogenesis of autoimmune thyroiditis, in
3 the context of an inflammatory *milieu*. When not counterbalanced by antioxidant mechanisms, excess ROS
4 trigger thyrocytes damage and the release of pro-inflammatory mediators, such as TNF-alpha and IL-1,
5 which in turn activate macrophages and promote the recruitment of immune cells (Th17, Th1 and CD8 cells
6 and neutrophils) via IL-23, IL-17, IL-22 and INF-alpha. The consequent intervention of pro-inflammatory
7 mediators sustains thyroid damage in a cyclic loop.
8
9
10
11
12
13
14
15

16 **Figure 2.** A) This simplified scheme highlights the connections between the main ROS produced at cellular
17 level. They can lead to several damages like the oxidation of lipids, cytosol/nuclear proteins and nucleic
18 acids, up to the generation of novel autoantigens exacerbating autoimmunity. B) The main actions of catalase
19 and glutathione peroxidase.
20
21
22
23

24 ROS: reactive oxygen species; e⁻: electron; pr⁺: proton; Fe: iron; SOD: superoxide dismutase; MPO:
25 myeloperoxidase; CAT: catalase; GPx: glutathione peroxidase; GR: glutathione reductase; NADPH
26 nicotinamide adenine dinucleotide phosphate.
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

LIST OF ABBREVIATIONS

1	
2	4-HNE: 4-hydroxynonenal
3	8-OHdG: 8-hydroxy-2'-deoxyguanosine
4	AGEs: advanced glycation end products
5	AMH: anti-Mullerian hormone
6	Antiox-cap: non-enzymatic antioxidants
7	AOPP: advanced oxidation protein products
8	ApN: adiponectin
9	ARE: arylesterase
10	ARS: enzymatic antioxidants
11	AT: autoimmune thyroiditis
12	BAP: biological antioxidant potential
13	CAT: catalase activity
14	C/EBP α and C/EBP β : CCAAT/enhancer-binding protein alpha and beta
15	CRP: C-reactive protein
16	DC: diene conjugate
17	d-ROMs: derived reactive oxygen metabolites
18	FRAP: ferric reducing antioxidant power
19	FT4: free thyroxine
20	FT3: free triiodothyronine
21	GD: Graves' disease
22	GO: Graves' ophthalmopathy
23	GPx: glutathione peroxidase
24	GR: glutathione reductase
25	GSH: Glutathione
26	IMA: ischemia-modified albumin
27	HT: Hashimoto's thyroiditis
28	¹³¹ I: ¹³¹ iodine
29	IL-6: interleukin 6
30	IL-37: interleukin 37
31	L-T4: levothyroxine
32	LOOH: lipid hydroperoxide
33	MDA: malondialdehyde
34	MMI: methimazole
35	MNTG: multinodular toxic goiter
36	MPO: myeloperoxidase
37	NO: nitric oxide
38	NOX2: Nicotinamide adenine dinucleotide phosphate oxidase, isoform 2;
39	NT: nitrotyrosine
40	oLab: anti-oxidized low-density lipoprotein (LDL) antibodies
41	ox-LDL: oxidized-low density lipoprotein
42	OHypo: overt hypothyroidism
43	OS: oxidative stress
44	OSI: oxidative stress index
45	PC: protein carbonyl
46	PON1: paraoxonase 1
47	PPAR γ : proliferator-activated receptor gamma
48	RAGE: advanced glycation end products receptor
49	SH: thiol groups;
50	SHypo: subclinical hypothyroidism;
51	SOD: superoxide dismutase;
52	sRAGE: soluble advanced glycation end products receptor;
53	TAC: total antioxidant capacity
54	TGF- β 1: transforming growth factor-beta 1
55	T3: triiodothyronine
	TAS: total antioxidant status

Tg-Ab: anti-thyroglobulin antibodies

TOS: total oxidative stress

TOS*: total oxidant status

TPO-Ab: anti-peroxidase antibodies

TRAP: total reactive antioxidant potential

VEGF: vascular endothelial growth factor

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55

PEER REVIEW COPY
Minerva Endocrinologica

1 TABLES

2

3

4 Table 1. An overview of the studies evaluating the relationship between thyroid autoimmunity, functional status and oxidative stress parameters in
5
6 patients affected by autoimmune thyroiditis.

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

Study (Ref.)	Year	Patients	Controls	Thyroid functional status	Oxidative stress indexes/parameters	Results
Ates et al (25)	2015	31 31 31	31	Euthyroidism SHypo OHypo	oxidants: ARE, OSI, SH, TOS antioxidants: PON1, TAS	antioxidants reduction progressively higher towards OHypo
Erdamar et al (37)	2008	20	20	OHypo HT	oxidants: MDA, MPO and nitrites	specific treatment revealed an amelioration in OS
Resch et al (39)	2002	34	34	OHypo	oxidants: oLAb, peroxides antioxidants: Antiox-cap, ARS	enhanced OS; higher oLAb in hypothyroid (atherosclerosis progression index)
Baskol et al (41)	2007	33	26	OHypo (18 euthyroid under therapy)	oxidants: MDA, NO antioxidants: PON1, SOD	significant pro-oxidative imbalance in hypothyroidism, lipid peroxidation linked to atherosclerosis
Torun et al (42)	2009	20 40	40	OHypo SHypo	oxidants: LOOH, MDA antioxidants: TAS other parameters: CRP	pro-oxidative imbalance in both conditions paired with altered lipid metabolism
Reddy et al (43)	2013	36 36	39	OHypo SHypo	oxidants: CAT, LOOH, MDA antioxidants: FRAP, GPx, GR, GSH, SOD, TAC	antioxidants deficiency linked to hypothyroidism severity, higher in overt forms
Baser et al (53)	2014	35	35	Euthyroidism	oxidants: IMA, ox-LDL, TOS* antioxidants: TAS	oxidative stress increased in HT, TAS negatively correlated with thyroid autoantibodies

1	Ruggeri et al (54)	2016	71	63	Euthyroidism	oxidants: AGEs, AOPP, d-ROMs antioxidants: BAP	TPO-Ab+ related imbalance favoring oxidants
2							
3							
4	Ruggeri et al (55)	2019	45	50	Euthyroidism	oxidants: AGEs, d-ROMS antioxidants: BAP other parameters: IL-37	Increased oxidative stress; increase in IL-37 as a potential protective factor
5							
6							
7							
8	Ruggeri et al (56)	2020	50	50	Euthyroidism	oxidants: AGEs antioxidants: sRAGE	high AGEs and low sRAGE levels both correlated with TPO-Ab levels
9							
10	Taddei et al (64)	2006	53	45	SHypo	oxidants: NO cytokines: IL-6 other parameters: CRP	increased OS markers, no effects of vitamin C on endothelium
11							
12							
13							
14	Vural et al (65)	2009	40	40	Euthyroidism	oxidants: NO other parameters: TGF- β 1, VEGF	increased OS
15							
16							
17	Lassoued et al (66)	2010	29	30	Untreated OHypo	oxidants: CAT, MDA antioxidants: GPx, SOD	high OS in untreated diseases
18							
19							
20	Öztürk et al (67)	2012	18 30	30	OHypo SHypo	oxidants: DC, MDA, NT, PC antioxidants: FRAP	increased OS, higher in overt forms
21							
22							
23	Rostami et al (68)	2013	44	58	Hypo	antioxidants: GSH	decrease of antioxidants correlated with TPO-Ab
24							
25							
26	Ates et al (69)	2018	40 40	-	Euthyroidism SHypo	oxidants: ARE, OSI, PON1, TOS	TSO independent predictor of progression towards OHypo
27							
28	Giannakou et al (70)	2017	96 109	95	Treated HT Untreated euthyroid HT	oxidants: RAGE	RAGE polymorphism linked to progression towards OHypo
29							
30							
31	Korkmaz et al (71)	2016	25	27	Euthyroidism under L-T4 therapy	oxidants: ARE, LOOH, PON1, SH	redox imbalance favoring oxidant compounds
32							
33							
34							
35	Erol et al (72)	2016	57	50	Euthyroidism	oxidants: AGEs, ARE antioxidants: PON1 other parameters: AMH	antioxidants reduction paired with higher AMH in female adolescents
36							
37							
38							

1	Korkmaz et al (74)	2015	25	27	Euthyroidism under L-T4 therapy	oxidants: OSI, prolidase, SH, TOS antioxidants: TAS	increase in prolidase correlated with TOS and OSI
2							
3							
4	Mseddi et al (76)	2017	43	65	SHypo and OHypo HT	oxidants: MDA, SH	high levels of MDA and high immunoreactivity towards oxidized thyroid antigens
5							
6							
7							
8							
9							

10 Abbreviations: AMH: anti-Mullerian hormone; CRP: C-reactive protein; FT4: free thyroxine; HT: Hashimoto's thyroiditis; IL-6: interleukin 6; IL-37: interleukin 37; L-T4:
11 levothyroxine; OHypo: overt hypothyroidism; OS: oxidative stress; SHypo: subclinical hypothyroidism; T3: triiodothyronine; TPO-Ab: anti-peroxidase antibodies.

12
13 OS indexes: BAP: biological antioxidant potential; FRAP: ferric reducing antioxidant power; OSI: oxidative stress index; TAC: total antioxidant capacity; TAS: total antioxidant
14 status; TOS: total oxidative stress; TOS*: total oxidant status; TRAP: total reactive antioxidant potential.

15
16 OS biomarkers: AGEs: advanced glycation end products; Antiox-cap: non-enzymatic antioxidants; AOPP: advanced oxidation protein products; ARE: arylesterase; CAT: catalase
17 activity; DC: diene conjugate; d-ROMs: derived reactive oxygen metabolites; GPx: glutathione peroxidase; GR: glutathione reductase; GSH: Glutathione; IMA: ischemia-modified
18 albumin; LOOH: lipid hydroperoxide; MDA: malondialdehyde; MPO: myeloperoxidase; NO: nitric oxide; NT: nitrotyrosine; oLab: anti-oxidized low-density lipoprotein (LDL)
19 antibodies; ox-LDL: oxidized-low density lipoprotein; PC: protein carbonyl; PON1: paraoxonase 1; RAGE: advanced glycation end products receptor; SH: thiol groups; SOD:
20 superoxide dismutase; sRAGE: soluble advanced glycation end products receptor; TGF- β 1: transforming growth factor-beta 1; VEGF: vascular endothelial growth factor.

21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38

1 **Table 2. An overview of the studies evaluating the relationship between thyroid autoimmunity, functional status and oxidative stress parameters in**
 2 **patients affected by Graves' disease.**
 3

Study (Ref.)	Year	Patients	Controls	Thyroid functional status	Oxidative stress indexes/parameters	Results
Abalovich et al (35)	2003	69	19	Hyperthyroidism	oxidants: CAT, hydroperoxide antioxidants: GPx, GSH, SOD, TRAP	all oxidants reduced after MMI; hydroperoxide remained high after 131I
Bednarek et al (36)	2005	47	24	Hyperthyroidism (22 with GO)	oxidants: CAT, hydroperoxide, ceruloplasmin, LOOH, thiobarbituric acid-reacting substances antioxidants: GPx, GR	MMI reduced markers in patients without GO
Aslan et al (38)	2011	36	30	Hyperthyroidism (21 GD, 15 MNTG)	oxidants: OSI, TOS* antioxidants: TAC	TAC significantly lower, TOS and OSI significantly higher in hyperthyroid patients
Resch et al (39)	2002	34	34	Hyperthyroidism	oxidants: oLAb, peroxides antioxidants: Antiox-cap, ARS	enhanced OS; higher POX in hyperthyroid (hypermetabolic state)
Lassoued et al (66)	2010	16	30	Untreated hyperthyroidism	oxidants: CAT, MDA antioxidants: GPx, SOD	high OS in untreated disease
Korkmaz et al (71)	2016	25	27	Euthyroid treated GD	oxidants: ARE, LOOH, PON1, SH	redox imbalance favoring oxidant compounds
Korkmaz et al (74)	2015	25	27	Euthyroid treated GD	oxidants: prolidase, OSI, SH, TOS antioxidants: TAS	increase in prolidase correlated with TOS and OSI
Agan et al (75)	2019	33	35	Untreated hyperthyroidism	oxidants: OSI, PC, SH, TOS	positive correlation between free thyroid hormones and thiol homeostasis

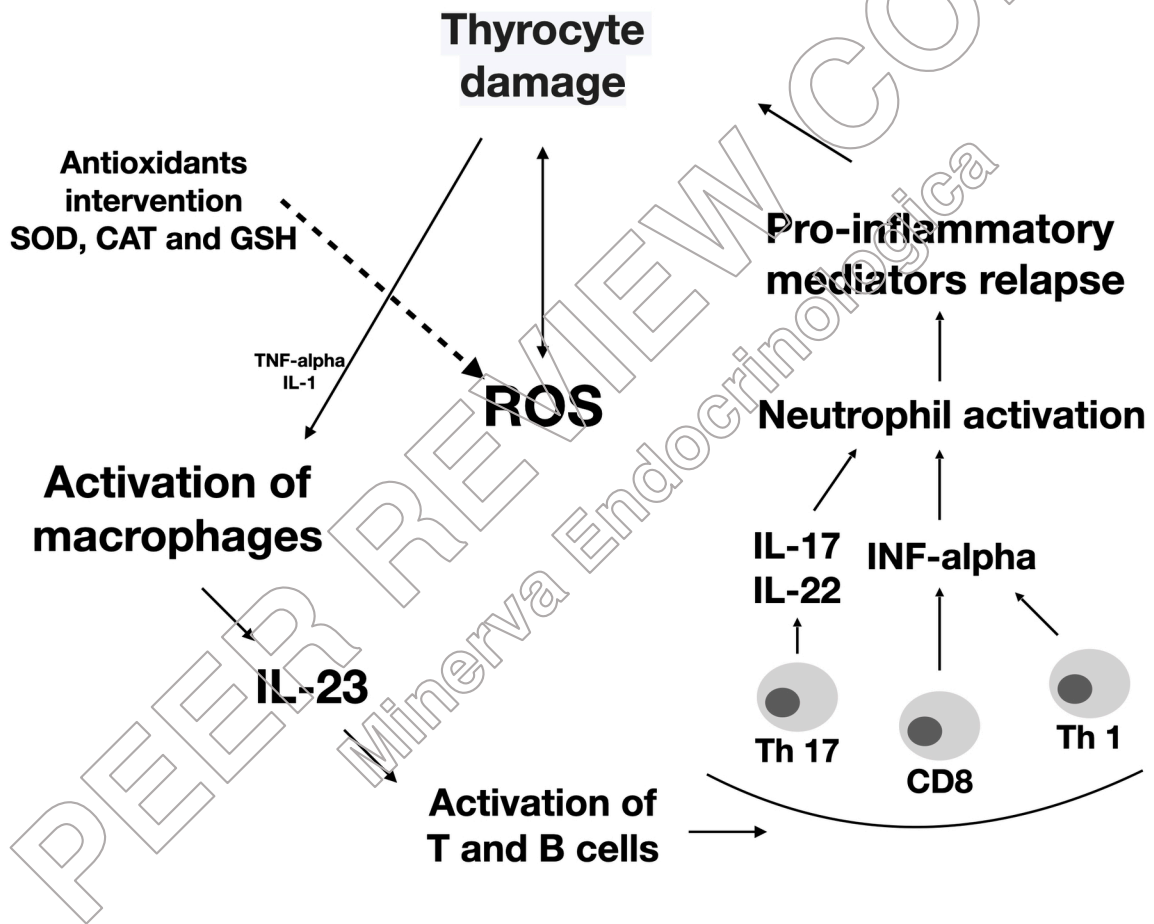
1 2 3 4	Mseddi et al (76)	2017	34	65	Untreated hyperthyroidism	oxidants: MDA, SH	high levels of MDA and high immunoreactivity towards oxidized thyroid antigens
5 6 7 8 9	Gargouri et al (77)	2019	34	65	Untreated hyperthyroidism	oxidants: CAT	positive correlation between T3 and immunoreactivity towards MDA-modified catalase
10 11 12 13	Choi et al (78)	2018	27 35	25	inactive GO GD active GO GD (both variable functional status)	oxidants: 8OH-dG (tears), MDA	increased OS markers in tear secretions from GD patients
14 15 16 17 18 19	Diana et al (80)	2018	54 19 5	42	Untreated hyperthyroid GD Euthyroid treated GD Hyperthyroid MNTG	oxidants: 4-HNE, NOX2	OS parameters higher in untreated GD and detectable in urine samples

20 Abbreviations: 131I: 131 iodine; FT4: free thyroxine; GD: Graves' disease; GO: Graves' ophthalmopathy; MMI: methimazole; MNTG: multinodular toxic goiter; OS: oxidative stress; T3: triiodothyronine.

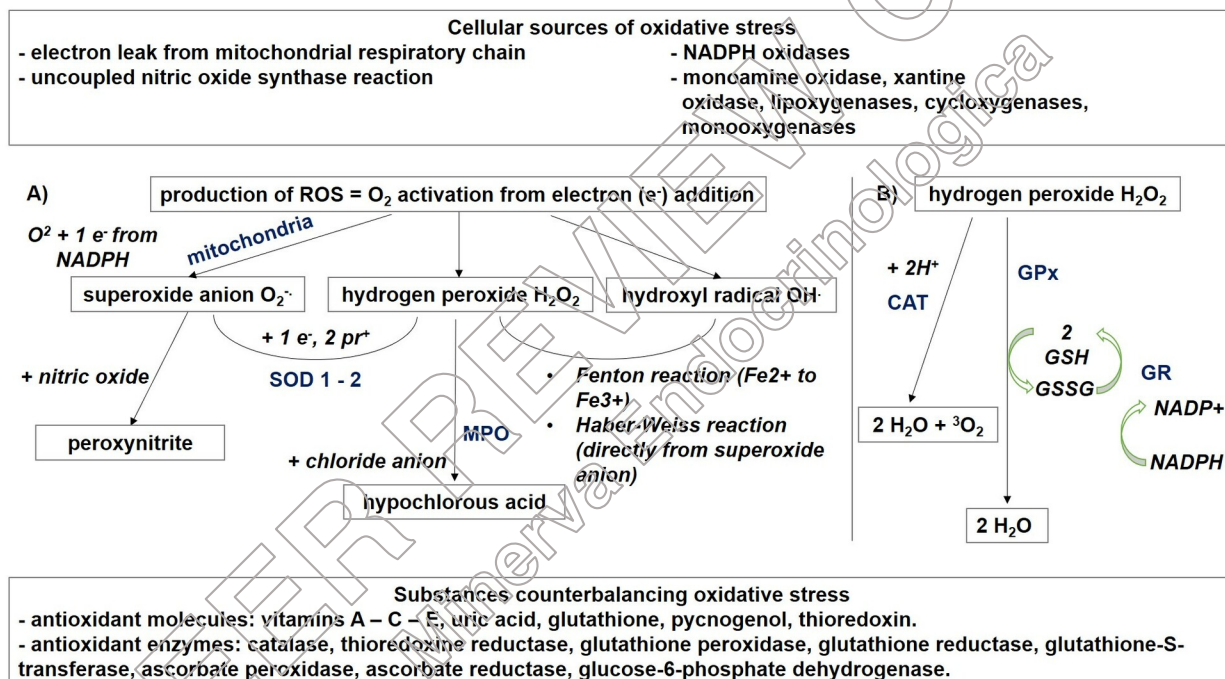
23 OS indexes: OSI: oxidative stress index; TAC: total antioxidant capacity; TAS: total antioxidant status; TOS: total oxidative stress; TOS*: total oxidant status; TRAP: total reactive antioxidant potential.

26 OS biomarkers: 4-HNE: 4-hydroxynonenal; 8-OHdG: 8-hydroxy-2'-deoxyquanosine; Antiox-cap: non-enzymatic antioxidants; ARE: arylesterase; ARS: enzymatic antioxidants; GPx: glutathione peroxidase; GR: glutathione reductase; GSH: Glutathione; LOOH: lipid hydroperoxide; MDA: malondialdehyde; NOX2: Nicotinamide adenine dinucleotide phosphate oxidase, isoform 2; oLab: anti-oxidized low-density lipoprotein (LDL) antibodies; ox-LDL: oxidized-low density lipoprotein; PC: protein carbonyl; PON1: paraoxonase 1; SH: thiol groups; SOD: superoxide dismutase.

30
31
32
33
34
35
36
37
38



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55