THE AGING THYROID: A REAPPRAISAL WITHIN THE GEROSCIENCE INTEGRATED PERSPECTIVE

Claudio Franceschi[1]*, Rita Ostan[2]*, Stefano Mariotti[3], Daniela Monti[4#]Giovanni Vitale[5,6#]

[1]Department of Applied Mathematics, Institute of Information Technology, Mathematics and Mechanics (ITMM), Lobachevsky State University of Nizhny Novgorod-National Research University (UNN), Nizhny Novgorod, Russia；e-mail: claudio.franceschi@unibo.it

[2]Interdepartmental Centre "L. Galvani" (CIG), Alma Mater Studiorum-University of Bologna, Bologna, 40126, Italy; e-mail: rita.ostan3@unibo.it

[3]Department of Medical Sciences and Public Health, University of Cagliari, Monserrato-Cagliari 09042 Italy; e-mail: s.mariotti@unica.it

[4]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, 50134 Florence, Italy; e-mail: daniela.monti@unifi.it

[5]Istituto Auxologico Italiano IRCCS, Laboratorio Sperimentale di Ricerche di Neuroendocrinologia Geriatrica ed Oncologica, Milan, 20100, Italy; e-mail: giovanni.vitale@unimi.it

[6]Department of Clinical Sciences and Community Health, University of Milan, Milano, Italy; email: giovanni.vitale@unimi.it

*Equally contributed

[#]Co-senior authorship

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Corresponding author: Claudio Franceschi, e-mail: claudio.franceschi@unibo.it

IRCCS, Institute of Neurological Sciences of Bologna, Bellaria Hospital, Via Altura 3, 40139 Bologna, Italy.

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Abstract

Thyroid plays a crucial and pervasive role in physiology (metabolism, thermogenesis and immunity, among others) and its aging and related changes in thyroid hormones production contribute to the common occurrence of thyroid diseases in elderly and to age-associated changes in other organs and systems. We address the complexity of thyroid aging following the basic suggestions of Geroscience. This integrative new perspective identifies few basic molecular mechanisms or "pillars" (inflammation, adaptation to stress, loss of proteostasis, stem cell exhaustion, metabolism derangement, macromolecular damage, and epigenetic modifications) as a unifying conceptual framework to understand the aging process and age-associated diseases. Within this scenario, we review available data on presence and role in the thyroid of alterations of such mechanistic pillars, paying particular attention to: i) inflammation, focusing on cellular senescence and age-associated dysbiosis (alteration of gut microbiota); ii) telomere shortening as an example of macromolecular damage; iii) proteasomal function including mitophagy and autophagy; iv) stem cells and cell renewal; v) energy metabolism and mitochondrial dysfunction; vi) age-related epigenetic changes, focusing on DNA methylation. Overall, the study of these topics in the thyroid is in its infancy and deserves much more attention. Finally, thyroid function in centenarians as a model of healthy aging is reviewed within the framework of possible adaptive mechanisms involving thyroid to attain longevity. Accordingly, the concept of "thyroid biography" is proposed to grasp the complex combination of factors (including endocrine disruptors and lifestyle habits) impinging lifelong on thyroid function at the individual level.

Précis

Thyroid aging is fully understood if the basic molecular mechanisms underpinning aging identified by Geroscience are investigated within a lifelong perspective but this knowledge is still in its infancy.

1. Introduction: conceptual background and scope of the review

1.1. The global aging of populations and the unifying perspective of Geroscience

The global aging of humans is considered one of the major challenge owing to its pervasive economic, medical and cultural implications. People over 85 years of age, namely the "oldest old", represent the segment of the population, which is growing faster worldwide, and one or more age-associated diseases (AADs) affect most of them. Thus, it is urgent to grasp the complexity of the aging process and its underpinning molecular mechanisms. According to the integrated view of Geroscience: i) aging is the predominant risk factor for AADs, and aging and AADs share a common set of basic mechanisms (1); ii) aging and AADs are not separate events but rather parts of a continuum where precise boundaries do not exist, and where the two extremes are the centenarians, who largely avoided or postponed most AADs and are characterized by decelerated aging, and persons who suffered by one or more severe AADs in their 60s-80s and show signs of accelerated aging, respectively (2,3); iii) AADs can be conceptualized as a manifestation of accelerated aging (2).

1.2. Thyroid aging within the new perspective of Geroscience

With age, changes occur in all body systems including the endocrine system, and thyroid function is particularly important owing to its central role in metabolism, thermogenesis and immunity, among others, and its contribution to most common chronic AADs. The network of basic mechanisms involved in the aging process identified by a group of international experts, and collectively indicated as the "pillars" of Geroscience, includes inflammation, adaptation

to stress, loss of proteostasis, stem cell exhaustion, metabolism derangement, macromolecular damage and epigenetic modifications (1). These pillars do not operate separately but are interconnected, influencing and modulating each other, thus constituting an integrated network (1).

Here we propose a reappraisal of thyroid aging and thyroid age-related dysfunction from the new Geroscience perspective, focusing on the above-mentioned mechanistic molecular pillars which drive the aging process, as illustrated in Figure 1, and on centenarians and their offspring as the best model of healthy aging in humans (4). Moreover, taking into account that a major characteristic of old subjects is the large heterogeneity of their phenotype, including thyroid function, and accepting the challenge of personalized medicine, we will propose the concept of "thyroid biography" to grasp the complex combination of lifestyle habits and environmental factors impinging lifelong on thyroid function at the individual level.

2. Thyroid Aging within the Context of the Basic Mechanisms of Biological Aging

In the following paragraphs, we will present a detailed analysis of the literature focused on the above-mentioned pillars of the Geroscience in order to check whether and how much the thyroid aging fits this unifying conceptual framework of the aging process.

2.1. Inflammation

Inflammation is one of the Geroscience pillars and accumulating evidence indicates that aging is associated with a chronic, low-level inflammation termed "inflammaging" that represents a major contributor to the pathogenesis of AADs (2). The peculiarity of inflammation is that alterations of anyone pillar converge and fuels inflammation, which in turn affects all the other pillars (2). Inflammaging involves basically the innate immune system but acquired immunity also contributes to this phenomenon which is deeply related to all the changes occurring with age in the immune system, collectively indicated as immunosenescence (5).

Recently we have proposed that inflammaging can be considered as a complex mechanism of adaptation depends on the context in which it develops, that can be interpreted as a negative (favoring age-associated diseases) or positive (promoting health) phenomenon. Therefore, inflammaging is an overall adaptation of the entire body within an integrated view of organs and systems and is the result of the continuous activation of mechanisms in order to establish progressively new homeostatic equilibria (6,7).

2.1.1. Immune effects of thyroid hormones

A plethora of data indicates that thyroid hormones (THs) influence innate and acquired immune functions (chemotaxis, phagocytosis, generation of reactive oxygen species (ROS), and cytokine synthesis/release) as particularly evidenced in hypo and hyperthyroid conditions. Although these data show that thyroid hormones modulate both innate and acquired immune responses it has not been clearly demonstrated if changes in thyroid function with age are correlated with an inflammatory state of the gland and/or with the systemic inflammation or inflammaging.

The available data regarding the effects of thyroid hormones on immune responses in old subjects can be summarized as follows:

i) in 93 healthy late-middle-aged euthyroid subjects THs concentration was positively correlated to immune functions, including the level of complement proteins C3 and C4, C-reactive protein (CRP), phagocyte activity, percentage and number of Natural Killer and T-cell, and IL-6 expression by activated monocytes (8).

ii) THs, particularly T3, increase metabolic activity and oxygen consumption and contribute to oxidative stress both in the short- and in the long-time range (9). ROS production may facilitate various immune functions, such as the bactericidal activity of macrophages through NADPH oxidase activation. Additionally, in immune cells, FT4 is able to increase ROS production, leading to cell migration in tissues in response to chemo-attractant molecules (10).

iii) THs can bind to integrin αvβ3 on the macrophages and activate phosphoinositide 3-kinase (PI3K) and extracellular signal-regulated protein kinase 1/2 (ERK1/2) pathways followed by the upregulation of inducible nitric oxide synthase (iNOS) favoring the intracellular killing of bacteria (11). Alternatively, FT4 enters in the macrophage through monocarboxylate transporters MCT8 or MCT10, where the prohormone T4 is converted to active hormone T3 by D2 deiodinase (D2) resulting in increased phagocytosis and cytokine response (12). These effects are mediated, at least in part, by TRα, which is the predominant TR isoform in macrophages, and knockout mice for TRα have an aberrant macrophage function (12).

iv) Macrophages contribute to immune system surveillance by sensing and adapting to local stimuli and micro-environmental signals (13). It has been recently shown that FT3 negatively contributes to the differentiation of bone marrow-derived monocytes into non-polarized macrophages (14). FT3 promotes the generation of M1 macrophages (pro-inflammatory phenotype), even after the differentiation and activation of monocytes into M2 macrophages (14). *In vivo*, FT3 increases the number of resident macrophages in the peritoneal cavity, whereas it reduces the content of the recruited monocyte-derived cells in the inflamed locus (potentially damaging). In an *in vivo* model of lipopolysaccharide (LPS)-induced endotoxemia, FT3 protects mice from developing endotoxic shock. While low FT3 levels increase inflammatory cell recruitment into tissues, an opposite phenomenon occurs when FT3 levels are restored (14).

v) In Leiden 85-plus Study, Rozing et al. demonstrated that higher levels of circulating CRP and IL-6 were significantly related to lower serum levels of FT3. On the contrary, after LPS stimulation of whole blood *in vitro*, higher levels of serum FT3 were associated with a higher production of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) (15). The authors postulate that serum FT3 stimulates the production of the pro-inflammatory cytokines, while pro-inflammatory cytokines, in turn, blunt the stimulatory effect of FT3 by lowering peripheral

THs levels. This influence of cytokines probably occurs through regulation of peripheral deiodinase activity, although this putative mechanism is not yet demonstrated. The stimulatory effect of T3 on cytokine production is likely mediated via nuclear receptors regulating genes involved in the cell-mediated immune response (15).

vi) TSH is able to increase IL-6 production from adipocytes derived from abdominal subcutaneous fat but not from the omental deposit. The basal IL-6 release is higher for pre-adipocytes than differentiated adipocytes, independently from their origin, indicating an effect of TSH on adipocyte differentiation (16).

Overall, circulating THs have profound effects on neutrophil, macrophage and dendritic cell function and generally, a rise of THs levels results in an amplification of the pro-inflammatory response of these cells. In this framework, a reduction of THs during aging could be considered a form of adaptation to reduce inflammation/inflammaging and could play a role in immunosenescence. Thus, thyroid disorders might be involved in immunosenescence (17), and the maintenance of normal thyroid function could, therefore, contribute to preserving immune responses in the elderly. THs can modulate inflammation even stimulating adipocytes to produce adipokines acting on several homeostatic aspects of metabolism and energy that influence body weight, thermogenesis and lipolysis (described in detail in 2.5.1 paragraph). From the available data, the link between thyroid function, inflammaging and immunosenescence is yet unclear and it is still uncertain whether the hypothyroidism of the elderly represents an anti-inflammatory adaptation.

2.2. Cellular Senescence, telomere shortening, DNA damage and thyroid

The accumulation of senescent cell with age is a hallmark of aging and a pillar of Geroscience. Such accumulation occurs in a variety of organs and tissues and represents another major stimulus that fuels inflammaging. Senescent cells are characterized by cell cycle arrest, telomere shortening as a consequence of DNA damage (18), and they develop a distinct

secretome profile characterized by a persistent pro-inflammatory phenotype (19,20) known as Senescence-Associated Secretory Phenotype (SASP), which includes a variety of pro-inflammatory cytokines as well as growth factors and extracellular matrix degrading proteins. "Chronic" SASP induces senescence in adjacent young cells, contributing to the propagation of inflammation and tissue dysfunction to neighbouring cells (21,22). Because of their low but chronic inflammatory phenotype, persistent senescent cells are thought to accelerate aging and the onset of age-related diseases (2). Cellular senescence not only plays a role in aging and contributes to the appearance of AADs (23,24) but is also an important anti-proliferative process that acts as a strong barrier against cellular transformation and cancer progression (25).

As far as we know, most of these main topics, such as the possible age-related accumulation of senescent cells in the thyroid, have not been directly addressed in humans despite their importance, for the physiopathology of the gland and taking into account that the age-associated THs changes can play a role in the accumulation of senescent cells in other organs and tissues.

The available data on this topic can be summarized as follows:

i) progressive telomere shortening, a well-known cellular senescence biomarker, has been evaluated in different tissues, including thyroid from individuals of different age (0-98 years)(26). Telomere erosion in the thyroid was evident after 50 years of age, likely owing to slow cell turnover rate, at variance with other human organs where an earlier reduction of telomere occurs, suggesting that the rate of telomere shortening is tissue-specific (26).

ii) THs appear to be able to induce senescence *in vitro* and *in vivo.* THs can activate metabolism by binding to two receptors, i.e.TRα and TRβ, but depending on which receptor is engaged, opposite effects can ensue. T3 induces DNA damage by oxidative stress and drives mouse embryonic fibroblasts to premature senescence, by binding to TRβ and not to TRα and

involving the DNA repair ataxia telangiectasia mutated (ATM) protein (27). ATM protein detects genomic damage, activates mitochondria to produce dangerous ROS, and consequently augments the numbers of DNA double-strand breaks, favouring cellular senescence. Similar results were obtained *in vivo*, using genetically altered mice lacking TRβ which displayed a reduced number of senescent cells in the liver in comparison to wild-type animals (27).

Overall, emerging hits suggest that the thyroid could play a role on the systemic accumulation of senescent cells with age but the crucial topic of the presence of senescent cells in normal thyroid gland during aging has not been addressed both in animal models and in humans. The occurrence of senescent cells in the thyroid can be predicted taking into account that thyroid epithelial cells are constantly exposed to ROS through dual oxidases for the synthesis of THs, thus producing large amounts of H_2O_2, which can induce genomic damage and telomere erosion. To our knowledge, this topic, which could help in clarifying the adaptive or maladaptive role of hypothyroidism in old and very old subjects (see section 3.3) and could pave the way for senolytic trials targeted either to thyroid or to AADs (28), has not been investigated in humans and represents an unmet need.

2.3. Thyroid hormones and stem cell renewal

One of the aging hallmarks and Geroscience pillar is the decline in the regenerative potential of organs and tissues thoroughly described in many organs and compartments, including bone marrow, intestine, brain, muscle, and bone, among others (29). There is a vast literature showing that THs have a major role in cell division and differentiation and in the development of the nervous system, intestine, bone and muscle during organogenesis and development as well as in the regulation of adult stem cell function in the intestine, muscle (30,31) and brain (32–35). However, the presence of thyroid stem/progenitor cells in the adult organ is controversial, even if a population of cells with stem properties, which are activated

upon tissue regeneration after partial thyroidectomy (36,37) has been described (38). This topic is difficult to address owing to the low turnover of adult thyrocytes, which can be estimated in several years (38).

The available data on this topic can be summarized as follows:

i) hypothyroidism reduced proliferation and apoptosis of stem cells in the subventricular zone as well as migration of transgene-tagged neuroblasts out of the stem cell niche, inhibiting the generation of new cells. These effects were mediated by TRα, but not TRβ (33).

ii) The regeneration of cardiotoxin-injured skeletal muscle of mice without D2 was markedly delayed in comparison to wild-type mice (35). D2 generates intracellular active thyroid hormone in muscle and is essential for normal mouse myogenesis and muscle regeneration. Indeed, D2-mediated increase in FT3 levels is essential for the enhanced transcription of myogenic differentiation 1 (MyoD) and for the execution of the myogenic program. Therefore, the retardation of regeneration of cardiotoxin-injured skeletal muscle of mice without D2 was associated with a failure of main markers of terminal differentiation (35). Moreover, in the same model, it has been demonstrated that the satellite cells augmented their proliferative capacity in response to attenuation of THs signaling, suggesting that low T3 levels are required in the early phase of muscle regeneration (39). Recently, Ambrosio et al. have published a very interesting and clarifying systematic review on the functional role of deiodinases in muscle stem cells and on the ability of THs to affect the composition, contraction force, glucose metabolism and energy metabolism (30).

iii) A variety of experimental data show that the stem cell population requires low levels of THs to maintain its stemness and renewal capacity (40). This consideration is particularly important taking into account the age-related modifications of THs that in turn can affect the adult stem cell renewal in a variety of organs and tissues.

Overall, the presence and function of thyroid stem/progenitor cells in aged thyroid is a rather neglected issue, despite its potential interest for thyroid function in the elderly as well as for thyroid pathologies such as cancer. Within this complex scenario, we can also hypothesize that the complex remodeling of thyroid function with age is adaptive and contributes to the new systemic homeostatic equilibrium involving several organs and tissues in advanced age, as suggested by centenarians' studies discussed in a subsequent paragraph.

2.4. Thyroid and the proteostasis network, including Ubiquitin-Proteasome System (UPS), autophagy and mitophagy.

An accumulation of damaged proteins is a hallmark of the aging process and proteostasis is one of the Geroscience pillars (1). All cells exploit a series of quality control mechanisms to preserve the stability and functionality of their proteomes. Proteostasis involves a set of molecular components and mechanisms devoted to protein clearance (proteostasis network) that prevents the toxicity associated with protein misfolding and accumulation of toxic aggregates in different subcellular compartments and tissues (41). Proteolytic systems like UPS, autophagy and mitophagy (the selective clearance of damaged mitochondria) are the main molecular components of the proteostasis network, and their decreased activity is a central characteristic of aging contributing to the onset of AADs (41). In particular, mitophagy eliminates dysfunctional or damaged mitochondria, thus counteracting degeneration, dampening inflammation, and preventing unwarranted cell loss. Overall, a combination of insufficient autophagy and mitophagy contributes to multiple AADs (41). In this scenario, T3 regulates lipid homeostasis by stimulating the shuttling of free fatty acids into mitochondria (β-oxidation) (42), and this process is coupled with induction of hepatic autophagy (43) and an increase in oxidative phosphorylation (OXPHOS)-generating ROS that damage mitochondria.

The topic of thyroid proteostasis is critical for thyroid physiology, including age-related changes, as well as for thyroid pathology, but few data focusing on thyroid are available and they can be summarized as follows:

i) in a study of transcriptomics on 322 normal thyroid glands from subjects of different age, the most significant age-related change was the downregulation of genes related to the mitochondrial and proteasomal functions, loss of differentiation, and activation of autoimmune processes (44). Cho et al. demonstrated that thyroid age-associated gene expression profile was associated with the upregulation of immune activity and overlapped with gene expression patterns in tissues affected by autoimmune thyroiditis (AITD). These data indicate a possible ‘‘link’’ between aging and AITD (44).

ii) In another study on age-dependent transcriptomic changes, Yang et al. reported similar results for multiple organs, but not for thyroid (45), a discrepancy likely due to a different analytic method and the size of the dataset (45).

iii) A study exploiting *in vitro* and *in vivo* hepatic cell models showed that T3 induces ROS production leading to initiation of mitophagy, a major mechanism to remove severely damaged mitochondria during cell stress or excess mitochondria during development and for sustaining efficient oxidative phosphorylation (46).

iv) In skeletal muscle, THs control cellular growth, regeneration, differentiation and induce autophagy by producing ROS, activating AMPK (5’ AMP-activated protein kinase), stimulating ULK1 (Unc-51 Like Autophagy Activating Kinase 1) and the autophagosome formation by inhibiting mTOR (mammalian target of rapamycin) (47). The THs-induced autophagy in skeletal muscle is essential for stimulation of mitochondrial biogenesis and activity (48).

Overall, the scarce above-mentioned data suggest that thyroid proteostasis is likely profoundly affected by aging, but its knowledge is still in its infancy, particularly in humans.

2.5. Thyroid hormones, metabolism, and aging.

THs strongly control key metabolic pathways responsible for energy balance by regulating energy storage and expenditure. THs act on liver, white and brown adipose tissue, skeletal muscle, and pancreas modulating plasma glucose levels, insulin sensitivity, and carbohydrate metabolism. Thus, understanding how age-associated THs changes affect central and peripheral mechanisms of metabolism in homeotherms, are essential.

2.5.1.Thyroid hormones, thermogenesis and mitochondria

THs have a role in the adaptation of the organism to changing environmental conditions, including cold acclimation by modulating metabolic rate, muscle force production and cardiac performance (40). THs are able to increase metabolic rate and thermogenesis, including maintenance of body temperature, directly modulating the transcription of nuclear and mitochondrial genes. During cold exposure, thyroid function is activated through the stimulation of TRH synthesis, mediated by the catecholaminergic neurons, and THs synergistically interact with the sympathoadrenal system to induce thermogenesis (49–51), while the activity of D2 is upregulated in brown adipose tissue (52). These THs functions are critical on aging as cold adaptation change in old animals, in association with alteration of thyroid function (53). It is possible to hypothesize that the increased sensitivity of old people to cold could be the price to pay for the possible beneficial effect of mild hypothyroidism on longevity by reducing metabolic rate, ROS generation, and oxidative damage. Mitochondrial metabolism is the best-recognized link between THs and longevity (40). THs are able to increase metabolic rate and thermogenesis through multiple mechanisms involving mitochondrial function (the “uncoupling hypothesis”) (54). A major geriatric syndrome is sarcopenia and it is important to note that in addition to its metabolic activity, T3 is considered an important regulator of muscle development as above described.

Many are the unanswered questions related to thyroid, thermogenesis and mitochondria as this topic regards domains critical for survival and healthy aging, such as the physiological metabolic and thermoregulatory condition of elderly, and particularly of the oldest old.

The available data on this topic can be summarized as follows:

i) old animals are more susceptible to cold stress than young ones, and cold-induced THs release occurs independently of TSH (55).

ii) The expression of Uncoupling Protein 1 (UCP1) in mitochondria is activated in response to cold by T3-dependent mechanisms driving the heat production by brown adipose tissue independently from shivering or other muscular processes (56,57). In humans, two variants located in the upstream enhancer region of UCP1 gene affect gene expression and are correlated with human longevity (58). Brown adipose tissue and mitochondrial uncoupling can be targeted for interventions to prevent and treat obesity and AADs (59).

iii) T3 increased fatty acid oxidation and mitochondrial respiration as well as autophagic flux, mitophagy, and mitochondrial biogenesis, with no significant induction of intracellular ROS despite high mitochondrial respiration and UCP1 induction by T3 (53). However, when cells were treated with Atg5 siRNA to block autophagy, induction of mitochondrial respiration by T3 decreased and was accompanied by ROS overproduction, demonstrating a critical role for autophagic mitochondrial turnover (60) (see paragraph 2.4).

iv) In male Wistar rats receiving T4 in drinking water, it was reported that the induced hyperthyroidism increased the content of mitochondria in liver, changed the structure of mitochondrial membranes and uncoupled OXPHOS with an increase of 50% in the generation of superoxide radicals, resulting in accelerated aging and decrease of lifespan (61). On the contrary, on the same animal model, calorie restriction was accompanied by an increase in lifespan and a reduction of body temperature significantly correlated with a decrease in T3 and T4 levels (61).

v) p43, the ligand binding form of thyroid hormone receptor α1 (THRα1), is located in the mitochondria and plays a major role in the crosstalk between nucleus and mitochondria. p43 responds to T3 acting as a transcription factor for nuclear and mitochondrial genome, and *in vivo* studies on murine models have shown that p43 overexpression in skeletal muscle increases mitochondrial transcription and biogenesis, inducing a stimulation of mitochondrial respiration and a shift in metabolic and contractile features of muscle fibres toward an oxidative phenotype (62). On the same model, p43 overexpression, after an early rise in mitochondrial DNA and mass, induces oxidative stress characterized by a strong increase of lipid peroxidation and protein oxidation in quadriceps muscle eventually resulting in muscle atrophy, probably through stimulation of the ubiquitin-proteasome pathway. Therefore, prolonged stimulation of mitochondrial activity by p43 contributes to the insurgence of muscle atrophy, stressing the importance of tight control of p43 expression by the mitochondrial pathway regulated by T3 as one of the processes involved in sarcopenia (62).

vi) Peroxisome Proliferator-Activated Receptor-γ Coactivator-1α (PGC-1α) is actively involved in the modulation of the mitochondrial biogenesis by THs. The expression of PGC-1α is activated endogenously by T3 and co-activates several nuclear transcription factors, including THR. Therefore, also PGC-1α plays a key role in the crosstalk between nuclear and mitochondrial aging pathways. During aging, telomere shortening causes p53-dependent repression of PGC-1α resulting in a reduction of mitochondrial biogenesis and an impairment of mitochondrial functions (63). These findings strongly support the importance of the strict interactions among PGC-1α, p53, and THs, and their role in the mechanisms by which the hypothalamic-pituitary-thyroid axis (HPT) affects and regulates metabolic homeostasis during aging (40).

THs affect a complex circuitry critical for adaptation to cold, metabolic rate and mitochondrial function/biogenesis, but the impact of aging on such molecular and cellular

cross talk is still unclear and deserves further studies in both animal models and humans. To this regard is interesting to note that lower basal body temperature appears to be associated with healthy aging (64) and long-term calorie restriction lowers core body temperature in humans (65). However, women appear to have a slightly higher body temperature and yet live longer than men (66). Thus, aged thyroids and age-related THs remodelling can have both detrimental and adaptive effects, but additional studies in humans, particularly in the oldest are warranted. In support of an adaptive hypothesis, it has been reported that mean temperature decreased with age, with a difference of 0.3°F between the oldest and youngest groups after controlling for many confounders. These results are consistent with low body temperature as a biomarker for longevity, but prospective studies are needed to confirm whether a lower body temperature lifelong is a survival advantage and potential relation with age-related changes in thyroid function (67).

2.5.2. Thyroid hormones and the gut microbiota (GM)

GM is a complex, highly dynamic and evolutionarily shaped ecosystem, recognized as an integral and active "organ" contributing to physiological, metabolic and immune functions. GM composition is affected lifelong by individual lifestyle (e.g. nutrition, physical activity) and environmental variables (68,69). GM and immune system establish a constant lifelong interplay, and conditions that alter GM homeostasis and increase the permeability of intestinal epithelial barrier have been associated with the onset of local and systemic inflammatory and autoimmune disorders (70)Another important gut function is the gastrointestinal absorption of iodine and selenium, essential nutrients for the maintenance of thyroid functions. The role of GM in iodine uptake is still poorly studied while the link between GM composition and host availability of selenium, an essential constituent of deiodinase isoforms, is stronger (71).

The available data on this topic can be summarized as follows:

i) the study of GM of young adults, elderly, and centenarians highlighted that the mutualistic changes in composition and diversity of GM occurring with age are non linear, remaining highly similar between young adults and older adults until 70 years of age and markedly changing in centenarians, suggesting that GM undergoes a profound and possibly adaptive remodelling in the last decades of life(72). In subjects of extreme age, the loss of important core components is accompanied by the gain of new microbial subdominant components, including potentially beneficial but also pathobionts and allochthonous bacteria, resulting in an overall increase in GM diversity which has been observed in centenarians from different ethnicities (68,73–77). Besides such commonality regarding the increased GM diversity, bacterial signatures that are common among centenarians of different ethnicities, such as Italian, Chinese and Japanese (78) as well Indians (79), have been reported, despite their consistent diversity regarding genetics, nutrition, and many other context-dependent variables. ii) Patients with chronic AITD showed alteration of the intestinal mucosal morphology (increased space between adjacent microvilli and augmented thickness of microvilli) and an impaired intestinal permeability (80). Autoimmune overt hypothyroidism is a risk factor for abnormal bacterial overgrowth in the small intestine (81). Hypothyroidism was associated with decreased frequency of rhythmic colonic activity and slower oro-cecal transit time(82) predisposing to bacterial overgrowth. In these patients, a decontamination therapy with rifamixin improved gastrointestinal symptoms often associated with hypothyroidism (81). In patients affected by small intestinal overgrowth, it was found that the strongest contributor to this condition is levothyroxine use (83). However, evidence for potential bacterial contribution to the onset and progression of AITDs (including Hashimoto Thyroiditis and Graves' Disease) is based on retrospective studies measuring bacterial antibodies (especially towards *Yersinia enterocolitica, Helicobacter pylori*,*andBorreliaburgdorferi*) (84).

Hyperthyroidism is associated with a decrease of *Bifidobacterium* and *Lactobacillus* and an increase of *Enterococcus*, compared to the control group (85).

iii) Duodenum and caecum play the major role in the adsorption of dietary selenium depending on its chemical form (86) but common GM components such as *Escherichia Coli*, Clostridia, and Enterobacteria, possess selenoprotein-encoding genes and can compete with the host for selenium uptake (87). The quantity of selenium not absorbed in the small intestine may be actively taken up in the colon where is metabolized by the resident microbial community, suggesting that a competition that potentially decreases selenium bio-accessibility likely exists (88).

iv) Studies on rats showed that deiodinases are present and active in diverse tissues including intestinal mucosa (89), and these enzymatic activities may be inhibited by the resident microflora (90). Human intestinal tract retains a relevant deiodinases activity and, due to its large surface, gives a noteworthy contribution to the whole body T3 pool. In addition, diluted human and rat faecal suspension was able to hydrolyse significant amounts of iodothyronine conjugates due to the presence of obligate anaerobic bacteria with glucuronidase activity such as *Peptococcusproductus* (91,92). The glucuronidase activity in faecal content indicates the presence of enterohepatic circulation for iodothyronines as suggested by data showing that GM allows the reabsorption of native T3 following the hydrolysis of conjugated forms of the hormone (93). The fraction of reabsorbed T3 escaping from liver extraction may re-enter in the general circulation contributing to the systemic pool of iodothyronines. The observation that plasma reabsorption of radiolabelled T3 is abolished in germ-free animals (93) support the key role of GM on the thyroid homeostasis through this enterohepatic cycle.

As far as we know, no studies have been performed evaluating directly the correlation between age-related thyroid physiological changes and age-related GM remodeling, despite a

variety of studies on the correlation between GM changes and thyroid diseases such as Hashimoto and Graves disease.

An interesting and unexpected novelty is that in centenarians a longevity-specific (common to different ethnicities) GM remodeling and signature and peculiar hypothyroidism (see section 3.3) are concomitantly present, potentially paving the way to develop a GM/thyroid-based biomarker for healthy aging/longevity even if the link between these two phenotypes is unclear at present.

Finally, the role of anaerobic enteric bacteria in humans is not yet supported by direct experiments, even if GM seems to be a further regulator of thyroid homeostasis acting directly through its metabolic enzymes as well as by modulating the chemical bioavailability of iodothyronines for reabsorption in the blood (94). Further studies, particularly in old subjects, on the bidirectional, lifelong crosstalk between host thyroid function and GM are urgently needed.

2.5.3. Thyroid hormones, epigenetic changes and aging

Epigenetic changes, involving histone modifications, noncoding RNAs and DNA methylation have a role in the modulation of aging and AADs (95–98). Although the role of THs to influence these processes remains still poorly elucidated, few pieces of evidence exist regarding the capacity of THs to modulate epigenetic profile (99,100).

The available data on this topic can be summarized as follows:

i) T3 treatment caused different effects in adult C57BL/6 mice (histone modifications involved in regulating transcription in liver and no significant changes in the DNA methylation) (101) and in postnatal day 6 C57BL/6J mice [increased transcription of *de novo* DNA methyltransferase 3a (DNMT3a) gene in the brain] (102). T3 increased DNMT3a mRNA expression during metamorphosis also in Xenopus tadpoles(102).

ii) A recent paper showed lower global DNA methylation levels and DNMT1 expression in T and B lymphocytes of Graves'disease patients compared to age-matched controls, and these parameters were restored by treating the hyperthyroidism (103).

iii) A mild maternal hypothyroxinemia during pregnancy in mice induced in the offspring damage in learning and memory in adulthood, probably due to a persistent DNA hypermethylation in the promoter region of brain-derived neurotrophic factor (BDNF) gene in the hippocampus, capable of suppressing BDNF expression and thus promoting cognitive disorders in adult offspring (104). Although it is difficult to establish a cause-effect relationship between these events in absence of specific human data, several epidemiological studies reported that children born from mothers with hypothyroxinemia showed cognitive and psychomotor deficits (105–108). Such studies focused on intellectual development during the school age and it is unknown whether epigenetic mechanisms related to maternal hypothyroidism during pregnancy predict an age-related change in cognitive ability in the offspring in their adulthood and old age through.

iv) We have characterized the DNA methylation profiles from peripheral leukocytes of female centenarians, their female offspring and female offspring of both non-long-lived parents. It is important to mention that centenarians' offspring have a consistently healthier phenotype than their age-matched control born from non-long-lived parents (4). Several genes involved in DNA/RNA synthesis, metabolism, and cellular signalling were differently methylated between centenarians'offspring and controls. More recently, in an epigenome-wide association study (using 450 Bead Chip array capable of assessing the methylation of more than half a million CpG) involving an Italian cohort of semi-supercentenarians (105 years old and over), their offspring and age-matched controls, we reported that 105+ and their offspring are biologically younger than their chronological age. We showed that according to the Horvath "epigenetic clock" (which consider 353 CpG in the entire genome) centenarians and

their offspring are younger than expected based on their chronological age (8.7 years and 5.2 years, respectively) (109). Although the cause-effect relation is difficult to be verified in such a cross-sectional study, we cannot exclude that a slower cell growing/metabolism and a better control in signal transmission through epigenetic mechanisms may be involved in the process of longevity (110). These aspects reflect previous observation on the potential benefits of a mild hypothyroidism in the elderly through its lowering effects on basal metabolic rate and oxidative metabolism, with a consequent reduction of ROS-induced DNA damage (111). Indeed, high levels of TSH (112) and low levels of FT4 (113) are associated with a better survival in elderly subjects. A mild thyroid failure may suppress processes involved in nucleotide biosynthesis and DNA replication(114,115), suggesting that a specific THs-related epigenetic modulation of genes involved in DNA metabolism and control of signal transmission may contribute to the longer lifespan and healthy aging of centenarians' offspring. In addition, epigenetic mechanisms may also influence thyroid landscape during aging.

v) The expression of THRβ in peripheral blood mononuclear cells obtained from healthy elderly and long-lived individuals was significantly lower than in young individuals, and likely related to the increased methylation of the CpG island located within the TRβ promoter (116).

vi) Maternal exposure to iodine excess of Wistar rats induces hypothyroidism in their adult male offspring, morphological alterations in thyroid follicles, increased thyroid oxidative stress and decreased expression of thyroid differentiation markers and transcription factors (117). Increased DNA methylation and DNA methyltransferases expression, hypermethylation of histone H3, hypoacetylation of histones H3 and H4, increased expression/activity of histone deacetylases and decreased expression/activity of histone acetyltransferases are involved in the repression of thyroid gene expression (117). Overall,

these epigenetic changes appear to be a kind of adaptive phenomenon to protect the offspring's thyroid from the deleterious effects of iodine excess.

vii) Finally, many evidence suggests a main role of the epigenetic network in the control of the expression of D1 in chicken (118), D2 in chicken (119) and rats (120) and D3 in mice (121,122), neonatal goats (123) and Siberian hamsters (124). Within this scenario and in absence of specific data in humans we cannot exclude that a modulation of the local levels of THs and metabolites occurs in aging through epigenetic mechanisms.

Overall, available data suggest that epigenetic modifications may represent another mechanism of the pervasive influence of THs on the aging process, but many mechanistic details are lacking.

3. The thyroid aging and the lesson from centenarians

3.1. Thyroid aging in the oldest old.

The evaluation of TRH–TSH–T4/T3 axis during aging presents many problems due to the concomitant presence of several confounding variables and difficulties in the correct definition of "healthy elderly" (125), and results of studies examining the influence of age on HPT axis also in the absence of thyroid disease remain controversial, as reported in Table 1.

As previously argued the oldest old will represent a considerable percentage of the elderly owing to their faster increase, suggesting that particular attention should be devoted to the thyroid status of this segment of the population as a rational pre-requisite for any type of possible intervention. The problem is that the oldest old are very heterogeneous regarding their overall phenotype and health status, a result of possible successful or unsuccessful adaptation, according to the remodelling theory of aging (144). For this reason, the oldest old necessitate a careful and specific study, and available data on their thyroid status will be reviewed and critically evaluated. Subjects older than 85 years have been only rarely included

556 in most of the studies exploring the effect of thyroid function on mortality and available
557 results are conflicting (40), as reported in Table 2 focused on the association between thyroid
558 function and all-cause mortality in euthyroid individuals.

559 **3.2. Centenarians and their offspring as a model of longevity in humans**

560 Studies on centenarians could help to better understand the role of THs in healthy aging and
561 longevity as they reached the extreme limits of life and have escaped or delayed the onset of
562 major AADs (161). Therefore, centenarians can be considered as the most successfully
563 remodelled people, presenting a complex and adaptive phenotype, but also characterized by a
564 precarious homeostatic balance (5). Centenarians are even more interesting considering that
565 they are a sort of gold standard of *H. sapiens*, i.e. people who exploited the maximum living
566 capacity of the species. Indeed, despite the number of centenarians is rapidly increasing
567 worldwide no one has been reported to live more than about 120 years, suggesting that the
568 lifespan of *H. sapiens* has a biological limit that cannot be overcome unless within the
569 uncanny nightmare of changing its basic genetics/biology (162). Table 3 highlights the main
570 features of centenarians taking into account the seven pillars of geroscience. This conceptual
571 framework applies also to possible intervention regarding alterations in the thyroid
572 functioning in the oldest old, as illustrated in paragraph 5.2.

573 A correlated model of healthy aging is represented by centenarians' offspring who can
574 overcome some limitations inherent in the study of centenarians (rarity, lack of an age-
575 matched control group and presence of frailty related to their extreme age) (166,181).
576 Centenarians' offspring can be compared to age-matched controls born from non-long-living
577 parents (181), and this comparison showed that they are healthier (4,181), biologically
578 younger (175) and with a higher probability to become long-lived than members of the same
579 demographic cohorts (181,182).

580 **3.3. Thyroid aging in centenarians and their offspring**

Aging is associated with a decreased volume of the thyroid gland and decreased levels of THs (183). As suggested by the remodeling theory of aging (144) such a situation may represent an adaptive phenomenon to attain "successful" aging and to prevent excessive catabolism in the elderly through a reduction in basal metabolic rate and, consequently, in the production of ROS and DNA damage. Despite conflicting results in the literature, most studies reported that higher TSH and/or lower FT4 concentrations within the euthyroid range are associated with lower mortality in old subjects (Table 2). Studies on the relationship between thyroid function and longevity also produced conflicting results (Table 4).

Mariotti et al. reported that healthy centenarians had lower serum TSH and FT3 levels and higher serum rT3 levels compared with those observed in other age control groups (185). In this centenarians' population, the prevalence of thyroid autoantibodies was not significantly different from that observed in controls aged less than 50 years, notwithstanding the age-related increase in the prevalence of thyroid autoantibodies observed with aging (184). These data were also confirmed by Magri et al. who found in centenarians lower TSH levels, higher rT3 levels and lower thyroid autoantibodies positivity as compared with 70/80 years old subjects (187,189). In another Italian population of centenarians, total T4 values were lower than the normal range in 60% of examined subjects (186). In Polish centenarians, Baranowska et al. found that serum TSH and T4 concentrations were comparable with those observed in younger women, while serum T3 levels were lower compared with the other groups (188). Atzmon et al. demonstrated that Ashkenazi Jews centenarians have significantly higher median serum TSH concentrations compared with younger Ashkenazi controls and with a population of thyroid disease-free individuals. An inverse correlation between FT4 and TSH levels in centenarians and Ashkenazi controls has been observed (190), and this phenotype appears to be heritable (193). Also in Chinese centenarians' families, a decline in thyroid function (high TSH and low FT3 concentrations) appears to be associated with age, and this

phenotype is heritable and likely contribute to longevity (191).In relatives of Italian centenarians (offspring or nieces/nephews) lower comorbidities, FT3, FT4 and TSH levels have been reported compared to age-matched controls(194). Lower plasma level of FT4 in centenarians' offspring compared to age-matched controls were confirmed in another Italian population (181). Rozing et al., in the Leiden Longevity Study, showed that when compared with their partners, the group of offspring of nonagenarian siblings showed a trend toward higher serum levels of TSH together with lower FT3 and FT4 levels (183). Lower mortality in the parents of nonagenarian siblings was associated with higher serum TSH levels, lower serum FT3 and FT4 levels in the nonagenarian siblings (196).

We have recently characterized thyroid function profile in an Italian cohort of 672 subjects consisting of centenarians, semi-supercentenarians (*i.e.* persons who reach the age of 105 years and over), centenarian's offspring and elderly subjects age-matched with centenarian's offspring. We have found an age-dependent decrease in FT3 level and FT3/FT4 ratio, while FT4 and TSH increased. In long-lived individuals, higher FT4 level and lower FT3/FT4 ratio were associated with an impaired functional status and an increased mortality. From this analysis, we excluded subjects with a thyroid profile suggestive of non-thyroidal illness syndrome. These results indicated that the age-related decrease in FT3/FT4 ratio could be due to a decline in 5'deiodinase activity. Centenarians and semi-supercentenarians with relatively high FT3/FT4 ratio are probably able to preserve D1 activity, likely maintaining a good hormonal negative feedback (192). This phenomenon could be relevant for preserving a good functional capability and survival optimization. During aging, a decline in serum T3 levels could be balanced by a compensatory increase in D1 activity. This adaptive ability, aimed at maintaining an adequate local production of T3, could allow preserving THs signaling and to counteract the aging-associated metabolic disturbances. Such interpretation is also supported by a recent prospective study showing that FT3/FT4 ratio represents an independent marker

of frailty and survival in a population of euthyroid older patients, hospitalized for an acute event (157).

In conclusion, the mild and progressive decrease in thyroid function observed with aging could be part of adaptive strategies involving also the endocrine system (183) that the body utilizes to survive in the last decades of life and that likely contribute to attaining the extreme limit of human life having largely avoided/postponed most AADs. . In particular, better preservation of local T3 concentration through a suitable peripheral T4 to T3 conversion may have a relevant role in assuring a remarkable longevity and healthy aging.

4. "Thyroid Biography" and thyroid aging within a lifelong perspective

Aging and longevity are complex traits, where each individual follows a different personal trajectory. The result is the high phenotypic heterogeneity which characterizes old subjects and which increases with age (197), mirrored by the large individual variability in the levels of serum TSH, T4 and T3, which in turn explain the difficulty encountered in the diagnosis of thyroid dysfunctions in the elderly and in the oldest old. This heterogeneity is a complex biomedical and public health problem as all the symptoms that characterize thyroid diseases diminish the working capacity and the quality of life. Moreover, thyroid pathologies in the elderly have systemic effects and can cause hypertension, cardiac insufficiency, adverse lipid profile, insulin resistance, endothelial dysfunction, among others, in turn posing an increased risk for atherosclerosis, cardiovascular disease (CVD), diabetes mellitus type 2 (T2D), cognitive impairment, depression, and mortality (198).

Thus, the crucial question becomes where does this heterogeneity come from? Starting from the period of life spent in utero each individual is exposed to a unique combination of stimuli, including hormonal ones, which can affect all organs of the body including the thyroid. We recently proposed the concept of "immunobiography" (2,197) to grasp the large heterogeneity

of immune system aging and responsiveness (immunosenescence and inflammaging) in different individuals (197). Similarly, we propose here to adopt the concept of "*Thyroid Biography*" in order to better understand the thyroid aging at individual level (Figure 2). The basic idea is to systematically and accurately collect and store data capable of reconstructing in each individual/patient the unique, lifelong combination of variables such as age, sex, place and geography of birth and of living, type of work, socio-economic and psychological status, lifestyle habits (nutrition and physical activity), diseases (with particular attention to the endocrinological ones including those of the parents), comorbidities, results of blood examination performed lifelong (including endocrinological data), drugs used, among others, that can have long and/or short-term effects on thyroid function and physiopathology. In particular, environmental factors, including the exposure to endocrine disrupting compounds as well as smoking and other conditions, could influence thyroid homeostasis lifelong (199).

5. Summary and Perspectives

The main message of this review is that, in order to fully understand the aging of the thyroid in humans, we have to follow the suggestions emerged in the field of aging research, and particularly those conceptualized/proposed by Geroscience, which can be summarized as follows:

i) there is an urgent need to investigate in depth at the thyroid level the main molecular and cellular mechanisms identified as key to understanding the aging process in animal models and humans. As far as we know, this approach, which starts to be systematically applied in many organs and systems in experimental animals and humans, represents a novelty in the studies on thyroid aging. Available data on these mechanisms or "pillars" have been scrutinized, and the emerging scenario is that the knowledge on these critical points is still scarce, suggesting that data on fundamental mechanisms such as the accumulation of

senescent cells in thyroid, the relationship between gut microbiota dysfunction and thyroid function, the role of inflammaging and its propagation within the thyroid and systemically, thyroid cells DNA methylation, are still largely unexplored;

ii) derangements in these basic mechanisms of aging can help in explaining the pathogenesis of age-related thyroid pathologies as well to better understand the role of thyroid aging on the aging of other organs and systems. To this regard a rather neglected but very interesting topic which deserves much more attention both from a basic and clinical perspective is the contribution of thyroid function abnormalities to the onset of chronic AADs;

iii) the thyroid status and function in the oldest old is particularly complex and heterogeneous, and this topic also needs further investigations;

iv) a variety of environmental factors and lifestyle habits have the capability to deeply interfere with thyroid function. The knowledge on this point of public health importance, particularly for the next generations, is still scarce and an effort is urgently needed.

v) the complex history behind thyroid status in the elderly, as well as their physiological and clinical heterogeneity regarding thyroidal function, could be better understood by adopting the comprehensive concept of "thyroid biography" and its inherent capability to grasp the combination of factors impinging lifelong on the thyroid at individual level (Figure 2). We envisage the difficulties in the present scenario of medical services and organizations to realize, starting from birth, such as "thyroid passport" for each citizen. At the same time, we consider our proposal a suggestion we hope useful for colleagues working in the public health sector to start building a personalized medicine as a prerequisite for a personalized aging.

Finally, besides such clarifications, we have the impression that a change of paradigm is emerging regarding the thyroid age-related changes occurring physiologically, i.e. in absence of overt clinically-relevant pathologies. These changes, as well as many others concomitantly happening in the immune system, such as immunosenescence and

inflammaging (5), are no more considered simply detrimental but can be conceptualized as part of the systemic, adaptive remodelling that helps survive in relatively good shape until the limit of human life. This new perspective could help in taking a decision regarding the treatment with THs of elderly and oldest old, which is at present a still controversial issue.

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Legends for Figures and Tables

Figure 1. Molecular mechanisms of aging ("pillars") involved in thyroid aging, according to the new Geroscience perspective.

Figure 2. The new concept of "Thyroid Biography" is proposed in order to better understand the heterogeneity of thyroid aging in each individual/patient as a consequence of the unique combination of variables impinging lifelong upon thyroid function.

Table 1. Summary of the results obtained in several studies evaluating the TRH–TSH–T4/T3 axis during aging in thyroid disease-free populations (n > 300). A review of the literature was conducted using PubMed database with the following keywords: "thyroid" and "ageing". The search included articles published in the English language between January 2000 and February 2019. (*) indicates a cross-sectional study and (**) indicate longitudinal study

Table 2. Summary of the results obtained in different studies evaluating the association between thyroid function and all-cause mortality in euthyroid individuals (n>300). The arrows ↑ ↓ indicate augments or decreases, respectively, of TSH and/or THs, but always within the euthyroid range. A review of the literature was conducted using PubMed database with the following keywords: "thyroid" and "mortality" and "euthyroid". The search included articles published in the English language between January 2000 and February 2019.

Table 3. The Seven Pillars of Aging in centenarians.

Table 4. Summary of results obtained in different studies evaluating the thyroid function in the centenarians. A review of the literature was conducted using PubMed database with the following keywords: "thyroid" and "centenarians". The search included articles published in the English language between January 1990 and February 2019.

Summary of results	Country (Ethnicity)	Participants	Ref.	Year
TSH and the prevalence of anti-thyroid antibodies are greater in females, increase with age, and are greater in whites and Mexican Americans than in blacks.	USA (White 35%, Black 32%, Mexican American 29%, Other 4%)	Thyroid disease-free population of 13,344 people (≥ 12 years of age)*	(126)	2002
Serum TSH gradually decreases with age, whereas after age 60, serum FT4 increases, possibly because of the development of thyroid autonomy after longstanding borderline sufficient iodine intake.	The Netherlands (96% White)	Population of 5,167 individuals (≥ 18years) selected by excluding those at risk for thyroid disease.*	(127)	2006
TSH distribution progressively shifts toward higher concentrations with age	USA (White, Black, Mexican American, Other)	Thyroid disease-free population of 14,376 people (≥ 12 years of age)*	(128)	2007
FT3 and TSH decreased with age. The TSH response to TRH was blunted in older subjects, especially in male individuals.	Germany	387 thyroid disease-free population (13-100 years, mean age 39.5 years)*	(129)	2008
Age was not associated with serum TSH levels	China (Asian)	2,237individuals of reference population (> 13 years of age)*	(130)	2008
A shift to higher TSH with ageing occurred in black and white subgroups.	USA (Black or African Americans 33%, White 15%, Hispanic 5%, Unknown 47%)	Population of 22,116 people (>10 years, median age 44 years) without clinical evidence of thyroid disease.*	(131)	2009
The TSH 2.5th, 50th, and 97.5th percentiles increased with age, with the most significant effects seen at the 97.5th percentile, which increases by 0.3 mIU/L with each 10-year increase in a subject's age	USA (White 35%, Black 31%, Mexican American 29%, Unknown 5%)	Thyroid disease-free population of 13,344 people (> 12 years of age)*	(132)	2011
A statistically significant increase in TSH (+12%) and FT4 (+2.5%), and a decrease in T3 (-13%) over the 13-yr period.	USA	Thyroid disease-free population of 533 participants (≥ 75years)**	(133)	2012
Aging was associated with increased serum TSH concentrations (+21%), with no change in fT4 over 13 yr of follow-up, suggesting an age-related alteration in TSH set point or reduced TSH bioactivity rather than occult thyroid disease.	Australia (predominantly White)	Thyroid disease-free population of 908 participants (mean age at baseline 45.5 years)**	(134)	2012
An increasing age is associated with an increase in median and 97.5th percentile for TSH.	Australia (predominantly White)	Thyroid disease-free population of 148,938 people (1-106 years, mean age 48.2 years in women and 53.8 years in men)*	(135)	2012
During the 11-yr follow-up, mean TSH increased significantly (+8.7%), particularly in	Denmark	2,203 participants (18-65 years) with no	(136)	2012

the area with the highest iodine intake.		previous thyroid disease**		
An increase in median and 97.5th centile TSH with increasing age.	UK	Thyroid disease-free population of 153,127 people (≥ 18years of age)*	(137)	2013
TSH and FT3 were inversely associated with age.	Germany	Thyroid disease-free population of 1,002 individuals (45-83 years).*	(138)	2014
TSH (+8.9%) and FT4 (+9.3%) values increase over the 11-yr period, particularly from the age of 50 yr. No significant change was observed for FT3	Spain	Thyroid disease-free population of 552 participants (18-65 years, mean age at baseline 41.7 years)**	(139)	2015
FT4 increased and TSH decreased at follow-up evaluation	Korea	A total of 313 euthyroid participants (5-year follow-up evaluation)**	(140)	2015
Until age 40, for each increase in TSH quartile, FT3 and the FT3/FT4 ratio increased and FT4 decreased significantly. In older age groups, increasing TSH was not associated with increased FT3/FT4 ratio. This could reflect a decrease in deiodinase activity and/or the development of TSH resistance with aging.	Israel	Thyroid disease-free population of 27,940 people (1-80 years)*	(141)	2016
TSH levels did not change over time, irrespective of age. FT4 levels increased over time, most prominently in those older than 65 years of age.	The Netherlands (predominantly White)	9,402 participants (≥ 45years, mean age 65.1years) from the Rotterdam Study not taking thyroid medication (longitudinal for 1,225 people with a follow-up of 6.5 years)**	(142)	2016
FT3 and FT4 decreased throughout life, while TSH declines until age 50 years and then increased slightly. FT4 declined, among females more than among males until middle age. After 60 years of age FT4 levels mildly increased only in females	Israel	Thyroid disease-free population of 27,940 people (≥ 1years of age)*	(143)	2017

Table 1. Summary of the results obtained in several studies evaluating the TRH–TSH–T4/T3 axis during aging in thyroid disease-free populations (n > 300). A review of the literature was conducted using PubMed database with the following keywords: "thyroid" and "ageing". The search included articles published in the English language between January 2000 and February 2019. (*) indicates a cross-sectional study and (**) indicate longitudinal study

Thyroid hormone changes significantly associated with increased all-cause mortality	Main measures of thyroid function	Follow up period (years)	Country	Participants	Ref.	Year
↑FT4	TSH, FT4, TT4, T3, rT3 and T4-binding globulin	4	The Netherlands	403 male participants (73-94 years old) of the Zoetermeer Study	(113)	2005
No association	FT4, FT3, and TSH	8.5	Germany	3651 individuals of the Study of Health in Pomerania (20-79 years old)	(145)	2010
No association	TSH, FT4	8.3	USA	1,387 euthyroid men of Osteoporotic Fractures in Men (MrOS) study (mean age 73.6 years)	(146)	2012
↓TSH	TSH	4.5	Israel	42,149 subjects (≥40 years old)	(147)	2012
↑FT4	TSH and FT4	6.4	Australian	3,885 euthyroid men (≥65 years old)	(148)	2013
↓FT4	FT4, FT3, and TSH	4.3	South Korea	212,456 middle-aged (40.2 years old) euthyroid participants of the Kangbuk Samsung Health Study	(149)	2014
↑FT4, ↑TSH	TSH, FT4 and peroxidase antibodies	9.4	The Netherlands	493 participants (≥80 years old) of the Nijmegen Biomedical Study	(150)	2014
↓TSH, ↑FT4	TSH, T3 and FT4	Over 17	USA	2843 participants (74.5 ± 5.1 years)	(151)	2015
↓TSH	TSH, FT3 and FT4	9	Italy	815 euthyroid participants of Aging In the Chianti Area (InChianti Study)	(152)	2016
↓↑TSH (U-shaped association)	TSH, FT4	19.1	USA	12,584 adults aged ≥20 years	(153)	2016
↑rT3	rT3, FT3, FT4 and TSH	9	UK	645 participants (85 years old) of the Newcastle 85+ Study	(154)	2016
↓FT3/FT4 ratio, ↓FT3, ↑FT4	TSH, FT3 and FT4	5/3.8	The Netherlands	805 nonagenarians from Leiden Longevity Study (median age 91 years) and 259 nonagenarians form Leiden 85-plus Study (median age 94 years)	(155)	2017
↓TSH and ↑FT4 in men; ↓FT3 in women	TSH, FT3 and FT4	10	Italy	933 participants (324 men and 609 women) of Milan Geriatrics 75+ Cohort Study with normal TSH (81.6± 4.6	(156)	2017

				years)		
↓ FT3/FT4 ratio	FT3 and FT4	2.5	Italy	643 geriatric patients (83.8 ± 7.4 years)	(157)	2018
↑TSH in women	TSH, FT3 and FT4	7.7	Turkey	614 hospitalized patients (40-79 years)	(158)	2018
No association	TSH, FT3 and FT4	13	The Netherlands	2431 participants of the PREVEND cohort, aged 28–75 years,	(159)	2017
↓FT3/FT4 ratio	TSH, FT3 and FT4	1	China	953 euthyroid patients with acute myocardial infarction	(160)	2018

Table 2. Summary of the results obtained in different studies evaluating the association between thyroid function and all-cause mortality in euthyroid individuals (n > 300). The arrows ↑↓ indicate augments or decreases, respectively, of TSH and/or THs, but always within the euthyroid range. A review of the literature was conducted using PubMed database with the following keywords: "thyroid" and "mortality" and "euthyroid". The search included articles published in the English language between January 2000 and February 2019.

Metabolism	Preserved glucose tolerance and insulin sensitivity and lower levels of serum IGF-I in centenarians with respect to elderly controls	(163–166)
Inflammation	The increased plasma levels of inflammatory molecules such as interleukin (IL)-6, interleukin (IL)-18, interleukin (IL)-15, C reactive protein (CRP), serum amyloid A, fibrinogen, von Willebrand factor, resistin and leukotrienes are counterbalanced by a concomitant large quantity of anti-inflammatory molecules (*i.e.* adiponectin, Transforming Growth Factor (TGF)-β1, interleukin (IL)-1 receptor antagonist (IL-1RA), cortisol, anti-inflammatory arachidonic acid compounds	(167–174)
Epigenetics	According to the "epigenetic clock", centenarians are younger (8.6 years) than expected based on their chronological age	(175)
Adaptation to Stress	Higher plasma levels of cortisol, ACTH and CRH than young subjects	(171)
Stemcells and regeneration	The basal hematopoietic potential (capability of CD34+ cells to respond to hemopoietic cytokines and to form erythroid, granulocyte, and macrophage and mixed colonies) is well preserved in healthy centenarians	(176)
Proteostasis	Cultures of fibroblast-derived from healthy centenarians have a functional proteasome	(177)
Macromolecular damage	Lymphocyte cell lines from centenarians preserve their capability of priming the mechanism of repair after H_2O_2 oxidative damage and in poly(ADP-ribosyl)ation capacity. Differences in BRCA1 genotype frequencies between the centenarians and controls. No difference in the number of spontaneous chromatid breaks in lymphocytes from healthy centenarians and controls but centenarian's cells show a higher sensitivity (DNA breaks per cell) to the radiomimetic agent bleomycin.	(178–180)

Table 3. The Seven Pillars of Aging in centenarians.

Summary of results	Main outcome measures	Population	Participants	Ref.	Year
The prevalence of thyroid autoantibodies increased with age until ninth decade of life. The prevalence of thyroid autoantibodies in centenarians was not significantly different from that in controls aged less than 50 yr.	Serum thyroid autoantibodies	Italian	34 healthy centenarians (100-108 years), 549 control subjects (7-85 years)	(184)	1992
FT3 and TSH decreased with age. FT4 did not change with age. rT3 was significantly higher in centenarians than in elderly and adult subjects. The prevalence of serum anti-Tg and anti-TPO antibodies was low and did not differ among centenarians, elderly and adult subjects.	Serum anti-Tg and anti-TPO antibodies, FT4, FT3, rT3 and TSH	Italian	Healthy centenarians (100-110 years), 33 healthy elderly subjects (65-80 years), 98 healthy adults (20-64 years) and 52 patients with miscellaneous nonthyroidal illness (28-82 years).	(185)	1993
All the parameters were within normal range, with the exception of TT4 values, which were reduced in 60% of centenarians.	total T3, total T4, FT3, FT4, TSH, anti-Tg and anti-microsomal antibodies	Italian	20 healthy centenarians (100–108 years), 40 healthy elderly subjects (70–84 years) and 50 healthy adults (38–62 years)	(186)	1997
TSH decreased significantly whereas rT3 slightly, but significantly, increased with age. FT3/FT4 ratio decreased with age suggesting a decline of the 5’ deiodinase activity. The incidence of thyroid autoantibodies was lower in centenarians than in elderly subjects.	TSH, FT3, FT4, rT3, anti-Tg, anti-TPO antibodies and nutritional markers	Italian	24 healthy centenarian women (100-106 years), 24 healthy elderly women (71-93 years) and 20 healthy young subjects (22-33 years).	(187)	2002
TSH did not differ significantly among centenarians, elderly and young women. T3 was significantly lower in centenarian women in elderly and young women.	T3, T4, glucose and lipid profiles, plasma leptin, NPY, insulin, TSH, GH, PRL, LH, FSH and cortisol	Poland	78 centenarian women (100–115 years), 21early elderly women (64–67 years), 21 postmenopausal women (50–60 years) and 35 younger women (20–50 years)	(188)	2007
TSH was significantly lower in centenarians than in healthy old and young controls. The FT3/FT4 ratio was significantly lower in elderly subjects and centenarians when compared to young controls. rT3 was higher in centenarians compared to both old and young controls.	Serum cortisol, dehydroepiandrosterone-sulfate (DHEAS), FT3, FT4, rT3 and TSH, urinary free cortisol and 6 hydroxymelatonin sulfate (aMT6s)	Italian	59 centenarians (100–107 years), 24 healthy old (mean age 85 years) and 20 young controls (mean age 28 years)	(189)	2008
TSH was significantly higher in centenarians than in controls. The TSH frequency distribution curve of centenarians shifted significantly to higher TSH values compared with controls.	TSH, FT4, and TSH frequency distribution curves	North American (Ashkenazi Jewish and U.S. National	Ashkenazi Jews centenarians (median age, 98 years), Ashkenazi controls (median age, 72 years), healthy NHANES	(190)	2009

FT4 was similar in centenarians and controls, and there was a significant inverse correlation between FT4 and TSH in both groups.		Health and Nutrition Examination Survey 1998–2002, NHANES)	controls (median age, 68 years)		
TSH increased with age. T3, FT3 and the FT3/FT4 ratio decreased with age. T4 and FT4 did not change with age. A significant association was found between TSH and FT3 levels of centenarians and those of their offspring suggesting that TSH and FT3 concentrations may be considered heritable phenotype.	TSH, T3, FT3, T4 and FT4	Chinese	61 centenarians (mean age 103 years), 63 centenarians' offspring (mean age 62 years), 47 spouses of the offspring (mean age 60 years), 25 centenarians' second-generation offspring (mean age 32 years) and 10 spouses of second-generation offspring (mean age 31 years)	(191)	2015
FT3 level and FT3/FT4 ratio decrease while FT4 and TSH increase with age. In CENT/105+, higher FT4 level and lower FT3/FT4 ratio are associated with an impaired functional status and an increased mortality. Cluster analysis identified three clusters of CENT/105+ based on their FT3, FT4 and TSH levels. Cluster3, characterized by lower FT3 and TSH and higher FT4, shows the worst health status and the shortest survival. A group of CENT/105+ showed a thyroid profile suggestive of non-thyroidal illness syndrome (NTIS) and are characterized by a worse functional and cognitive status and an increased mortality with respect to CENT/105+ without NTIS	TSH, FT3 and FT4	Italian	672 well-characterized Italian subjects (age range: 52–113 years), including of 144centenarians (mean age 100 years) 70 semi-supercentenarians (mean age 105.9 years), as well as 308 centenarian's offspring (mean age 71 years) and 150 age-matched elderly (mean age 70 years)	(192)	2018

Table 4. Summary of results obtained in different studies evaluating the thyroid function in the oldest old and centenarians. A review of the literature was conducted using PubMed database with the following keywords: "thyroid" and "centenarians". The search included articles published in the English language between January 1990 and February 2019.

Figure 1.

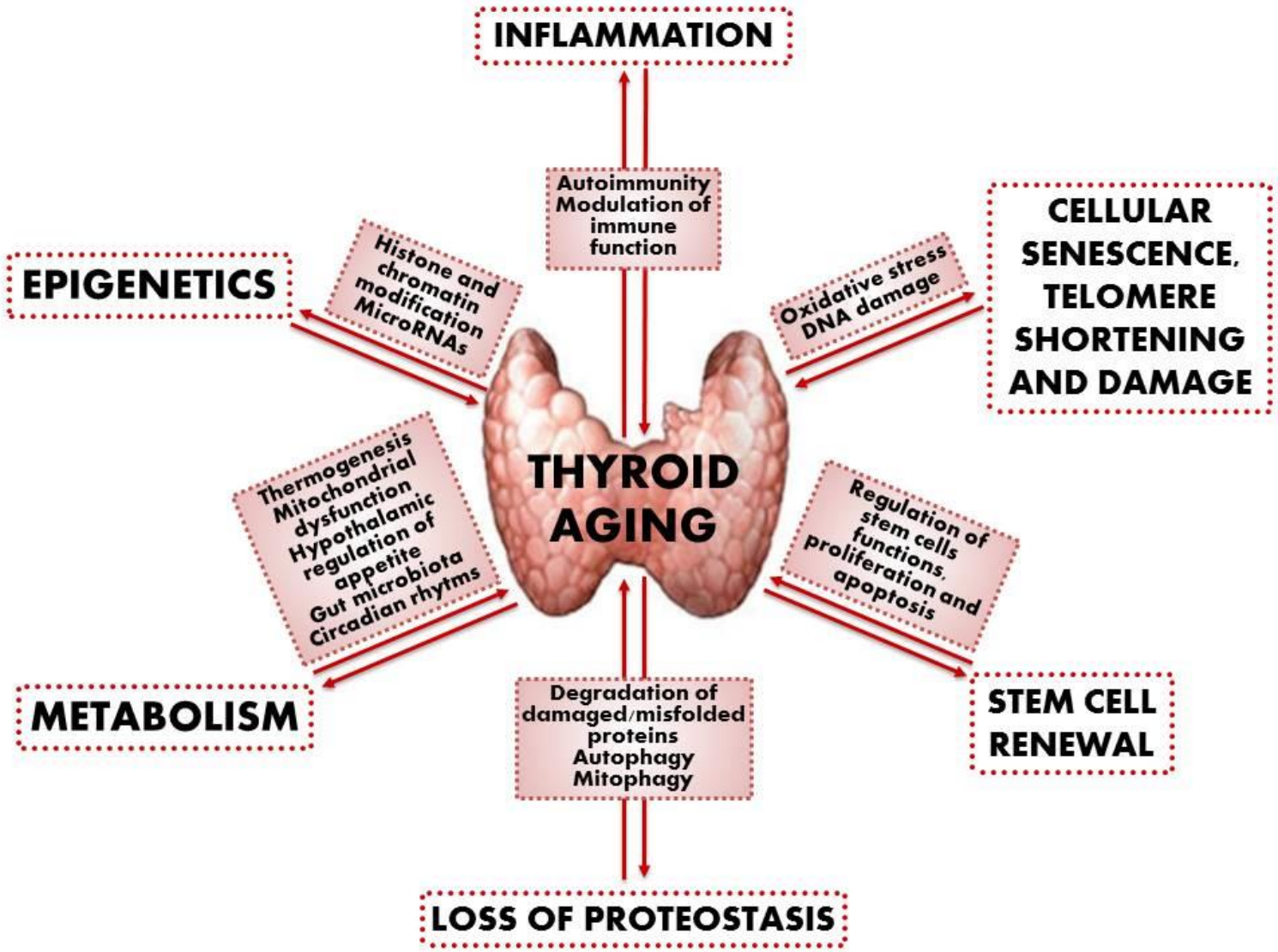
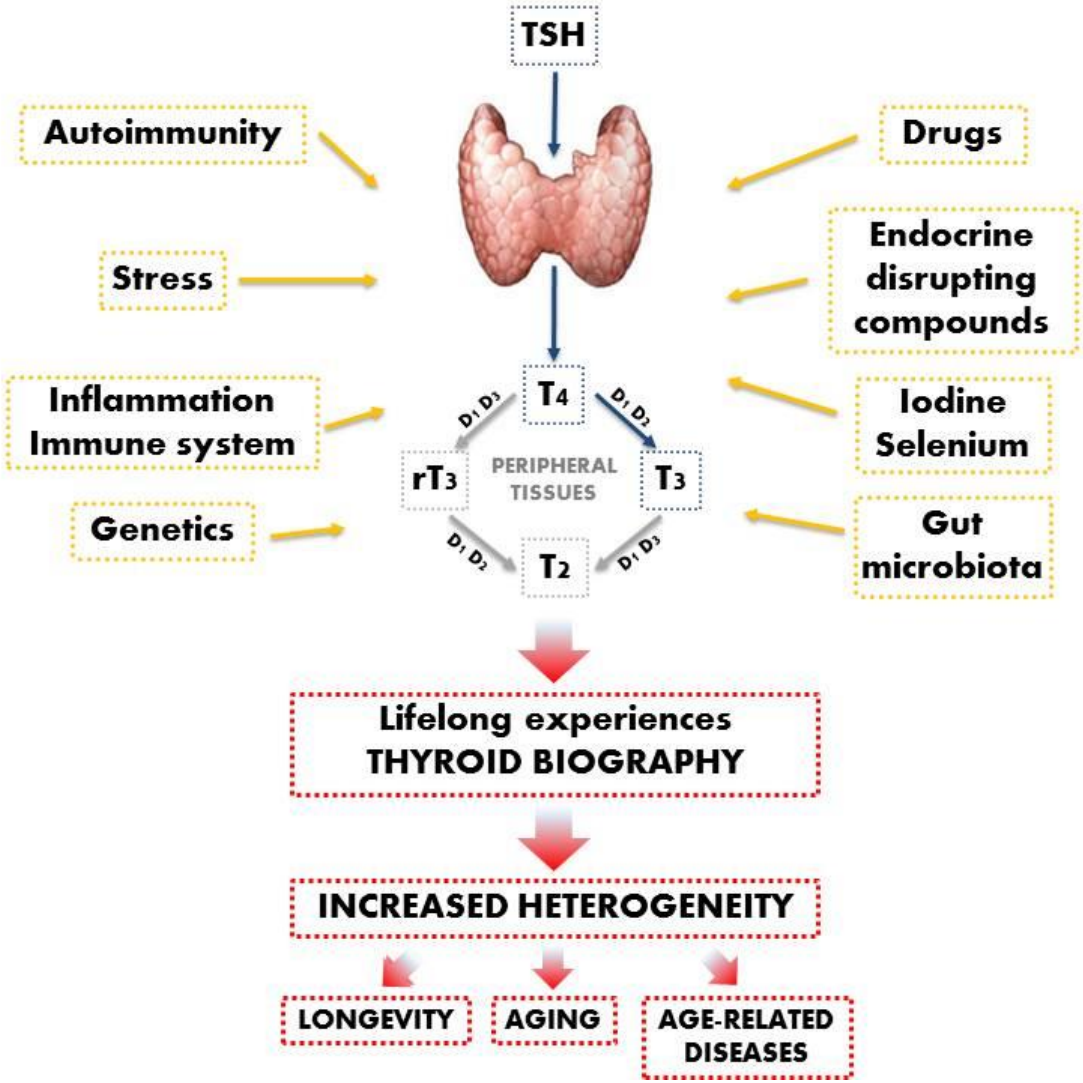

INFLAMMATION
Autoimmunity
Modulation of immune function
EPIGENETICS
Histone and chromatin modification
MicroRNAs
CELLULAR SENESCENCE, TELOMERE SHORTENING AND DAMAGE
Oxidative stress
DNA damage
THYROID AGING
Thermogenesis
Mitochondrial dysfunction
Hypothalamic regulation of appetite
Gut microbiota
Circadian rhytms
METABOLISM
Regulation of stem cells functions, proliferation and apoptosis
STEM CELL RENEWAL
Degradation of damaged/misfolded proteins
Autophagy
Mitophagy
LOSS OF PROTEOSTASIS

Figure 2


TSH
Autoimmunity
Drugs
Stress
Endocrine disrupting compounds
Inflammation Immune system
Iodine Selenium
Genetics
Gut microbiota
T4
rT3
PERIPHERAL TISSUES
T3
T2
Lifelong experiences
THYROID BIOGRAPHY
INCREASED HETEROGENEITY
LONGEVITY
AGING
AGE-RELATED DISEASES