Thyroid axis challenges in Leiden Longevity Study

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Chapter 1

General introduction

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As the older population makes up a continuously increasing fraction of the whole demographic (1), understanding healthy ageing and deciphering the mechanisms underlying it, is now more valuable than ever. The sheer number of persons aged 60 years and older worldwide is expected to more than double by the year 2050, to an astounding 2.1 billion, from 962 million in 2017(2). Furthermore, the worldwide number of persons aged 80 years and older is expected to triple in this same period, to 425 million from 137 million in 2017(2). In the Netherlands, both the percentage of the population aged 65 and older, as well as the percentage of the population aged 80 years and older, are expected to greatly increase by the year 2050 compared to year 2015 (3), and with them the size of the population aged > 85 years, the 'oldest old'.

Considering that age is the main risk factor for development of chronic diseases due to the time-dependent functional decline that occurs with it(4), detailed investigation of factors contributing to healthy ageing and of the mechanisms underlying the ageing process is not only significant for the individual but also crucial for sustainability of health care and the welfare of society, not only locally but globally. However, healthy ageing is a complex process.

Thyroid axis

One of the systems influencing healthy ageing might be the thyroid axis (figure 1). The thyroid axis is a complex network that regulates circulating levels of thyroid hormones, which in turn are major regulators of multiple physiological processes - from mood and cognition, to growth and development, to immunity and metabolism (5,6).

Feed forward and feedback regulation

The thyroid axis consists of several hormones which create a tightly regulated feed forward and feedback loop. Thyrotropin releasing hormone (TRH) is secreted by the hypothalamus and stimulates the secretion of thyroid stimulating hormone (TSH) from the anterior pituitary gland. TSH in turn stimulates the thyroid gland, through

TSH receptors (TSH-R), to produce and release thyroid hormones into the circulation. The circulating thyroid hormones (TH) inhibit the release of TRH and TSH through negative feedback, thereby maintaining an euthyroid state.

The thyroid gland itself is the place of thyroglobulin (Tg) production and storage and, from Tg, thyroid hormone production and secretion into the systemic circulation upon signalling from TSH. The thyroid gland produces mostly (90%) the prohormone thyroxine (T4) and partially also the active hormone, triiodothyronine (T3).

Circulating thyroid hormones are mostly bound by transport proteins (thyroglobulin binding protein (TBG), thyroxin-binding albumin (TBA), and albumin), although a small fraction is in free form (fT4 and fT3).

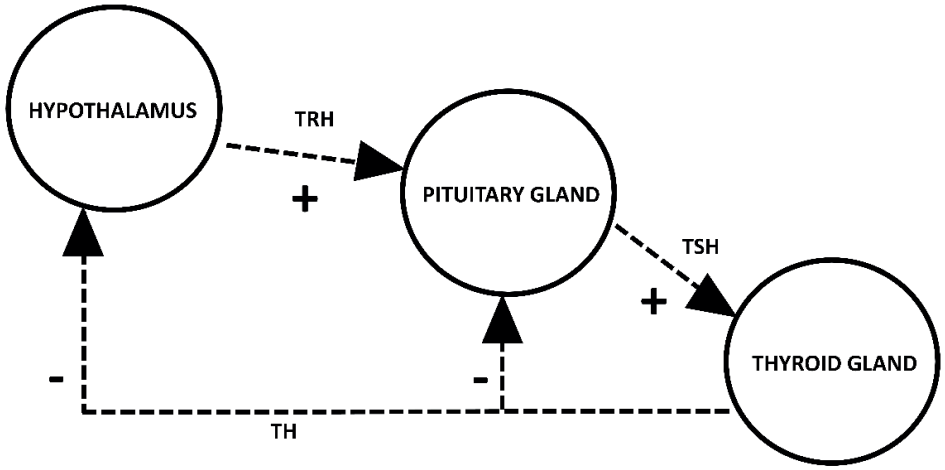

Figure 1. The negative feedback and feedforward system of the thyroid axis. TRH: thyrotropin releasing hormone, TSH: thyroid stimulating hormone, TH: thyroid hormones.

Tissue-specific regulation

Thyroid hormones can be taken up by cells from the circulation via specific transporter proteins. After cellular uptake, thyroid hormones can be activated and,

following binding to nuclear thyroid hormone receptor complexes, affect transcription. Deiodinase enzymes in tissues such as the liver, kidney, skeletal muscle and the thyroid itself, can activate the prohormone T4 into the active hormone T3 or deactivate the prohormone T4 into inactive reverse T3 (rT3) (7).

Pathophysiology

The influence of the thyroid axis is clearly apparent in its pathophysiology; the symptoms associated with hypo- (deficiency in circulating TH) and hyperthyroidism (surplus of circulating TH) are severe, devastating for the health and quality of life of the patient, and potentially fatal (5,6). Symptoms can range from weight gain, xerosis, hair loss, tiredness, dyslipidaemia, mood impairment and even myxoedema coma in the case of hypothyroidism; to weight loss, mood impairment, atrial fibrillation, muscle wasting and osteoporosis in hyperthyroidism (5,8).

Thyroid axis and ageing

Considering the vast implications of the thyroid axis in essential physiological processes, it is not surprising that it is also implicated in healthy ageing and longevity. Ageing is considered to be based on specific hallmarks: primary (genomic instability, telomere attrition, epigenetic alternations, loss of proteostasis), antagonistic (deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence) and integrative (stem cell exhaustion, altered intercellular communication)(4). The thyroid axis could influence multiple of these processes and thereby affect the rate of ageing. Indeed, various studies have shown a relationship between the thyroid axis and ageing and longevity (9-11).

In animal models, it has been shown that TH themselves may influence ageing adversely, as lower TH levels in various model organisms were associated with extended lifespan (10,12,13). The beneficial effects of low TH levels are attributed to lower basal metabolic rate and thereby lower levels of reactive oxygen species (ROS) and oxidative stress, the effects of which could lead to increased senescence (14). TH

can also adversely affect cell membranes as well as cell oxidant status, and even directly lead to increased cell senescence.

Moreover, the identification of TSH-R expression in tissues other than the thyroid, such as bone and adipose tissue (15,16), leaves possibility for TSH to exercise independent effects as well, not only through TH regulation but directly on tissues other than the thyroid gland.

In human studies, even within euthyroid range there is an association between lower thyroid status and ageing (11,17). Moreover, there is an increased prevalence of elevated TSH in the older population (18). Treating subclinical hypothyroidism (elevated circulating TSH in the absence of TH deficiency) in patients aged > 65 years has been shown through an extensive randomized controlled trial to have no apparent benefits (19) and other studies showed that familial longevity is actually associated with (genetic predisposition to) higher (normal) circulating TSH (9,20).

This thesis focuses on a subset of the participants from the Leiden Longevity Study (LLS, figure 2). The LLS was founded in 2002 specifically to study genotypes and phenotypes in healthy ageing (21). This unique cohort recruited long-lived siblings in the Netherlands without demographic restrictions other than age. Men, aged 89 and over, and women, aged 91 and over, respectively, were recruited in the study if they had another sibling of the same age criterium, thereby considering their family enriched for longevity. The offspring from this generation was also included in the cohort, as middle-aged subjects enriched for familial longevity, with their current partners as controls of similar age and with similar socio-economic background. In total, 421 families were included (22).

It has been confirmed that these long-lived siblings, as well as the offspring from these families, show familial (genetic and/or epigenetic) enrichment for extreme survival (21). Moreover, it was found that the offspring show less morbidity and mortality compared to controls (21).

Moreover, in LLS, a relationship was found between familial longevity and lower thyroid function (11) as well as low triiodothyronine levels (17). This does not seem to be the case in all familial longevity. In Ashkenazi centenarians and their offspring, another example of familial longevity, this relationship between the thyroid and longevity was not found (20). It could be that part of the LLS population has a lower thyroid setpoint, on which they thrive on lower TH levels and reap the benefit of longevity.

In 2015, the Switchbox consortium was established to investigate hormonal axes in longevity and healthy ageing. In a subgroup of 20 offspring and 18 controls from the LLS, a detailed observational study of thyroid hormone levels was performed - blood was sampled at high frequency (every 10 minutes) over a period of 24 hours and thyroid axis hormones measured at each time point. It was found that throughout the whole study period, offspring had on average a 0.8 mU/L higher circulating TSH levels than controls, despite an absence in differences in TH levels between the two groups (23). This unexpected finding sparked questions about the mechanism underlying this difference. The TSH bioactivity was not different between members of long-lived families and controls (23).

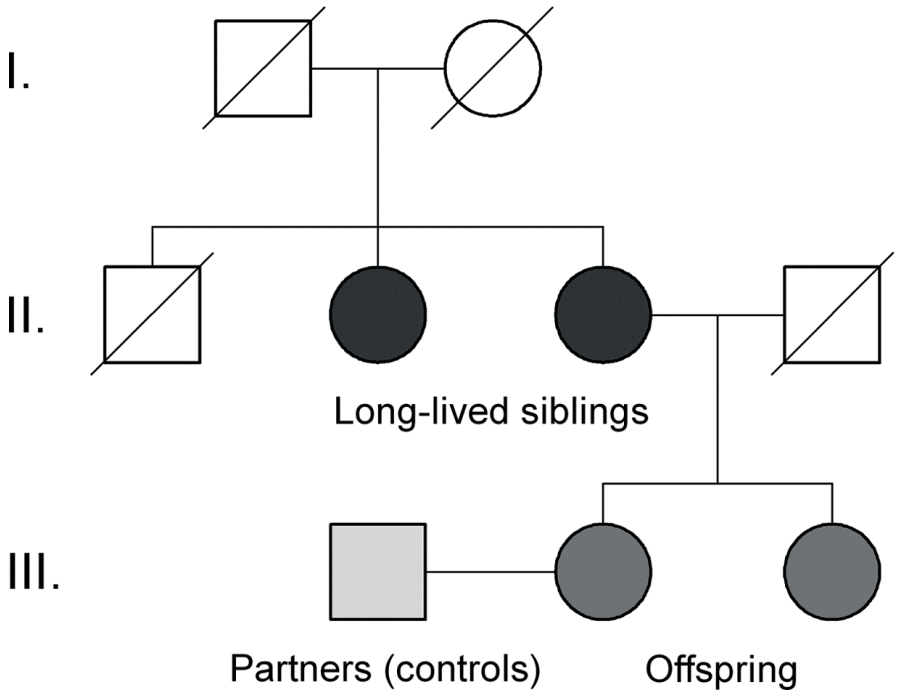

Figure 2. Leiden Longevity Study (LLS) – schematic representation of the design and family tree. Long-lived siblings (men aged 89 years or older, or women aged 91 years or older, with at least one sibling of the same age criterium) were considered enriched for familial longevity and were included in the LLS. They are descendants of the first generation. The offspring from these families are the third generation enriched for longevity, and their partners were included in the LLS as controls.

Hypothesis

In order to investigate the mechanism underlying the different thyroid axis status in members of long-lived families compared to controls, two hypotheses were formulated. In members of LLS where offspring had higher TSH than controls in the absence of differences in TH levels, we hypothesized that:

1) Offspring from long-lived families might have lower responsivity of the thyroid gland to stimulation by TSH, thereby needing higher levels for the same TH secretion,

and/or

2) Offspring from long-lived families might have higher turnover of TH in the circulation, thereby needing higher TSH levels in order to maintain adequate TH levels.

Objectives and rationale of this thesis

This thesis attempts to investigate the mechanism(s) underlying previously confirmed differences in thyroid status in offspring of long-lived families compared to controls in the Leiden Longevity Study.

The primary objective of these studies was to experimentally test the two hypotheses relating to TSH responsivity of the thyroid and TH turnover in members of long-lived families compared to controls. This was done by performing two thyroid axis challenge studies: one with recombinant human TSH (rhTSH, Thyrogen) and one with synthetic thyroid hormone triiodothyronine (T3) known as liothyronine (Cytomel).

The sec ondary objective of these studies was to explore secondary effects of these challenge studies on general physiology, tissue regeneration and the immune system through, during the challenge studies, obtaining data and performing analyses of: electrocardiography (ECG), metabolic parameters in blood and morning urine, markers of tissue regeneration (such as bone), immunological analysis through the collection of peripheral blood mononuclear cells (PBMCs), and biobanking of blood and urine samples for future analyses.

This thesis describes the first findings of two clinical challenge studies of the thyroid axis within the members of long-lived families and controls from the LLS. The layout is as follows: study design and data collection (Chapter 2), pilot study following the rhTSH challenge (Chapter 3), analysis of the primary outcomes in rhTSH challenge (Chapter 4), the primary outcomes in T3 challenge (Chapter 5) and first secondary outcomes - bone markers in rhTSH challenge study (Chapter 6). It is concluded with a general discussion placing the findings into context and providing future perspectives (Chapter 7).

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