Thyroid axis challenges in Leiden Longevity Study

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Chapter 7

General discussion and future perspectives

As the fraction of the older population increases, and with it the prevalence of chronic diseases and costs of health care, great effort is being made to understand healthy ageing. The thyroid axis plays an essential role in multiple physiologic processes and has been vastly implicated in ageing (1), making it one of the prime candidates of research and candidates for innovation.

The Leiden Longevity Study (LLS) (2) is an established cohort for investigating differences between offspring of long-lived families and controls of similar age and socio-economic background, and previous studies among LLS participants showed that families with a lower mortality history score had the highest thyroid stimulating hormone (TSH) and lower thyroid hormone (TH) levels (3). When the thyroid axis in LLS offspring and controls was investigated in more detail through a series of measurements every 10 minutes over a period of 24 hours, it was found that the circulating TSH levels were consistently higher in offspring than in controls (4). Interestingly, throughout the whole study period, the circulating TH levels were not higher but were comparable between offspring and controls (4). Theoretically, this finding could be explained by a lower TSH bioactivity in offspring. However, the TSH bioactivity was investigated and found not to be different between offspring and controls (4). Two other possible underlying mechanisms were formulated that were fundamental for the work described in this thesis, namely that:

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

and/or

ii) Offspring from long-lived families might have higher turnover of TH in the circulation, thereby requiring higher circulating TSH for adequate circulating TH levels.

Through two challenge studies, one with 0.1mg recombinant human TSH (rhTSH) in 30 LLS participants, and one with 100mcg synthetic triiodothyronine (T3) in 27 LLS participants, we found that offspring have lower thyroidal responsivity than controls, and that offspring have similar TH concentrations and T3 feedback on TSH levels. These findings support the first hypothesis, dismiss the second hypothesis, and make the combination of the two superfluous.

These findings indicate that the mechanism behind the different thyroid status in familial longevity found among the offspring of LLS lies in the responsivity to TSH of the thyroid gland. Although the precise mechanism causing the difference in TSH responsivity remains unknown, this could be related to the TSH receptor (TSH-R) rather than to a difference in the metabolism of TH.

The full study design used to investigate these hypotheses is outlined in **Chapter 2**. The inclusion of participants shows that almost two thirds of the initially approached participants were not eligible for the study, underlining the difficulty of performing intervention studies in a healthy, older population even in a dedicated cohort, due to high prevalence of chronic diseases, especially cardiovascular disease, in older age. Significant cardiovascular disease as well as other significant comorbidities were one of the main exclusion criteria for our study in order to minimise the risk of adverse events such as atrial fibrillation upon administration of rhTSH or TH. In future challenge studies with LLS participants, caution should be employed to prevent selection bias; due to the fact that morbidity is more prevalent among controls(2), selecting only healthy participants could lead to the selection of the healthiest controls, who might most resemble offspring in their phenotype, and not be truly representative of the control population, thus diluting potential differences between groups.

In **Chapter 3** we determined the optimal frequency of thyroid parameter measurements in a healthy, older population following a challenge with 0.1mg i.m. rhTSH by measuring samples from all 17 study time points in six participants. We

show that in the first 8 hours following 0.1mg i.m. rhTSH administration, TSH concentrations vary at relatively high frequency and may best be measured often (every 15 minutes), while measurement of TH is adequate at lower frequency of every two hours. All parameters may be measured at 24, 48 and 72 hours following rhTSH administration to observe their return to baseline. Thyroglobulin (Tg) displayed very low variability in the first 8 hours, and may be measured at baseline, and then 24 hour intervals following rhTSH administration. The measurement points suggested are comparable to those previously used in healthy younger and middle-aged populations, where parameters were measured at 2-4 hours intervals and for up to 72 hours(5,6). In future studies, as an addition to our time points, we advise repeated baseline measurements for calculation of mean baseline hormone levels.

The primary outcomes of the rhTSH challenge as measured in the whole cohort are outlined in **Chapter 4**. Following intramuscular administration of 0.1mg rhTSH, the ratio of area under the curve (AUC) of free T4 to AUC of TSH (AUC fT4/AUC TSH) as well as AUC Tg/AUC TSH ratio were lower in offspring than in controls. These novel findings indicate that offspring have lower thyroidal responsivity to TSH than controls.

The primary outcomes of the T3 challenge as measured in the whole cohort are outlined in **Chapter 5**. In a healthy, older population, following a challenge with 100mcg T3, offspring and controls had similar circulating T3 levels and similar circulating TSH levels, indicating similar turnover of T3 and similar negative feedback on TSH, respectively. In future studies, labelled T3 could be administered in a smaller sub group to indeed confirm similar turnover between offspring and controls.

In **Chapter 6**, exploratory measurements are reported of bone metabolism in offspring from long-lived families and controls at baseline and following the rhTSH challenge. TSH receptor has been identified on bone cells and is likely to enhance bone resorption. In our study, following the rhTSH challenge, the increase in bone marker levels was not different between offspring and controls. However, baseline

levels of circulating bone markers CTX and P1NP were lower in offspring than in controls, as was previously briefly shown in Switchbox study. This could be due to a preserved regenerative capacity in offspring compared to controls, in this case of bone tissue. Interestingly, our findings in bone markers following rhTSH administration do not show a clear difference in direct effects of TSH on bone tissue between offspring and controls, as could have been the case with differences in the TSH receptor. It is possible that any effect of the TSH receptor has been confounded by also increased circulating thyroid hormones. Unfortunately, in a study of a healthy human population it is not possible to discern increasing TSH from increasing TH. However, by performing bone marker measurements following a rhTSH challenge in a population of patients with thyroidectomy, direct effects of TSH on bone markers, regardless of TH, could be measured. It is not clear whether these patients also have variations of the TSH receptor.

In this thesis, for the first time, we describe in detail the circulating TSH and TH levels in a healthy, euthyroid, older population following 0.1mg i.m. rhTSH administration and also following 100 mcg oral T3 administration. The two challenge studies performed are complementary: one investigating the feed forward mechanism and one investigating the feedback mechanism of the thyroid axis in offspring of long-lived families and controls. The conclusions drawn align with results of previous work related to thyroid function and ageing in larger populations. In the Leiden 85 study, with 599 participants aged 85 years and followed-up for four years, higher circulating TSH has not been associated with adverse effects and has been associated with lower mortality rate(7). Moreover, in centenarians, there is an increased prevalence of higher circulating TSH(8), indicating its possible beneficial effects on longevity. A randomized controlled trial in an older population with subclinical hypothyroidism, where circulating TSH is elevated but TH are within euthyroid range, showed that treatment with TH levothyroxine is not beneficial compared to placebo(9).

The results of the two thyroid axis challenge studies for the first time implicate lower thyroidal responsivity to TSH as the mechanistic underpinning for the previously

found higher circulating TSH in the absence of differences in TH between offspring from long-lived families and controls(4). These findings indicate the likely importance of TSH and/or the TSH receptor in longevity. It is possible that the lower TSH receptor responsivity in offspring results in higher circulating TSH levels necessary for adequate TH release in offspring, and that this elevated, albeit within euthyroid range, circulating TSH carries longevity enhancing benefits. The TSH itself might have an effect on the activity of deiodinase enzymes in tissues and thereby regulate tissue specific TH availability. Through these effects, TSH could indirectly play an important role in local maintenance and repair processes, and thereby influence the chance of developing age-related diseases. Although tissue-specific TH levels are challenging (if not impossible) to manipulate in humans, animal models can be used to study tissue-specific inactivity or overexpression of TH and its effects on development of tissue-specific disease. Similar work has been performed by THYRAGE partners in bone, muscle and brain tissue(10-12). In the future, these studies could contribute to using TSH as means of tissue-specific delivery of TH.

Moreover, the TSH receptor has recently been identified on both bone tissue as well as in the thymus(13,14). Further investigation of the TSH receptor as well as TSH specific effects on tissues other than the thyroid are necessary. In future studies, thyroid biopsy in offspring from long-lived families and controls and TSH receptor genotyping could help identify differences between the TSH receptors in the two groups.

This thesis explored the primary outcomes of the thyroid axis challenge studies. However, direct effects of TSH on tissues other than the thyroid, as well as the secondary objectives of both challenge studies (such as cardiovascular effects, metabolomics and immunological factors such as the effect on peripheral blood mononuclear cells), remain to be investigated.

It is also possible that other hormonal axes, which influence the thyroid axis, are indirectly implicated in the differences in TSH status found between offspring and

controls. For example, dopamine regulates TSH secretion and could be lower in familial longevity, thereby contributing to higher circulating TSH levels. The longevity benefits attributed to higher circulating TSH could in part be due to lower dopamine levels. To test this hypothesis, future studies should measure circulating dopamine in relation to familial longevity, possibly in the study participants investigated in this thesis, or in other (healthy, older) populations with higher circulating TSH.

Identifying higher circulating TSH as implicated in familial longevity might also be of value for clinical practice, since higher circulating TSH might be of therapeutic importance. In animal studies, it has been shown that TSH administration can improve bone health by preventing and restoring bone loss(15). Since recombinant human TSH can be administered intramuscularly and intravenously in humans, and we show here that it does not cause adverse events at a low dose in a healthy older population, it may be useful for treating bone loss, for example in post-menopausal women under risk for developing osteoporosis, but also as a long-term supplement for enhancing healthy ageing. Of course, further studies are needed to investigate the benefits, length of intervention, number needed to treat and side-effects of such a treatment.

Healthy ageing remains a complex and nuanced subject not easily answered with a one-for-all solution(16). This thesis emphasizes the notion that the thyroid axis is vastly implicated in longevity and healthy ageing, through a great role of TSH and thyroid gland responsivity to TSH, possibly through the TSH receptor. Through this work of studying healthy ageing and deciphering its underlying mechanisms, we advance understanding of healthy ageing, and thereby strive to facilitate innovation in the field that will benefit individuals as well as society in the coming decades.

References

1. Bowers J, Terrien J, Clerget-Froidevaux MS, Gothie JD, Rozing MP, Westendorp RG, van Heemst D, Demeneix BA. Thyroid hormone signaling and homeostasis during aging. Endocr Rev. 2013;34(4):556-589.
2. Westendorp RG, van Heemst D, Rozing MP, Frolich M, Mooijaart SP, Blauw GJ, Beekman M, Heijmans BT, de Craen AJ, Slagboom PE, Leiden Longevity Study G. Nonagenarian siblings and their offspring display lower risk of mortality and morbidity than sporadic nonagenarians: The Leiden Longevity Study. J Am Geriatr Soc. 2009;57(9):1634-1637.
3. Rozing MP, Houwing-Duistermaat JJ, Slagboom PE, Beekman M, Frolich M, de Craen AJ, Westendorp RG, van Heemst D. Familial longevity is associated with decreased thyroid function. J Clin Endocrinol Metab. 2010;95(11):4979-4984.
4. Jansen SW, Akintola AA, Roelfsema F, van der Spoel E, Cobbaert CM, Ballieux BE, Egri P, Kvarta-Papp Z, Gereben B, Fekete C, Slagboom PE, van der Grond J, Demeneix BA, Pijl H, Westendorp RG, van Heemst D. Human longevity is characterised by high thyroid stimulating hormone secretion without altered energy metabolism. Sci Rep. 2015;5:11525.
5. Nielsen VE, Bonnema SJ, Hegedus L. Effects of 0.9 mg recombinant human thyrotropin on thyroid size and function in normal subjects: a randomized, double-blind, cross-over trial. J Clin Endocrinol Metab. 2004;89(5):2242-2247.
6. Torres MS, Ramirez L, Simkin PH, Braverman LE, Emerson CH. Effect of various doses of recombinant human thyrotropin on the thyroid radioactive iodine uptake and serum levels of thyroid hormones and thyroglobulin in normal subjects. J Clin Endocrinol Metab. 2001;86(4):1660-1664.
7. Gussekloo J, van Exel E, de Craen AJ, Meinders AE, Frolich M, Westendorp RG. Thyroid status, disability and cognitive function, and survival in old age. JAMA. 2004;292(21):2591-2599.
8. Sawin CT, Chopra D, Azizi F, Mannix JE, Bacharach P. The aging thyroid. Increased prevalence of elevated serum thyrotropin levels in the elderly. JAMA.

1979;242(3):247-250.
9. Stott DJ, Rodondi N, Kearney PM, Ford I, Westendorp RGJ, Mooijaart SP, Sattar N, Aubert CE, Aujesky D, Bauer DC, Baumgartner C, Blum MR, Browne JP, Byrne S, Collet TH, Dekkers OM, den Elzen WPJ, Du Puy RS, Ellis G, Feller M, Floriani C, Hendry K, Hurley C, Jukema JW, Kean S, Kelly M, Krebs D, Langhorne P, McCarthy G, McCarthy V, McConnachie A, McDade M, Messow M, O'Flynn A, O'Riordan D, Poortvliet RKE, Quinn TJ, Russell A, Sinnott C, Smit JWA, Van Dorland HA, Walsh KA, Walsh EK, Watt T, Wilson R, Gussekloo J, Group TS. Thyroid Hormone Therapy for Older Adults with Subclinical Hypothyroidism. N Engl J Med. 2017;376(26):2534-2544.
10. Williams GR, Bassett JHD. Thyroid diseases and bone health. J Endocrinol Invest. 2018;41(1):99-109.
11. Sagliocchi S, Cicatiello AG, Di Cicco E, Ambrosio R, Miro C, Di Girolamo D, Nappi A, Mancino G, De Stefano MA, Luongo C, Raia M, Ogawa-Wong AN, Zavacki AM, Paladino S, Salvatore D, Dentice M. The thyroid hormone activating enzyme, type 2 deiodinase, induces myogenic differentiation by regulating mitochondrial metabolism and reducing oxidative stress. Redox Biol. 2019;24:101228.
12. Gothie JD, Demeneix B, Remaud S. Comparative approaches to understanding thyroid hormone regulation of neurogenesis. Mol Cell Endocrinol. 2017;459:104-115.
13. Abe E, Marians RC, Yu W, Wu XB, Ando T, Li Y, Iqbal J, Eldeiry L, Rajendren G, Blair HC, Davies TF, Zaidi M. TSH is a negative regulator of skeletal remodeling. Cell. 2003;115(2):151-162.
14. Dutton CM, Joba W, Spitzweg C, Heufelder AE, Bahn RS. Thyrotropin receptor expression in adrenal, kidney, and thymus. Thyroid. 1997;7(6):879-884.
15. Sun L, Vukicevic S, Baliram R, Yang G, Sendak R, McPherson J, Zhu LL, Iqbal J, Latif R, Natrajan A, Arabi A, Yamoah K, Moonga BS, Gabet Y, Davies TF, Bab I, Abe E, Sampath K, Zaidi M. Intermittent recombinant TSH injections prevent ovariectomy-induced bone loss. Proc Natl Acad Sci U S A. 2008;105(11):4289-4294.
16. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153(6):1194-1217.