CHAPTER 9

General discussion

GENERAL DISCUSSION

The overall hypothesis of the Switchbox study is that maintenance of homeostasis is pivotal for maintenance of health in old age. Therefore, the aim of this thesis as part of the Switchbox study, was to expand our knowledge of homeostatic mechanisms at old age, thus trying to unravel underlying mechanisms of healthy human longevity. The primary endocrine systems of investigation of this thesis were the hypothalamic-pituitary-thyroid (HPT) and the hypothalamic-pituitary-adrenal (HPA) axes.

Previous research, done on thyroid function in relation with human longevity in the Leiden Longevity Study (LLS) found that families with the lowest family mortality history score, had the highest levels of thyroid stimulating hormone (TSH) and the lowest levels of free thyroxine (fT4) and free triiodothyronine (fT3)(1). Moreover in the Leiden 85-plus study, survival advantages were associated with higher TSH levels in 85 and 90 years old participants(2, 3). Also in other cohorts high TSH was associated with longevity(4). Taken together, this may imply that lower thyroid status is a heritable phenotype that contributes to exceptional longevity. However, underlying mechanisms and the effects of changes in HPT-axis function on familial longevity remained elusive.

The HPA-axis is the most important neuro-endocrine stress response system of our body and is of critical importance for survival. Changes in HPA-axis function are associated with different diseases including diabetes, hypertension, high blood pressure and insulin resistance(5-9); however no data is available on the changes in HPA-axis function and human longevity.

Hypothalamic-pituitary-thyroid axis function in longevity

Although we and others have previously found multiple indications for changes in the HPT-axis in human longevity, the precise mechanisms behind these findings and their physiological effects were not yet established(3). In chapter 4 we found that familial longevity was characterized by higher thyroid stimulating hormone (TSH) secretion, in the absence of differences in thyroid hormone (TH) levels and energy metabolism. In this study we explored a number of different candidate mechanisms that might underlie the increased TSH secretion (Figure 1A). One potential mechanism was reduced bioactivity of TSH (Figure 1B) in the offspring; however in both offspring and partners TSH was equally bioactive. Moreover, we considered diminished sensitivity of the thyrotrophs to negative feedback by thyroid hormones less likely, because if thyrotrophs would be less sensitive, the levels of fT4 would also increase(10). Furthermore, the regularity of TSH secretion (assessed by approximate entropy (ApEn) of TSH) was comparable between groups, which is indicative of intact thyroid hormone mediated feedback on TRH and TSH secretion (Figure 1C)(11). Another explanation of the increased TSH secretion was enhanced thyroid hormone turnover in peripheral tissues (Figure 1D). This is likely not the case in our study

population, since the T3/reverse T3 (rT3) ratio, which is correlated with deiodinase 1 activity in the liver(12), was not different between the groups. **In chapter 5** we further investigated the underlying mechanisms for the increased total TSH secretion by studying ultradian and circadian rhythmicity of TSH. There were no differences between offspring and partners in the pulsatile secretion of TSH or in the TSH circadian rhythmicity, both measures which have previously been associated with ageing and disease(11, 13, 14). We found that the increase in total TSH secretion was fully attributable to increased basal (non-pulsatile) TSH secretion (Figure 1A). Besides Thyrotropin releasing hormone (TRH), TSH is also under the influence of somatostatin (SST), glucocorticoids and dopamine. We considered changes in SST and glucocorticoids as causes of enhanced TSH secretion unlikely, since there were no differences in leptin levels and body weight or in ACTH and cortisol levels between the groups (**chapter 7**). Taken together, three remaining possible underlying mechanisms for the increased TSH secretion might be 1) diminished responsiveness of the thyroid gland to TSH, thus overall more TSH would be needed to ensure the same amount of thyroid hormone output (Figure 1 E) diminished central dopaminergic tonus or 3) a combination of both. It is a hypothetical possibility that offspring from their birth onwards have a lower dopaminergic tone, leading to decreased suppression of TSH (more TSH) secretion and that the decreased thyroidal sensitivity to TSH is a compensatory mechanisms to maintain fT4 and fT3 within the normal range to maintain energy homeostasis. To test these hypotheses, future experiments should focus on challenge experiments with a low dose of thyrogen (recombinant TSH), which significantly increases TSH and TH levels in healthy volunteers(15) or bromocriptine(16), a dopamine agonist, which lowers TSH secretion, in offspring of long-lived siblings and their partners. Many ageing theories have linked energy metabolism to the ageing process, including the 'rate of living theory' which states that the positive correlation between lifespan and size implicates that species differences in resting metabolic rate and the 'free radical theory of ageing' which proposed that byproducts of oxidative metabolism may underlie the negative correlation between life span and resting metabolic rate. In **chapter 4 and 5** we did not find differences in circulatory thyroid hormone levels (fT3, fT4 and T3) between the groups of offspring and partners included in the Switchbox study. In line in **chapter 4** we did not find differences between groups in resting metabolic rate or in core body temperature, implying that differences in energy metabolism are not likely an underlying mechanisms for healthy familial longevity. However besides metabolism, the HPT-axis plays also a key role in developmental processes. In adult mammalian tissues, damaged and worn-out mature cells are continuously being replaced during normal tissue homeostasis and in response to stress or damage. The decline in regenerative capacity of tissues is a characteristic of ageing as is stated in the stem cell theory of ageing(17). This process is dependent on differentiation of

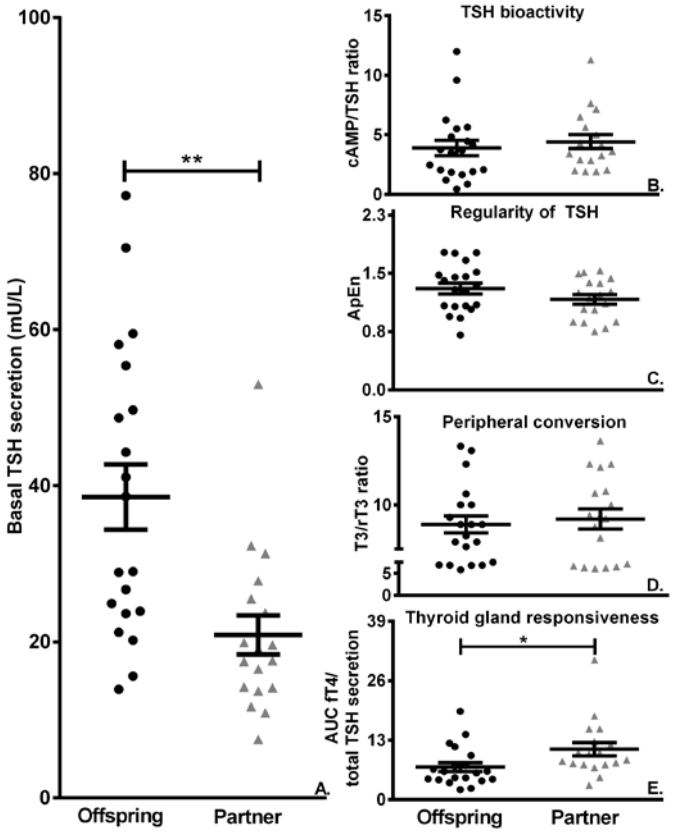

Figure 1 Exploration of mechanisms underlying increased levels of TSH secretion in offspring from long-lived siblings and partners.

Black circles represent 20 offspring, grey triangles represent 18 partners. Solid lines represent mean with standard error of the mean (SEM) of **A**. basal TSH secretion **B**. cAMP/TSH ratio **C**. ApEn **D**. T3/rT3 ratio **E**. AUC fT4/total TSH secretion. AUC= area under the curve. * $P < 0.05$ ** $P \leq 0.01$.

self-renewing, tissue-specific stem cells. Recently, it was found that the TSH-receptor was expressed on bone marrow-derived mesenchymal stem cells in humans(18). Moreover, TSH was found to induce gene expression of mediators involved in self-renewal, maintenance, development and differentiation(18). If TSH may play a role in self-renewal, maintenance, development and differentiation of (mesenchymal) stem cells, we hypothesize that high TSH might prevent precocious depletion of tissue specific stem cells by slowing tissue turnover rates. For example, during bone

remodeling, bone is renewed by a balanced process of resorption of old bone and new bone formation preventing damage accumulation and maintaining bone strength and mineral homeostasis. To investigate our hypothesis we measured bone turnover markers (e.g. β-crosslaps, P1NP and Alkaline Phosphatase) and found indications that osteoclastogenesis may be suppressed by high circulatory TSH levels. Besides on bone cells and mesenchymal stem cells, the TSH-receptor is also expressed in brown adipose tissue (BAT), skeletal muscles and the brain. Therefore we suggest that pleiotropic effects of the HPT-axis may protect long-lived families by extra-thyroidal effects of TSH on target tissues and cells that are important for maintenance of health up to old age.

Hypothalamic-pituitary-adrenal axis function in longevity

Many different adverse conditions have been associated with changes in HPA-axis function, however no data is available on HPA-axis function in relation with longevity. Therefore, in **chapter 6,** in 330 offspring and partners from the LLS, cortisol was measured from saliva samples collected in a home based setting in the morning, to assess the awakening response, and in the evening. Moreover, to test HPA-axis feedback sensitivity an 0.5 mg overnight dexamethasone test was performed. We observed that offspring from long-lived siblings had a slightly lower cortisol awakening response and lower cortisol levels in the evening. However, no differences were found in the HPA-axis feedback sensitivity. This may indicate that offspring from long-lived siblings had a slightly lower HPA-axis function. To further explore the HPA-axis function in relation with familial longevity, we measured in 38 offspring and partners from the LLS, 24-hour levels of both cortisol and ACTH. Since cortisol alone does not reflect HPA-axis activity. In **chapter 7,** we used state of the art mathematical models to study secretion profiles of ACTH and cortisol in a relative small subgroup under resting conditions in a laboratory setting. We found no significant differences between offspring and partners in 24-hour mean plasma concentrations of ACTH and serum cortisol concentrations, although we did find some modest, sex-specific differences, including non-significantly higher mean plasma ACTH levels in female offspring and significantly higher basal ACTH secretion in male offspring. Likewise, offspring and partners did not exhibit major differences in secretory regularity of ACTH and cortisol or feedforward and feedback synchrony and the endogenous ACTH-cortisol dose-response relationship. These results were seemingly conflicting with the differences observed in saliva cortisol in a much larger study sample (**chapter 6**). One of the explanations may be the strict inclusion and exclusion-criteria for the 24-hour blood sampling study that may have resulted in the inclusion of very healthy offspring and partners. Another explanation may be the difference in study setting; the study described in **chapter 6** was in a home-based setting while the study in **chapter 7** was performed under laboratory conditions. A third possible

explanation is the difference in sampling methods. In **chapter 6** we investigated saliva cortisol levels, which correlate most with free cortisol levels, while in **chapter 7**, serum cortisol levels were measured which predominantly reflect total cortisol. Under resting conditions, 70% of the cortisol is bound to Corticosteroid Binding Globulin (CBG), 20% is bound to albumin and only 10% of the cortisol is unbound and in its free form(19). Possibly, the proportion between bound and unbound cortisol is different between offspring and partners. Moreover, the sample size in the 24-hour ACTH and cortisol measurements study was small compared to that of the saliva cortisol sample study. As a consequence, it may thus only have been possible to detect relatively large differences between groups, such as the 60% higher TSH secretion in the offspring group (**chapter 4** and **chapter 5**).

Overall we may conclude that familial longevity is not associated with major differences in the HPA-axis activity under resting conditions, although modest, sex-specific differences may exist between the groups that are clinically relevant. Since the differences in resting conditions were small, in **chapter 8** we challenged the HPA-axis using the Trier Social Stress Test (TSST), to induce acute social stress. The TSST is a well validated laboratory stress test also up to higher ages(20). We found that male offspring enriched for familial longevity compared to male non-offspring may have a slightly lower overall physiological response to a psychological stressor. Moreover, we found that offspring were more relaxed than non-offspring in the run-up to participating in the experiment, although during the stress experiment both scored comparable for their subjective stress. Cortisol acts through 2 types of receptors which both have a different function during the stress reaction. The mineralcorticoid receptor (MR) is involved in the appraisal process and the onset of the stress response, the glucocorticoid receptor (GR) is only activated by large amounts of corticosteroids and is involved in termination of the stress reaction(21). Different SNP`s of the MR are associated with positive appraisal of a stressor(22). Moreover, during ageing the MR expression is lower resulting in increased ACTH secretion. As mentioned above we found **in chapter 7** in female offspring a non-significant tendency towards higher mean plasma ACTH concentrations compared to female controls and in male offspring higher basal ACTH secretion compared to male controls. In line, the Brown Norway rat, who are long-living, is characterized by unchanged serum corticosterone levels with amplified ACTH secretion and a faster recovery after restraint stress(23, 24). Based on the findings of the TSST another explanation for the differences in findings under resting conditions emerges. In the home based settings, participants had to do the experiment themselves, and partners might have worried more in anticipation of the experiment while the offspring were more relaxed and therefore had lower cortisol levels. In the study under laboratory conditions, both offspring and partners were already in the hospital the day before and were both adapted to their new situation.

Thus, offspring enriched for longevity tended to have slightly lower salivary cortisol levels in a home based setting. This may be a reflection of their more healthy phenotype. In addition offspring may tend to worry less prior to and have a lower peak when confronted with an actual stressor. During one`s life one is repetitively exposed to social stressors, such as (negative) social interactions with -or evaluations from- family or (voluntary) work. So offspring from long-lived families may tend to worry less prior to events, which together with a lower starting point in physiological responses, and a lower peak when confronted with an actual stressor, might limit damage due to stress over a lifetime.

Future perspectives

This thesis explored homeostatic mechanisms, in particular the hypothalamic-pituitary –thyroid- and –adrenal axis, and gave insight in healthy human longevity. First we found that offspring from long-lived siblings had a 60% higher TSH secretion, without changes in thyroid hormones (fT4 and fT3). In line, there were no differences between offspring and partners in available measures of energy metabolism (basal metabolic rate and core body temperature). Second, offspring tended to have lower saliva (free) cortisol in the morning and evening compared to partners. However, no major differences comparable to those observed for TSH, were detected in the regulation of ACTH and cortisol over 24 hours under resting conditions. However, offspring tended to have a smaller overall cortisol output in response to stress and tented to worry less prior to a stressful event. These results indicate that subtle differences in the HPA-axis between groups may exist and underpin the important role of challenge experiments in amplifying such subtle differences.

Future studies should aim to disentangle underlying mechanisms of the increased TSH secretion and lower overall cortisol output during a stress experiment in offspring. One underlying mechanism that might explain both the increased TSH levels and lower overall cortisol response to stress in offspring compared to their partners is a lower dopamine release. Future experiments may therefore focus on dopamine release in relation with familial longevity. One way in humans is to measure prolactin secretion, which is mainly regulated by dopamine. Moreover, futures studies should focus on performing challenge experiments using recombinant TSH (Thyrogen) to test the resistance of the thyroid gland for TSH, which also might be an underlying mechanism of the increased TSH. Moreover the extra-thyroidal effects of TSH should be studied in more detail, since this may result in therapeutic options. For example, recently it was found that intermittent injections with recombinant TSH can prevent and restore bone loss, at least in mice(25). Moreover we should explore the underlying mechanisms, that explain why offspring tend to worry less prior to stress full events and have a smaller overall cortisol output during a stress experiment as these are also of clinical relevance in our 24.7 society.

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