The endocrinology of familial longevity : time series analyses of different hormonal axes and their interrelationships

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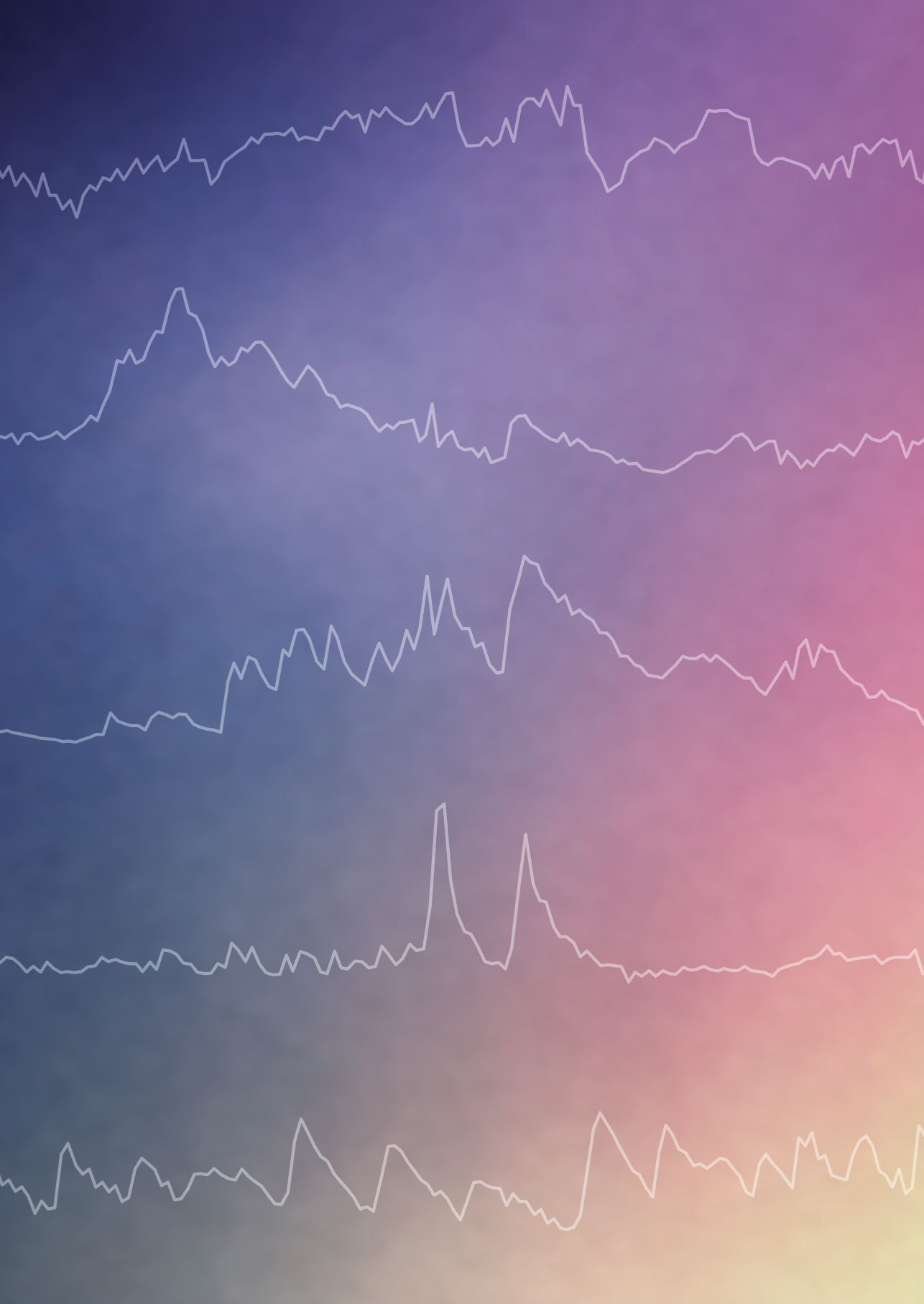

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General discussion

GENERAL DISCUSSION

Ageing

Ageing has been described as "a progressive, generalized impairment of function, resulting in an increasing vulnerability to environmental challenge and a growing risk of disease and death" [1]. It is hypothesized that due to evolved limitations in maintenance and repair, molecular damage will accumulate gradually, and interfere with the integrity of cells and tissues, thus driving functional decline and risk of disease and death. From this hypothesis, it follows that the rate of ageing is largely determined by the balance between damage accumulation and defence and repair mechanisms. Many external and internal factors can affect this balance. Among these, neuro-endocrine pathways, which are centrally regulated by the brain, are thought to play an important regulatory role [2]. The common goal of these neuro-endocrine pathways is to maintain homeostasis in the body by detecting changes in the environment and by orchestrating a coordinated physiological response to these signals. In this context, one of the best known neuro-endocrine pathways is the insulin/insulin-like growth factor 1 (IGF-1) signalling (IIS) pathway. This pathway regulates metabolism and growth in invertebrates, including yeast, *Caenorhabditis Elegans* (*C. Elegans*), and *Drosophila melanogaster*, but also in mammals, including mice and human. In invertebrates, central regulation of the IIS pathway occurs via the production of different insulin/IGF-1 like neuropeptides. In mammals, the IIS pathway is divided into two separate, but partly overlapping, signalling pathways; the insulin signalling pathway regulating primarily glucose and lipid metabolism and the growth hormone (GH)/IGF-1 pathway, regulating primarily linear and cellular growth. The upstream regulatory hypothalamic factors and the secretion of GH from the pituitary are present in mammals, but not in invertebrates. Despite differences in complexity and regulation, the core downstream components of the IIS pathway are conserved between invertebrates and mammals.

Neuro-endocrine control of ageing in animal models

Reduced insulin/IGF-1 signalling has been consistently associated with longevity in various model organisms, from yeast to mice [3-10]. For example, the lifespan of *C. Elegans* can be prolonged from 2-3 weeks up to 6 months when mutations in the daf-2 gene, which is homologous to the insulin receptor, were made [11]. Actually, mutations in almost all core components of the IIS are associated with extended lifespan in *C. Elegans*. Also, a loss of function mutation in the insulin receptor of *Drosophila* has been associated with prolonged lifespan [10]. As another example, the health- and lifespan of mice lacking functional growth hormone releasing hormone (GHRH) or GH receptors is prolonged

compared with wildtype controls [12, 13]. In these animal models, the prolonged lifespan comes at the costs of reduced reproductive capacity and growth, indicating the existence of trade-offs. These studies indicate that not only the IIS pathway is evolutionary conserved, but that also the link between reduced insulin/IGF-1 signalling and longevity is an evolutionary conserved mechanism. The ligands, receptors and downstream signalling molecules that were found to have a major impact on longevity in yeast, fruit flies, and worms are homologous to proteins from the insulin/IGF-1 pathway in mammals. Moreover, these conserved proteins are regulated by GH in mammals.

Neuro-endocrine control of human ageing

The studies in animal models demonstrated the important role of neuro-endocrine pathways in the regulation of ageing. However, the relations between neuro-endocrine parameters and the human ageing process are complex and not fully understood. The main neuro-endocrine pathways in human are the hypothalamic-pituitary-target gland axes, consisting of the somatotropic, thyroid, adrenal, gonadal, and prolactin axes (Figure 1). In previous studies with participants of the Switchbox Study, a sub-study of the Leiden Longevity Study, associations between familial longevity and hormones of the hypothalamic-pituitary-thyroid (HPT) and the hypothalamic-pituitary-adrenal (HPA) axes have been investigated. It was found that offspring of long-lived families and their partners had no major differences in HPA axis activity, but the offspring had higher total thyroid-stimulating hormone (TSH) secretion compared to partners [14, 15]. Whether familial longevity is also associated with altered endocrine features in other hypothalamic-pituitary-target gland axes or their interplay had not been investigated yet. Since offspring and partners had similar thyroid hormone levels, resting metabolic rate, and core body temperature, we hypothesized that pleiotropic effects of the HPT axis cause the favourable effects on lifespan, for example on tissue maintenance. Interestingly, preliminary data indicated that levels of biomarkers of bone turnover differed between offspring of long-lived families and partners. In this thesis, we focussed on the roles of the hypothalamic-pituitary-somatotropic and hypothalamic-pituitary-gonadal axes in familial longevity, the interrelationships between hormones of the hypothalamic-pituitary-target gland axes, and the 24-h profiles of bone turnover markers in healthy older subjects.

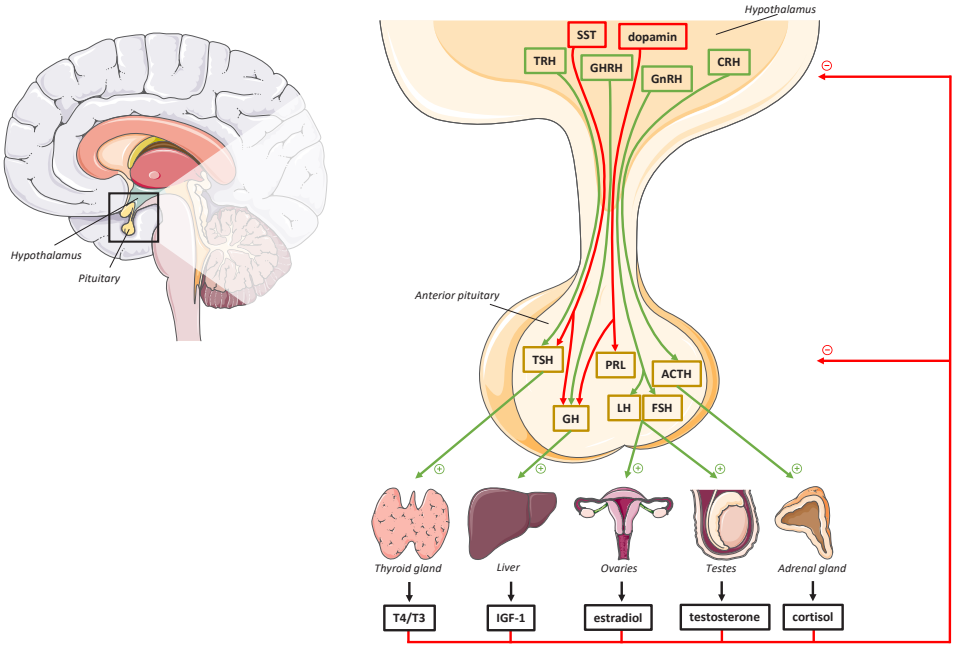

Figure 1. Hypothalamic-pituitary-target gland axes.

Schematic representation of the (regulation of the) human hypothalamic-pituitary-target gland axes; hypothalamic-pituitary-thyroid axis, hypothalamic-pituitary-somatotropic axis, hypothalamic-pituitary-gonodal axis, hypothalamic-pituitary-prolactin axis, and hypothalamic-pituitary-adrenal axis. The green arrows indicate the stimulation of hormone secretion and the red arrows the inhibition of hormone secretion. Figures of tissues/organs were adapted from Servier Medical Art, *https://smart.servier.com/*. Abbreviations:

ACTH: adrenocorticotropic hormone
CRH: corticotropin-releasing hormone
FSH: follicle-stimulating hormone
GH: growth hormone
GHRH: GH-releasing hormone
GnRH: gonadotropin-releasing hormone
IGF-1: insulin-like growth factor 1
LH: luteinizing hormone
PRL: prolactin
SST: somatostatin
T3: triiodothyronine
T4: thyroxine
TRH: thyrotropin-releasing hormone
TSH: thyroid-stimulating hormone

Hypothalamic-pituitary-somatotropic axis and familial longevity

The hypothalamic-pituitary-somatotropic axis (GH/IGF-1 axis) consists of hypothalamic growth hormone releasing hormone (GHRH), GH from the pituitary and IGF-1 mainly produced by the liver (Figure 1). In **Chapter 2** of this thesis, we investigated whether circulating IGF-1 axis parameters were associated with old age survival and functional status in nonagenarian siblings from the LLS. Studies on circulating IGF-1 and mortality have yielded inconclusive results since both low and high IGF-1 concentrations are associated with increased mortality. It is important to note that circulatory levels of IGF-1 predominantly reflect IGF-1 produced by the liver. Also other tissues produce IGF-1, which

is mainly GH-independent, and these paracrine IGF-1 concentrations are likely to be much higher than plasma concentrations. Unfortunately, these are difficult, if not impossible, to measure in human. Furthermore, GH was not included in this study since GH is secreted in a pulsatile manner and has a short half-life and should therefore be measured in blood which was sampled frequently over the day to obtain reliable information. This type of study was however not possible in this study population. Circulatory IGF-1 is more stable over a 24-h period than GH. Both GH and IGF-1 are bound to binding proteins, but only 40% of GH is bound to binding proteins while 99% of IGF-1 is bound to binding proteins, of which IGFBP3 is most abundant, which largely explains the longer half-life of IGF-1. In Chapter 2, it was observed that lower IGF-1/IGFBP3 molar ratios, which are indicative of reduced IGF-1 availability, were associated with improved survival. Furthermore, nonagenarians in the lowest quartile of IGF-1/IGFBP3 ratios had the highest scores for both Activities of Daily Living (ADL) scales and Instrumental ADL scales, indicating better functional status. It is unknown whether these long-lived nonagenarians only had reduced IGF-1 availability at old age or whether these nonagenarians already had reduced IGF-1 availability at younger age. Unfortunately, we do not have circulating levels of their IGF-1 parameters at young age, but we did include their offspring in the Switchbox Study, of which the results are discussed in **Chapter 3**.

In Chapter 3, we found an association between lower GH secretion and familial longevity. No significant differences were observed in circulating levels of IGF-1 and IGFBP3 between offspring and controls, although these tended to be somewhat lower in the offspring. As the study discussed in Chapter 2, this study was also a cross-sectional study, so we do not know whether these healthy older individuals had a reduction in their GH secretion at older age or already had low GH secretion at younger age. These results could indicate that GH secretion is more important for longevity than circulating IGF-1. In support of this hypothesis, the impact of disrupting GH signalling on longevity is larger than the impact of disrupting IGF-1 signalling or events downstream from IGF-1 receptors in laboratory mice [16].

Although GH and IGF-1 are part of the same signalling pathway and stimulate growth, the short-term effects of GH and IGF-1 are complementary. Acute effects of GH can be seen as anti-insulinogenic effects, since GH inhibits glucose uptake by muscle, stimulates lipolysis in adipose tissue, and stimulates gluconeogenesis in the liver, while IGF-1 has similar effects as insulin, especially on the muscle by stimulating protein synthesis and the uptake of glucose and amino acids. Moreover, GH has no direct effect on longitudinal growth, but only indirect via IGF-1. This could explain the fact that the offspring had

similar height as their partners, since offspring had lower GH secretion, but similar IGF-1 levels compared to partners. The lifespan-extending mutations in yeast, fruit flies, worms and mice lead to an accumulation of glycogen and/or fat. Surprisingly, although all these mice are obese, mice with reduced GH action are insulin sensitive and the mice with reduced IGF-1 action are insulin resistant. This observation supports the fact that GH and IGF-1 also have opposite/independent effects besides their similar effects.

The mechanisms by which reduced GH/IGF-1 signalling is leading to ageing and longevity have mainly been investigated in mouse models and reviewed by Bartke *et al.* [8, 17, 18]. It was among others observed that mice with reduced GH signals had beneficial alterations in their anti-oxidant defence mechanisms, stress resistance, and oxidative damage. Furthermore, reduced GH signals led to increased fatty acid oxidation and insulin sensitivity, but a decrease in both IGF-1 and mTOR signalling. Moreover, the number of senescent cells and inflammatory processes were decreased in mice with reduced GH signalling. All these processes are integrated in an interactive network that is associated with decreased risk of cancer, diabetes, and other age-related diseases, and also with longevity. Whether the same mechanisms will be present in humans is not completely clear. Unravelling these mechanisms in human could lead to potential starting points for intervening in the ageing process and hopefully eventually lead to healthy human ageing.

Studies in patients with a genetic alteration in the GH/IGF-1 pathway causing GH-deficiency or GH-resistance show contradictory results on health and longevity. Laron syndrome dwarfs with GH receptor gene mutations causing congenital growth hormone resistance were found to be protected against cancer and have relatively long lifespans [19, 20]. However, the lifespan of a group of 11 patients with untreated late onset isolated GH deficiency in Switzerland were relatively short compared to their unaffected siblings and these patients had an increased risk of cardiovascular disease [21]. Patients and animal models carry mutations that have a large impact on GH action, while subjects from the Switchbox Study do not have this extreme phenotype but rather display a more subtle alteration in their GH/IGF-1 axis. It is therefore an interesting observation that already a small difference in GH secretion is associated with familial longevity. The association between the GH/IGF-1 axis and familial longevity have also been investigated in other human studies. For example, a study in Italy showed that the IGF-1 bioactivity, levels of total IGF-1, and the IGF-1/IGFBP3 ratio were lower in the offspring of centenarians compared to an age-matched control group [22]. Furthermore, the female offspring of Askenazi Jewish centenarians displayed several signs of relative IGF-1 resistance, including higher serum IGF-1 levels and a smaller stature [23]. Since these

studies were cross-sectional and group-based, it makes it more difficult to unravel mechanisms underlying the link between reduced GH action and longevity in this population. It is important to keep this in mind when comparing (our) observations in humans to the studies in animal models.

Taken together, these observations led to the strong and convincing conclusion that reduced GH/IGF-1 signalling leads to a prolonged health- and lifespan. However, since GH levels decline with age and because of beneficial effects of GH on muscle and adipose tissue, reduced GH levels can also be viewed as a result, or even a cause, of the ageing phenotype. In general, it is assumed that most of the age-related changes are not beneficial for health- and lifespan. For example, ageing is also associated with a decline in cognitive functioning, mobility, and insulin sensitivity. However, anything that is beneficial at young age does not necessarily have to be beneficial at old age as well, and maybe it is sometimes even the other way around. Some changes with age could actually be beneficial, especially if these reflect an adaptive response of the body to survive, since these survival responses could lead to prolonged health- and lifespan. The decline in circulating GH and IGF-1 levels could reflect such a survival response and it could indicate that more energy is invested in maintenance and repair instead of in growth. However, studies in mice showed that especially reduced GH signalling during early life is important for longevity. For example, exposure to GH treatment early in life reduced longevity of dwarf mice [24].

Besides lower GH secretion, we also found in Chapter 3 that GH secretion was more tightly controlled in the offspring of long-lived families compared with their partners. This could indicate that the timing and regulation of a hormone is just as, or even more, important than total hormone levels. When the right amount of a hormone is secreted at the right moment, no waste of hormone resources occurs. More importantly, saving resources of the body could potentially lead to a longer health- and lifespan since it can be argued that less energy is invested in growth and development and more energy can be invested in maintenance and repair. This is in line with our hypothesis that longevity is associated with a prolonged ability to preserve the optimal balance between investments in growth and development versus maintenance and repair throughout life. Both low GH secretion as well as its tighter regulation could lead to preserved hormone resources. To test this hypothesis in future studies, the association between GH/IGF-1 signalling and biomarkers of tissue maintenance could be investigated.

Hypothalamic-pituitary-gonadal axis and familial longevity

Another hormonal axis is the hypothalamic-pituitary-gonadal (HPG) axis, which consists of hypothalamic gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary, and gonadal secretion of testosterone in men and oestrogen in women (Figure 1). As GH and IGF-1 levels, testosterone and oestrogen levels also decrease with age, with an acceleration after menopause in women. A later age of menopause was associated with reduced female mortality [25]. In model organisms, many long-lived mutants have a reduced reproductive output. Also in humans, decreased reproduction was found to associate with exceptional human longevity in both men and women [26]. Longevity inducing interventions, such as fasting, led to a decline in LH secretory burst mass and testosterone concentrations and to an increase in LH release pattern orderliness [27, 28]. In **Chapter 4**, we investigated in healthy male middle-aged participants whether familial longevity is associated with altered LH and testosterone secretion. We found no major differences in features of the HPG axis between male offspring of long-lived families and the control group. This could be related to the selection criteria of the study population, which were based on the presence of at least two long-lived siblings, so we may have selected on fertility, and the strict inclusion criteria on health. We also did not observe differences in single measurements of oestradiol and prolactin in a 24-h pool between offspring of long-lived families and controls, neither in men nor in women. Although this was a relatively small study, and therefore possibly underpowered, these results suggest that the HPG axis is not involved in familial longevity.

Interrelationships between hormones of the hypothalamic-pituitary-target gland axes

Up to now, most research on the associations between neuro-endocrine pathways and longevity is performed for each neuro-endocrine pathway separately. The interplay between neuro-endocrine pathways have rarely been addressed, which is surprising since neuro-endocrine pathways are highly linked with each other. For example, anterior pituitary cells share the same embryonic origin; the anterior pituitary is derived from oral ectoderm [29]. During the embryonic development of the anterior pituitary, specific genes direct the cells toward a particular fate. For lactotrophs, somatotrophs, and thyrotrophs, the same genes are involved in their development until the final differentiation. This means that lactotrophs, somatotrophs, and thyrotrophs largely share the same developmental cascade. Moreover, there is evidence for crosstalk between pituitary cells [30]; studies in rats showed that there is functional overlap between the different anterior pituitary cell types and many anterior pituitary cells respond to more than one

hypothalamic-releasing hormone [31, 32]. Furthermore, the main goal of these neuro-endocrine pathways is to maintain homeostasis in the body by detecting changes in the environment and by responding to these signals. It is therefore very likely that these pathways interact and/or have shared actions. When investigating each hormonal axis separately, we found in the Switchbox Study that altered endocrine features of the thyroid and somatotropic axes were associated with familial longevity. These associations might reflect separate mechanisms, but these hormonal changes could also be synchronized with each other and their concerted impact might be larger than the sum of their individual impacts on ageing and longevity. Also in other systems and organs of the body, interplay, interaction, and networks are highly important for maintenance of homeostasis and proper functioning. Additionally, the current understanding is that all hormones in a certain hormonal axis have the same primary actions. However, for example within the somatotropic axis this is not the case since GH and IGF-1 have diverse effects. This could therefore also be the case for the other hypothalamic-pituitary-target gland axes. The interrelationship between TSH and adrenocorticotropic hormone (ACTH) could for example be different from the interrelationship between TSH and cortisol.

Therefore, in **Chapter 5** of this thesis, we investigated the interrelationships between hormones of hypothalamic-pituitary-target gland axes within axes as well as between axes. We confirmed that within interlinked hormonal axes, ACTH and cortisol concentrations are correlated. The correlations found between hormonal axes, so between cortisol and TSH concentrations, between TSH and GH concentrations, and the joint pattern synchrony between GH and cortisol are indications that there is interplay between hormonal axes in healthy older individuals. These interconnected hormones are also the hormones mostly affected by survival responses, including calorie restriction. However, no major differences in the interrelationships between hormones were found between offspring of long-lived families and partners. This could indicate that this interplay between hormones is crucial for survival and if this interconnection would disappear, it would lead to illness. Participants in this study were selected based on their health status which resulted in a group of healthy older individuals and this could have influenced the results. The strong correlation found between GH and TSH concentrations measured simultaneously, which were the only two hormones of which the secretion differed between offspring and partners, could indicate that a common upstream regulator may regulate both hormones. The increased TSH secretion and reduced GH secretion in offspring of long-lived families could be pleiotropic effects of this upstream regulator while this regulator influences longevity via another mechanism.

Biomarkers of tissue maintenance

The mechanisms by which hormones of the hypothalamic-pituitary-target gland axes are associated with longevity still need to be elucidated. We hypothesize that (the interplay of) hormones of the different hypothalamic-pituitary-target gland axes are key regulators in adjusting the balance between investments in growth, development, and reproduction versus maintenance and repair to its optimal state (see Figure 2). The optimal balance between these processes will be different for the different phases of the life cycle and we hypothesize that longevity is associated with a prolonged ability to preserve an optimal balance throughout the different phases of life. To test this hypothesis, valid biomarkers of tissue maintenance should be assessed, and it needs to be determined whether biomarkers of tissue maintenance are indeed early markers of the ageing process. Subsequently, the association between hormones and biomarkers of tissue maintenance should be investigated.

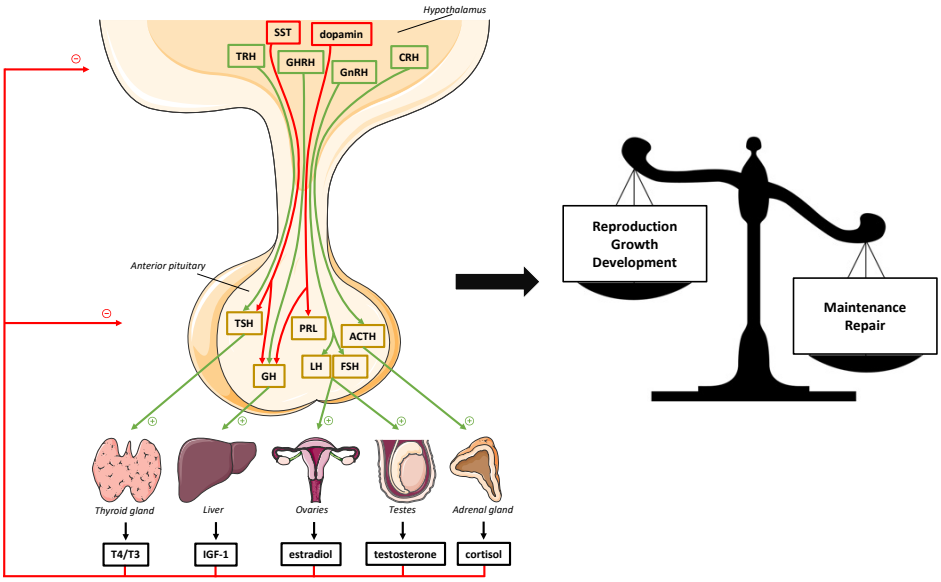

Figure 2. Hypothesis: hormones of the hypothalamic-pituitary-target gland axes regulate the balance between investments in growth, development, and reproduction versus maintenance and repair.

A review on a proposed panel of biomarkers of bone, cartilage, muscle, and brain tissue maintenance has been written during this PhD project (manuscript in press, not included in this thesis). At this moment it is unknown whether it is beneficial to have elevated levels of biomarkers of tissue maintenance or reduced levels (assuming that the amount of damage is equal). Higher levels could indicate that the tissue is capable to regenerate but

it could also indicate that eventually the capacity of regeneration will decrease faster, leading to an earlier onset of age-related functional decline. Lower levels could indicate that many cells are already in senescence, so fewer cells are able to regenerate, which is undesirable. On the other hand, lower levels could also indicate that the tissue is only regenerating itself when this is necessary leading to prolonged ability for maintenance and repair, thus increased lifespan. Moreover, it could be that it is beneficial to have elevated levels at young age but reduced levels at old age. To determine this, the proposed biomarkers could for example be measured at different timepoints in their life in humans with different rates of ageing, including people showing delayed biological ageing compared to accelerated biological ageing, or offspring of long-living families compared to age-matched controls. However, before validated biomarkers can be generally used in research or the clinic, measurement of these biomarkers should also be standardized [33]. Whether reduced or elevated levels of biomarkers of tissue maintenance are beneficial might also be dependent on the context, so on the (micro)environment, communication with other tissues and cells, and/or circulating factors. Therefore, when measuring biomarkers of tissue maintenance, one should consider to include other regulatory factors to get a more comprehensive picture. Moreover, these regulating factors might even be more informative than a single biomarker of tissue maintenance. Therefore, besides validating proposed biomarkers of tissue maintenance, future research should include identification of circulating factors crucial for tissue maintenance.

One other important aspect that needs to be determined before the association between hormones and biomarkers of tissue maintenance could be investigated, is the circadian rhythm of these biomarkers. If these biomarkers vary over the day, these biomarkers should be measured frequently over the day to obtain reliable data. In **Chapter 6** of this thesis, we confirmed the circadian rhythm of the bone resorption marker C-terminal cross-linked telopeptide of type 1 collagen (CTX) and found that postmenopausal women had a larger amplitude than men. Osteocalcin, which is mostly a biomarker of bone formation, showed higher levels during night-time compared to day-time in both men and women. For the bone formation marker N-terminal propeptide of type 1 procollagen (P1NP) levels we observed a small but significant increase in the night in men. Sclerostin and Dickkopf-related protein 1 (DKK1), which are negative regulators of bone formation, did not show a circadian rhythm, but sclerostin levels differed between time points. Because of the large intraindividual variation, DKK1 as measured in this study cannot be considered a reliable marker for diagnostic or research purposes.

Hormone replacement therapy

It is thought that the age-related changes in hormone levels, including GH, TSH and sex hormones, are possible contributors to many problems in older persons. For example, it even has been suggested that since the ageing phenotype has similarities with the clinical picture of patients with GH-deficiency, that the age-related decline is the cause of the ageing phenotype. It is therefore an ongoing debate whether there should be replacement therapy for GH in elderly to prevent age-related loss in functioning. Although GH therapy did have small beneficial effects on body composition, overall it did not result in increased muscle strength or bone mineral density. Moreover, several randomized controlled trials have shown that GH therapy in healthy elderly is associated with increased rates of various side effects [34].

Also the prevalence of subclinical hypothyroidism, which is defined as an elevated TSH level together with an fT4 level within the normal range, is increasing with ageing [35]. It has been suggested that levothyroxine supplementation would provide clinical benefits in these older persons with subclinical hypothyroidism. However, a recent randomized clinical trial in older persons with subclinical hypothyroidism showed that although TSH levels in participants receiving levothyroxine treatment decreased, there was no beneficial effect on their thyroid-related symptoms compared to the placebo group [36]. Also a systematic review and meta-analysis provided evidence that the use of thyroid hormone therapy was not associated with improvements in general quality of life or thyroid-related symptoms [37]. The offspring of long-lived families from the Switchbox study also had, albeit still in the normal range, higher TSH secretion compared to their partners [14]. Also the offspring of Ashkenazi Jewish centenarians and these centenarians themselves had elevated TSH levels compared to controls [38, 39]. These studies support the idea that elevated TSH are not necessarily unfavourable.

The decline in oestrogen with ageing in women, most predominantly during menopause, is one of the risk factors for osteoporosis. Therefore, hormone replacement therapy (HRT) is often given to postmenopausal women to lower their risk of developing osteoporosis. Although HRT was indeed associated with decreased fracture risk, it was also associated with adverse effects including an increased risk of certain types of cancer [40, 41]. Also testosterone replacement therapy is a popular intervention; testosterone replacement therapy use has been three to four times increased between 2003 and 2013 in the United States [42]. Meta-analyses showed that lean mass and health-related quality of life increased in older men using testosterone therapy [43, 44] but the risk of prostate cancer and haematocrit were among others increased compared to the placebo group [45].

Circulatory levels do not always represent tissue-specific levels of hormones. For example, many tissues produce paracrine IGF-1 that does not contribute to circulating IGF-1. Interestingly, in Ames dwarf mice with reduced GH secretion, brain IGF-1 levels were elevated compared to control mice [46]. This could indicate that tissue-specific levels of hormones are more important for ageing and longevity than circulatory levels. Moreover, tissue-specific hormone supplementation could lead to health benefits in contrast to general hormone therapy.

Future perspectives

Future studies should aim to disentangle underlying mechanisms of the altered endocrine features of the thyroid and somatotropic axes in human longevity. One question is whether longevity is primarily achieved via the thyroid axis or somatotropic axis or whether both hormonal axes collaborate in achieving longevity. The somatotropic axis has been more consistently associated with longevity in animal models than the thyroid axis. However, studies are primarily performed in mice with a central TSH-deficiency leading to hypothyroidism, but the TSH secretion was higher, although still in the normal range, in offspring of long-lived families compared with the control group while their thyroid hormone levels were similar. To our knowledge, no studies have been performed assessing the lifespan in animal models with increased TSH secretion but with normal thyroid hormone levels. In this thesis we showed that TSH and GH concentrations were positively correlated without any delay. This could indicate that these hormones collaborate in achieving longevity. However, as mentioned in the discussion, another possibility is that both TSH and GH secretion are not causally related to longevity, but that the altered secretion of GH and TSH are caused by a common upstream regulator. The increased TSH secretion and reduced GH secretion in offspring of long-lived families could be pleiotropic effects of this upstream regulator while this regulator influences longevity via another mechanism. However, no major differences in the interrelationships between hormones were found between offspring of long-lived families and partners.

Since the Switchbox Study was an observational cross-sectional study, direct effects between hormones could not be determined. For this purpose, intervention studies in which a certain pituitary (or hypothalamic) hormone is administrated and other hormones are frequently measured need to be performed. In order to investigate the importance of the interplay of hormones in ageing and longevity, future studies could include offspring of long-lived families and partners with less strict exclusion criteria on health. Ideally, participants would be followed over time to determine changes in both hormone secretion and hormonal interrelationships with ageing.

Our main hypothesis is that the underlying mechanism of the relationship between pituitary hormones and longevity is tissue maintenance. To test this hypothesis, the effect of pituitary hormones on tissue maintenance should be assessed. For this, as discussed earlier in this discussion, biomarkers of maintenance of tissues that are crucial in the ageing process can be determined in relation to levels of hormones. Prevalent age-related diseases are associated with a reduced musculoskeletal system function, notably osteoporosis, osteoarthritis and sarcopenia or with reduced cognitive function, notably several neurodegenerative diseases. Since especially the function of bone tissue, cartilage, skeletal muscle, and the brain are crucial for maintenance of independence into old age, acquiring biomarkers of loss of function of these tissues and organs is particularly desired. Besides the effect of pituitary hormones on tissue maintenance, the effect on physiological parameters, including temperature and ECG measures, could be investigated. Furthermore, tissue-specific levels and effects of these hormones could be determined to identify underlying mechanisms of longevity. To investigate this, tissue-specific knockout mice could be created. Moreover, tissue-specific hormone supplementation could lead to health benefits in contrast to general hormone therapy. It would therefore be worthwhile to investigate the effects of tissue-specific hormone supplementation in animal models.

Studies in laboratory mice already showed that the impact of disrupting GH signalling on longevity is larger than the impact of disrupting IGF-1 signalling or events downstream from IGF-1 receptors, which is in line with our observation that GH secretion was significantly lower in offspring of long-lived families while IGF-1 levels were not-significantly lower. However, nonagenarians with lower IGF-1 bioavailability had improved survival compared to nonagenarians with higher IGF-1 parameters. Future studies should aim to determine whether a reduction in GH or IGF-1 is more important for the longevity phenotype.

Disentangling the underlying mechanisms of the link between reduced GH signalling and longevity is less complicated in animal models than in human. However, how applicable are findings in mice for human? Besides the fact that mice are different organisms than human, laboratory mice live under standardized conditions while human health and lifespan are influenced by many known and unknown factors.

One of the remaining questions is whether the offspring from the Switchbox Study, but also the nonagenarians with improved survival from the Leiden Longevity Study, already had reduced GH/IGF-1 signalling at younger age or whether the offspring and the long-

lived nonagenarians had a steeper decline in their GH/IGF-1 signalling parameters with age compared to controls. When time and money would be no limitation, it is a possibility to follow-up a large number of individuals over time and measure their GH/IGF-1 parameters regularly to look at the change in GH in relation to health and mortality. This is also an option for TSH/TH levels to investigate the change over time and their relationship to health parameters.

The tighter control of GH secretion in offspring of long-lived families is an interesting finding since it suggests that the timing and regulation of a hormone is just as, or even more, important for longevity than total hormone levels. It would be interesting to test in animal models whether this is indeed the case. Moreover, if this stronger regulation leads to preserved resources by secreting the right amount at the right moment and this is a mechanism relevant for longevity, this could also be the case for other systems of the body. This would be an interesting hypothesis to investigate in future studies.

Unravelling the mechanisms of the altered endocrine features of the thyroid and somatotropic axes in human longevity could lead to potential starting points for intervening in the ageing process and will hopefully lead to the improvement of human health at old age.

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