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

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General introduction and
outline of this thesis

BACKGROUND

Studies in animal models indicate that altered central hormone signalling is associated with delayed ageing and longevity [1]. For example in the roundworm, insulin is secreted from neurosecretory cells in response to food cues, and single mutations in the insulin/insulin-like growth factor 1 (IGF-1) signalling pathway can double lifespan [2]. Also in mammals, mutations in the evolutionarily conserved growth hormone (GH)/IGF-1 pathway are associated with increased lifespan. Ames dwarf mice, which have a combined GH, prolactin, and thyroid-stimulating hormone (TSH) deficiency, live approximately 50% longer than wildtype controls [3]. Healthy ageing and longevity in humans are challenging to investigate, because of the relatively long lifespans and the difficulty to determine causality. Furthermore, proper controls are lacking in old age. Older persons are often compared to younger persons, but it is unclear whether differences thus identified are caused by differences between birth cohorts, selective survival or whether these reflect age-induced changes. To circumvent some of these methodological concerns the Leiden Longevity Study (LLS) was designed, in which 421 long-living families were included [4]. Along with nonagenarian siblings, their offspring, who have the propensity to reach old age in good health, together with their partners, as an environmental and age-matched control group, were included (see Figure 1 for the study design). Among the key findings from the LLS were the observations that the offspring had lower prevalence of myocardial infarctions, diabetes mellitus, hypertension, and metabolic syndrome compared to their partners [5, 6]. We also observed several differences in glucose and lipid metabolism as well as in endocrine features. Specifically, it was found that total secretion of TSH was higher in the offspring compared to their partners, but that there were no differences in the circulating thyroid hormone levels free triiodothyronine (fT3) and free thyroxine (fT4), nor in metabolic rate [7]. Familial longevity was found to be associated with a strong TSH-fT3 relationship, but not with major differences in hypothalamic-pituitary-adrenal (HPA) axis activity [8, 9].

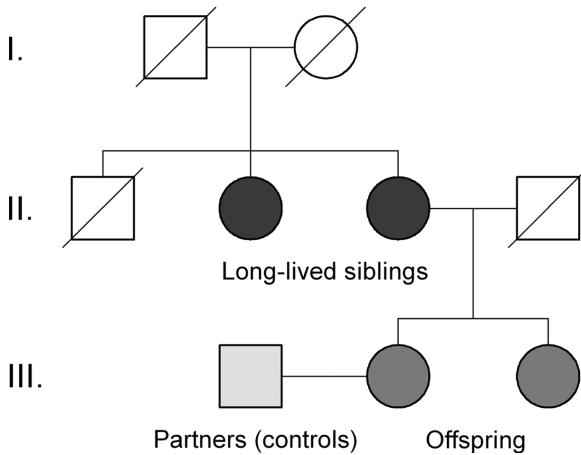


Figure 1. Study design of the Leiden Longevity Study.

Long-lived families with at least two Caucasian siblings fulfilling the age criteria (men ≥ 89 years and women ≥ 91 years) were included in the LLS, together with their offspring and partners of the offspring. Adapted from *PE Slagboom et al. Phil Trans R Soc Lond B Biol Sci. 2011 Jan 12;366(1561):35-42.*

GENERAL HYPOTHESIS

Maintenance and repair is of key importance for the proper functioning of cells, tissues, and integrated physiology. We hypothesize that the balance between investments in growth, development, and reproduction versus maintenance and repair is regulated by the brain. Specifically (the interplay of) hormones of the different hypothalamic-pituitary-target gland axes seem to be key regulators in constantly adjusting this balance to its optimal state. The optimal balance between these processes will be different for the different phases of the life cycle. Due to the accumulation of damage over time, requirements for maintenance and repair are hypothesized to increase with age. Furthermore, we hypothesized that longevity is associated with a prolonged ability to preserve an optimal balance throughout the different phases of life.

STUDY DESIGN

This PhD project was embedded into two International Consortia, Switchbox and Thyrage, funded by the European Union [10, 11]. In Switchbox, various physiological data and biomaterials have been collected over 24 h in 20 offspring and 18 partners from the LLS. Because pituitary hormones are secreted in a pulsatile manner and some exhibit a circadian rhythm, these hormones were measured in blood that was withdrawn every 10 min during 24 h to obtain reliable and informative data on pituitary hormone secretion. Concentrations of adrenocorticotrophic hormone (ACTH) and cortisol from the HPA axis,

and of TSH had been measured before the start of this PhD project. Concentrations of luteinizing hormone (LH) and testosterone from the hypothalamic-pituitary-gonadal (HPG) axis, and of GH were measured during this PhD project. LH and testosterone concentrations were measured in blood withdrawn from 20 men only, of which 10 offspring and 10 partners. In the H2020 project Thyrage, one of the aims is to associate biomarkers of tissue maintenance with parameters of the thyroid axis in offspring and partners from the LLS. For this, an overview of possible and reliable biomarkers of tissue maintenance was written during this PhD project. For some of these biomarkers of tissue maintenance, especially bone turnover markers, it is known that they fluctuate over time. Before it is possible to associate biomarkers of bone turnover with parameters of the thyroid axis (and other pituitary hormones), we first need to determine the 24-h profile of bone turnover markers. To this end, bone turnover markers were measured in blood sampled every 4 h over 24 h during this PhD project.

OBJECTIVES

In this PhD project, I aimed to answer the following research questions:

1. Is familial longevity associated with altered endocrine features in the hypothalamic-pituitary-somatotropic axis?
2. Is familial longevity associated with altered endocrine features in the hypothalamic-pituitary-gonadal axis?
3. What are the interrelationships between hormones of the hypothalamic-pituitary-target gland axes in healthy older subjects?
4. What are the 24-h profiles of bone turnover markers in healthy older men and women?

METHODOLOGY

To examine these research questions, time series data on various hormone concentrations were collected. To analyse this type of data, specific methods for time series analysis are needed. Which method to use depends on the type of data and the research question. Below, the four time series analysis methods used in this PhD project are explained.

Cosinor analysis

To determine whether endocrine parameters display a sinusoidal circadian rhythm, cosinor analyses were performed. Cosinor analysis is a model-dependent method which fits a cosinor model to the raw data (see Figure 2 for an example). First, the rhythm detection test, also called the zero-amplitude test, was performed to test the overall

significance of the cosinor model. One of the circadian parameters calculated by the cosinor analysis is the midline estimating statistic of rhythm (MESOR), which is a circadian rhythm-adjusted mean based on the parameters of a cosine function fitted to the raw data. In addition, the amplitude is provided, which is the difference between the maximum and MESOR of the fitted curve. The acrophase represents the phase of the maximal value assumed by the curve [12].

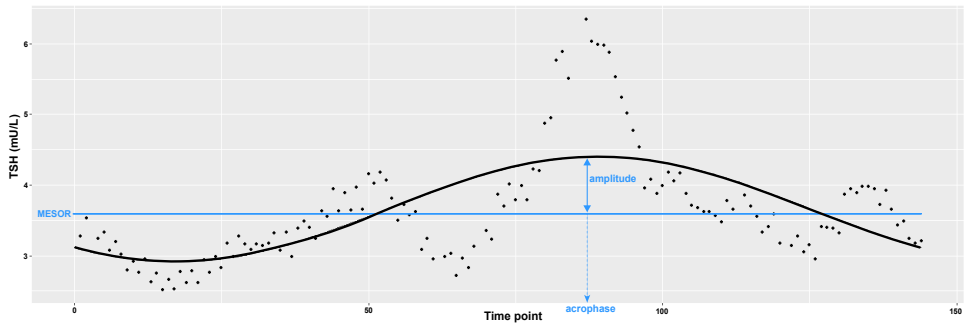


Figure 2. TSH concentration profile over 24 h of one participant with cosinor analysis.

The fit of the cosinor model is significant, indicating that this TSH concentration profile exhibits a circadian rhythm. The MESOR is indicated by the horizontal line, the amplitude by the solid arrow, and the acrophase by the dotted arrow.

Deconvolution analysis

By deconvolution analysis [13], a 24-h hormone concentration profile is decomposed into underlying secretory bursts, basal secretion, elimination of previously secreted hormone and random experimental variability using the Matlab software program. The algorithm first detrends the data and normalizes concentrations to numbers within the interval 0 to 1. Thereafter, successive potential pulse-time sets, each containing one fewer burst, were created by a smoothing process. Finally, a maximum-likelihood expectation deconvolution method estimated all secretion and elimination rates simultaneously for each candidate pulse-time set. Outcome parameters of main interest are basal (non-pulsatile) secretion, pulsatile secretion, the sum of basal and pulsatile secretion (total secretion), number of pulses per 24 h (secretory-burst frequency), interpulse regularity (Weibull gamma), mean pulse mass, and (fast and) slow half-life. Figure 3 presents an example of a GH concentration profile with indicated deconvolution parameters.

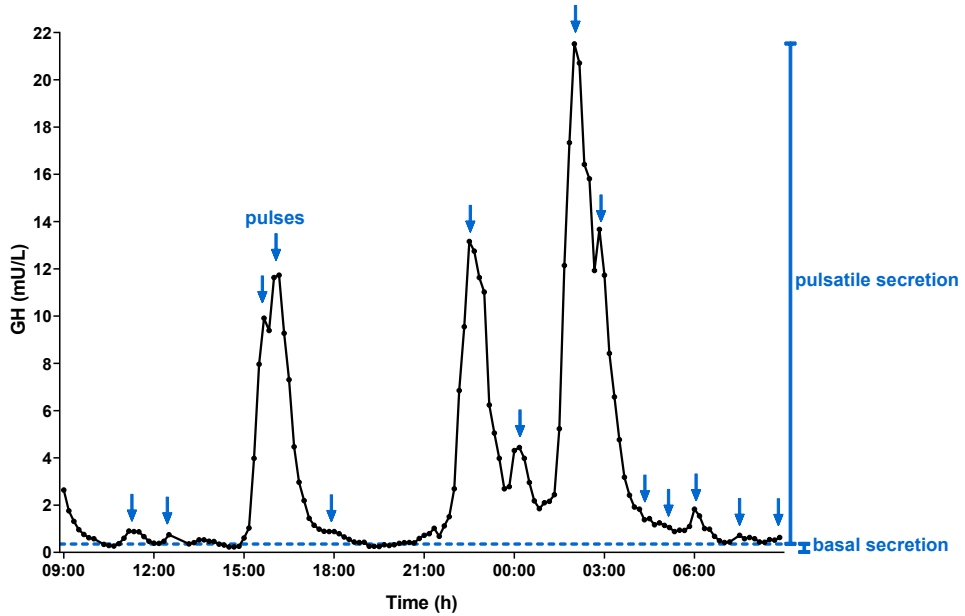


Figure 3. GH concentration profile over 24 h of one participant with deconvolution analysis parameters.

Pulses are indicated by the arrows, basal secretion by the dotted line and pulsatile secretion by the vertical line.

(Cross) Approximate Entropy

Approximate entropy (ApEn) is a measure for the strength of feedforward and feedback control signals in a hormone system. It is a scale- and model-independent statistic that quantifies the regularity of consecutive time-series data using the Matlab software program [14]. ApEn has high sensitivity and specificity (both > 90%) for analysis of hormone concentration measurements over 24 h. Low ApEn values imply that the sequence of time-series data is regular and that it contains many repetitive patterns, such as a sinus wave. High ApEn values indicate greater irregularity and randomness. Figure 4 presents the GH concentration profiles of two participants, one with a low ApEn value and one with a high ApEn. In neuro-endocrine time-series of a length of 50-300 data points, m (window length) = 1 is preferred, and for lengths $N \geq 60$, r (criterion of similarity) should be set to the predetermined value of 20% of the standard deviation (SD) of the individual subject time series [15]. Subsequently, the Jack-knifed ApEn (JkApEn) was calculated, which is a rigorous and objective cross-validation test that gives less bias in smaller samples than regular ApEn and it is more applicable for hormone data [16].

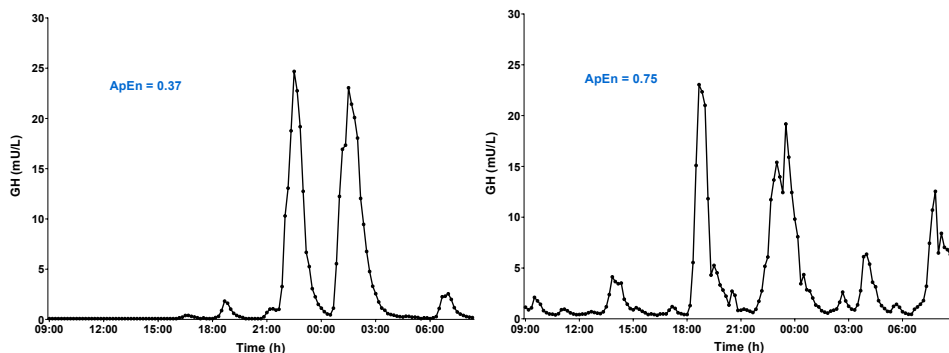


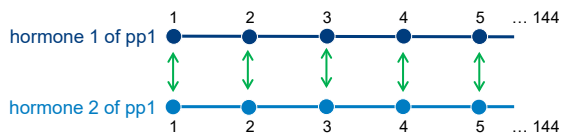
Figure 4. GH concentration profiles over 24 h of a participant (top) with a low ApEn, indicating a regular pattern, and a participant (bottom) with a high ApEn, indicating greater irregularity.

Bivariate cross approximate entropy (Cross-ApEn) quantifies joint pattern synchrony between two simultaneously measured time series, with lower cross-ApEn values signifying greater synchrony [17, 18]. Synchrony refers to pattern similarity, so to what extent sub patterns of window length m in time series A appear in time series B with a criterion of similarity r . Changes in the cross-ApEn reflect feedback and/or feedforward alterations within an interlinked axis [18].

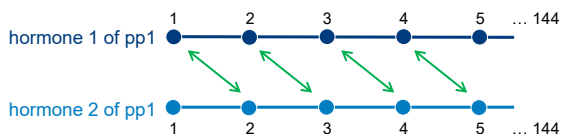
Cross-correlation

Cross-correlation assesses the relative strength between two simultaneously measured hormonal time series for all possible time shifts by calculating linear Pearson's correlation coefficients [19, 20]. Hormone concentrations in time series A are compared pairwise with those of series B measured simultaneously (zero lag) or measured earlier or later (with a time lag). The unit of one lag time is the interval between two sampling points, so a lag time of 1 means that there is a delay of 10 min between two time series. Figure 5 presents a visual explanation of the cross-correlation procedure.

Lagtime 0



Lagtime 1



Lagtime 2

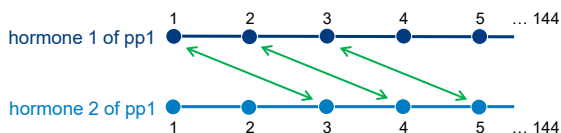


Figure 5. Explanation of cross-correlation.

The cross-correlation at lag time 0 is obtained when concentrations of hormone 1 are correlated with those of hormone 2 measured simultaneously in the same participant. The cross-correlations at lag time 1 and 2 are obtained when concentrations of hormone 1 are correlated with those of hormone 2 measured with a time lag of 10 and 20 min, respectively.

OUTLINE OF THIS THESIS

In **Chapter 2**, the question is addressed whether circulating IGF-1 axis parameters associate with old age survival and functional status in nonagenarians from the LLS. In **Chapter 3**, we use GH concentrations measured every 10 min over 24 h to derive and compare GH secretion parameters between offspring of long-lived families and their partners. In **Chapter 4**, we investigate the association between HPG axis parameters and familial longevity. In **Chapter 5**, we use 24-h time series data of pituitary hormones to investigate how changes in the different hormonal axes are correlated with each other over time. In **Chapter 6**, we determine the circadian rhythm of bone turnover markers in healthy older subjects.

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