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

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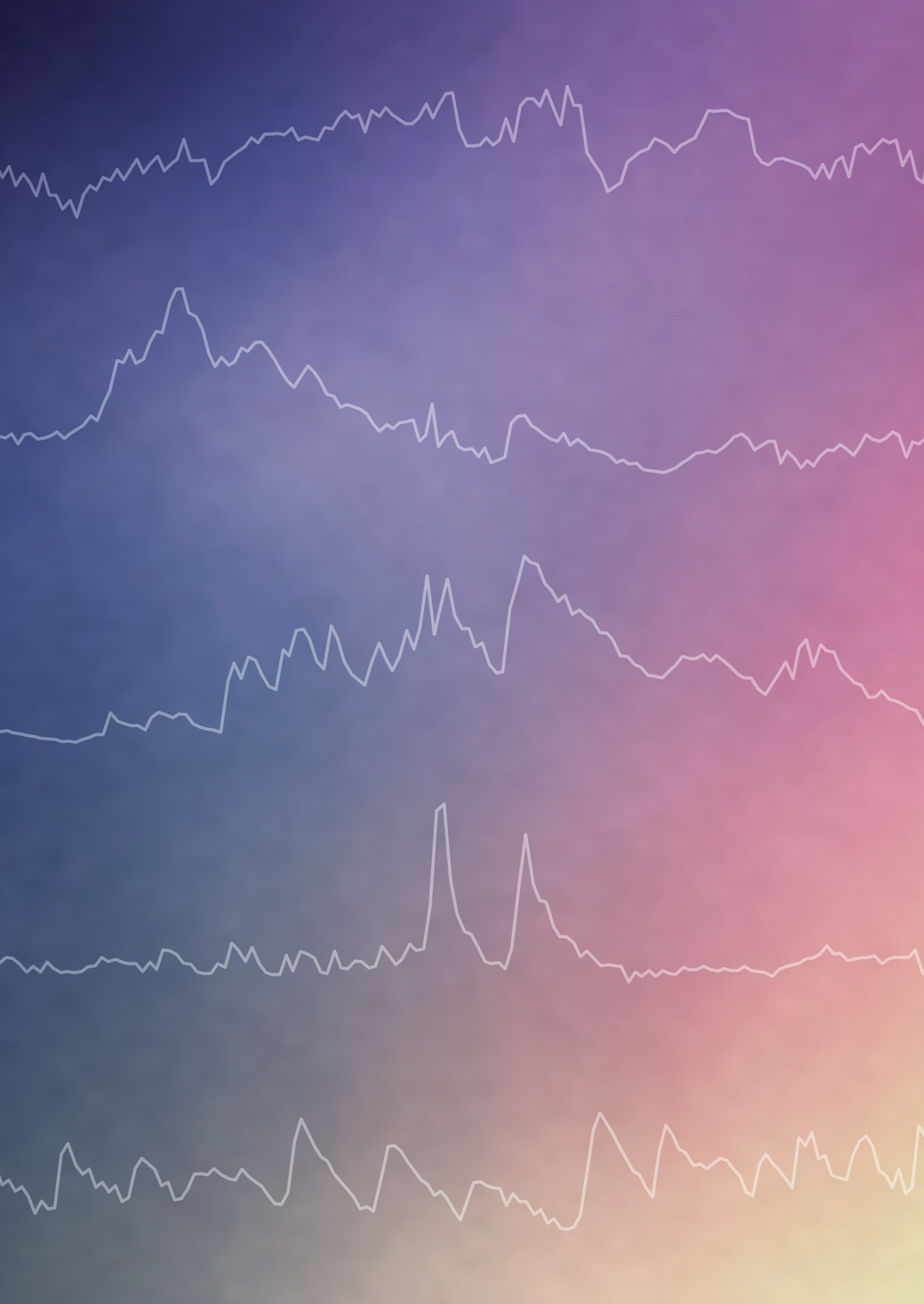

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Interrelationships between pituitary hormones as assessed from 24-h serum concentrations in healthy older subjects

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ABSTRACT

Context: Hormones of the hypothalamic-pituitary-target gland axes are mostly investigated separately, while the interplay between hormones might be as important as each separate hormonal axis.

Objective: Our aim is to determine the interrelationships between GH, TSH, ACTH, and cortisol in healthy older individuals.

Design: We made use of 24-h hormone serum concentrations assessed with intervals of 10 min from 38 healthy older individuals with a mean age (SD) of 65.1 (5.1) years from the Leiden Longevity Study. Cross-correlation analyses were performed to assess the relative strength between two 24-h hormone serum concentration series for all possible time shifts. Cross-approximate entropy was used to assess pattern synchronicity between two 24-h hormone serum concentration series.

Results: Within an interlinked hormonal axis, ACTH and cortisol were positively correlated with a mean (95% CI) correlation coefficient of 0.78 (0.74 – 0.81) with cortisol following ACTH concentrations with a delay of 10 min. Between different hormonal axes, we observed a negative correlation coefficient between cortisol and TSH of -0.30 (-0.36 – -0.25) with TSH following cortisol concentrations with a delay of 170 min. Furthermore, a positive mean (95% CI) correlation coefficient of 0.29 (0.22 – 0.37) was found between TSH and GH concentrations without any delay. Moreover, cross-ApEn analyses showed that GH and cortisol exhibit synchronous serum concentration patterns.

Conclusions: This study demonstrates that interrelations between hormones from interlinked as well as different hypothalamic-pituitary-target gland axes are observed in healthy older individuals. More research is needed to determine the biological meaning and clinical consequences of these observations.

INTRODUCTION

Hormones of the hypothalamic-pituitary-target gland axes are regulated by central and peripheral feedforward and feedback signals. The interplay among these regulators in time dictates the hormone secretion pattern, which will be adapted depending on the changing needs of the organism, such as during the circadian rhythm, sleep, activity, food intake, stress, and inflammation. Although hormones of the hypothalamic-pituitary-target gland axes are each regulated by different factors and respond to different signals, the common goal of all these hormonal axes is to maintain homeostasis in the human body. Furthermore, anterior pituitary cells share the same embryonic origin – the anterior pituitary is derived from oral ectoderm – and pituitary hormones carry out their actions in similar ways [1]. Moreover, there is evidence for crosstalk between pituitary cells [2]; 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. These shared features have however rarely been addressed in human studies, while the interplay between hormones might be as important, or more important, as each separate hormonal axis. For example, with ageing or after menopause, levels of several hormones change concomitantly. While this might reflect separate mechanisms, these hormonal changes could also be synchronised with each other and their concerted impact might be larger than the sum of their individual impact on the ageing phenotype. Also, in other systems and organs of the body, interplay, interaction, and networks are highly important for maintenance of homeostasis and proper functioning of the human body.

Little is known about the interrelationships of hormones from different hypothalamic-pituitary-target gland axes, especially over time, since patients, or healthy individuals, are rarely sampled multiple times during the day. Some studies did collect hormonal time series data and investigated associations between pituitary hormones and/or hormones from an endocrine target gland. For example, in patients with Cushing syndrome, who display excessive production of cortisol, pulsatile TSH secretion is suppressed and irregular [3]. TSH secretion is also decreased in patients with acromegaly who display excessive production of GH [4]. However, these studies were performed in patients, so the observed relationships could be influenced by other aspects of their illness and not only by the altered hormone secretion. Few studies have been performed in healthy individuals. For example, Vis *et al.* assessed hormonal relationships in 18 obese women and found among others relationships between ACTH and cortisol, TSH and GH, TSH and cortisol, and between TSH and ACTH [5]. Furthermore, glucocorticosteroid administration

directly supressed pulsatile TSH secretion in healthy men [6] and a positive cross-correlation between GH and cortisol was found in older men and women [7].

In the present study, we aimed to determine the interrelationships between GH, TSH, ACTH, and cortisol in healthy older men and women. To this end, we analysed 24-h hormone concentration series assessed at intervals of 10 min from 38 healthy older participants from the Leiden Longevity Study [8]. Moreover, we examined whether interrelationships between hormones differ between men and women or between offspring of long-lived families and their partners. Furthermore, differences between interrelationships during the lights-on and -off periods were determined. We performed cross-correlation analyses to assess the relative strength between two 24-h hormone concentration series for all possible time shifts and cross-approximate entropy (ApEn) to assess pattern synchronicity between the different 24-h hormone concentration series.

METHODS

Study participants

In the Switchbox Leiden Study, we collected 24-h blood samples from 38 healthy older (range 52-76 years) individuals comprising 20 men and 18 women between June 2012 and July 2013 [9]. Participants were recruited from the Leiden Longevity Study, which is a family-based study consisting of 421 families with at least two long-lived siblings (men ≥ 89 years and women ≥ 91 years) together with their offspring and the offspring's partners without any selection on health or demographics [10]. The Switchbox Leiden Study comprised of 20 offspring of long-lived families, including 10 men and 10 women, and 18 partners of the offspring as a control group, including 10 men and 8 women. Participants had a stable body mass index (BMI) between 20-34 kg/m^2 and although not formally asked, based on the age range, the majority of women was most likely postmenopausal. Exclusion criteria were having a fasting plasma glucose above 7 mmol/L, having chronic renal, hepatic or endocrine disease, or using medication known to influence lipolysis, thyroid function, glucose metabolism, GH or IGF-1 secretion and/or any other hormonal axis. Hence, none of the participants were using estrogen-containing compounds. Participants were excluded if they had a recent trans meridian flight or when they recently performed shift work. To be able to safely perform the 24-h blood sampling, other exclusion criteria were difficulties to insert and maintain an intravenous catheter, anaemia (haemoglobin below 7.1 mmol/L), and blood donation within the last two months. The Switchbox Leiden Study protocol was approved by the Medical Ethical

Committee of the Leiden University Medical Centre and performed according to the Helsinki declaration. All participants gave written informed consent for participation.

Study protocol

Participants were admitted to the Research Centre at 08:00 h where a catheter was placed in a vein of the forearm of the nondominant hand. Blood sampling started around 09:00 h and every 10 min, 2 ml of blood was collected in a serum tube and 1.2 ml in an EDTA tube [8]. The participants received standardized feeding consisting of 600 kcal Nutridrink (Nutricia Advanced Medical Nutrition Zoetermeer, The Netherlands) at three fixed times during the day (between 09:00 and 10:00 h, 12:00 and 13:00 h, and 18:00 and 19:00 h). Lights were switched off between 23:00 and 08:00 h to allow the participants to sleep and except for lavatory use, no physical activity was allowed during the study period. All participants were sampled in the same research room. Anthropometric measurements, comprising weight, height, waist circumference, fat mass, and lean body mass were performed in the Research Centre using a scale, measuring tape, and Bioelectrical Impedance Analysis at a fixed frequency of 50 kHz (Bodystat® 1500 Ltd., Isle of Man, British Isles). Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of height (in meters). Data on habitual bedtime and getting up time during the past month were obtained using the Pittsburgh Sleep Quality Index questionnaire [11].

Biochemical assays

All laboratory measurements were performed with fully automated equipment and diagnostics from Roche Diagnostics (Almere, The Netherlands) and Siemens Healthcare diagnostics (The Hague, The Netherlands) at the Department of Clinical Chemistry and Laboratory Medicine of the Leiden University Medical Center in The Netherlands. Full details on the procedures of the hormone assays have been described previously [9, 12, 13]. Levels of GH, TSH, cortisol, and ACTH were all measured in blood samples collected every 10 min from all 38 participants. Human growth hormone with a molecular mass of 22 kDa was measured in serum samples using Siemens reagents and an IMMULITE® 2000 Xpi Immunoassay system (Siemens Healthcare diagnostics). TSH and cortisol were measured in serum samples by ECLIA (ElectroChemoLuminescence ImmunoAssay) using cobas reagents and a Roche Modular E170 Immunoanalyser. ACTH was measured in EDTA samples using Siemens reagents and an IMMULITE® 2000 Xpi Immunoassay system. The data was checked for obvious outliers by visual inspection of a graphical display of individual hormone profiles from all 38 participants [14]. This was performed by four reviewers with expert knowledge in endocrinology. After reviewing the data

individually, a consensus meeting was held to reach agreement on data points which only one or two reviewers had marked as an outlier. In total for 28 out of 38 participants, 1.1 (SD = 1.8) data points per hormonal concentration series were on average detected as outliers and excluded from the dataset. Glucose and insulin were measured in a fasting serum sample withdrawn around 08:30 h at the second day of the 24-h blood sampling. Glucose was measured using Roche Hitachi Modular P800 and insulin was measured using IMMULITE® 2000 Xpi Immunoassay.

Cross-correlation

Cross-correlation assesses the relative strength between two 24-h hormone concentration series for all possible time shifts, by calculating linear Pearson's correlation coefficients as explained in more detail elsewhere [15, 16]. For example, 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. Cross-correlation analyses were performed using the ccf function in the software program R, version 3.4.3 (The R Foundation for Statistical Computing, Vienna, Austria). The range of tested lag times depends on the number of data points in one time series; the range is lag -18 to 18 (360 min in total) for 144 data points. A correlation is considered significant when the absolute value is greater than $2/(\sqrt{n - |k|})$, where n is the number of data points in one time series and k is the maximal possible lag [17]. For a time series of 144 data points and a maximal lag of 18, the significance level is 0.18. Cross-correlation analyses were also performed after stratifying the 24-h data for lights-on period, which is the data from time point 09:00 h up to and including 22:50 h, and lights-off period (23:00 to 08:00 h). For these sub analyses, the lag range and the significance level changed accordingly to a lag range of -16 to 16 (320 min) and significance level of 0.24 for the lights-on period, and a lag range of -14 to 14 (280 min) and significance level of 0.31 for the lights-off period.

Cross-ApEn

Bivariate cross-approximate entropy (Cross-ApEn) quantifies joint pattern synchrony between two simultaneously measured time series, with lower cross-ApEn values signifying greater synchrony [18, 19]. 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 certain margin (r). Cross-ApEn was calculated for $m = 1$ and $r = 0.2$ (20% of the SD of the individual subject's hormone time series) with standardized data using the Matlab software program (Mathworks, Inc., Natick, MA, USA). Subsequently, jackknifing was

performed, which is a rigorous and objective leave-one-out cross-validation test that gives less bias in smaller samples than regular cross-ApEn, and it is more applicable for hormone data. It is important to note that a cross-ApEn of hormones A-B is different from a cross-ApEn of hormones B-A, since A is leading in the first case and following in the second. Cross-ApEn analyses were also performed after stratifying the 24h data for lights-on period, which is the data from time point 09:00 h up to and including 22:50 h, and lights-off period (23:00 to 08:00 h). Since cross-ApEn analyses cannot deal with missing data, missing data points were linearly interpolated.

Statistical analysis

Characteristics of the study participants were calculated using descriptive statistics. Normally distributed variables were presented as mean with standard deviation and differences between men and women and between offspring and partners were assessed by independent-samples t-tests. Not normally distributed variables were presented as median with interquartile ranges, using the nonparametric independent-samples Mann-Whitney U test to assess differences between subgroups. All statistical analyses were performed using SPSS for Windows, version 23 (SPSS, Chicago, IL, USA). Tukey box plots were made using GraphPad Prism version 7 (GraphPad, San Diego, CA, USA).

RESULTS

Characteristics of study participants

Characteristics of study participants are presented in Table 1 for all participants and stratified for sex and offspring-partner status. The number of men is equal in offspring and partner groups. Men and women were also similar in their offspring-partner distribution. Participants had a mean (SD) age of 65.1 (5.1) years, which was similar for men and women and for offspring and partners. The observed median (IQR) BMI of 24.8 (22.2 – 28.0) kg/m^2 is normal for this age category and was similar for all subgroups. Fasting glucose and insulin levels were for all participants within the reference range of our laboratory, with similar levels between groups. As expected, men and women differed in measures of body composition, with men being taller, having less fat mass, more lean body mass, and larger waist circumference than women. Groups of offspring and partners were similar in body composition. Participants were normal nocturnal sleepers in the month prior to the study day with a median (IQR) habitual bedtime of 23:30 (23:00 – 00:00) h and getting up time of 08:00 (07:30 – 08:15) h, which is similar to the time schedule of the study protocol during the 24-h blood sampling. Habitual bedtimes and getting up times were similar for men and women and for offspring and partners.

Table 1. Characteristics of study participants, for all subjects and stratified for sex and family history

		Stratified for sex			Stratified for family history		
	All subjects *n* = 38	**Men *n* = 20**	**Women *n* = 18**	***P* value**	**Offspring *n* = 20**	**Partners *n* = 18**	***P* value**
Male, *N* (%)	20 (52.6)	NA	NA	NA	10 (50)	10 (55.6)	0.76
Offspring of long-lived family, *N* (%)	20 (52.6)	10 (50)	10 (55.6)	0.76	NA	NA	NA
Age (years)[a]	65.1 (5.1)	65.6 (5.3)	64.6 (5.0)	0.56	65.6 (5.4)	64.6 (4.9)	0.52
BMI (kg/m^2)[b]	24.8 (22.2 – 28.0)	25.2 (23.3 – 27.4)	23.1 (21.6 – 29.9)	0.21	24.8 (22.3 – 29.3)	25.1 (22.1 – 27.7)	0.96
Height (cm)[b]	175 (165 – 181)	178 (175 – 182)	165 (162 – 168)	<0.001	175 (164 – 180)	175 (167 – 182)	0.58
Fat mass (kg)[b]	20.5 (18.5 – 27.0)	19.1 (18.0 – 24.1)	23.5 (19.7 – 34.7)	0.02	21.9 (18.7 – 27.5)	20.4 (18.4 – 29.1)	0.78
Lean body mass (kg)[b]	53.3 (41.5 – 62.2)	60.5 (57.6 – 66.0)	41.5 (37.4 – 44.8)	<0.001	52.4 (41.8 – 62.8)	54.3 (40.4 – 63.0)	0.73
Waist circumference (cm)[b]	94 (82 – 100)	97 (92 – 106)	82 (80 – 95)	0.001	92 (82 – 101)	94 (83 – 98)	0.96
Fasting glucose [mmol/L][a]	4.9 (0.6)	4.9 (0.7)	4.9 (0.5)	0.98	4.9 (0.7)	4.8 (0.4)	0.51
Fasting insulin [mU/L]	5.7 (3.7 – 7.9)	6.2 (3.4 – 10.1)	5.1 (3.9 – 6.3)	0.44	4.5 (3.5 – 8.0)	5.9 (3.8 – 7.8)	0.78
Habitual bedtime (h)	23:30 (23:00 – 00:00)	23:30 (23:00 – 23:45)	23:30 (23:00 – 23:45)	0.68	23:30 (23:00 – 00:00)	23:30 (23:00 – 23:30)	0.92
Habitual getting up time (h)	08:00 (07:30 – 08:15)	07:45 (07:00 – 08:15)	08:00 (07:30 – 08:15)	0.23	08:00 (07:30 – 08:30)	07:45 (07:00 – 08:15)	0.35

Unless indicated otherwise, data are presented as median with interquartile ranges. [a]Data are presented as mean with standard deviation. [b]Data were not available for one male partner. [c]Data were not available for one female offspring. NA = not applicable.

Cross-correlations of GH, TSH, ACTH, and cortisol

Figure 1 presents the cross-correlations between TSH and GH (a), cortisol and TSH (b), ACTH and cortisol (c), cortisol and GH (d), ACTH and GH (e), and ACTH and TSH (f) in all 38 participants. For TSH and GH, the maximal correlation was found at lag time 0 with a mean (95% CI) correlation coefficient of 0.29 (0.22 – 0.37). All cross-correlations between lag time -90 and 80 were positive. The strongest correlation between cortisol and TSH was found at lag time 170 with a mean (95% CI) correlation coefficient of -0.30 (-0.36 – -0.25). Also between lag times 90 and 180, cortisol and TSH were significantly negatively correlated, indicating that cortisol concentrations are negatively followed by TSH with a delay of 90 to 180 min. For ACTH and cortisol, the mean (95% CI) maximal correlation coefficient was 0.78 (0.74 – 0.81) at lag time 10, indicating that cortisol concentrations follow ACTH concentrations with a delay of 10 min. No significant cross-correlations between cortisol and GH, nor between ACTH and GH, were found. For ACTH and TSH, a weak maximal cross-correlation was observed at lag time 180 with a mean (95% CI) correlation coefficient of -0.19 (-0.24 – -0.15), which indicated that ACTH concentrations are negatively followed by TSH concentrations after 180 min.

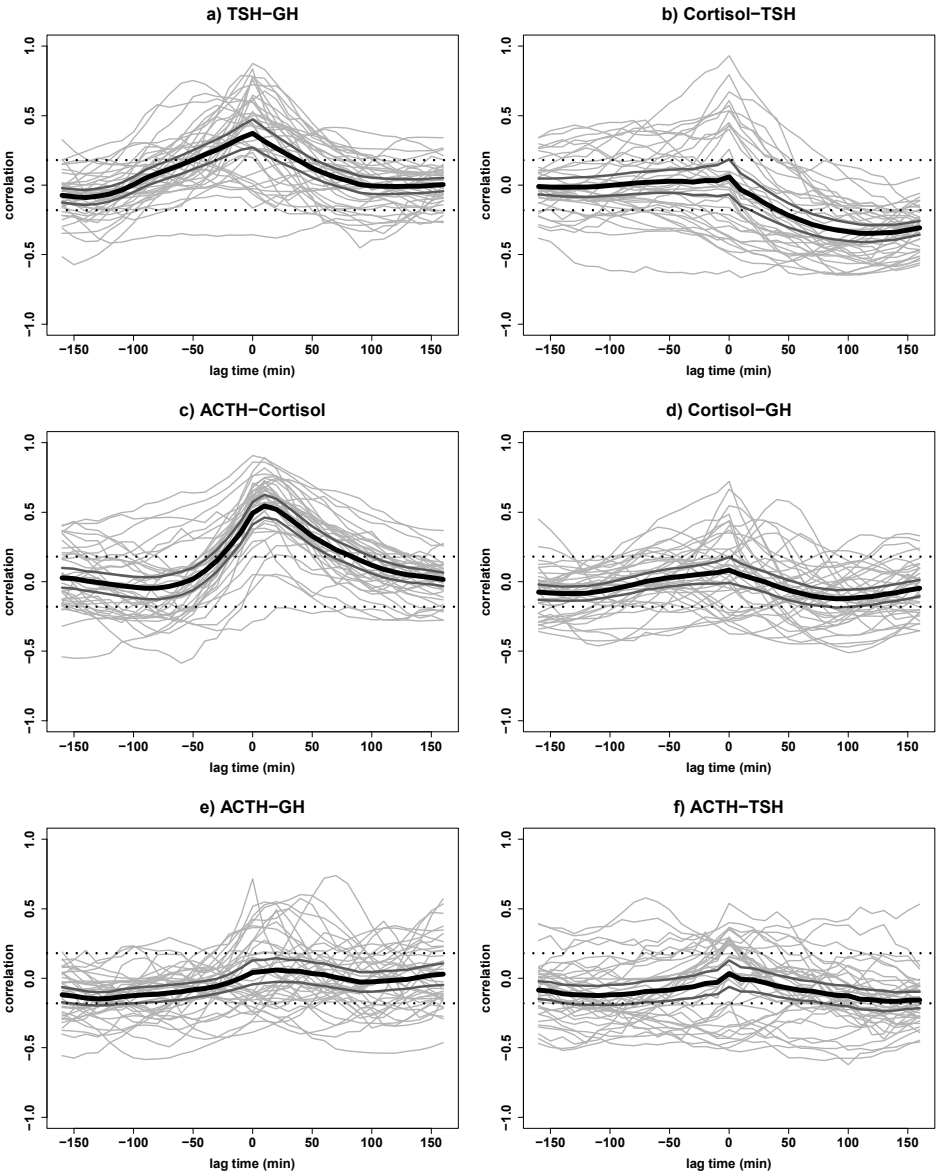
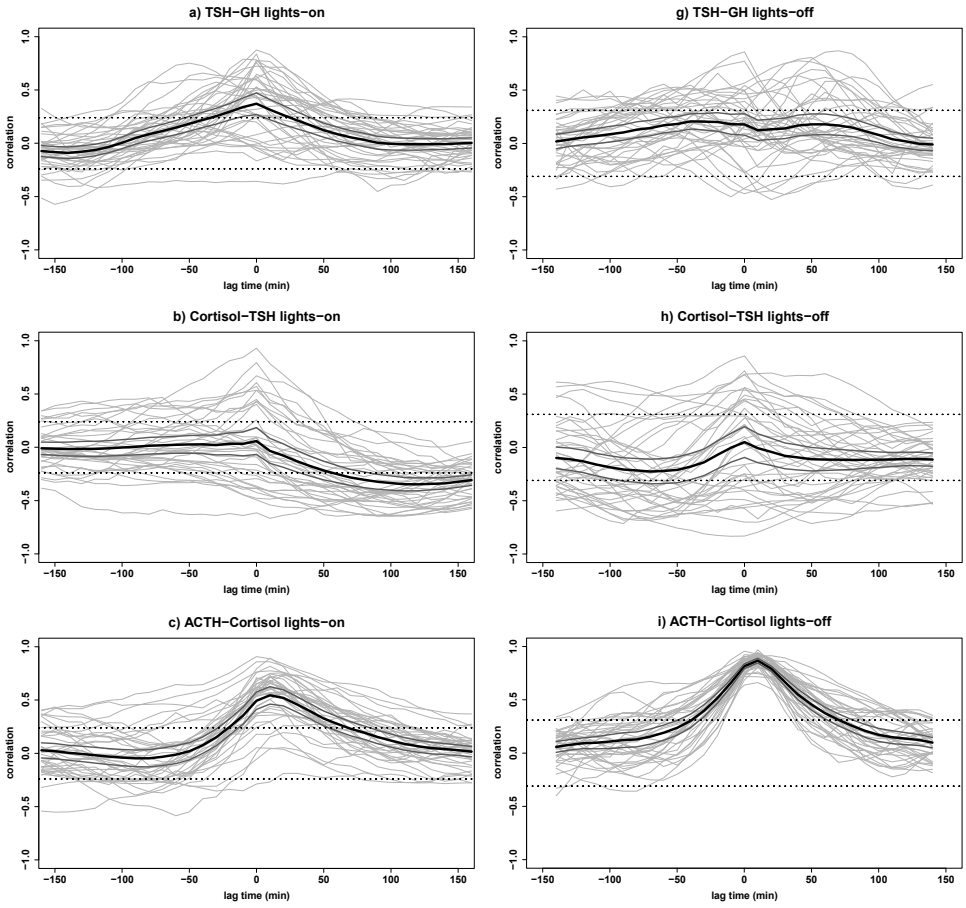


Figure 1. Cross-correlations of GH, TSH, ACTH, and cortisol.

Cross-correlations of a) TSH and GH, b) cortisol and TSH, c) ACTH and cortisol, d) cortisol and GH, e) ACTH and GH, and f) ACTH and TSH in all 38 participants. Cross-correlation assesses the relative strength between two hormone time series for all possible time shifts. The graph displays the correlation (y-axis) at a lag time in minutes (x-axis) with each grey line corresponding with one participant. The black line indicates the mean correlation for all participants and the two dark grey lines indicate the 95% confidence interval. The significance level is indicated by two straight dotted lines at correlations -0.18 and +0.18. Negative lag times represent a correlation in which hormone 2 is followed by hormone 1 and positive lag times represent a correlation in which hormone 1 is followed by hormone 2.

Cross-correlations stratified for lights-on and lights-off periods

Figure 2 presents the cross-correlations of GH, TSH, ACTH, and cortisol stratified for lights-on and lights-off periods. In line with the correlation found between TSH and GH concentrations over the complete 24-h period, we observed a strong positive correlation at lag time 0 (0.37 (0.18 – 0.35)) during the lights-on period. However, the correlation between TSH and GH disappeared in the lights-off period. Also for cortisol and TSH, we observed similar results during the lights-on period as during the complete 24-h period. A negative correlation at positives lag times was found during the lights-on period, but no significant correlation was found during the lights-off period. The cross-correlation between ACTH and cortisol is stronger during the lights-off period (0.87 (0.85 – 0.89)), than during the lights-on period (0.55 (0.39 – 0.53)). No significant cross-correlations between cortisol and GH, between ACTH and GH, and between ACTH and TSH concentrations, were found after stratifying the 24-h data for lights-on and -off periods.



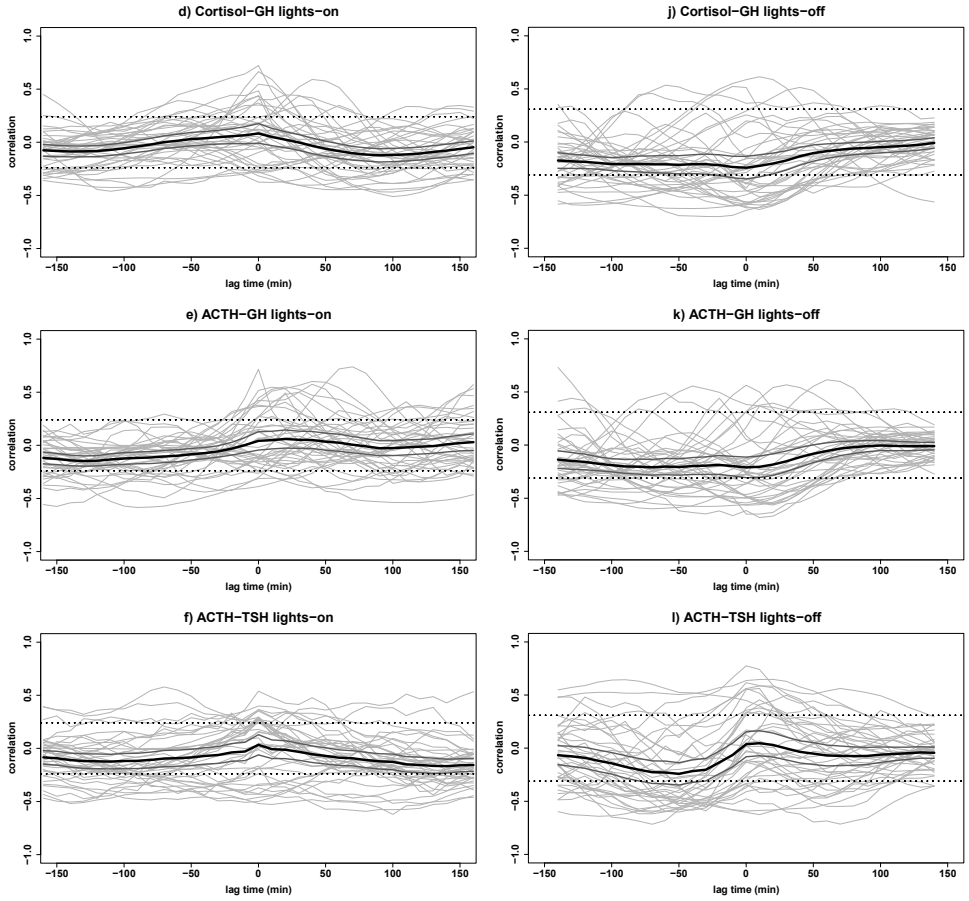


Figure 2. Cross-correlations of GH, TSH, ACTH, and cortisol stratified for lights-on and lights-off periods.

Cross-correlations of hormone combinations of GH, TSH, ACTH, and cortisol in all 38 participants stratified for lights-on period (a-f) from 09:00-22:50 h and lights-off period (g-l) from 23:00-08:00 h. Cross-correlation assesses the relative strength between two hormone time series for all possible time shifts. The graph displays the correlation (y-axis) at a lag time in minutes (x-axis) with each grey line corresponding with one participant. The black line indicates the mean correlation for all participants and the two dark grey lines indicate the 95% confidence interval. The significance level is indicated by the two straight dotted lines at correlations -0.24 and +0.24 for the lights-on period and at -0.31 and +0.31 for the lights-off period. Negative lag times represent a correlation in which hormone 2 is followed by hormone 1 and positive lag times represent a correlation in which hormone 1 is followed by hormone 2.

Cross-correlations stratified for men and women

Cross-correlation results of GH, TSH, ACTH, and cortisol were stratified for men and women. In Figure 3, a graphical summary of main findings from cross-correlation analysis are displayed for all participants (a) and for men (b) and women (c) separately. For TSH and GH, the maximal correlation in women was found at lag time 0 with a mean (95% CI) correlation coefficient of 0.27 (0.15 – 0.39). In men, the strongest cross-correlation (0.33 (0.24 – 0.43)) between GH and TSH was found at lag time -40 indicating that TSH concentrations are following GH concentrations with a delay of 40 min. However, also in men there were positive correlations at all lag times between -100 and 120 min. The strongest correlation between cortisol and TSH in men (-0.35 (-0.42 – -0.28)) was found at lag time 170, but in women, the strongest cross-correlation (0.32 (0.20 – 0.44)) was found at lag time 0, indicating that cortisol and TSH concentrations were strongly positively correlated without a delay. However, also in women we observed a weak but significant negative correlation at lag times 120 until 180 min. For ACTH and cortisol, similar results were observed in men (0.78 (0.73 – 0.83)) and women (0.78 (0.73 – 0.82)). No significant cross-correlations between cortisol and GH, and between ACTH and GH, were found after stratifying for men and women. In men, a weak mean correlation coefficient of -0.21 (-0.27 – -0.15)) at lag time 180 min was found between ACTH and TSH concentrations. In contrast, a weak positive correlation coefficient of 0.22 (0.11 – 0.33) was found at lag time 0 in women.

Cross-correlations stratified for offspring and partners

When cross-correlation results were stratified for offspring and partners, similar results were observed in offspring (0.32 (0.21 – 0.44)) and partners (0.26 (0.16 – 0.35)) for the cross-correlation of TSH and GH concentrations (data not shown). Also for cortisol and TSH, similar results were observed in offspring (-0.30 (-0.36 – -0.23)) and partners (-0.31 (-0.40 – -0.22)). The strongest cross-correlation coefficient for ACTH and cortisol concentrations in offspring was 0.75 (0.70 – 0.81), which was similar to their partners (0.80 (0.76 – 0.85)). No significant cross-correlations between cortisol and GH, and between ACTH and GH, were found after stratifying for offspring and partners. In partners, a mean negative correlation coefficient of -0.22 (-0.29 – -0.14) was found between ACTH and TSH concentrations. In contrast, no significant correlation between ACTH and TSH was observed in the offspring.

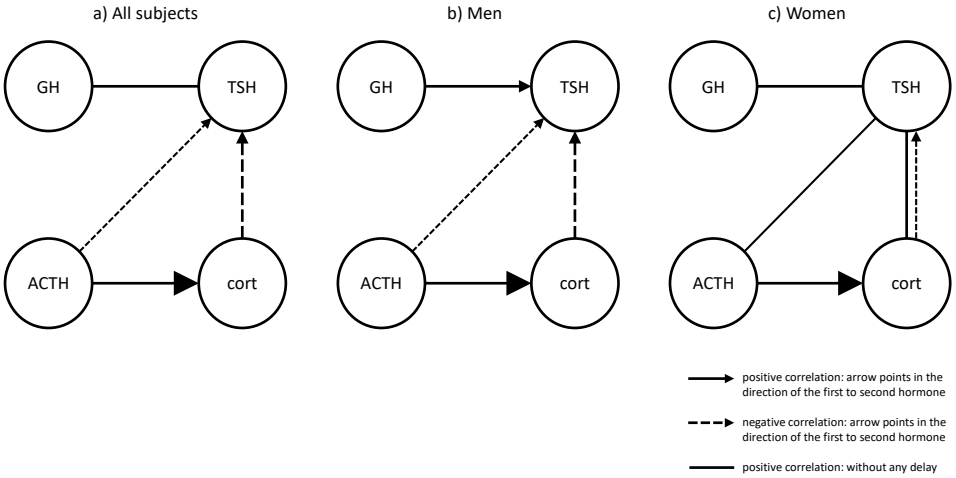


Figure 3. Summary of cross correlations in a) all subjects, b) men, and c) women.
A graphical summary of cross-correlation analyses in a) all 38 participants, b) 20 male participants, and c) 18 female participants. Solid lines represent positive correlations between hormones which is strongest at lag time 0, so without a delay. Solid arrows represent positive correlations between hormones which is strongest at a certain lag time, with the arrow directed towards the hormone which is following the leading hormone. Dotted arrows represent negative correlations between hormones which is strongest at a certain lag time, with the arrow directed towards the hormone which is (negatively) following the leading hormone. The weight of the line/arrow represents the strength of the correlation.

Cross-ApEn of GH, TSH, ACTH, and cortisol

Figure 4 presents box plots of cross-approximate entropy (cross-ApEn) results for hormone combinations of GH, TSH, ACTH, and cortisol. Values of cross-ApEn ranged from 0.5 to 2.3 and mean values ranged from 0.9 to 1.4 in all participants, with lower cross-ApEn values signifying greater joint pattern synchrony between two hormone concentration time series. The cross-ApEn between GH and cortisol was the lowest of all hormone combinations with a mean (95% CI) of 0.9 (0.8 – 1.0). Also the cross-ApEn values of GH-TSH, GH-ACTH, and cortisol-GH were lower than those of other hormone combinations. After stratifying for lights-on and lights-off periods, cross-ApEn values were significantly lower during the lights-on period compared with the lights-off period for the following hormone combinations: cortisol-TSH, GH-TSH, and GH-cortisol (data not shown). For cortisol-TSH, a mean (SE) difference of -0.17 (0.07) with a *P* value of 0.03 was found between lights-on and lights-off periods. The mean (SE) difference in cross-ApEn of GH-TSH was -0.21 (0.08) with $P = 0.01$ and for GH-cortisol, the mean (SE) difference was -0.15 (0.07) with $P = 0.04$. For the hormone combinations ACTH-GH, ACTH-cortisol, ACTH-TSH,

and TSH-ACTH, cross-ApEn values were lower during the lights-off period compared with the lights-on period where lower cross-ApEn signifies stronger synchronicity. For ACTH-GH, the mean (SE) difference in cross-ApEn between lights-on and lights-off periods was 0.24 (0.06) with a *P* value < 0.001. The difference in cross-ApEn between lights-on and lights-off periods was greatest for ACTH-cortisol with a mean (SE) difference of 0.27 (0.07) and a significance of *P* < 0.001. A mean (SE) difference of 0.15 (0.07) (P=0.03) for ACTH-TSH cross-ApEn values between lights-on and lights-off periods was found. Also the cross-ApEn of TSH-ACTH was lower during the lights-off period compared with the lights-on period with a mean (SE) difference of 0.17 (0.07) and *P* = 0.02. No significant differences between men and women were found, but in general men tended to have lower cross-ApEn values than women (data not shown). Also between offspring and partners no significant differences were observed (data not shown).

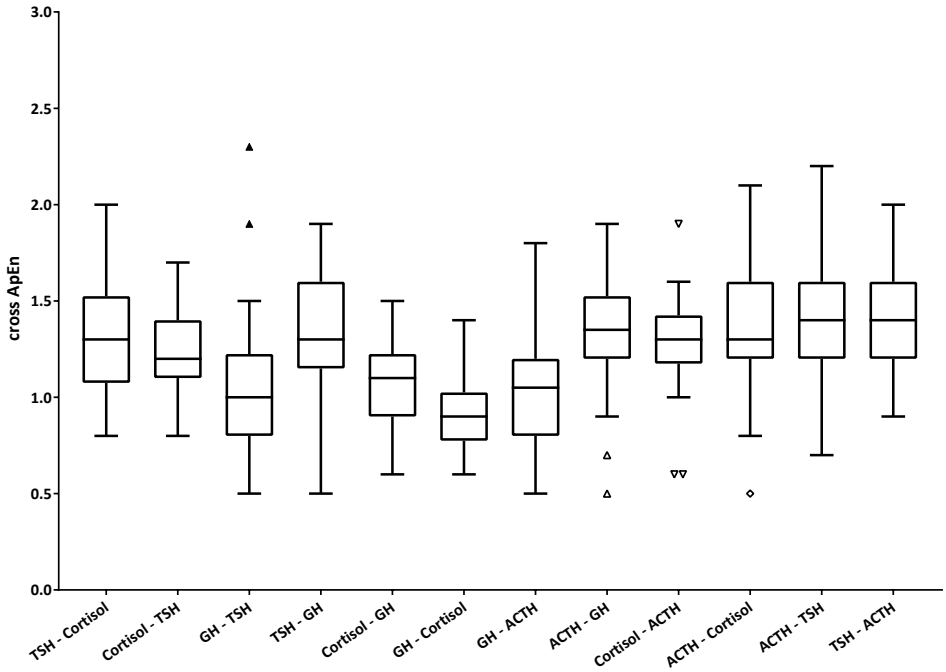


Figure 4. Cross ApEn for GH, TSH, ACTH, and cortisol.

Tukey box plots of the cross approximate entropy results of combinations of the hormones GH, TSH, ACTH, and cortisol in all 38 participants. Lower cross ApEn values signify greater synchrony between two hormone time series.

DISCUSSION

In this study, we aimed to determine the interrelationships between serum concentrations of GH, TSH, ACTH, and cortisol in healthy older individuals using 24-h hormone concentration series with intervals of 10 min. We confirmed that ACTH is positively correlated with cortisol, where cortisol follows ACTH with a delay of 10 min [20-22], and demonstrated that this correlation was stronger during night hours. Furthermore, we corroborate previous observations that cortisol and TSH concentrations are negatively cross-correlated in healthy older individuals [6, 23], where TSH follows cortisol concentrations with a delay of 170 min. Not earlier reported, a positive correlation between TSH and GH without any delay was found, which was more strongly during daytime. The cross-ApEn analyses showed that GH and cortisol exhibit synchronous serum concentration patterns. Several differences in cross-ApEn values were found between lights-on and -off periods, indicating that the pattern synchronicity between hormones is dependent on the time of the day. No major differences in cross-correlations were found between men and women, except for the positive correlation without any delay between cortisol and TSH concentrations, which was found in women but not in men. In general, men tended to have lower cross-ApEn values than women which was similar to other studies and which could indicate that postmenopausal women have reduced hormone pattern synchrony compared to men [9, 29]. No major differences in the interrelationships between hormones were found between offspring and partners.

Although cross-correlation and cross-ApEn analyses are complementary methods, the strong cross-correlation found between concurrent GH and TSH concentrations were strengthened by a relatively low cross-ApEn of GH-TSH pointing to strong synchronization between the two hormone concentration series. This strong interrelationship can probably not be explained by circadian synchronicity, since GH is mostly influenced by sleep and is less under circadian control [24]. Both TSH and GH play important roles in the regulation of energy metabolism, growth, and development, which could explain the presence of the interrelationship between TSH and GH concentrations. Additionally, we could speculate that this interrelationship between TSH and GH is established by the dopaminergic or somatostatinergic system, since these systems play regulatory roles in both the TSH and GH secretion [25]. Moreover, thyrotropin-releasing hormone (TRH) might stimulate, besides TSH, the secretion of GH, which was observed in a culture of rat cells [26]. 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. Therefore, one might expect stronger correlations between TSH, GH, and prolactin than for example with ACTH, LH, or FSH. This might explain the strong correlation between GH and TSH.

The strong negative cross-correlation between cortisol and TSH indicates that cortisol concentrations are negatively followed by TSH with a delay of 90 to 180 min. Literature shows that glucocorticoids indeed suppress TSH secretion [6, 23, 27, 28] and it is believed that glucocorticoids exert a suprapituitary action. For both GH-TSH and cortisol-TSH, the cross-correlation as well as the pattern synchronicity were stronger during daytime than during night-time. One explanation why some of the significant cross-correlations disappeared after stratifying for lights-off periods could be that there are less data points during the lights-off period. This dilutes any effects and increases the threshold value for significance. Interestingly, we observed an even stronger correlation between ACTH and cortisol during the lights-off period, which potentially could be explained by the fact that the ACTH-cortisol system is maximally active during night-time. Similarly, cross-ApEn values of ACTH and cortisol were lower, indicating higher synchrony, during the night in this study, but also in healthy young subjects [20].

There is no cut-off value for significance for cross-ApEn values, but when comparing hormone pairs with each other, we found that the combination of GH and cortisol had the greatest joint pattern synchrony of all hormone combinations with higher synchrony during daytime. Another study found similar results for GH-cortisol cross-ApEn in healthy older men and women [7, 29]. Also other studies have shown a link between cortisol and GH in human [30, 31]. Other cross-ApEn values of hormone combinations with GH were relatively low as well, including the GH-TSH and GH-ACTH combinations, which could indicate that GH is interlinked with many different hormonal axes. We did not find a significant cross-correlation between GH and cortisol, which demonstrates that cross-correlation and cross-ApEn analyses are complementary methods. Cross-correlation assesses the strength between paired time series for all possible time shifts, resulting in linear lag-specific correlations, while cross-ApEn quantifies joint pattern synchrony between paired time series, which is lag-independent [32]. These methods therefore reflect different aspects of interrelationships between hormones.

This is one of the first studies in which interrelationships between hormones from different hypothalamic-pituitary-target gland axes over 24 h in healthy older subjects have been investigated by cross-correlation and cross-ApEn analyses. This innovative approach is a strength of the study although it makes it exploratory in nature as its novelty limits the

availability of similar studies. Cross-correlations between hormone concentrations are not evidence for a direct causal relationship between hormones. Furthermore, the potential day-to-day intra-subject variation remains unknown. Cross-ApEn is a validated tool to determine the joint pattern synchrony in a closed hormone system with known feedforward and feedback signals. However, cross-ApEn is rarely applied to combinations of hormones from different hormonal axes which makes it harder to interpret the biological meaning. Therefore, this study is more descriptive than conclusive. Nonetheless, it may promote the generation of new hypotheses on which future research can build.

It is assumed that offspring of long-lived families are biologically younger than their partners since among the key findings from the Leiden Longevity Study were the observations that the offspring had lower prevalence of myocardial infarctions, diabetes mellitus, hypertension, and metabolic syndrome compared to their partners [10, 33]. Therefore, we hypothesized that the offspring of long-lived families would have stronger hormonal interrelationships than controls. However, no major differences in the interrelationships between hormones were found between offspring 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.

Hormones of the hypothalamic-pituitary-gonadal and the hypothalamic-pituitary-prolactin axes were not considered in this article. However, GH, TSH, ACTH, and cortisol might also interact with these hormones. Especially since lactotrophs, somatotrophs, and thyrotrophs largely share the same developmental cascade. Indeed, studies showed a positive association between the hypothalamic-pituitary-thyroid axis and prolactin; TRH regulates the synthesis and release of prolactin [34, 35], and Saini *et al.* found concurrent pulses of TSH and prolactin in young men [36]. Furthermore, prolactin was positively correlated with GH, TSH, and ACTH without any delay and with cortisol at a lag of 10 min in obese women [5]. Also the hypothalamic-pituitary-gonadal axis seems to be associated with other hormonal axes; long-term testosterone administration resulted in increased overnight GH secretion in healthy older men [37] and prolactin concentrations increased in response to oestrogen treatment in postmenopausal women [38].

CONCLUSION

This study demonstrates that interrelations between hormones from interlinked as well as different hypothalamic-pituitary-target gland axes are observed in (older) individuals. In particular, the correlations between cortisol and TSH concentrations, between TSH and GH concentrations, and the great joint pattern synchrony between GH and cortisol, are indications that distinct hormonal axes interact in healthy older individuals. No major differences were found between men and women, except for the positive correlations between cortisol and TSH concentrations found in women. Also no major differences between offspring of long-lived families and partners were found. The cross-correlation and pattern synchronicity between TSH and GH, and the pattern synchronicity between cortisol and TSH, were stronger during daytime than during night-time, but the cross-correlation and pattern synchronicity between ACTH and cortisol were stronger during night-time. More research is needed to determine the biological meaning and clinical consequences of these interrelationships between pituitary hormones.

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