



# Cortisol serum levels in familial longevity and perceived age: The Leiden Longevity Study

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## KEYWORDS

Cortisol;  
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## Summary

**Background:** Cortisol levels are strongly associated with a person's health. Familial longevity and age assessment of facial photographs (perceived age) are both associated with morbidity and mortality. The present study aimed to investigate morning cortisol levels in familial longevity and the association of these levels with perceived age.

**Methods:** Perceived age and serum morning cortisol levels were measured for 138 offspring from long-lived families and 138 partners from the Leiden Longevity Study. Considered confounding factors were chronological age, gender, body mass index, current smoking habits, antidepressant drug use, antihypertensive drugs and diabetes medication.

**Results:** In the fully adjusted model, which was restricted to participants who did not use antidepressant drugs, offspring had similar serum cortisol levels compared to their partners (0.54 and 0.55  $\mu\text{mol/L}$ , respectively;  $p = 0.54$ ). Using a similar model taking offspring and partners together, an increase of 0.1  $\mu\text{mol/L}$  in morning cortisol levels was associated with an 0.42 (95% CI 0.0–0.84,  $p = 0.048$ ) year increase in perceived age. This association was significantly attenuated in the offspring group (0.01, 95% CI –0.58 to 0.59,  $p = 0.98$ ) compared to the partner group (0.81, 95% CI 0.20–1.41,  $p = 0.009$  year increase in perceived age per 0.1  $\mu\text{mol/L}$  increase in cortisol respectively) ( $p$  for interaction = 0.042).

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**Conclusion:** This study demonstrates that high levels of cortisol are associated with a higher perceived age. This association was attenuated in offspring from long-lived families compared to their partners, suggesting enhanced stress resistance in these subjects. Future research will be aimed at elucidating potential mechanisms underlying the observations in this study.

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## 1. Introduction

Chronic psychological stress has a major effect on a person's health, as it is associated with both an increased incidence of cardiovascular morbidity and mortality (Kiecolt-Glaser and Glaser, 1999; Ohlin et al., 2004) and with a poorer immune response (Kiecolt-Glaser et al., 1996; McEwen et al., 1997; Vedhara et al., 1999). One of the hormones secreted under acute stress is the glucocorticoid cortisol. Cortisol secretion is tightly regulated by the hypothalamus–pituitary–adrenal axis (HPA axis) (Lundblad and Roberts, 1988). Moreover, cortisol itself inhibits upstream HPA signaling by a negative feedback mechanism, resulting in a diminished secretion of cortisol (Beyer et al., 1988). Chronically high serum cortisol levels are frequently observed in patients with Cushing syndrome (Boscaro et al., 2001), depression (Tafet et al., 2001), and obesity (Bjorntorp and Rosmond, 2000). Subjects having chronically high levels of cortisol have a higher prevalence of muscle weakness, osteoporosis, hypertension and diabetes mellitus type 2 and have a higher mortality risk (Walker et al., 1998; Manelli and Giustina, 2000; Schoorlemmer et al., 2009). Thus, high levels of cortisol are potentially damaging for tissues over time and, conversely, low levels might be indicative of healthy aging.

The Leiden Longevity Study was set up to study biological mechanisms associated with familial longevity and healthy aging. Families were included in this study when at least two siblings had reached the age of 89 years (men) or 91 years (women) (Schoenmaker et al., 2006). The middle aged offspring of these long-lived siblings have a lower prevalence of diabetes mellitus type 2, myocardial infarction, and hypertension compared to their partners (married and cohabitating), who were not part of a long-lived family (Westendorp et al., 2009). It is unknown, however, whether cortisol associates with familial longevity and, therefore, whether it could be partly responsible for the beneficial profile of the offspring.

Another marker of health aging is how old individuals look in facial photographs (their, so-called, "perceived age"). A higher perceived age (*i.e.* an older looking facial appearance) associates with both morbidity and mortality (Christensen et al., 2009). In addition, environmental factors known to influence health also associate with perceived age. For example, smoking and low body mass index both associate with an older looking appearance, whereas a high social class and high education associate with a younger looking appearance (Rexbye et al., 2006). Research on identical twins suggests also a genetic contribution to perceived age (Shekar et al., 2005; Gunn et al., 2009). However, specific genes associated with perceived age have yet to be described. Furthermore, biological mechanisms associated with an older (or younger) looking appearance still need to be elucidated. Previous

research has hinted toward an association between cortisol and perceived age, as depression is associated with both (Tafet et al., 2001; Rexbye et al., 2006), but a direct associative relationship has yet to be investigated.

In this study we aimed to investigate whether cortisol levels are associated with familial longevity and perceived age. To assess this, three research questions were addressed. First, we assessed whether offspring from long-lived families have lower levels of serum morning cortisol compared to their partners. Second, we determined whether higher levels of serum morning cortisol levels were associated with a higher perceived age. And third, we assessed whether the association between morning cortisol levels and perceived age was different between offspring from long-lived families and their partners. To answer these research questions we measured morning cortisol levels and assessed perceived age in a sample of 276 middle aged subjects (138 offspring and 138 partners thereof) from the Leiden Longevity Study.

## 2. Materials and methods

### 2.1. Study design

The Leiden Longevity Study was designed to find phenotypic and genetic markers related to familial longevity. An extended description about the longevity phenotype and inclusion strategy has been published previously (Westendorp et al., 2009). In short, a total of 421 families were recruited consisting of long-lived Caucasian siblings together with their offspring and the partners thereof. Inclusion was only performed when at least two long-lived siblings were still alive and fulfilled the age criteria of 89 years in case of males and 91 years for females. The siblings were not selected on health conditions or demographics. Because proper controls at high age are lacking, the offspring from these nonagenarian siblings were asked to participate along with their partners who act as age-matched controls (Schoenmaker et al., 2006) who did not come from a long-lived family. Partners are hereafter called controls.

In total, 190 couples (an offspring living with a control), who were living in the near district of the research center (less than 45 min by car), were asked to come fasted at 0830 h to the research center for fasted blood sampling and an oral glucose tolerance test. In total 140 couples approved and participated in this study. Four subjects (2 offspring and 2 controls) were excluded because of use of oral corticosteroids at the time this study was conducted, leaving 276 subjects (138 offspring and 138 controls) for analyses. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center and written informed consent was obtained from all subjects.

## 2.2. Cortisol measurements

Cortisol levels were measured in serum using a cortisol assay (ECLIA) on the Modular E170 (Roche Diagnostics, Mannheim, Germany). The reference range of the measurement for morning cortisol in serum was between 0.1 and 0.6  $\mu\text{mol/L}$ .

## 2.3. Perceived age assessment

The method for assessing a person's perceived age has been described and validated previously (Gunn et al., 2008, 2009; Christensen et al., 2009). In short, participants were asked to come to the research center without any make-up or hair-styling products. From all participants we took one facial photograph from the front and one at 45 degrees. Photographs, with hair and clothing concealed, were assessed to determine perceived age by 60 independent assessors. Assessors were predominately British and had no previous age assessment experience. There was some variation in age and gender among the assessors, however, this was previously shown to have a negligible effect on the average age assessment when a high number of assessors is used (Gunn et al., 2008). The mean of all 60 assessments was used for analyses and is herein called the perceived age. Inter-rater reliability of the perceived age assessment was determined using Cronbach's alpha and was 0.99.

## 2.4. Other variables

At the study center, weight and height were measured by research nurses. Information on smoking habits was obtained using a questionnaire. Information on disease history was obtained from subjects' general practitioner and medication use by subjects' pharmacies. Body mass index was calculated by dividing weight (in kilograms) with length (in meters) squared.

## 2.5. Statistical analysis

A series of linear regression models were estimated. In each case, model 1 adjusted for age and gender; model 2 additionally adjustment for body mass index, current smoking, antidepressant drug use, antihypertensive drugs, and diabetes medication; and model 3 was restricted to those not taking anti-depressant medication. The first set of analyses compared serum morning cortisol levels between offspring and controls. A second set of analyses examined associations between cortisol levels and perceived age, for the full sample and separately for offspring and controls.

We then investigated a possible statistical interaction in the association between cortisol levels and perceived age between the offspring and the partner groups. For this, we included a two-way interaction term of cortisol level and group (offspring or control) in the regression model, and examined whether the association between cortisol levels and perceived age was statistically different in the offspring compared to the control group.

The association between cortisol levels and perceived age is presented as the change in years in perceived age per 0.1  $\mu\text{mol/L}$  increase in serum morning cortisol. For a graphical interpretation of the association between morning cortisol levels and perceived age in a normal population, we divided the controls in tertiles based on their morning cortisol levels. All statistical analyses were performed using the SPSS program for Windows (version 17.0, USA). A  $p$ -value below 0.05 was considered statistically significant.

## 3. Results

### 3.1. Characteristics of the study population

Characteristics of the study population are presented in Table 1. On average, the perceived age of the subjects was lower compared to their chronological age. The offspring group was

**Table 1** Characteristics of the study population.

	All (N = 276)	Offspring (N = 138)	Controls (N = 138)
Females, no. (%)	137 (49.6)	66 (47.8)	71 (51.4)
Chronological age (years)	63.0 (6.7)	63.5 (6.1)	62.6 (7.4)
Perceived age (years) <sup>a</sup>	59.2 (7.7)	59.3 (7.4)	59.0 (8.0)
Body mass index ( $\text{kg/m}^2$ )	26.6 (3.9)	26.5 (4.0)	26.8 (3.9)
Current smoking, no. (%)	30 (10.9)	15 (10.9)	15 (10.9)
Disease history, no. (%)			
Diabetes mellitus type 2	19 (6.9)	7 (5.1)	12 (8.7)
Hypertension	71 (25.7)	34 (24.6)	37 (26.8)
Myocardial Infarction	4 (1.4)	2 (1.4)	2 (1.4)
Stroke	4 (1.4)	4 (2.9)	0 (0.0)
Malignancies	19 (6.9)	9 (6.5)	10 (7.2)
COPD	10 (3.6)	4 (2.9)	6 (4.3)
Rheumatoid arthritis	3 (1.1)	2 (1.4)	1 (0.7)
Medication use, no. (%)			
Diabetes medication	15 (5.4)	7 (5.1)	8 (5.8)
Anti-hypertensives	78 (28.3)	34 (24.6)	44 (31.9)
Anti-depressants	14 (5.1)	7 (5.1)	7 (5.1)

All continuous data presented as mean (standard deviation), except when indicated otherwise.

<sup>a</sup> As measured by age assessment of facial photographs. Abbreviations: COPD, chronic obstructive pulmonary disease.

**Table 2** Morning cortisol levels in offspring and controls.

	Offspring		Controls		T-value	p-Value
	N	Mean cortisol (95% CI)	N	Mean cortisol (95% CI)		
Model 1	138	0.49 (0.47–0.52)	138	0.51 (0.48–0.53)	0.74	0.46
Model 2	138	0.53 (0.47–0.59)	138	0.55 (0.48–0.61)	1.06	0.29
Model 3	131	0.54 (0.49–0.59)	131	0.55 (0.50–0.61)	0.62	0.54

Data presented as mean cortisol concentrations (in  $\mu\text{mol/L}$ ) with a 95% confidence interval (95% CI). Model 1 is adjusted for age and gender; model 2 is adjusted for chronological age, gender, body mass index, current smoking, antidepressant use, antihypertensive drugs and diabetic medication. Model 3 is the same as model 2 but restricted to participants without antidepressant medication use.

not different to the control group with respect to their chronological age, perceived age, body mass index, gender and current smoking habits. Offspring had a slightly lower prevalence of type 2 diabetes and hypertension, but were similar with regard to prevalence of myocardial infarction, stroke, chronic obstructive pulmonary disease (COPD) and rheumatoid arthritis. Both groups were similar with regard to antidepressant drug use.

### 3.2. Serum morning cortisol levels and familial longevity

The comparison in morning cortisol levels between offspring and partners is presented in Table 2. There was no difference in serum morning cortisol levels between the offspring and controls. Similar results were obtained when analyses were performed within males and females separately (results not shown), when analyses were adjusted for possible confounding factors (0.53  $\mu\text{mol/L}$  for offspring and 0.55  $\mu\text{mol/L}$  for controls;  $p = 0.29$ ) and when excluding those who used antidepressant drugs (0.54  $\mu\text{mol/L}$  for offspring and 0.55  $\mu\text{mol/L}$  for controls;  $p = 0.54$ ).

### 3.3. Association of morning cortisol with perceived age

Results on the association between morning cortisol levels and perceived age, for offspring and controls combined are presented in Table 3. When adjusted for chronological age and gender, a 0.1  $\mu\text{mol/L}$  increase in serum morning cortisol level was associated with a 0.51 year increase in perceived age (model 1, 95% CI 0.11–0.92,  $p = 0.014$ ). Additional adjustment for possible confounding factors, including body mass index, current smoking habits, use of antidepressant drugs, antihypertensive drugs, and diabetes medication diminished the association to some extent, but was still near

statistical significance (0.40 year increase in perceived age per 0.1  $\mu\text{mol/L}$  increase in morning cortisol levels (model 2, 95% CI –0.0 to 0.81,  $p = 0.052$ )). Restriction of the analysis to participants who did not use antidepressant drugs did not materially change the size of association, a 0.42 year increase per 0.1  $\mu\text{mol/L}$  increase in morning cortisol levels, although the association now reached statistical significance (model 3, 95% CI 0.0–0.84,  $p = 0.048$ ).

### 3.4. Serum morning cortisol in offspring and partners separately

To assess whether the association between morning cortisol levels and perceived age was different in offspring as compared to controls, we repeated all analyses for offspring and partners separately. Results from these analyses are presented in Table 4. After adjusting for chronological age and gender, the association between cortisol levels and perceived age was significant in the partners but not in the offspring, 0.81 year increase in perceived age per 0.1  $\mu\text{mol/L}$  increase in morning cortisol ( $p = 0.007$ ) in the partners compared to a 0.18 year increase per 0.1  $\mu\text{mol/L}$  increase in morning cortisol ( $p = 0.52$ ) in the offspring. However, the difference between the offspring and controls with regard to the association of cortisol level and perceived age was not statistically significant (model 1,  $p$  for interaction = 0.14). The difference in association increased, however, when the analysis was adjusted for additional confounding factors (model 2,  $p$  for interaction = 0.054). When those on antidepressant drugs were excluded, an increase of 0.1  $\mu\text{mol/L}$  morning cortisol levels was associated with an increase in perceived age of only 0.01 years ( $p = 0.98$ ) in the offspring, whereas the same increase in cortisol levels in the controls resulted in an 0.81 year increase in perceived age ( $p = 0.009$ ). The difference in association was statistically significant (model 3,  $p$  for

**Table 3** Association of cortisol levels and perceived age.

	N	Change in years per 0.1 $\mu\text{mol/L}$ cortisol increase (95% CI)	T-value	p-Value
Model 1	276	0.51 (0.11–0.92)	2.48	0.014
Model 2	276	0.40 (–0.0 to 0.81)	1.95	0.052
Model 3	262	0.42 (0.0–0.84)	1.99	0.048

Model 1 is adjusted for chronological age and gender. Model 2 is adjusted for chronological age, gender, body mass index, current smoking, antidepressant use, antihypertensive drugs and diabetic medication. Model 3 is the same as model 2 but restricted to participants without antidepressant medication use.

**Table 4** Association of cortisol levels and perceived age in offspring and controls separately.

	<i>N</i>	Change in years per 0.1 μmol/L cortisol increase (95% CI)	<i>T</i> -value	<i>p</i> -Value	<i>p</i> for interaction
Model 1					
Offspring	138	0.18 (−0.39 to 0.75)	0.63	0.53	0.14
Controls	138	0.81 (0.22–1.40)	2.73	0.007	
Model 2					
Offspring	138	−0.00 (−0.57 to 0.56)	0.01	0.99	0.054
Controls	138	0.75 (0.16–1.35)	2.50	0.014	
Model 3					
Offspring	131	0.01 (−0.58 to 0.59)	0.02	0.98	0.042
Controls	131	0.81 (0.20–1.41)	2.64	0.009	

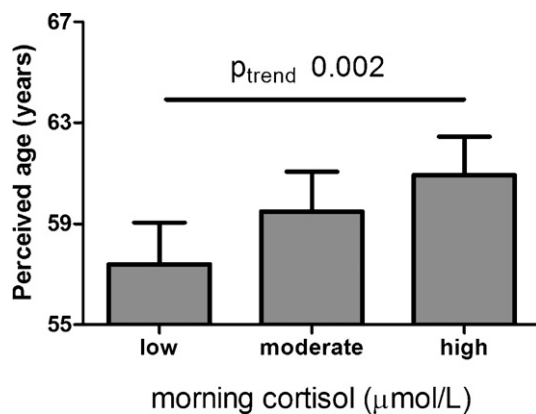
Model 1 is adjusted for chronological age and gender. Model 2 is adjusted for chronological age, gender, body mass index, current smoking, antidepressant use, antihypertensive drugs and diabetic medication. Model 3 is the same as model 2 but restricted to participants without antidepressant medication use. The *p* for interaction states whether the slopes of the association between morning cortisol levels and perceived age are statistically different in the offspring compared to the partners.

interaction = 0.042). A graphical presentation of the relationship between cortisol levels and perceived age in controls is given in Fig. 1.

## 4. Discussion

### 4.1. Main findings

The main findings of this study are threefold. First, we found that morning cortisol levels in serum were similar in offspring from long-lived families compared to their age and environmental matched controls. Second, we showed that high serum cortisol levels were associated with a higher perceived age. And third, we showed that the association between cortisol levels and perceived age was attenuated in offspring from nonagenarian siblings compared to the controls.



**Figure 1** Perceived age dependent on tertiles of morning cortisol levels in the control group. Figure depicts the perceived age in tertiles (low, medium and high) of serum morning cortisol levels in the control group only. Mean perceived age increases from 57.4 (SE = 1.65) years in the lowest tertile to 59.5 (SE = 1.57) years in the second tertile to 60.9 (SE = 1.54) years in the highest tertile, *p* for trend = (0.002). Data is presented as means with the standard error of the mean. The analysis was adjusted for chronological age, gender, body mass index, current smoking, antidepressant drug use, antihypertensive drugs and diabetes medication.

### 4.2. Serum morning cortisol levels and familial longevity

Morning cortisol levels were similar for the offspring and control groups. The lack of a trend in this comparison suggests that sample size might not explain the lack of a difference in cortisol levels. In support of this finding, a previous study also showed no difference in serum morning cortisol levels between groups of young, healthy aged and centenarian subjects (Ferrari et al., 2008). However, nocturnal cortisol levels in elderly subjects, but not morning levels, were found to be higher than in young individuals (Dodt et al., 1994). This latter study indicates that evening or nocturnal cortisol levels might be a better estimate of cortisol levels than levels in the period after awakening. Additional studies on cortisol rhythm will clarify whether cortisol in the evening or at night are lower in offspring compared to controls.

### 4.3. Association of morning cortisol with perceived age

In the present study, high levels of serum morning cortisol were associated with a higher perceived age. It has been previously reported that subjects with a higher depression score also had a higher perceived age (Rexbye et al., 2006). Moreover, higher cortisol levels are associated with depression (Tafet et al., 2001). In this study, we showed that the association between morning cortisol levels and perceived age was largely independent from confounders including antidepressant drug use, suggesting that the association was not confounded by depression.

Skin aging is a major component of facial aging and is strongly associated with perceived age (Gunn et al., 2009). Chronically high levels of cortisol, as is the case in Cushing syndrome, have severe adverse effects on almost all tissues throughout the body, but particularly skin tissue, which results in skin atrophy and impaired wound healing (Boscaro et al., 2001). *In vivo* studies have shown that exposing skin to cortisol (and other glucocorticoids) results in reduced pro-collagen production (Autio et al., 1994; Oikarinen et al., 1998). In addition, by the inhibition of the



transcription factors AP-1, cortisol inhibits the expression of MMP-1 (collagenase) which breaks down damaged collagen (Karin and Chang, 2001). This suggests that high levels of cortisol could lead to premature aging of the skin via a reduced production and repair of the collagen matrix. In addition, collagen repair occurs in other collagenous tissues including liver and kidney. Thus, previous links between perceived age and morbidity and mortality (Christensen et al., 2009) could be due to the effects of cortisol on collagen repair in skin and other tissues. However, skin is only one of a few tissues that influence perceived age (Gunn et al., 2009) and more research is required to elucidate the biological mechanisms that link cortisol levels to perceived age.

#### 4.4. Serum morning cortisol in offspring and partners separately

In this study, we additionally showed that the association between morning cortisol levels and perceived age was less pronounced in offspring compared to their partners, suggesting that familial longevity might be marked by enhanced stress resistance. One possibility is that the above described link between cortisol and MMP-1 is less effective in offspring from long-lived families compared to their partners. We have previously reported that skin fibroblast cultures from the offspring group exhibit a more resistant phenotype to a chemical stressor (Dekker et al., 2009), supporting the notion that cells from offspring might be more resistant to cellular insults. More research on the stress response, including a dexamethasone suppression test, should further elucidate whether the offspring truly have a cortisol resistant phenotype.

#### 4.5. Limitations

This study has a few limitations to address. Because of the cross-sectional study design, it was not possible to infer whether cortisol and perceived age are causally linked. Second, the cortisol samples were only measured at a single time point in the morning, whereas cortisol varies greatly over the day with the mean peak just after awakening. However, because the enrolled offspring were asked to come to the research center at the same time point together with their partners, the average time at which the blood was drawn from the subjects was similar. Nevertheless, this strategy has possibly resulted in an increased variation within the cortisol data, which attenuated the associations between morning cortisol levels and perceived age. More accurate cortisol data might give more precise point estimates. Third, no data on depression was available for the current study. Instead we made use of pharmacy data on antidepressant drug use. The limitation of this strategy is that patients with, for example, anxiety disorders and neuropathic pain, who also take antidepressant medication, were also excluded from our analyses. However, considering the small number of participants taking antidepressant medication, the influence of this misclassification will have been negligible. Despite these shortcomings, the significant associations in a relatively

small sample size suggest a relationship between cortisol levels and perceived age.

#### 4.6. Conclusion

The main conclusions of the current study are twofold. First, higher levels of serum morning cortisol levels associated with a higher perceived age. Second, although the controls with high cortisol levels looked significantly older than those with lower levels, such a relationship was not seen in the offspring, suggesting these offspring have a more stress-resistant phenotype. Additional rhythmic cortisol studies will provide greater sensitivity for confirming the relationship between cortisol levels, perceived age and familial longevity in the future.

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#### Conflict of interest

All authors declare no conflict of interest.

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