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Loneliness in older adults is associated with diminished cortisol output

N. Schutter [a,*], T.J. Holwerda [a], M.L. Stek [b], J.J.M. Dekker [c,d], D. Rhebergen [b], H.C. Comijs [b]

[a] Arkin Mental Health Care, Department of Geriatric Psychiatry, Nieuwe Kerkstraat 156, 1018 VM Amsterdam, The Netherlands
[b] GGZ InGeest, Department of Psychiatry, Amsterdam Public Health Research Institute, VU University Medical Center Amsterdam, The Netherlands
[c] Department of Psychology, VU University Amsterdam, Amsterdam, The Netherlands
[d] Department of Clinical Research, Arkin Mental Health Care, Amsterdam, The Netherlands

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ABSTRACT

Objective: Loneliness in older adults has been associated with increased mortality and health problems. One of the assumed underlying mechanisms is dysregulation of the hypothalamic-pituitary-adrenocortical axis (HPA–axis). The purpose of this study was to investigate whether loneliness in older adults is associated with HPA-axis dysregulation and whether this association differs between depressed and non-depressed persons.
Methods: Cross-sectional data of 426 lonely and non-lonely older adults in the Netherlands Study of Depression in Older Persons (NESDO) were used. Linear regression analyses and multinominal logistic regression analyses were performed to examine the association between loneliness and morning cortisol, cortisol awakening response, diurnal slope and dexamethasone suppression ratio. In all analyses, confounders were introduced. In order to examine whether the association between loneliness and cortisol measures is different in depressed versus non-depressed persons, an interaction term for loneliness x depression diagnosis was tested.
Results: Cortisol output in the first hour after awakening and dexamethasone suppression ratio was lower in lonely participants. There were no significant interactions between loneliness and depression diagnosis in the association with the cortisol measures.
Conclusion: This study is the first to investigate the association between the HPA–axis and loneliness in a large group of older adults aged 60–93 years. We found lower cortisol output in the first hour after awakening and lower dexamethasone suppression ratio in lonely older depressed and non–depressed adults. Whether diminished cortisol output is the underlying mechanism that leads to health problems in lonely older adults is an interesting object for further study.

1. Introduction

Loneliness is one of the main indicators of social well-being in all age categories [1]. It is described as a situation that occurs from a lack of quality relationships and is considered to be an expression of negative feelings of missing relationships in contrast with the actual absence of relationships [1]. Loneliness in older adults has been associated with increased mortality and a wide range of health problems such as less sleep, depression, decreased cognitive functioning over time and age-related increases in systolic blood pressure [2–7]. Previous studies have investigated the mechanisms through which loneliness affects health. One of the assumed underlying mechanisms is dysregulation of the hypothalamic-pituitary-adrenocortical axis (HPA-axis) [3,8–10].

The HPA-axis is a hormonal response system that can be activated by a broad array of mental and physical stressors [11,12]. Cortisol output usually shows a diurnal rhythm, with the highest levels in the early morning and lowest levels in the evening [13]. Cortisol levels are high upon waking, show a substantial increase in the 30–45 min after waking (called the cortisol awakening response or CAR) and decline over the remainder of the day (diurnal slope) [14,15]. These different features of cortisol levels can be influenced by a variety of sociodemographic variables and health indicators, such as sex, age, smoking and cardiovascular disease [14,16]. Additionally, chronic or severe stress may lead to dysregulation of the HPA-axis [13]. For instance, Heim et al. described several studies that have found that chronic stress, such as found in Post Traumatic Stress Disorder (PTSD), may lead to decreased urinary cortisol secretion and to low cortisol levels in plasma or saliva samples [17]. Yehuda [18] in her overview of HPA-axis alterations in PTSD concluded that PTSD patients show evidence of a highly sensitized HPA-axis with decreased basal cortisol levels and increased negative feedback. Seedat et al. found that 'intimate partner violence' encompassing physical and sexual abuse frequently in the context of emotional abuse, leads to decreased mean plasma cortisol levels [19].

Loneliness is considered a chronic source of stress [20]. Various researchers have studied the effects of loneliness on the HPA- axis, mostly in middle-aged adults. They found that loneliness is associated with a higher cortisol awakening response (CAR) [8,10]. In adolescents and

* Corresponding author.
E-mail address: natasja.schutter@mentrum.nl (N. Schutter).

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young adults, loneliness has been found to be associated with flattening of the diurnal cortisol rhythm, but not with changes in CAR [21,22]. However, Doane and Adam did find that prior day increases in loneliness predicted a greater cortisol awakening response the following morning. Elevated mean salivary cortisol levels across the course of a day were found in undergraduate students who were chronically lonely [23]. These outcomes in middle-aged (higher cortisol awakening response) and younger adults (flatter cortisol rhythm) suggest that there is a possible age-related difference in the effects of chronic loneliness on HPA-axis activity. Thus far, the effect of loneliness on the HPA-axis in older adults has not yet been extensively investigated. The only study that included older adults is the one by Adam et al. [8], who included older adults up to 68 years old.

Next, loneliness has been found to be a significant risk factor for depressive symptoms in a group of adults aged 54 years and older [4]. Loneliness and depression have strong reciprocal influences in middle-aged and older adults [4]. In depression, a fairly consistent biological finding is an altered activity of the HPA-axis, such as higher basal cortisol levels in the morning and high post-dexamethasone levels [24]. The dexamethasone suppression test examines the function of the negative feedback loop: dexamethasone, a synthetic glucocorticoid, decreases cortisol levels by acting on the pituitary [25]. With adequate feedback, cortisol levels are suppressed after dexamethasone intake. In depression, this negative feedback decreases, leading to higher post-dexamethasone cortisol levels (non-suppression). Depression in older adults is also associated with changes in cortisol output. Rhebergen et al. [26] found higher morning cortisol levels and lower AUCi (area under the curve with respect to the increase) in depressed older patients. Lower AUCi signifies a decreased ability to respond dynamically to the stress of awakening, thus indicating a less dynamic cortisol awakening response [26]. Belvederi Murri et al. [24] found a high degree of dysregulation of HPA-axis activity, with higher basal cortisol levels in older depressed patients compared to younger ones during all phases of the diurnal cycle and high post-dexamethasone cortisol levels in plasma. These findings possibly indicate a specific pattern of dysregulation of HPA-axis activity in geriatric depression.

Since loneliness and depression are strongly associated, and depression is associated with HPA-axis dysregulation, the effect of depression has to be taken into account when studying the association between loneliness and the HPA-axis. The aim of our study is to investigate whether loneliness in older adults is associated with dysregulation of the HPA-axis. Based on previous research in middle-aged and older adults [8,10,24,26], we hypothesize that loneliness is associated with higher cortisol awakening response and that the effects of loneliness on HPA-axis functioning are more pronounced in depressed participants.

2. Methods

2.1. Study sample

Study participants were derived from the Netherlands Study of Depression in Older Persons (NESDO), a large cohort ($N = 510$) study designed to investigate in a prospective design the course of late-life depression and comorbidities. Detailed information on the NESDO design, recruitment and methods is described elsewhere [27]. In short, respondents were recruited from general practitioners and mental health institutions. Non-depressed participants were recruited from general practices and were included when there was no lifetime diagnosis of depression. General exclusion criteria were: a primary clinical diagnosis of dementia or other severe psychiatric disorder, a Mini-Mental State Examination-score (MMSE) [28] below 18 (out of a maximum of 30 points) indicating severe cognitive impairment, and not being fluent in the Dutch language. The final study population consisted of 378 depressed and 132 non-depressed persons aged 60 through 93 years ($n = 510$). A diagnosis of depression included a 6-month diagnosis of a Major Depressive Disorder (MDD) (95% of depressed persons) and/or a 6-month dysthymic disorder (26.5% of depressed persons), or a minor depression (5% of depressed persons) according to DSM-IV-TR criteria [29]. The ethical review boards of participating study sites approved of the research protocol and all respondents provided written informed consent.

For the present study, an exclusion criterion would be the use of corticosteroid medication, as corticosteroid use would influence cortisol levels. However, none of the participants used corticosteroids. Persons with at least one cortisol measure were included. Since 84 persons had missing values on all the cortisol measures, the final study population consisted of 426 participants [26].

2.2. Measurements

2.2.1. Psychopathology

Psychopathology was assessed with the Composite International Diagnostic Interview (CIDI; WHO version 2.1; life-time version). The CIDI is a structured clinical interview that is designed for assessing mental disorders according to the definitions of ICD-10 and DSM-II-R, to be used in research settings, and has a high validity and reliability [30–32]. Due to its high degree of standardization of symptom questions, it improves consistency of symptom assessment and reliability of diagnostic decision and can be administered reliably by non-clinicians after a relatively brief training [32].

2.2.2. Cortisol

Cortisol levels were obtained through saliva sampling. Respondents were instructed to collect saliva samples at home on two consecutive days shortly after the interview at baseline. For each sample, participants were instructed to refrain from eating, drinking or brushing teeth within 15 min before sampling. No dental work up to 24 h prior to sampling was allowed [26]. Six saliva samples were taken: at the time of awakening (T1), 30 minute post-awakening (T2), 45 min post-awakening (T3), 60 minute post-awakening (T4) and at 22:00 h (T5). Furthermore, dexamethasone suppression was measured by sampling the next morning at awakening (T6) after dexamethasone ingestion of 0.5 mg the night before (directly after T5). The Dexamethasone Suppression Test (DST) is a measure of HPA-axis regulation and normally shows a decrease of morning cortisol concentrations due to inhibition of adrenocorticotropic hormone (ACTH) secretion after ingestion of dexamethasone the night before [25,33]. The salivettes were stored in the refrigerator in a tube labeled with date and time. After collecting all six samples, the participants were asked to return the samples by regular mail to the research center. After receipt, salivettes were centrifuged at 2000*g* for 10 min, aliquoted and stored at −80 °C. Cortisol analysis was performed by competitive electrochemiluminescence immunoassay (E170 Roche, Switzerland). The functional detection limit was 2.5 nmol/L and the intra- and inter-assay variability coefficients in the measuring range were <10% [26].

2.2.2.1. Cortisol awakening response (CAR). From the four saliva samples taken within 1 h after awakening (T1 through T4), the areas under the curve with respect to the increase (AUCi) and with respect to the ground (AUCg) were calculated using Pruessner's formulas [34]. The AUCg is an estimate of the total cortisol secretion over the first hour after awakening, whereas the AUCi represents the dynamics of the cortisol awakening response (CAR), more related to the sensitivity of the system, emphasizing changes over time after awakening (see also [26]). AUCi and AUCg could be calculated for all persons from whom all four morning cortisol samples were available (AUCg: $n = 369$; AUCi: $n = 371$). For area under the curve (AUC) analyses, at least three samples had to be available [15,26,35]. For those with one missing cortisol value ($n = 53$), the missing value was imputed using linear regression analyses including information on the three available cortisol levels, sex, age, awakening time and smoking status (see also [26]).

For all cortisol measures, outliers with values of >2 standard deviations from the mean were excluded from the analyses [14,15,26,35].

2.2.2.2. Evening cortisol and diurnal slope. The diurnal slope was calculated by subtraction of the evening sample (T5) from the sample at awakening (T1), resulting in the decline in cortisol level during the day. Due to missing samples at T1 or T5, the diurnal slope could be calculated for 383 persons [26]. A frequently used method for obtaining the diurnal slope is subtracting the wakeup cortisol value from the bedtime value, dividing by the total time awake [16]. Since we did not measure the total time awake, we could not calculate diurnal slope in this way.

2.2.2.3. Dexamethasone suppression test (DST). We calculated the dexamethasone suppression ratio in the persons for whom morning cortisol values on day 2 were available and who had taken 0.5 mg of dexamethasone the night before. The dexamethasone suppression ratio was calculated dividing the cortisol value at awakening on day 1 (T1) by the cortisol value at awakening the day after dexamethasone ingestion (T6). The dexamethasone suppression ratio could be calculated for 341 persons [26].

2.2.3. Loneliness

Loneliness was assessed with the De Jong Gierveld Loneliness Scale [36]. This scale was developed for use in scientific survey research. It is a valid and reliable measurement instrument for overall, emotional and social loneliness in older adults [37]. This scale consists of 11 items. The five-category responses are transformed into dichotomous responses, resulting in a maximum total score of 11. The cut-off score of 3 distinguishes between lonely and not lonely persons, whereas a cut-off score of 9 distinguishes between severely lonely people and not or moderately lonely persons [37]. In this study we used cut-off scores of 3 and 9.

2.2.4. Covariates

Since previous studies found associations between cortisol measures and various socio-demographic, biological, life style and sampling factors, it is important to adjust for these possible covariates [14,15,26].

Socio-demographic factors included age, sex, partner status, social network and years of education. Social network was assessed with the Close Persons Questionnaire [38].

Clinical characteristics included psychiatric diagnoses of depressive disorders, severity of depressive symptoms and use of antidepressant medication. A depression diagnosis included a primary diagnosis of major depression, dysthymia or minor depression according to DSM-IV criteria in the past 6 months and was assessed with the Composite International Diagnostic Interview (CIDI; WHO version 2.1; life-time version) [30,31]. The severity of depressive symptoms in the past week was measured using the 30-item Inventory of Depressive Symptomatology–Self Report version (IDS-SR). The IDS-SR is a valid and reliable instrument, also for use in older persons [39]. Antidepressant use was determined by inspection of the medication containers and classified according to the Anatomical Therapeutic Chemical (ATC) classification [40]. The use of selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs) and other antidepressants were dichotomized into yes/no (see also [26]).

Health characteristics included the number of chronic diseases. These were assessed by means of a self-report questionnaire that has previously been used in the Longitudinal Aging Study Amsterdam [41]. Participants were asked whether they currently or previously had any of the following chronic diseases: cardiac disease, peripheral atherosclerosis, stroke, diabetes mellitus, COPD, arthritis or cancer, or any other disease.

Lifestyle characteristics included Body Mass Index (BMI), smoking status (no smoker or current smoker) and physical activity (measured with the International Physical Activity Questionnaire (IPAQ)) in MET-minutes [42].

Sampling factors of cortisol consisted of time of awakening and season on the first sampling day. Season was categorized by months with less daylight (October–February) and more daylight (March–September).

2.3. Statistical analyses

To examine the distribution of characteristics across non-lonely, lonely and severely lonely participants we used chi-square statistics for categorical variables, Student's *t*-tests for continuous variables and Mann-Whitney *U* tests in case of non-normal distributions.

To detect relevant confounders, we performed linear regression analyses between loneliness and cortisol measures and consecutively introduced possible confounders [43]. When the value for B changed >10%, the variable was considered a confounder.

To examine the association between loneliness and cortisol measures, linear regression analyses were performed with loneliness as a continuous measure. In case of non-normality, we performed multinominal regression analyses. We performed analyses with loneliness as a continuous variable and loneliness in three dummy groups: non-lonely, lonely and severely lonely. In order to study whether the association between loneliness and cortisol measures was dependent on the presence of depression, we entered the interaction terms of "loneliness x depression diagnosis (a primary diagnosis of major depression, dysthymia or minor depression according to DSM-IV criteria in the past 6 months)" in the fully adjusted models. Analyses were conducted using SPSS 22.

Table 1
Characteristics of the study population ($n = 426$).

	Not lonely ($N = 135$)	Lonely ($N = 166$)	Severely lonely ($N = 125$)	*p*-Value*
Age, mean (SD)	70 (7.4)	71 (7.2)	71 (7.3)	0.58
Female, %	90 (67)	98 (59)	80 (64)	0.38
Years of education, mean (SD)	12.0 (3.6)	10.7 (3.6)	10.5 (3.6)	**0.02**
Partnerstatus, % with partner	104 (77)	99 (60)	53 (42)	***0.01**
Depressed, %	57 (42)	132 (80)	122 (98)	***0.01**
Total IDS scores, mean (SD)	13.0 (11.4)	24.8 (14.7)	31.8 (12.7)	***0.01**
No. of somatic illnesses, mean (SD)	1.9 (1.3)	2.3 (1.5)	2.7 (1.7)	***0.01**
BMI, mean (SD)	26.7 (4.1)	26.2 (4.3)	27.0 (4.6)	0.26
Smoking (%)	16 (12)	40 (24)	32 (26)	**0.01**
Physical activity MET-min/week (SD)	3044 (2813)	2683 (2691)	2349 (2518)	**0.17**
MMSE-score, mean (SD)	28 (3.0)	28 (1.7)	28 (2.0)	0.96
Social network, number of persons above 18, %				
0–1	0 (0)	18 (11)	26 (21)	***0.01**
2–5	40 (30)	65 (39)	68 (54)	
6–10	51 (38)	51 (31)	22 (18)	
≥11	43 (32)	31 (19)	8 (6)	
Loneliness scores, mean (SD)	0.9 (0.8)	5.3 (1.8)	10.0 (0.9)	***0.01**
Sampling factors				
Time of awakening (SD)	7.29 (0.52)	7.31 (1.02)	7.23 (1.11)	0.54
More daylight (%)	98 (73)	102 (61)	76 (61)	0.79
Cortisol values				
T1 median (IQ)	15.5 (10.1)	16.5 (10.3)	15.9 (11.0)	0.60
AUCg, μg/dL/h median (IQ)	17.7 (8.7)	17.9 (11.2)	16.7 (10.4)	0.46
AUCi, μg/dL/h median (IQ)	1.1 (8.7)	−0.09 (7.7)	0.77 (8.8)	0.37
Diurnal slope median (IQ)	12.3 (9.7)	12.2 (11.4)	12.4 (10.9)	0.95
Cortisol suppression ratio median (IQ)	2.8 (2.3)	2.7 (2.0)	2.4 (2.1)	0.32

Significant p-values are in bold type.
* using chi-square statistics for categorical variables, Student's t-tests for continuous variables and Mann-Whitney U-tests in case of non-normal distributions.

3. Results

Sociodemographic and clinical characteristics of the study sample according to loneliness status are summarized in Table 1. The mean age of the study sample was 70.3 (SD 7.3) and 62.9% was female. There were no significant differences in age and gender between the lonely and non-lonely participants. Compared to non-lonely participants, lonely participants had a slightly lower level of education, had more somatic diseases, smoked more often, were more often without a partner and were more often depressed or anxious. Social network size was larger in the non-lonely participants.

For 426 participants, at least one cortisol measure was available. There were no significant differences in sampling factors. The mean number of salivettes returned was 5.48. 70% of the participants returned all six samples. 215 participants (61%) showed an increase in cortisol in the first 30 min after awakening. Mean values for cortisol measures in non-lonely, lonely and severely lonely participants are shown in Table 1. Cortisol levels were not sufficiently normally distributed: two of the four cortisol measures (diurnal slope and DST) appeared to be skewed: for AUCg, skewness was 0.73 and kurtosis was 0.58, for AUCi these values were 0.36 and 1.30, for DST ratio these values were 1.17 and 1.71 and for diurnal slope they were 1.09 and 1.98 Log transformation did not ameliorate this. Therefore we performed linear regression analyses (AUCg and AUCi) and multinominal logistic regression analyses (diurnal slope and DST) with loneliness as independent variable and cortisol measures as outcome variables.

To detect confounding variables, we introduced these possible confounders one by one in the regression analyses: age, gender, partner status, years of education, social network size, depression diagnosis, total IDS-SR score, smoking status, total MET-minutes per week, number of chronic diseases, BMI, MMSE-score, use of antidepressant medication, time of awakening, month of cortisol sampling [43]. These possible confounders were chosen based on theory and previous research [14,16]. Partner status, years of education, depression diagnosis, severity of depressive symptoms, network size, smoking status and total MET-minutes per week were found to be confounders. Because of the overlap between depression diagnosis and severity of depressive symptoms and the risk of multicollinearity we decided to consider only severity of depressive symptoms as confounder. Therefore, we subsequently executed linear regression analyses and multinominal regression analyses with loneliness as independent variable and cortisol measures as outcome variables and adjusted the analyses for partner status, years of education, severity of depressive symptoms, network size, smoking status and total MET-minutes per week.

3.1. Loneliness as a continuous variable

First, we analyzed the association between loneliness and the cortisol measures AUCg and AUCi with loneliness as a continuous variable, using the total score on the De Jong Gierveld Loneliness Scale. The results are shown in Table 2.

Loneliness was significantly associated with a lower area under the curve with respect to the ground (AUCg) (Table 2). We found no associations with AUCi. To test our hypothesis that the effects of loneliness on HPA-axis functioning are more pronounced in depressed participants, we entered the interaction term loneliness x depression diagnosis in the fully adjusted model. Because of the risk of collinearity, when we introduced this interaction term we removed total IDS-score from the model. This interaction term was not significant for all of the cortisol measures (Table 2).

Because of non-normality of cortisol measures, we analyzed diurnal slope and DST with multinominal logistic regression. The results are presented in Table 3. We did not find an association of loneliness with diurnal slope and DST nor significant interaction terms with depression.

Table 2
Linear regression analyses for loneliness as a continuous variable with cortisol measures as outcome.

Outcome	B	95% CI for B	*p*-Value
T1			
Loneliness	−0.10	−0.32–0.12	0.40
Loneliness[a]	−0.21	−0.52–0.11	0.20
Loneliness × depression[b]	−0.46	−1.29–0.38	0.28
AUCg			
Loneliness	−0.14	−0.36–0.07	0.19
Loneliness[a]	**−0.36**	**−0.66** to **−0.07**	**0.02**
Loneliness × depression[b]	−0.03	−0.82–0.77	0.94
AUCi			
Loneliness	−0.04	−0.25 to 0.17	0.73
Loneliness[a]	0.01	−0.29–0.31	0.97
Loneliness × depression[b]	0.29	−0.51–1.08	0.48

T1 = morning cortisol; AUCg = area under the curve with respect to the ground; AUCi = area under the curve with respect to the increase.
[a] Controlled for partner status, years of education, total IDS-score, network size, total MET-minutes per week, smoking status.
[b] Controlled for depression diagnosis, partner status, years of education, network size, total MET-minutes per week, smoking status.

3.2. Loneliness in three groups

We repeated the analyses with three groups of loneliness: non-lonely, lonely and severely lonely. These results were comparable to the analyses with loneliness as a continuous measure: a significant negative association between loneliness and AUCg. However, the significant association exists only in the severely lonely group. Additionally, we found an association between loneliness and the dexamethasone suppression test, both in the lonely and in the severely lonely group (Table 4).

We could not analyze interaction terms between the loneliness groups and depression diagnosis because the group of severely lonely non-depressed persons was too small (3 persons only).

4. Discussion

The objective of this study was to investigate whether loneliness in a large sample of older adults is associated with dysregulation of the hypothalamic-pituitary-adrenocortical axis. We found lower cortisol output the first hour after awakening (AUCg) in lonely participants. We also found diminished dexamethasone suppression in lonely participants. These findings are in contrast with our hypothesis that loneliness

Table 3
Multinominal regression analyses for loneliness as a continuous variable with cortisol measures.

Outcome	Low versus high cortisol		Middle versus high cortisol	
	Exp (B)	95% CI for exp (B)	Exp (B)	95% CI for exp (B)
Diurnal slope				
Loneliness	1.00	0.93–1.06	0.99	0.93–1.06
Loneliness[a]	0.99	0.90–1.08	1.00	0.91–1.09
Loneliness × depression[b]	1.05	0.82–1.33	1.15	0.88–1.50
DST				
Loneliness	1.05	0.98–1.13	1.01	0.95–1.09
Loneliness[a]	1.09	0.98–1.20	1.10	0.99–1.21
Loneliness × depression[b]	1.09	0.79–1.52	0.92	0.68–1.24

Diurnal slope = subtraction of evening cortisol from cortisol at awakening; DST = dexamethasone suppression ratio.
[a] Controlled for partner status, years of education, total IDS-score, network size, total MET-minutes per week, smoking status.
[b] Controlled for depression diagnosis, partner status, years of education, network size, total MET-minutes per week, smoking status.

Table 4
Multinominal regression analyses for three groups of loneliness with cortisol measures.

Outcome	Low versus high cortisol		Middle versus high cortisol	
	Exp (B)	95% CI for exp (B)	Exp (B)	95% CI for exp (B)
T1				
Lonely	0.93	0.63–1.36	1.07	0.74–1.56
Severely lonely	1.22	0.79–1.90	1.03	0.65–1.63
Lonely[a]	1.27	0.62–2.59	1.62	0.81–3.23
Severely lonely[a]	1.47	0.64–3.38	1.70	0.75–3.88
AUCg				
Lonely	0.98	0.66–1.45	0.90	0.61–1.34
Severely lonely	1.40	0.88–2.24	1.00	0.60–1.66
Lonely[a]	1.56	0.73–3.36	1.04	0.51–2.13
Severely lonely[a]	**2.50**	**1.03–6.07**	1.19	0.49–2.87
AUCi				
Lonely	1.21	0.81–1.83	1.26	0.84–1.89
Severely lonely	0.95	0.60–1.49	0.82	0.51–1.31
Lonely[a]	1.25	0.58–2.69	1.56	0.75–3.26
Severely lonely[a]	1.05	0.44–2.51	1.00	0.42–2.40
Diurnal slope				
Lonely	0.96	0.66–1.41	0.96	0.66–1.41
Severely lonely	1.03	0.65–1.64	1.00	0.63–1.60
Lonely[a]	1.12	0.54–2.32	0.97	0.48–1.95
Severely lonely[a]	1.14	0.48–2.72	0.97	0.42–2.21
DST				
Lonely	0.93	0.61–1.43	1.16	0.77–1.74
Severely lonely	1.36	0.83–2.21	1.11	0.66–1.85
Lonely[a]	1.25	0.54–2.87	**2.64**	**1.16–6.00**
Severely lonely[a]	2.15	0.83–5.52	**3.22**	**1.21–8.53**

T1 = morning cortisol; AUCg = area under the curve with respect to the ground; AUCi = area under the curve with respect to the increase; diurnal slope = subtraction of evening cortisol from cortisol at awakening; DST = dexamethasone suppression ratio.
[a] Controlled for partner status, years of education, total IDS-score, network size, total MET-minutes per week, smoking status.

would be associated with higher cortisol awakening response compared to cortisol values in older adults who are not lonely. We also hypothesized that HPA-axis dysregulation associated with loneliness would be even more pronounced in depressed participants. This hypothesis was not supported by our findings.

The analyses in three groups of loneliness show that lower AUCg is significantly associated with severe loneliness (Table 4). Dexamethasone suppression was significantly lower in both lonely and severely lonely groups (middle dexamethasone suppression ratio versus high dexamethasone suppression ratio, Table 4).

Previous studies found higher cortisol awakening response in the first 30 min after awakening in lonely middle-aged adults (47–59 years old) and the youngest old (50–68 years old) [8,10]. In lonely students, elevated mean salivary cortisol levels across the course of a day were measured [23], as well as flattening of diurnal cortisol rhythm, but no changes in CAR [21,22]. Hence, there seems to be a difference in HPA-axis dysregulation between younger lonely adults and older lonely adults. The middle-aged and older adults in these studies were between 47 and 59 years [10] or between 50 and 68 years old [8], whereas our study included persons varying in age from 60 to 93 years old. Since our study included a large group with a larger age-range, age-related alterations in HPA-axis function may play a role in these differences.

Age-related alterations in HPA-axis functioning have been described before [44,45], suggesting enhanced HPA-axis reactivity, resulting in higher morning cortisol levels. Ferrari et al. [46] also described age-related changes in cortisol secretion. They measured serum cortisol levels every 4 h during the day and every 2 h during the night in five different groups, ranging from young healthy participants to centenarians and old demented patients. Results showed that in comparison to young controls, morning cortisol levels were lower and the circadian rhythm of serum cortisol was flattened mostly due to higher nocturnal cortisol values in healthy elderly subjects, and even more so in demented patients. This finding suggests that aging itself may have a diminishing effect on diurnal cortisol levels. Enhanced HPA-axis reactivity with increasing age could explain the differences found between HPA-axis reactions to loneliness in middle-aged adults as compared with young adults. However, this does not explain our findings in lonely older adults, which showed lower cortisol levels. The finding that diurnal cortisol levels may diminish with increasing age could play a role in the difference between our findings of lower cortisol values in elderly lonely persons and Steptoe et al. [10] findings of higher cortisol levels in middle-aged lonely persons.

A review and meta-analysis performed by Miller et al. [13] indicated that generally, chronic stress is associated with lower morning cortisol levels, flattening of the diurnal variation with greater concentrations of afternoon/evening cortisol and higher daily volume of cortisol output. The authors identified a number of factors potentially relevant in HPA-axis function. For instance, it has been shown that morning cortisol levels and daily cortisol volume are inversely associated with the number of months since the onset of stress. With increasing age, loneliness could be of longer duration, resulting in diminishing activity or extinguishing of the HPA-axis. This may explain our finding that loneliness is associated with decreased cortisol output the first hour after awakening, suggesting exhaustion of HPA-axis function.

In this study we also measured dexamethasone suppression. We could not find other studies examining dexamethasone suppression in relation to loneliness in older adults. In one study, the dexamethasone suppression test was given to older adults suffering from bereavement [47]. These older adults also suffered from major depression. Although depression has been found to be associated with higher morning cortisol levels after dexamethasone ingestion the night before and more non-suppression [24,48], the authors found that severe depressive symptoms during bereavement were not associated with non-suppression on the dexamethasone suppression test. A positive association between age and dexamethasone non-suppression in a population of depressed patients but not in healthy controls has been found [48,49], suggesting an interaction between depression and aging. In our study, we found higher cortisol levels the morning after ingestion of 0.5 mg of dexamethasone in lonely and severely lonely older adults compared to non-lonely older adults, suggesting that loneliness may be related to non-suppression. We found no difference between depressed and non-depressed participants: the interaction term loneliness x depression diagnosis was not significant for DST.

Lower AUCi has been found in older depressed participants compared to non-depressed participants [26], indicating a less dynamic cortisol awakening response. Our results did not show a greater decline in AUCi related to loneliness in the depressed group than in the non-depressed group. However, the group of lonely non-depressed persons consisted of only 21 persons. This is possibly too small a group to detect significant differences between depressed and non-depressed lonely persons.

A strong point in our study is the NESDO-design, which provides the opportunity to study loneliness and cortisol in the context of a large well-defined representative cohort of depressed and non-depressed older adults. Compared to previous studies, we included older participants. Moreover, our study population consisted of 426 subjects, whereas previous studies investigating HPA-axis function in lonely people included only 25 up to 240 participants [2,3,8,21,10,50]. Another strength is that we were able to adjust for a large amount of covariates.

There are some limitations in this study. First, the design is cross-sectional. In order to be able to make causal inferences, longitudinal studies should be performed. Second, we only assessed cortisol levels on one day. This may have contributed to lower reliability of cortisol measurement [16]. However, most of the current large-scale collections of cortisol are obtained in this way and more research is needed to quantify the unreliability this method introduces [16]. Third, we did not assess compliance with the study protocol with an objective indicator. We did however provide extensive advice to the participants as suggested by Adam and Kumari [16]. Furthermore, previous evidence suggests

participants do collect morning cortisol samples accurately in relation to objectively determined waking times [52]. Fourth, as mentioned above, the group of lonely non-depressed persons was rather small. This group consisted of 21 persons and was therefore possibly too small to detect differences with respect to the association between loneliness and cortisol values between depressed and non-depressed participants. Fifth, a rather large group of people ($n = 125$, 39% of total population) did not show a rise in cortisol in the first 30 min after waking up. Other studies show a similar amount of people (32.5% and 31% respectively) not showing a rise in cortisol within the first hour of awakening [14,15,53]. One possible explanation is a delay between waking up and taking the first saliva sample, resulting in non-adherence to the time-protocol for cortisol collection. However, in studies with strict monitoring of compliance with the cortisol sampling procedure, still 14.7% of people do not show a rise in cortisol in the first hour after awakening (Dockray et al. 2008). Another explanation is the presence of cardiovascular disease, which has been found to be negatively associated with 1-h awakening cortisol levels [14,15,54]. We performed a chi-square test between loneliness and rise in cortisol in the first hour of awakening and found no significant association. Finally, we did not measure total sleep time in the night before taking cortisol samples and therefore were not able to calculate diurnal slope as suggested by Adam and Kumari [16]. However, by administering the Insomnia Rating Scale, we did ask participants what the mean number of hours sleep per night was. We were therefore able to calculate diurnal slope by subtracting wakeup from bedtime cortisol values and dividing by the mean total time awake. The results from regression analyses with these diurnal slope values did not differ from the results with analyses using diurnal slope values as described in the methods sections (presented in Tables 2, 3 and 4).

5. Conclusion

This study is the first to investigate the association between loneliness and HPA-axis regulation in a large group of older adults aged 60–93 years. We have found that loneliness in older adults is associated with diminished cortisol output and diminished dexamethasone suppression. HPA-axis dysregulation resulting in lower cortisol levels may lead to disease in various ways. Inadequate responses by one physiologic system (for example cortisol secretion) may lead to compensatory increases in others (for example secretion of inflammatory cytokines) [12]. Also, adrenal steroids play an important role in trafficking immune cells [11]. Hypocortisolism has been described as a relevant factor in the pathogenesis of autoimmune disorders, inflammation, chronic pain, asthma and allergies [17]. Finally, frailty has been found to be associated with lower cortisol levels 30 min after awakening [51]. These observations provide interesting starting points for further study. Another interesting finding is the diminished dexamethasone suppression associated with loneliness in older adults. Together, these two findings suggest that loneliness as a source of stress may influence biologic systems through dysregulation of the HPA-axis.

Conflict of interest statement

This manuscript has not been publicized previously, nor is it being considered for publication elsewhere. All the authors have contributed to and approved of this manuscript. The authors declare no conflict of interest.

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