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Cumulative Load of Depressive Symptoms is Associated with Cortisol Awakening Response in Very Old Age

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This research was supported by grants from the National Institute on Aging (AG 08523-02), the Australian Research Council (DP 0879152; LP 100200413), and the National Health and Medical Research Council (229922). Christiane Hoppmann gratefully acknowledges the support of the Michael Smith Foundation for Health Research.

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Abstract

Concurrent associations between depressive symptoms and markers of hypothalamic-pituitary-adrenal activity, including cortisol, are well established. However, little is known about the potential cumulative burden of older adults' past history of depressive symptoms on alterations in the cortisol diurnal profile as older adults engage in their everyday lives. We used five waves of longitudinal data over a 15-year period from 50 older adults (*M* age = 89.05 years; 64% female) who participated in the Australian Longitudinal Study of Ageing (ALSA) and who also provided seven cortisol samples per day over a one-week period as part of the ALSA Daily Life Time-Sampling Study (ADuLTS). Of interest were links between cumulative and present depressive symptoms and the present cortisol diurnal profile in oldest-old adults. Findings revealed an effect of cumulative depressive symptoms on the cortisol awakening response (CAR) in the oldest-old. Specifically, individuals who had reported more past depressive symptoms over the previous 15 years showed a lower CAR compared to individuals with fewer past depressive symptoms. Interestingly, present depressive symptoms were not associated with the CAR. These findings suggest that cumulative depressive symptoms are associated with a blunted cortisol diurnal profile in the oldest-old. The integration of research on long-term developmental changes in depressive symptoms and cortisol diurnal profiles informs our understanding of distal health factors in very old age.

Keywords: Salivary cortisol, HPA axis, depressive symptoms, oldest-old

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Psychological models of health and aging emphasize the inter-relations between *distal* and *proximal* factors, and health outcomes (Aldwin & Gilmer, 2013). For example, the integrative approach to health (B. H. Singer & Ryff, 2001) identifies the need to link information across diverse levels of analysis, including biological and psychosocial, and across time. Empirically, Wrosch, Miller, Lupien, and Pruessner (2008) showed that an increase in physical symptoms over a two-year period (*distal* factor) was associated with a higher level of cortisol across three days, but only among those who experienced high negative affect and poor sleep (*proximal* factors). Longitudinal studies measure long-term changes in individuals over years, i.e., on a macro-time scale (Nesselroade & Baltes, 1979). In contrast, intensive time-sampling studies measure short-term processes in individuals over days or multiple times within a day, i.e., on a micro-time scale (Salthouse & Nesselroade, 2010). The understanding of *distal* and *proximal* factors in health benefits from an embedded study design by integrating intensive time-sampling studies within a longitudinal study (Ram, Gerstorf, Lindenberger, & Smith, 2011; Ram, Lindenberger, & Blanchard-Fields, 2009). Specifically, the embedded study design affords the examination of the inter-relations in changes in health that manifest in multiple levels, e.g. biological and psychosocial, and different timescales, e.g. years and days.

The present study implemented this integrated approach to examine both long-term changes in depressive symptoms (over a 15-year period) and cortisol diurnal profiles over seven days in oldest-old adults. Given individual differences in the patterns of change in depressive symptoms in old age, oldest-old adults who have the same current level of depressive symptoms may have a different history of depressive symptoms (Burns, Butterworth, Luszcz, & Anstey,

2013; Schilling, Wahl, & Reidick, 2013). Thus, the examination of depressive symptoms over time may provide important insight to understanding individual differences in the cumulative wear and tear in the aging process, and its effect on current cortisol diurnal profile.

Recent research suggests that cortisol, a stress hormone that is secreted when the hypothalamic-pituitary-adrenal (HPA) axis is activated, may provide valuable evidence for the dynamic interactions between stress and health in the aging process (Piazza, Almeida, Dmitrieva, & Klein, 2010). Cortisol can be reliably measured in saliva. The diurnal profile of cortisol has a characteristic shape with relatively low levels at waking and a peak about 30 minutes later. The increase after waking is termed the cortisol awakening response (CAR). Cortisol then gradually declines until bedtime, which is typically described by the diurnal slope (Pruessner et al., 1997). Past findings suggest that the CAR and the diurnal decline may be associated with chronic stress and burnout (Agbedia et al., 2011; Pruessner, Hellhammer, & Kirschbaum, 1999). Chronic alterations in cortisol responses have been associated with increased physical and mental disease risks (Tsigos & Chrousos, 2002). For example, increased and prolonged production of cortisol is manifested in depression and immunosuppression (Tsigos & Chrousos, 2002). However, evidence linking cortisol and health is inconclusive (Saxbe, 2008). Some studies have found that a higher level of cortisol is associated with more adverse health outcomes (Wrosch, Schulz, Miller, Lupien, & Dunne, 2007); others that a lower level of cortisol is associated with more stress and adverse health outcomes (Miller, Cohen, & Ritchey, 2002).

A large body of research has examined individual differences in cortisol responses using experimental paradigms (Dickerson & Kemeny, 2004). However, in the context of aging research, the acute laboratory stress inductions may have limited ecological validity and may not necessarily map onto real life everyday stressors or major stressful life events encountered over

one's lifespan (Saxbe, 2008). As such, recent research incorporating ambulatory cortisol assessments in the context of daily life provides an important supplement to researching the inter-relations between cortisol and health in older adults (Hoppmann & Riediger, 2009).

Leading models of stress and aging, such as the allostatic load model (Karatsoreos & McEwen, 2013; Seeman & Gruenewald, 2006) and the integrated approach to health (B. H. Singer & Ryff, 2001), focus on adaptation and vulnerability in shaping physical and mental health over time (Miller, Chen, & Zhou, 2007). Although adaptation and vulnerability over time are major foci in these models, empirical examination of stress accumulation over the life course and its manifestation in the daily lives of very old adults is under-developed (Wethington & Exner, 2011). For instance, most studies on cortisol and chronic stress compare the cortisol profiles of a healthy control group to those of a group that experiences high levels of chronic stress (for a review, see Miller et al., 2007). There is little evidence for longitudinal changes in health and the associated cortisol profiles (for an exception, see Wrosch et al., 2008).

The present study used historical and current data on depressive symptoms and current cortisol data to examine the extent to which experience of distal depressive symptoms and proximal depressive symptoms are associated with current diurnal profiles of salivary cortisol in oldest-old adults. Stress occurs throughout life, but advanced old age and approaching the end of life often bring frequent and severe physical and social losses that induce a higher level of depressive symptoms (Baltes & Smith, 2003). From a lifespan perspective, stress experienced in the past may cumulate and maintain an effect on older adults' current health status (Ensel, Peek, Lin, & Lai, 1996). Furthermore, although findings of age-related changes in cortisol are well established, i.e. more blunted (lower CAR and flatter slope of diurnal decline) throughout the day as age increases (Piazza et al., 2010), most studies used samples of young-old adults (e.g. Ice,

Katz-Stein, Himes, & Kane, 2004). To our knowledge, it is not known whether the characteristic cortisol diurnal profile persists into oldest-old age.

We draw from the integrative approach to health (B. H. Singer & Ryff, 2001) to increase our understanding of the inter-relations between depressive symptoms and cortisol in the oldest-old, linking information across biological and psychosocial levels of analysis, and across macro- and micro-time scales. The goals of the study reported here are both substantive and methodological. The primary goals are substantive. First, we provide the descriptive cortisol diurnal profiles in a sample of oldest-old adults. Second, we examine how past and present depressive symptoms are associated with the present cortisol diurnal profiles in the oldest-old. The third goal is methodological. Specifically, we apply multilevel modeling to illustrate the potential and challenges of linking data from multiple levels of analyses and different time-scales.

Based on past findings that chronic stressors are associated with depressive symptoms (McGonagle & Kessler, 1990) and a blunted cortisol diurnal profile (Chida & Steptoe, 2009; Miller et al., 2007; Saxbe, 2008), we tested two hypotheses. First, we expected that a higher current level of depressive symptoms would be associated with a blunted cortisol diurnal profile, i.e., a lower CAR and flatter slope of decline in the oldest-old. Second, we expected that a higher level of cumulative depressive symptoms would be associated with a blunted cortisol diurnal profile, above and beyond the effect of the current level of depressive symptoms.

Methods

Participants

Fifty-one participants were successfully recruited (64.7% women; *M* age = 88.96 years; *SD* = 2.59; range = 83.55 – 96.54) in 2010 from the 168 active participants in the Australian Longitudinal Study of Ageing (ALSA; Luszcz et al., 2007) to the ALSA Daily Life Time

Sampling Study (ADuLTS; Luszcz, et al., 2011, November). Screening criteria utilized ALSA Wave 10 data: (a) vision and hearing sufficient to independently complete diaries; (b) scores on the Mini-Mental State Examination ≥ 24 (MMSE; Folstein, Folstein, & McHugh, 1975); and (c) sound overall functioning as judged by a clinical assessor. Analyses were based on 50 individuals' data (18 men and 32 women; *M* age = 89.05 years; *SD* = 2.50; range = 84.77-96.54) because one participant did not provide salivary samples or other information after the baseline assessment. The vast majority (49 out of 50) of participants lived in the community. Most participants were widowed (62%), others were married (28%), de facto (4%), never married (4%), or divorced (2%). The average participant reported having a diagnosis of 2.48 (*SD* = 1.63) chronic conditions in the last 12 months and taking 7.02 (*SD* = 4.22) medications. They also reported reasonably good health (*M* = 2.68; *SD* = .87; 1 = *Excellent*; 5 = *Poor*).

Attrition analyses showed that ADuLTS participants were positively selected (Luszcz et al., 2011, November), compared to the whole ALSA sample at Wave 1. ADuLTS participants were younger and better educated as well as having better cognitive functioning, self-rated health, functional health, and fewer depressive symptoms and experiencing less decline in health and functioning over the years. Attrition was primarily due to mortality as opposed to experimental-based selectivity, such as refusal to participate. Across 15 variables representing cognitive, emotional, and everyday functioning, mortality explained more than 50% of total selectivity on 12 variables (e.g. Boston Naming Test Incidental Recall (BNTIR): 67%). However, compared to all active ALSA participants in 2010, ADuLTS participants were not positively selected from the non-participants in 29 of 32 variables examined. ADuLTS participants had better memory than non-participants, BNTIR, $t(64) = 2.48$, and MMSE free recall, $t(84) = 2.44$; and did more daily activities involving domestic chores, $t(79) = 2.14$, all ps $< .05$.

Procedure

In addition to the ALSA protocol, participants took part in an ambulatory assessment study, ADuLTS, to capture the cortisol diurnal profile in very old age. The ADuLTS consisted of a baseline session, followed by seven within-day self-assessments, including samplings of saliva, for seven consecutive days. The day after completing all self-assessments, research assistants returned to participants' homes for debriefing, feedback and to collect all materials.

During the baseline assessment, the 10-item Center for Epidemiologic Studies Depression Scale (CES-D; Andresen, Malmgren, Carter, & Patrick, 1994; Radloff, 1977) was administered. Participants also received instructions on the use of a beeper (which they carried throughout the week), when and how to fill out the within-day self-reports (Chui, Hoppmann, Gerstorf, Walker, & Luszcz, in press), and provide saliva samples. Research assistants met the participants again at home on Day 2 to make sure they followed the instructions without difficulties. They were encouraged to contact the research assistant by telephone should the need arise. Each day, the first self-reports and saliva collections were completed shortly after waking up, and were self-initiated. The second self-reports and saliva collections were completed 30 minutes after waking, using a kitchen timer provided to remind them to do this. The remaining five daily assessments were completed in response to a beep that occurred at three-hourly intervals. Beep intervals were adjusted to reduce conflicts with participants' daily routines and covered the entire day. Participants were instructed to respond to the beep as soon as possible and within two hours. To ensure compliance with the protocol, i.e. no back-filling, participants used an electronic time stamp to record the time that they began and finished each self-report. Participants put each questionnaire into an envelope and stamped the time again across the seal. Analyses were based on the baseline data and seven within-day self-reports of daily activities

(see Control variables under Measures), and cortisol samplings for the consecutive seven-day assessment period. Participants provided a total of 2,313 samples of salivary cortisol. Compliance was 94% (*M* = 47, *SD* = 2.62). In particular, out of the total 344 cortisol samples collected at Time 2 during the day, 297 (86%) were collected within the 15-45 minutes post-awakening window (*M* = 31.5 minutes; *SD* = 4.1 minutes; range = 18-45 minutes).

Measures

Salivary cortisol. Saliva samples were collected using synthetic Salivettes (Sarstedt Rommelsdorf, Germany). Participants were asked to record the time of saliva collection using electronic time-stamps. Participants were instructed not to exercise, take a cold shower, brush teeth, eat, smoke, consume caffeine or alcohol, or fall back to sleep before they completed the second saliva collection each morning. Cortisol assays were performed by the Kirschbaum Lab at the Technical University of Dresden, Germany, using a commercial chemiluminescence immunoassay (IBL Hamburg, Germany). Outliers (more than 3 standard deviations above the sample mean for that time of day) were substituted with the respective individual's mean cortisol value of that time of day, provided that the first cortisol sample of the day was not missing and at least three other cortisol samples were available for the respective day.

Cumulative depressive symptoms. The Center for Epidemiologic Studies Depression Scale (CES-D; Radloff, 1977) consists of 20 items of depressive symptomology and was used in the ALSA: Wave 1 (1992), Wave 3 (1994), Wave 6 (2000), Wave 7 (2003), and Wave 9 (2007). Participants rated their depressive symptoms in the last two weeks using a 4-point scale (0 = *Rarely or none of the time*; 3 = *Most or all of the time*). Across the five waves, Cronbach's alpha ranged from .71 to .83. One item missing in Wave 9 was replaced by the individual's mean in that wave. For cases where participants missed a wave, 9 (4%) missing assessments were

substituted using individuals' mean across waves. The score of cumulative depressive symptoms was calculated by summing the scores across waves. Table 1 provides the descriptive statistics of cumulative depressive symptoms. Figure 1a shows the trajectories of the 50 ADuLTS participants and the mean trajectory of depressive symptoms in the 15-year period.

Present depressive symptoms. A 10-item short form CES-D (Andresen et al., 1994) was used in ADuLTS baseline. Participants rated their depressive symptoms in the last two weeks using the same 4-point scale above. One participant did not provide data on one item and mean substitution was performed. Present depressive symptoms was the sum score of 9 items (M = 4.69, SD = 3.46). The item "My sleep was restless" was dropped because Cronbach's alpha increased from .62 to .65 using the remaining 9 items. The lower Cronbach's alpha of the 10-item CES-D in ADuLTS was comparable to that using the same items from ALSA (range = .65-.77).

Control variables. Preliminary analyses were performed to examine the associations between potential control variables and cortisol values. Multiple variables at different levels that showed associations with cortisol were controlled, including between-person, day, and within-day levels. The between-person variables included gender, body mass index (BMI), number of chronic conditions, and use of corticosteroids or medications for depression, anxiety, or thyroid conditions, in the last two weeks (0 = *not taken*; 1 = *taken*). Day level variables included wake-up time and sleep duration. Within-day level variables included use of nicotine, medicine, caffeine, alcohol, having a cold shower, food intake, brushed teeth, and physical exercise (0 = *No since waking or last beep*; 1 = *Yes since waking or last beep*).

Statistical analysis

We present results from two-level models rather than three-level models. Due to non-significance, the day-level random effects were first estimated and later removed. The basic two-level model to capture the cortisol diurnal profile can be represented using these equations:

Level 1 (Within-day model):

$$Cortisol_{ti} = \pi_{0i} + \pi_{1i} \times Time\,since\,waking_{ti} + \pi_{2i} \times Time\,since\,waking_{ti}^2 + \pi_{3i} \times CAR_{ti} + e_{ti}$$

$Cortisol_{ti}$ is the cortisol value at within-day assessment *t* of individual *i*. The intercept, π_{0i}, represents the person-specific cortisol level at waking of individual *i*. Cortisol level was predicted by the time since waking (in hours). Both linear and quadratic terms for time since waking were included to account for curvilinearity in the diurnal decline. The slope π_{1i} represents the person-specific linear change in cortisol across the day from waking to the last assessment of the day of individual *i* and π_{2i} represents the person-specific rate of curvilinearity in the change of cortisol across the day of individual *i*. The 30-minute peak after awakening, i.e. Cortisol Awakening Response (CAR), was indicated with a dummy variable (1 = CAR, cortisol was sampled within 15-45 minutes after waking; 0 = not CAR, cortisol was sampled at other times). π_{3i} represents the person-specific CAR effect of individual *i*. e_{ti} is the error term, that is, the deviation of cortisol level at assessment *t* of individual *i* from the model.

Level 2 (Individual model):

$$\pi_{0i} = \beta_{00} + r_{0i}$$
$$\pi_{1i} = \beta_{10} + r_{1i}$$
$$\pi_{2i} = \beta_{20} + r_{2i}$$
$$\pi_{3i} = \beta_{30} + r_{3i}$$

β_{00} is the mean cortisol level at waking. β_{10} and β_{20} are respectively the mean linear and quadratic effect of time since waking. β_{30} is the mean effect of CAR. The *r's* are error terms that

represent the deviation of individual *i*'s level-1 coefficients from the predicted values based on the level-2 model.

The two-level multilevel approach allowed the modeling of moment-to-moment changes in cortisol diurnal profile. The model also allowed the test of effects of (a) individual characteristics on cortisol by including gender, BMI, medication use (for depression, anxiety disorders, thyroid disorders, and corticosteroids), cumulative and present levels of depressive symptoms, (b) day-level variables including wake-up time and sleep duration, and (c) within-day control variables including use of nicotine, medicine, alcohol, food and caffeine intake, having a cold shower, brushed teeth, and physical exercise. Cumulative and present levels of depressive symptoms were grand-mean centered. Control variables of BMI, wake-up time, and sleep duration were grand-mean centered. Gender was dummy-coded (men = -.5; women = .5). Following Singer and Willett (2003), residuals analyses showed that residuals did not deviate substantially from the normality assumption. Thus, cortisol values were not log-transformed for the more straight-forward interpretation of linear effects.

Results

Descriptive analyses

The mean cortisol levels across the day showed the characteristic diurnal profile, with the wake-up level (M = 14.53; SD = 6.86), the CAR peak (M = 18.66; SD = 9.13), and a gradual decline towards bedtime (M = 3.52; SD = 2.26), indicating that the well-known cortisol diurnal profile continues into oldest-old age. Table 1 provides more descriptive statistics for the cortisol diurnal profile. Cumulative and present CES-D were significantly correlated, r = .57, p < .001.

Multilevel models

Table 2 presents the series of multilevel models fitted to examine the diurnal cortisol profile and the associations with present and cumulative depressive symptoms. First, to examine the cortisol diurnal profile, Model 1 was fitted where time since waking, time since waking^2, and CAR were entered to predict cortisol level. Second, to test the effect of present depressive symptoms, present depressive symptoms and its interaction with time since waking and CAR were entered into Model 2. Third, we examined the effect of cumulative depressive symptoms in Model 3 where effects of cumulative depressive symptoms and its interaction with time since waking and CAR were entered. Finally, we tested whether cumulative depressive symptoms would be associated with cortisol, above and beyond present depressive symptoms, using Model 4. All control variables were entered in these models.

The AIC indicated that Model 4 is the best model but the BIC indicated that Model 1 is the best model. Because Model 1 is nested within Model 4, the Likelihood Ratio test was used to compare Models 1 and 4, $\chi^2 = 20.64$, $df = 6$, $p < .01$. Model 4 was selected as the best-fitting model. Figure 1b gives a graphical presentation of results of Model 4. The significant effect of cumulative depressive symptoms × CAR, $\beta_{32} = -.10$, $SE = .04$, $t(1929) = -2.5$, $p < .05$, indicates that a higher cumulative load of depressive symptoms was associated with a lower CAR. At waking (intercept), individuals with more cumulative depressive symptoms did not differ significantly in their cortisol level. At 30 minutes post-waking (CAR), individuals with more cumulative depressive symptoms had a lower level of cortisol, compared to individuals with fewer cumulative depressive symptoms. The linear and quadratic effects of time since waking did not differ between individuals with varying levels of cumulative depressive symptoms. The effects of current depressive symptoms on cortisol were not statistically significant.

Follow-up Analyses

We performed three sets of additional analyses to evaluate the robustness of the above findings. The first set concerns outliers, control variables, and back-filling. First, outliners in wake-up time were examined. The earliest wake-up time was 1 am. To control for the potential effect of unusually early waking on cortisol, observations 3 *SD* away from mean wake-up time, i.e. before 3:52 am, were removed from analyses (59 observations from 9 participants and 13 days). Results were essentially the same as those reported herein. Second, non-significant control variables were removed from the models described in Table 2. Anxiety disorder medication and wake-time continued to have significant fixed effects on cortisol and were retained in additional analyses. Results of models retaining all control variables and models dropping non-significant control variables were essentially identical. Third, we controlled for possible back-filling based on the time indicated by the electronic time stamps. Questionnaires that were not sealed with a legible time stamp on the envelopes were classified as invalid. In addition, the time on the seal had to be consistent with the finish time (within five minutes) on the questionnaire. Based on these criteria, 90% of all observations were classified as valid cases. Results of analyses using only the valid observations and results based on all observations were substantially identical.

The second set of additional analyses concerned the alternative measures of cortisol diurnal profiles. Person-level aggregates of the components of cortisol diurnal profile were calculated, including the mean area under curve (AUC), mean wakeup level, mean CAR, and mean diurnal slopes (Wakeup-Bedtime and CAR-Bedtime), and their associations with cumulative depressive symptoms were examined. Given the small sample size, we examined the associations between these person-level aggregates and cumulative depressive symptoms using bootstrapped confidence intervals. A set of 500 bootstrapped samples (with replacement) from the original sample was obtained using the boot package in R (Canty & Ripley, 2013). Results of

the 95% confidence interval of the bootstrapped correlation coefficients showed consistent results. More cumulative depressive symptoms were significantly associated with a lower CAR, r = -.18 [-.63, -.0001], $p < .05$, and a flatter diurnal slope (CAR-Bedtime), r = .31 [.06, .56], $p < .05$.

The third set of additional analyses explored five alternative measurements of cumulative depressive symptoms. First, we modeled the developmental trajectory of depressive symptoms using multilevel modeling. Depressive symptoms increased .23 points per year, starting at the initial value of 4.72 at the beginning of ALSA. Individuals' intercepts and slopes were extracted for further analysis. Although results of the multilevel models showed evidence of individual differences in the intercept and slope, the estimated intercepts and slopes were very similar across individuals. Using the estimated intercepts and slopes as measurements of cumulative depressive symptoms resulted in model nonconvergence. Second, we calculated the number of waves in which participants reported high levels of depressive symptoms, separately using prescribed CES-D cutoffs of 16 and 12. A majority of the ADuLTS participants reported low depressive symptoms (74%, CES-D < 16; or 58%, CESD-D < 12) consistently across all five ALSA waves. Using either cutoff, the number of waves with high depressive symptoms was associated with a lower CAR but the effect did not reach statistical significance at $p < .05$. Third, cumulative depressive symptoms were examined for each of the CES-D subscales, i.e., somatic symptoms, depressive mood, positive affect, and interpersonal relationships. Results showed that somatic symptoms, depressive mood, and positive affect were associated with a lower CAR, however, the effects did not reach statistical significance at $p < .05$. Using interpersonal relationships, the model did not converge. The more limited number of items in these subscales might have underestimated their effects on cortisol. Fourth, alternative scores of cumulative

depressive symptoms were calculated (a) ALSA Waves 3, 6, 7, and 9, (b) Waves 6, 7, and 9, and (c) Waves 7 and 9. Consistent with results reported, a higher level of cumulative depressive symptoms from Waves 3, 6, 7, and 9 was significantly associated with a lower CAR, $p < .05$. Using the other two alternative scores of cumulative depressive symptoms, results were in the same direction although did not reach statistical significance. Fifth, because of the use of the different versions of CES-D in ALSA (20-item) and ADuLTS (10-item), we calculated the cumulative depressive symptoms from the ALSA data using the 10-item version. To be consistent with the scoring of present depressive symptoms from ADuLTS, the item "My sleep was restless" was dropped. Results showed a larger effect size of the cumulative CES-D × CAR effect, β_{32} = -.20, *SE* = .06, $t(1929) = -3.11$, $p < .01$, compared to the results reported for the 20-item version. Otherwise, results using the 10-item or 20-item CES-D were essentially identical.

Taken together, results of these additional analyses showed that a higher cumulative load of depressive symptoms was associated with a blunted cortisol diurnal profile, i.e. a lower CAR. The interaction effects of depressive symptoms (both present and cumulative) and time since waking on cortisol, although not statistically significant, were in the expected direction, i.e., a flatter slope of decline during the day for those with more depressive symptoms.

Discussion

The present study addressed two substantive issues that are key to health and aging. First, our findings indicate a maintenance of the typical cortisol diurnal profiles in very old age, with the characteristic CAR peak followed by a gradual decline as the day progresses. This has not been shown previously. The finding appears to be robust in light of a relatively small and positively-selected sample. Second, we found that an accumulation of depressive symptoms in the past 15 years was associated with lower CAR in the oldest-old. We discuss how the

integration of different time-scales in this study offers novel insights that no other method would have been able to provide.

Our results are in accord with the integrative approach to health (B. H. Singer & Ryff, 2001), such that more cumulative depressive symptoms are associated with changes in diurnal cortisol profiles in old age. Although our sample size is relatively small, the present study is the first in which depressive symptoms were observed over a 15-year period and the cortisol diurnal profile associated with them was observed in a natural setting in the oldest-old.

Our results complement previous findings that lifetime vulnerabilities, e.g., lower socio-economic status, is associated with the blunted cortisol diurnal profiles (Agbedia et al., 2011; Chida & Steptoe, 2009; Gerritsen et al., 2010). Specifically, cumulative depressive symptoms were associated with a lower CAR in oldest-old adults; whereas present depressive symptoms were not associated with any parameters of the cortisol diurnal profile. Drawing on the model of allostatic load (McEwen, 1998; Seeman & Gruenewald, 2006), stress occurring over a long period of time contributes to allostatic load, i.e. cumulative burden resulting in wear and tear of HPA axis. Although the definitive function of CAR is currently not known, it is speculated that CAR plays a critical role in mobilizing energy at the start of the day, which enables individuals to respond to forthcoming daily challenges and demands (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010). Our results of the association between higher past depressive symptoms and lower CAR may inform research on burnout and fatigue, i.e. lack of energy, associated with chronic stress (Pruessner et al., 1999). Despite limitations in statistical power, current depressive symptoms were not significantly associated with cortisol. Present depressive symptoms in very old age may be more related to the frequent and severe physical and social losses normative in the last life stage (Baltes & Smith, 2003), rather than to between-person differences in cortisol.

In contrast, depressive symptoms in earlier older adulthood may constitute a qualitatively different phenomenon that shows considerable between-person differences, reflecting earlier adversity or stress, which are indeed linked with altered cortisol (Gerritsen et al., 2010).

Methodologically, the robustness of our findings shows that the application of multilevel modeling facilitates the examination of the complex interplay between multiple levels of health in different time-scales. Specifically, the macro-time scale of change in depressive symptoms is reflected in the sum of depressive symptoms over 15 years. The micro-time scale of within-day changes in cortisol is reflected in the effects of CAR and the linear and quadratic effects of time since waking. We believe that the use of the embedded design of longitudinal and intensive time-sampling studies is a fruitful way to examine the argument that health is best conceptualized as a multifaceted process across multiple timescales (B. H. Singer & Ryff, 2001).

Our findings need to be considered in light of several limitations. First, given the screening criteria to ensure that ADuLTS participants were able to follow the intensive time-sampling study protocol, our findings are based on a somewhat homogeneous sample of relatively healthy and high functioning oldest-old adults. Despite this limitation, the significant results obtained illustrate the potential the embedded study design might hold in the understanding of distal and proximal factors of health in the aging process (Ram et al., 2011). Attrition analyses showed that although ADuLTS participants reported a lower level of depressive symptoms across all five ALSA waves, compared to participants who were not in ADuLTS, they showed few differences compared to the pool of possible participants from Wave 10. Thus, results of the present study may not be generalizable to oldest-old adults in poorer health and/or with more depressive symptoms, but they well-characterize long-term ALSA participants. Future research is needed to examine the patterns of cortisol diurnal profile in a

larger and more diverse population of oldest-old adults varying in health status and past depressive symptoms (Suzman, Willis, & Manton, 1992).

Second, stress reactivity and depressive symptoms are multifaceted, each involves the nature and timing of stressors, personality, and coping strategies (Hoppmann & Klumb, 2006). The present study did not examine the effects of stressful life events, personality, or coping. Future studies will need to incorporate these measures to further illuminate the link between depressive symptoms and HPA axis activity across older adulthood. For example, the experience of stressful life events has been shown to be associated with an increase in neuroticism (Riese et al., 2013). In addition, individuals higher in neuroticism tend to perceive more stress (Bolger, Davis, & Rafaeli, 2003), and show a higher level of cortisol (Nater, Hoppmann, & Klumb, 2010). Thus, future research linking long-term changes in neuroticism and individual differences in cortisol diurnal profile may shed light on the role personality plays in health outcomes.

Third, our findings showed that a higher level of cumulative depressive symptoms is associated with a lower CAR in the oldest-old. Normatively, individuals showed the peak of cortisol increase within the first 30 minutes after waking at 50% over individual baselines (Wüst et al., 2000). Although deviations from the typical cortisol diurnal profile have been associated with multiple physical and psychological health conditions, no optimal level or profile of salivary cortisol has been established (Saxbe, 2008). We cannot ascertain whether the lower CAR found in individuals higher in cumulative depressive symptoms means adaptation or maladaptation in these individuals in the face of stressful life events and age-related challenges (Seeman & Gruenewald, 2006). In addition, CAR was measured in the present study only once at 30 minutes post-waking each day. Although a myriad of studies have reported between-person difference correlates of the CAR when using the exact same CAR sampling time as used in our

study (e.g. Karlamangla, Friedman, Seeman, Stawksi, & Almeida, 2013), the CAR may not be as reliably measured compared to studies where CAR was measured multiple times during the first hour of waking (Roberts, Wessely, Chalder, Papadopoulos, & Cleare, 2004). Future studies adopting a prospective approach will be needed to examine how components of the cortisol diurnal profile may predict health outcomes in old age.

Fourth, the 10-item version of CES-D was used to reduce participant burden in ADuLTS. Compared to the original 20-item version, the limited number of items and the lower internal consistency of the 10-item CES-D might have led to an underestimation of the association between present depressive symptoms and components of the cortisol diurnal profile. Despite these limitations, additional analyses showed that results were consistent with those reported when the same short version items were used to calculate cumulative depressive symptoms. Finally, the small sample size may have precluded observation of the interaction effects of depressive symptoms (both present and cumulative) and time since waking; yet these were in the direction expected. That is, more depressive symptoms were expected to be associated with a flatter slope of decline during the day.

Conclusion

Findings of the present study showed that an accumulation of past depressive symptoms is associated with a lower CAR in the oldest-old. Although past research has focused on the association between depressive symptoms, stressful life events, and cortisol in old age, our study is the first to examine the effect of an accumulation of depressive symptoms over 15 years on the cortisol diurnal profiles in the oldest old. Our findings speak to differential associations between cumulative and present depressive symptoms and cortisol diurnal profiles in older adulthood and suggest pathways to further elucidate the role of depressive symptoms in HPA axis functioning

in very old age. Results are in line with the assertion that an integration of a time-sampling study within a longitudinal study can inform the understanding of developmental changes that manifest on different timescales (Ram et al., 2009), such as distal and proximal factors associated with health in very old age (Aldwin & Gilmer, 2013).

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Table 1.

Descriptive statistics of cumulative depressive symptoms and cortisol samples by time

Depressive symptoms	Wave 1	Wave 3	Wave 6	Wave 7	Wave 9	Cumulative	Current
	M (SD)	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*
	4.67 (4.32)	5.11 (5.56)	7.44 (5.86)	6.67 (6.68)	8.26 (6.74)	31.61 (23.04)	4.69 (3.46)

Cortisol sample	Sampling time	Cortisol in nmol/L
	M (SD)	*M (SD)*
Wake-up	6:43 (00:57) h	14.53 (6.86)
30 minutes later	7:16 (00:58) h	18.66 (9.13)
3 hours later	9:22 (00:55) h	10.70 (5.55)
6 hours later	12:14 (01:02) h	6.94 (3.83)
9 hours later	15:16 (01:03) h	5.71 (3.24)
12 hours later	18:10 (01:03) h	5.00 (3.28)
15 hours later	21:09 (01:03) h	3.52 (2.26)

Note: h = hour. *M* = mean; *SD* = Standard deviation.

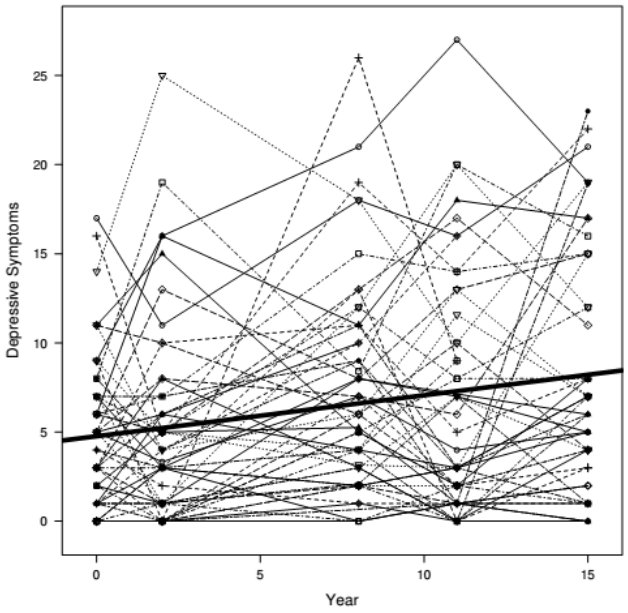
Table 2.

Fixed Effects Estimates of Multilevel Models for Effects of Present and Cumulative Depressive Symptoms on Cortisol

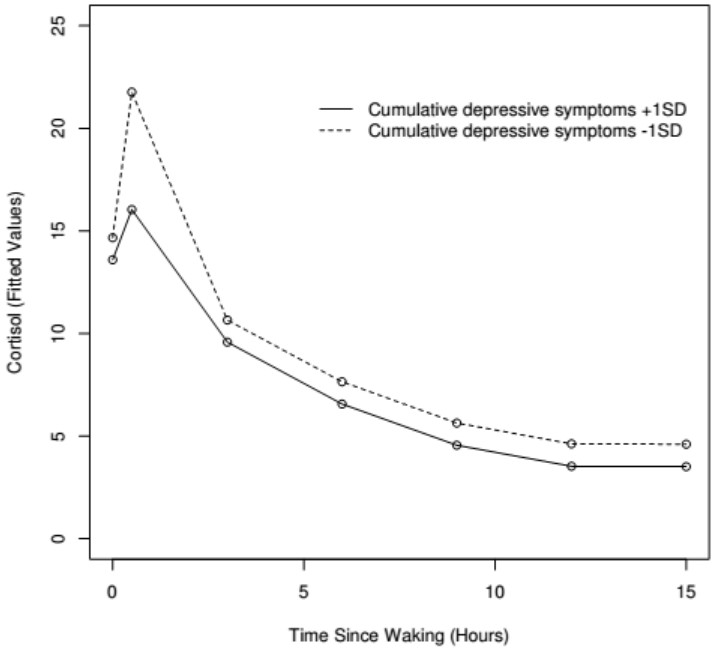
	Parameter	Model 1	Model 2	Model 3	Model 4
Fixed effects					
Intercept	β_{00}	14.15 (.45)***	14.18 (.45)***	14.15 (.45)***	14.13 (.45)***
Time since waking	β_{10}	-1.49 (.09)***	-1.51 (.09)***	-1.49 (.09)***	-1.50 (.09)***
Time since waking^2	β_{20}	.05 (.01)***	.06 (.01)***	.05 (.01)***	.06 (.01)***
CAR	β_{30}	5.53 (.82)***	5.56 (.82)***	5.49 (.77)***	5.51 (.77)***
Present CES-D	β_{01}		-.06 (.12)		.04 (.15)
Present CES-D × Time since waking	β_{11}		.02 (.01)		.02 (.01)
Present CES-D × CAR	β_{31}		-.20 (.23)		.18 (.27)
Cumulative CES-D	β_{02}			.02 (.02)	-.02 (.02)
Cumulative CES-D × Time since waking	β_{12}			.002 (.002)	.00002 (.002)
Cumulative CES-D × CAR	β_{32}			-.09 (.03)**	-.10 (.04)*
Anxiety disorder medication	β_{03}	5.02 (1.38)***	5.68 (1.34)***	4.95 (1.41)**	5.58 (1.25)***
Wake-up time	β_{04}	.29 (.05)*	.29 (.14)*	.29 (.15)*	.35 (.14)*
Random effects					
SD					
Intercept	σ_0	2.55	2.54	2.50	2.52

Time since waking	σ_1	.21	.20	.21	.20
CAR	σ_3	5.07	5.02	4.68	4.65
Level 1 residual	σ_ε	4.43	4.43	4.43	4.43
Correlation					
Intercept- time since waking	ρ_{01}	-.92	-.94	-.92	-.96
Intercept-CAR	ρ_{03}	.37	.335	.34	.29
Time since waking-CAR	ρ_{13}	-.43	-.41	-.39	-.46
Goodness of fit					
-2 log-likelihood		11803.45	11,794.76	11,796.66	11,782.81
AIC		11,855.45	11,852.76	11,854.66	11,846.81
BIC		12,001	12015.1	12017	12,025.94
Explained variance					
In intercept			.01	.04	.02
In time since waking			.09	.00	.09
In CAR			.02	.15	.16
Residual		.66	.66	.66	.66

Note. Standard errors are in parentheses. CAR is cortisol awakening response. *SD* = Standard deviation. Day-level random effects were first estimated and later removed for model simplicity due to non-significance. All control variables were entered in these models. Effects of non-significant control variables are not presented for simplicity. Only random effects in the intercept, time since waking, and CAR were estimated. Random effect in time since waking^2 was not estimated because of model non-convergence. AIC is Akaike Information Criterion and BIC is Bayesian Information Criterion. Explained variances in intercept, time since waking, and CAR, are calculated as proportional reduction in residual variance from Model 1.
*$p < .05$, **$p < .01$, ***$p < .001$.



(a)



(b)

Figure 1a. Depressive symptoms trajectories of the 50 participants. The thin lines represent the individuals' raw depressive symptoms scores. The bold line represents the predicted trajectory of the average individual.

Figure 1b. Cortisol diurnal profile plotted separately for higher (+1 SD) and lower (-1 SD) cumulative depressive symptoms.