Contents lists available at ScienceDirect

Psychoneuroendocrinology

Intra-individual cortisol variability and low-grade inflammation over 10 years in older adults

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ABSTRACT

Objective: This study examined the associations between intra-individual variability in, and inter-individual levels of, diurnal cortisol secretion with a marker of low-grade inflammation (i.e., C-Reactive Protein; CRP). Reasoning that greater day-to-day cortisol variability could reflect a dysregulation of the HPA axis, we hypothesized that it would predict higher levels of CRP, above and beyond inter-individual differences in cortisol levels.
Methods: A 10-year longitudinal study of 130 older adults examined diurnal cortisol secretion on three different days across each of the 6 waves of data collection and levels of CRP during the last 3 waves. Indicators of mean cortisol levels, short-term cortisol variability, and long-term cortisol variability were analyzed.
Results: Hierarchical linear modeling showed significant main effects, linking baseline mean cortisol levels, $T\text{-}ratio = 2.25$, $p = 0.03$, and long-term cortisol variability, $T\text{-}ratio = 2.63$, $p = 0.01$, with higher CRP values six to ten years after study entry. In addition, a two-way interaction demonstrated that short-term variability in cortisol were associated with higher levels of CRP among individuals who secreted relatively high, $T\text{-}ratio = 2.68$, $p = 0.01$, but not low, $T\text{-}ratio = -1.09$, $p = 0.28$, baseline levels of cortisol. Finally, a three-way interaction, $T\text{-}ratio = 2.24$, $p = 0.03$, suggested that the effect of long-term cortisol variability on CRP became stronger over time among participants who secreted high average levels of cortisol, whereas it became weaker among their counterparts who secreted low average levels of cortisol.
Conclusion: Variability in cortisol secretion across days forecasts low-grade inflammation, and this association is paramount among older adults who secrete high levels of diurnal cortisol.

1. Introduction

Theory and research suggest that cortisol disturbances could link the experience of stress and disease (Cohen et al., 2007; Lupien et al., 2009; McEwen, 2007). In recent years, there has been a surge of interest in cortisol's role in modulating low-grade chronic inflammation (Miller et al., 2002; Raison and Miller, 2003), a process that is increasingly recognized as a major pathway to a number of age-related illnesses, such as coronary heart disease, diabetes, or certain cancers (Allin and Nordestgaard, 2011; Danesh et al., 2004). In fact, older adults may be at a particular risk for exhibiting patterns of cortisol and immune disturbances. For example, aging is

characterized by inconsistent patterns of cortisol response to stress (Nicolson et al., 1997; Rohleder et al., 2002), greater daily cortisol levels (AUC; Nater et al., 2013; Otte et al., 2005), and greater levels of chronic inflammation and inflammatory disease (Franceschi, 2007). Thus, age-related changes in the HPA axis may influence (and be influenced by) inflammatory processes (Heffner, 2011; Straub et al., 2000) and increase vulnerability to inflammation-related diseases.

The extant research examining health-related consequences of cortisol dysregulation predominantly focused on inter-individual differences in cortisol secretion, by comparing cortisol patterns between individuals obtained over the course of one day or averaged across multiple days (e.g., levels of area under the curve [AUC] or diurnal cortisol slope). This approach has revealed important insights, for example, various aspects of cortisol secretion have been shown to predict morbidity and mortality, such as high daily cortisol volume (Heim et al., 2000), both high and low serum cor-

tisol levels (Marklund et al., 2004; Rotman-Pikielny et al., 2006), a flatter diurnal cortisol slope (Kumari et al., 2011; Sephton et al., 2013), and elevated night cortisol (Schrepf et al., 2015).

The specific links between cortisol secretion and inflammatory activity have also been considered. Synthetic preparations of cortisol have long been used in clinical settings to suppress inflammation associated with allergic, autoimmune, and other diseases. However, the doses administered in these settings are pharmacologic, and not reflective of how endogenously produced cortisol operates under physiologic conditions. In vivo, cortisol does modulate the cellular and molecular processes that underlie inflammation, but the patterns are complex (Busillo and Cidlowski, 2013). This complexity may explain why empirical studies of cortisol secretion and inflammatory processes in humans have yielded mixed findings (Chrousos, 1995; Elenkov and Chrousos, 2002; Karlović et al., 2012).

Another explanation for the mixed findings, which we address here, is that studies to date have focused on inter-individual variations in cortisol secretion, and overlooked the role of intra-individual variations. Indeed, by focusing on cortisol secretion over a single day, or aggregating scores across multiple days, potentially important differences in cortisol patterns that occur across days within different individuals are not captured (Hruschka et al., 2005). Studies that have teased apart within- and between-person differences in cortisol output over time have shown that within-person variability accounted for about 50% of the overall variation in cortisol output, indicating that there are substantial intra-individual differences in cortisol output that could be associated with health-related processes (Ross et al., 2014).

Little is known about intra-individual variability in cortisol patterns across days, or how they relate to downstream biological processes like inflammation. A degree of variability in cortisol output could mean a number of things. For example, it might reflect adaptive adjustments to changing environmental demands (e.g., as a response to a specific threat, Adam et al., 2006) and thus could be a result of co-occurring variability in stress exposure. Alternatively, unstable patterns of cortisol output could suggest an HPA axis that is responding erratically to environmental demands (Sannes et al., 2016); a possibility that would be consistent with research linking variability in successive cortisol measurements throughout the day, time-points across several days, and linear time trends with poor mental health and mood disorders (Havermans et al., 2011; Peeters et al., 2004; Sannes et al., 2016). Variability could also be part of the normative aging process, as research shows that intra-individual variability in the cortisol awakening response is greater among older as compared to younger adults (Almeida et al., 2009; Ice et al., 2004). Regardless of the cause, instability in outflow could have downstream implications for the bodily tissues that cortisol regulates, including the immunologic processes that underlie inflammation (McEwan and Stellar, 1993).

Based on the foregoing observations, the current study examined whether intra-individual cortisol variability is associated with a biomarker of low-grade inflammation, using data from a 10-year longitudinal study of community-dwelling older adults. Given the possibility that a person's typical level of cortisol might also have regulatory consequences for inflammation, these analyses simultaneously considered mean cortisol levels to account for between-person (inter-individual) differences. In addition, they explored whether mean cortisol level and intra-individual cortisol variability could interact in predicting CRP. More specifically, we predicted levels of C-reactive protein (CRP), observed 6–10 years after baseline, as an indicator of low-grade inflammation. Because intra-individual cortisol variability may increase among aging populations (Almeida et al., 2009), we compared the effects of two different indicators of cortisol variability: (1) short-term (based on three days at baseline) and (2) long-term (based on all days across waves) intra-individual cortisol variability. In addition, inter-individual differences in baseline mean levels of daily cortisol output (average AUC at T1), average mean cortisol levels across waves (average AUC across waves) and relevant covariates were included into the analyses. AUC was used as an indicator of cortisol secretion because of its previously shown associations with health-relevant outcomes (e.g., Heim et al., 2000).

2. Methods

2.1. Participants

This study is based on a sample of community-dwelling older adults who participated in the 10-year longitudinal *Montreal Aging and Health Study* (MAHS). Participants were recruited through newspaper advertisements in the Montreal area. To obtain a normative sample, the only inclusion criterion was that participants had to be older than 60 years at the time of recruitment. At baseline (T1), a total of 215 participants were assessed in their homes or in the laboratory. Participants were assessed again approximately 2 years (T2: $n = 184$), 4 years (T3: $n = 164$), 6 years (T4: $n = 136$), 8 years (T5: $n = 125$), and 10 years (T6: n = 95) after baseline. Study attrition was attributable to death ($n = 44$), refusal in study participation ($n = 17$), lost contact ($n = 21$), or withdrawal due to personal reasons ($n = 12$).[1]

We considered all participants for analysis who participated at T4, T5, or T6 (when CRP was assessed, $n = 143$). Thirteen participants were subsequently excluded because they did not provide useable data for either baseline cortisol ($n = 4$) or for CRP at T4, T5, or T6 ($n = 9$). The analytic sample thus included 130 participants. Excluded participants were significantly older at baseline ($M = 73.94$, $SD = 6.91$) than those who remained in the study ($M = 71.42$, $SD = 4.93$; $t[139.10] = -2.92$, $p < 0.01$). Excluded participants did not significantly differ from other participants on any of the other baseline measures used in this study ($|ts| < 1.09, ps > 0.05$).

2.2. Materials

2.2.1. C-Reactive protein (CRP)

C-reactive protein (CRP) was assessed as a measure of low-grade inflammation at T4–T6 by collecting dried blood spots. Single-use lancets were used to deliver a uniform puncture to the finger and up to three drops of blood were collected on a filter paper (McDade et al., 2004). The filter paper was allowed to dry and stored in a freezer at $-20\,^\circ$C degrees. After completion of each study wave, the samples were analyzed in the Laboratory for Human Biology Research at Northwestern University, using a high-sensitivity enzyme immunoassay (Williams and McDade, 2009). Validation studies measuring CRP from finger-prick dried blood spots have shown high correlations (e.g., r > 0.85) with matched CRP samples derived from venous blood, as well as good sensitivity and reliability (McDade et al., 2004). The averaged inter-assay coefficient of variation across waves was less than 8.7%. Three single CRP scores were excluded from the analysis because they were higher than 10 mg/L, which is likely to indicate acute infections (Pearson et al., 2003). Of the included participants, 120 participants at T4 reported valid CRP scores, 102 participants at T5, and 81 participants at T6.

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[1] Associations with CRP have been reported in a previous manuscript from the MAHS (Rueggeberg et al., 2012). However, the previous study only included CRP values from wave 4, and did not examine cortisol variability as a main predictor variable.

Table 1
Means, Standard Deviations and Frequencies of Main Study Variables (N = 130).

Constructs	Mean (SD) or Percentage
CRP (mg/L) T4	1.49 (1.47)
CRP (mg/L) T5	1.29 (1.23)
CRP (mg/L) T6	1.32 (1.13)
Short-term cortisol level (AUC in log nmol/L × h) (T1)	12.26 (2.48)
Long-term cortisol level (AUC in log nmol/L × h) (T1-T6)	11.64 (1.64)
Short-term cortisol variability (log nmol/L × h) (T1)[a]	1.54 (1.01)
Long-term cortisol variability (log nmol/L × h) (T1-T6)[b]	2.67 (.83)
Short-term stress level (T1)	0.28 (.41)
Long-term stress level (T1 – T6)	0.42 (.39)
Short-term stress variability (T1)[a]	0.19 (.26)
Long-term stress variability (T1–T6)[b]	0.45 (.30)
Education (%) (T1)	
None	4.0
High School	29.6
College/Trade	30.4
Bachelor	24.0
Masters/PhD	12.0
Income $CAD (%) (T1)	
Less than $17,000	20.7
$17,001-$34,000	38.0
$34,001-$51,000	19.8
$51,001-$68,000	14.9
$68,000–85,000	3.3
>$85,000	3.3
Perceived social status (T1)	6.23 (1.82)
Married or cohabitating (%) (T1)	53.8
Cortisol-related medication (%) (T1)	78.46
Mortality risk index (T1)	5.49 (2.22)
Age (y)	71.42 (4.93)
Male (%)	46.9
Diabetes (%)	15.4
Cancer (%)	3.1
Lung or other respiratory disease (%)	10.8
Heart condition (%)	14.6
BMI < 25 (%)	46.9
Current smoker (%)	7.8
Difficult bathing (%)	2.3
Difficult walking around the home (%)	1.5
Difficulty managing finances (%)	2.3
Difficulty doing heavy housework (%)	19.2

[a] Short term variability was operationalized as the standard deviation of baseline measures.
[b] Long-term variability was operationalized as the standard deviation of measures across all assessments.

2.2.2. Diurnal cortisol

Cortisol was assessed at each wave on three non-consecutive days because we were interested in assessing normative patterns of diurnal cortisol. Participants collected five saliva samples throughout the day using salivettes: at awakening, 30 min after awakening, 2 P.M., 4 P.M., and bedtime. The first saliva sample was taken upon awakening and participants collected the second saliva sample thirty minutes after awakening by using a timer. Participants were contacted by phone to facilitate compliance with the afternoon saliva collection (i.e., at 2 P.M. and 4 P.M). They collected the last saliva sample by themselves at the time they went to bed. Participants recorded the exact time of day for each sample collected. They were further instructed not to brush their teeth or eat prior to saliva collection to prevent contamination with food or blood. Samples were stored in participants' home refrigerators until they were returned to the lab 2–3 days after collection was completed, and they were frozen until completion of each wave. Cortisol analysis was performed at the University of Trier using a time-resolved fluorescence immunoassay with a cortisol-biotin conjugate as a tracer. The intra-assay variability at baseline was less than 5% and inter-assay variability from cortisol analyses performed at the University of Trier has been found to be routinely below 10%.

Samples that deviated three standard deviations or more from the mean cortisol level for a given time of day were further excluded from the analysis as those samples could have been contaminated with food or blood. We calculated cortisol scores only for participants who provided at least four usable cortisol scores on each of the three days for a given wave of assessment. To stabilize variance, all cortisol scores were log-transformed. As indicators of daily cortisol levels, we calculated the area-under-the-curve with respect to ground (AUC) across each day separately, using the trapezoidal method based on hours after awakening (Pruessner et al., 2003). We excluded the 30-min measure from the calculation of daily AUC because early morning increase of cortisol has been shown to be relatively independent from overall cortisol level (Chida and Steptoe, 2009). On each of the assessment days, cortisol levels significantly declined from awakening to bedtime, $ts > 12.49$, $ps < 0.001$. Across waves, cortisol levels exerted a curve-linear effect ($t = -1.97$, $p = 0.05$). Cortisol mean levels increased over the first three waves ($t = 2.03$, $p < 0.05$) and declined afterwards ($t = -6.85$, $p < 0.001$; T1: $M[SD] = 12.37$ [2.55], T2: $M[SD] = 12.85$ [2.45], T3: $M[SD] = 12.87$ [2.62], T4: $M[SD] = 10.09$ [2.65], T5: $M[SD] = 10.65$ [2.32], T6: $M[SD] = 10.59$ [2.67]).

To obtain a reliable indicator of mean *baseline cortisol level*, we averaged the three daily AUC scores at T1. To compute an indicator of mean *cortisol level across waves*, we averaged all daily AUC scores across all waves (T1-T6). To obtain indicators of cortisol variability, residual scores for the three baseline AUC scores and the eighteen cortisol AUC scores across all waves were obtained in hierarchical linear modeling (estimating an empty model in HLM and saving the error terms). Subsequently, for *short-term cortisol variability*, we computed the standard deviation of the three resid-

Table 2
Zero-Order Correlations of Main Study Variables (N = 130).

	1	2	3	4	5	6	7	8	9	10	11	12
1. Short-term cortisol level (T1)												
2. Long-term cortisol level (T1–T6)	0.63**											
3. Short-term cortisol variability (T1)	0.11	0.11										
4. Long-term cortisol variability (T1–T6)	0.21*	0.24**	0.27**									
5. Short-term stress level (T1)	−0.00	0.03	0.12	0.25**								
6. Long-term stress level (T1–T6)	−0.02	−0.04	0.14	0.17*	0.58**							
7. Short-term stress variability (T1)	0.04	0.02	0.13	0.19*	0.78**	0.51**						
8. Long-term stress variability (T1–T6)	−0.03	−0.01	0.15	0.09	0.33**	0.83**	0.39**					
9. Socioeconomic status (T1)	0.02	0.12	0.07	0.03	−0.11	−0.07	−0.11	−0.07				
10. Mortality risk index (T1)	0.13	0.15	0.09	0.09	0.08	−0.06	−0.01	−0.09	0.02			
11. Partnership status (T1)	−0.19*	−0.14	−0.06	−0.01	−0.18*	−0.14	−0.13	−0.03	0.26**	0.07		
12. Cortisol-related medication	−0.02	−0.02	0.02	−0.04	0.21*	0.07	0.13	−0.05	0.04	0.40**	0.06	
13. Averaged CRP (T4–T6)	0.18*	0.03	0.10	0.31**	0.04	−0.09	−0.09	−0.11	−0.22*	0.15	−0.01	0.07

Note. Higher values on partnership status indicate being married or cohabiting as compared to being single, divorced or widowed.

* $p \leq 0.05$.
** $p \leq 0.01$.

ual baseline AUC scores.[2] The intra-class coefficient (ICC) was 0.58 suggesting that 42% of the variance was related to intra-individual changes in cortisol across days. Finally, we computed an indicator of *long-term cortisol variability* by calculating the standard deviation of all available daily residual AUC scores from T1 to T6 (ICC = 0.22; see Table 1 for means and standard deviations). Cortisol variability was computed only if participants had at least three valid measures of cortisol level (short term variability = 123 out of 130, long-term variability = 130 out of 130).

2.2.3 Covariates

Covariates included participants' mean levels of and variability in perceived stress, socioeconomic status (SES), partnership status, mortality risk index, and a cortisol-related medication usage. Indicators of perceived stress were included as covariates to control for their potential association with intra-individual cortisol variability. Perceived stress was assessed on three non-consecutive days across waves. At the end of each day, participants were asked to report to what extent they experienced being "stressed" or "overwhelmed" during the day, using a 5-point Likert scale from 1 (*very slightly/not at all*) to 5 (*extremely*). Average stress levels were calculated by taking the mean across the three baseline days (average stress T1), and across all waves (average stress T1–T6). Intra-individual variability in stress was calculated by using the same method described for computing indicators of cortisol variability. To obtain an indicator of short-term stress variability the standard deviation of the three daily residual stress baseline scores was computed. To obtain an indicator of long-term stress variability the standard deviation of the 18 daily residual stress scores across all waves (T1–T6) was computed (but see Footnote 2). Note, however, that this approach does not directly address whether intra-individual changes in stress are associated with intra-individual changes in cortisol. In our data, this possibility does not appear to be the case, as there were no significant within-person associations between stress and cortisol at T1 or T1–T6 (*T*-ratios < |1.35|, *p*s > 0.18). In addition, SES, partnership status, and a mortality risk index were used as covariates because of their known health-related effects. SES was assessed using three variables; highest education completed, yearly family income, and perceived social status (Adler and Ostrove, 1999). The three standardized SES measures were correlated (*r*s = 0.39 to 0.50, *p*s < 0.001) and averaged to obtain a reliable indicator of SES. Partnership status was measured by categoriz-

ing participants into two groups: (1) single/separated/widowed or (2) married/cohabitating. The previously validated mortality index counted participants' weighted risk factors: age, being male, presence of diabetes cancer, lung or other respiratory disease, heart condition, body-mass-index <25, smoking, and problems with functional aspects of aging such as, bathing, walking around the home, managing finances, and heavy housework (for further details, see Lee et al., 2006). To control for the possibility that use of certain medications could affect cortisol section, a baseline measure of *cortisol-related medication* use was computed by counting the number of different medications participants took that could influence HPA axis activity (e.g., antidepressants, beta-blockers, or anti-inflammatory drugs, cf. Wrosch et al., 2007).

2.3. Data analyses

Preliminary analyses were conducted to describe the sample (by calculating means, standard deviations, and percentages) and examine associations between the main predictor variables (by conducting correlation analyses). The hypotheses were tested in two separate growth-curve models utilizing HLM 6.0 (Raudenbush, 2004). Separate models were estimated because the single AUC scores used for computing short-term cortisol variability (Model 1) were also used for calculating long-term variability in cortisol (Model 2). For both models, at Level 1 we estimated variance in participant's CRP levels at T4, T5 and T6 as a function of an intercept, person-centered scores of time in study, and a residual term. Note that the coefficient of interest is the intercept, which represented averaged levels of CRP across T4–T6. The estimation of the slope coefficient (time) further explored potential changes in levels of CRP over time.

At Level 2, we predicted the intercept and slope of CRP as a function of cortisol level (Model 1: mean baseline cortisol level at T1; Model 2: mean cortisol level across T1-T6), and cortisol variability (Model 1: short-term cortisol variability; Model 2: long-term cortisol variability). Both Level 2 models also included mean levels of and variability in perceived stress, mortality risk, SES, cortisol-related medication, and relationship status as covariates. We finally included the interaction terms between mean cortisol levels and cortisol variability (Model 1: short-term; Model 2: long-term) in the last step of the analyses. Significant interaction effects were followed up by estimating the effects of cortisol variability on CRP for one standard deviation above and below of the distribution of cortisol level. Level-2 predictors were standardized prior to conducting the analysis, and the reported effects are based on models

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[2] We also conducted analyses using variances instead of standard deviations as indicators of intra-individual variability (Wang et al., 2012), which showed highly similar pattern of findings.

Table 3
Results of Growth-Curve Analysis Predicting CRP by Short-Term Cortisol Variability, Short-Term Cortisol Levels, and Covariates (N = 130).

	CRP			
	Intercept (Average levels)		Slope (Time)	
	Coefficient (SE)	T-Ratio	Coefficient (SE)	T-Ratio
Level 1 (β_0; β_1)[a]	1.40 (0.10)**	13.96**	−0.02 (0.08)	−0.20
Level 2: Main effects				
Short-term cortisol level (AUC)	0.23 (0.10)*	2.25*	−0.07 (0.10)	−0.66
Short-term cortisol variability (VAR)	0.09 (0.09)	1.06	−0.01 (0.09)	−0.10
Short-term stress level	0.23 (0.19)	1.21	−0.04 (0.14)	−0.30
Short-term stress variability	−0.33 (0.14)*	−2.45*	0.04 (0.12)	0.36
Socioeconomic status	−0.32 (0.09)**	−3.49**	0.03 (0.10)	0.28
Mortality Index	0.08 (0.11)	0.74	0.11 (0.10)	1.15
Relationship status	0.09 (0.09)	1.01	−0.02 (0.09)	−0.18
Cortisol-related medication	0.02 (0.10)	0.19	0.16 (0.10)	1.57
Level 2: Interaction effect				
AUC × VAR	0.32 (0.12)**	2.62**	0.04 (0.13)	0.30

The Level 1 model had 129 *df*s, and the Level 2 models had 121 *df*s (main effects) and 120 *df*s (interaction).
SE = standard error.
* $p \leq 0.05$.
** $p \leq 0.01$.
[a] The first parameter (e.g., β_0) estimated the intercept, which represents participants' levels of CRP across T4, T5, and T6, and the second parameter (e.g., β_1) estimated the slope, which represents the within-person associations between years in study from T4-T6 and participants' CRP.
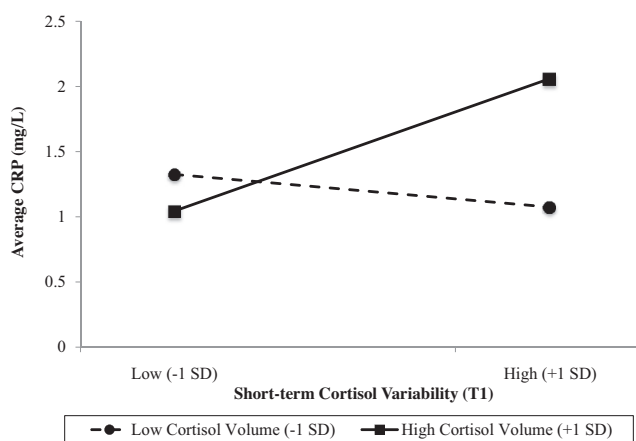

Fig. 1. Levels of CRP six to ten years after study entry as a function of short-term cortisol variability (based on baseline cortisol measures), separately for participants who secreted high versus low baseline levels of diurnal cortisol.

using restricted maximum likelihood estimation and robust standard errors.

2.3.1 Missing data

HLM is capable of handling missing data at Level 1 (i.e., CRP across time), and Level 1 missing data were therefore not replaced. However, HLM cannot accommodate missing data at Level 2. At T1, seven participants had missing data for short-term cortisol variability and baseline cortisol mean levels and four different participants had missing data for short-term stress variability baseline mean stress levels. To ensure that we could include these participants into our analyses, we used multiple imputations for replacing missing data of predictor variables including short-term cortisol variability and short-term stress variability. Prior to data imputation we conducted Little's MCAR test, demonstrating that these data were missing completely at random ($X^2 = 18.56, df = 11, p = 0.07$). We then imputed five data sets using SPSS 21. We chose to impute five data sets because the number of imputed data sets created should be equal to the percentage of missing data (5% in our case; Graham et al., 2007). Each of these imputed data sets were then analyzed separately by HLM 6.0, and the coefficients were pooled by HLM to obtain overall estimates and unbiased standard errors.

3. Results

3.1. Preliminary analyses

Participants represented diverse socioeconomic backgrounds with approximately 55% receiving less than $34,000 (CAD) yearly family income and 35% having obtained an undergraduate degree or higher (see Table 1). The sample average for perceived social status was slightly above the midrange of the scale. 54% of participants were married or cohabitating. The majority of participants took medication that could influence cortisol function (78%). The average mortality risk index was 5.49, which corresponds to 8–9% mortality risk over 4 years in Lee et al.s' (2006) validation sample. At baseline, participants were on average 71 years old and approximately half of them were female (53%). The average BMI of the sample was 25.56, including 47% of participants who had a BMI less than 25. A minority of participants smoked (8%). Between 3% and 16% of participants reported having cancer, diabetes, respiratory illness or a heart condition. Less than 3% had difficulty bathing, walking around at home, or managing their finances, and 19% had difficulty doing heavy housework. Overall, the sample exhibited socio-demographic and health-related characteristics that are within the normative range of community-dwelling older adults (National Advisory Council on Aging, 2006).

Levels of CRP at T4–T6 were significantly correlated (*range* = 0.35 to 0.47, *ps* < 0.01), indicating some stability in CRP. In addition, CRP was associated with higher baseline levels of cortisol, greater long-term cortisol variability, and a lower socioeconomic status (see Table 2). Long-term cortisol variability was also positively associated with higher baseline mean cortisol level, cortisol mean levels across waves, short-term cortisol variability, perceived stress mean levels at baseline and across waves, and short-term variability in perceived stress. Cortisol-related medication was positively associated with levels of perceived stress and mortality risk at baseline. Participants who were married or cohabitating had a higher socioeconomic status than participants who were single, separated, or widowed.

3.2. Short-term cortisol variability

The results of the analysis using short-term cortisol variability as a predictor of CRP are reported in Table 3. The Level 1 model showed a significant effect for the intercept, indicating that par-

Table 4
Results of Growth-Curve Analysis Predicting CRP by Long-Term Cortisol Variability, Long-Term Cortisol Levels, and Covariates (N = 130).

	CRP			
	Intercept (Average levels)		Slope (Time)	
	Coefficient (SE)	T-Ratio	Coefficient (SE)	T-Ratio
Level 1 (β_0; β_1)[a]	1.40 (0.10)**	13.96**	−0.02 (0.08)	−0.20
Level 2: Main effects				
Long-term cortisol level (AUC)	−0.02 (0.10)	−0.18	0.09 (0.12)	0.76
Long-term cortisol variability (VAR)	0.40 (0.15)**	2.63**	−0.05 (0.10)	−0.51
Long-term stress level	−0.17 (0.16)	−1.04	−0.01 (0.15)	−0.10
Long-term stress variability	−0.04 (0.17)	−0.22	0.03 (0.16)	0.16
Socioeconomic status	−0.29 (0.09)**	−3.19**	0.01 (0.09)	0.11
Mortality Index	0.10 (0.11)	0.94	0.10 (0.09)	1.07
Relationship status	0.02 (0.10)	0.17	0.01 (0.09)	0.15
Cortisol-related medication	0.06 (0.10)	0.57	0.17 (0.10)	1.67
Level 2: Interaction effect				
AUC × VAR	0.10 (0.11)	0.88	0.19 (0.08)*	2.24*

The Level 1 model had 129 *df*s, and the Level 2 models had 121 *df*s (main effects) and 120 *df*s (interaction).
SE = standard error.
* $p \leq 0.05$.
** $p \leq 0.01$.
[a] The first parameter (e.g., β_0) estimated the intercept, which represents participants' levels of CRP across T4, T5, and T6, and the second parameter (e.g., β_1) estimated the slope, which represents the within-person associations between years in study from T4-T6 and participants' CRP.

ticipants' levels of CRP across waves were significantly different from zero. However, the CRP slope was not statistically different from zero, implying that levels of CRP were relatively stable across time in the entire sample. Finally, the results from the Level 1 model showed that there was significant variance around participants' average intercept $\chi^2 = 420.97$, $df = 109$, $p < 0.001$, and slope $\chi^2 = 170.79$, $df = 109$, $p < 0.001$. The LR test was significant, $\chi^2(4) = 9.48$, $p < 0.05$, indicating that the model estimating a random effect for the CRP slope provides a better fit to the data compared to a fixed-effect only model.

In the Level 2 model, we attempted to predict the observed variance in the intercept and slope of participants' CRP scores. Of the covariates, SES significantly predicted the intercept of CRP, indicating that lower SES was associated with higher levels of CRP six to ten years later. Short-term variability in perceived stress also significantly predicted the intercept of CRP, suggesting that less stress variability was associated with higher levels of CRP six to ten years later. None of the covariates were associated with changes in CRP (time slope). In addition, the analysis revealed a significant main effect for baseline cortisol mean level on the intercept (but not the slope) of CRP scores, demonstrating that higher baseline levels of cortisol were associated with higher levels of CRP. The main effect of short-term cortisol variability did not predict the intercept or slope of CRP values. The final step of the analysis demonstrated a significant interaction effect between short-term cortisol variability and baseline levels of cortisol in predicting the intercept (but not slope) of CRP scores. After controlling for the alternate predictors, the interaction including cortisol level explained additional 7.74%, of the variability in levels of CRP.

To illustrate the significant interaction effect, we applied recommended growth-curve techniques (Preacher et al., 2006), and plotted in Fig. 1 the associations between short-term cortisol variability and averaged levels of CRP separately for one standard deviation above and below the baseline levels of cortisol. The shape of the obtained interaction suggests that to the extent participants secreted higher baseline levels of cortisol, short-term cortisol variability became more strongly associated with high levels of CRP six to ten year later. In addition, Fig. 1 indicates that the highest levels of CRP were obtained among participants' with high levels of short-term cortisol variability and high levels of cortisol. Follow-up analyses of the simple slopes supported this interpretation of the data. While short-term cortisol variability significantly predicted levels of CRP among participants who secreted high levels of cor-

tisol at baseline, coefficient = 0.51, *SE* = 0.19, *T-ratio* = 2.68, $p < 0.01$, no significant association between short-term cortisol variability and CRP was obtained among their counterparts who secreted relatively less cortisol at baseline, coefficient = −0.13, *SE* = 0.12, *T-ratio* = −1.09, $p = 0.28$.

3.3. Long-term cortisol variability

Table 4 summarizes the results of the analysis using long-term cortisol variability as a predictor variable. The significance and direction of effects for the Level 1 model were identical to the previously reported analyses and are therefore not reported again. Similar to the previous model, SES remained a significant predictor of lower CRP concentrations. However, there was no significant main effect for mean cortisol level across waves or stress variability on the intercept (or slope) of CRP values. Moreover, a significant main effect of cortisol variability predicted the intercept (but not the slope) of CRP scores, demonstrating that higher levels of long-term cortisol variability were associated with higher levels of CRP. Finally, the analysis revealed a significant cross-level 3-way interaction between long-term cortisol variability, mean cortisol levels across waves, and the time slope (but not the intercept) in predicting CRP scores. After controlling for alternate predictors, the main effect of long-term cortisol variability explained 11.40% of the variability in mean levels of CRP, and the interaction explained 4.39% of the variability in changes of CRP.[3]

Fig. 2 displays the effects of long-term cortisol variability on changes in CRP from T4 to T6, separately for participants who reported relatively high (upper panel) versus low (lower panel) mean levels of cortisol across waves. Note that none of the four simple slopes calculated was significant (plotted one standard deviation above and below the distribution of the predictor variables as reference points, *T-ratios* < |−1.83|, *ps* > 0.07), implying that the observed pattern needs to be interpreted in relative terms. Given these considerations, the results from the interaction analysis suggest that the adverse effect of long-term cortisol variability on increased levels of CRP became increasingly stronger over time

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[3] The long-term indicator of cortisol variability was not detrended (Wang et al., 2012). Note that the main effect of long-term cortisol variability on CRP (but not the interaction) was no longer significant if cortisol values were detrended (see Discussion section).
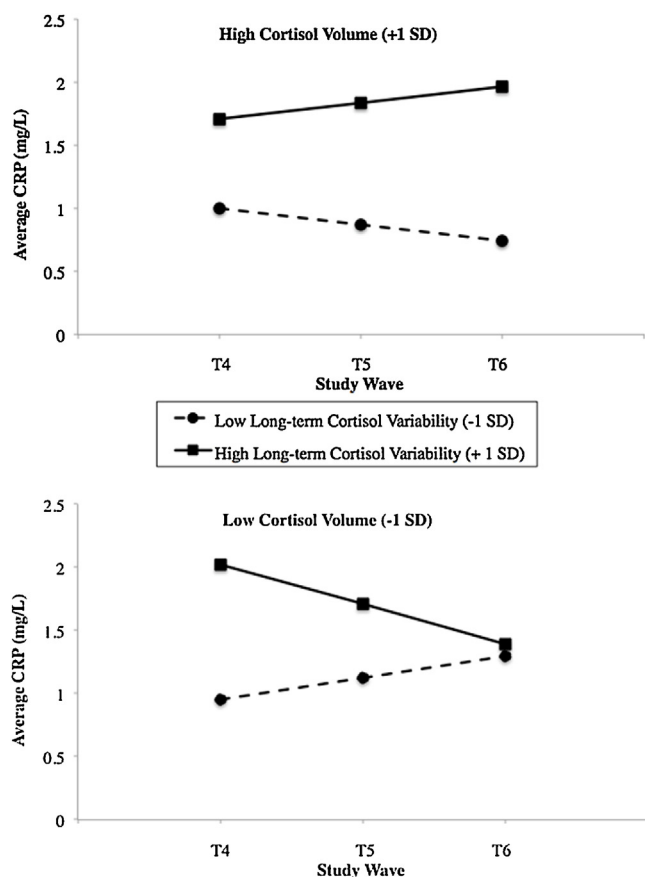
Fig. 2. Levels of CRP at T4–T6 as a function of long-term cortisol variability (based on all cortisol measures across waves), separately for participants who secreted low (*lower panel*) versus high (*upper panel*) mean levels of diurnal cortisol across waves.

among participants who secreted high mean levels of cortisol across waves (upper panel of Fig. 2). By contrast, among their counterparts who secreted low mean levels of cortisol across waves, the effect of long-term cortisol variability on levels of CRP became increasingly weaker over time (lower panel of Fig. 2).

4. Discussion

The present study showed in a normative sample of older adults that intra-individual variability in, and inter-individual levels of, diurnal cortisol secretion were both associated with higher levels of CRP, a biomarker of low-grade inflammation. In addition, it demonstrated that associations between short-term and long-term cortisol variability with mean levels and changes in CRP, respectively, were enhanced among older adults who secreted high mean levels of cortisol, but absent or reversed among their counterparts who secreted low mean levels of cortisol. These results were statistically independent of sociodemographic factors, a validated mortality risk index, average levels of, and variability in, perceived stress, and cortisol-related medication. Of note, a lower socioeconomic status was associated with higher levels of CRP.

Together, these findings suggest that in older adulthood, intra-individual variability in cortisol secretion is associated with low-grade inflammation, a biological pathway to a number of age-related diseases (Allin and Nordestgaard, 2011; Danesh et al., 2004). While much of the extant research on the health-related consequences of cortisol secretion has focused on between-person differences in cortisol secretion, our findings extend this approach by examining patterns of cortisol secretion that occur across multiple days within individuals (Hruschka et al., 2005). To this end,

the reported results suggest that in addition to inter-individual differences in levels of cortisol secretion, intra-individual cortisol variability across days may also reflect individual differences that foreshadow or contribute to health-relevant inflammatory processes.

We reasoned that greater intra-individual cortisol variability across days could be indicative of a dysregulated HPA axis that may not respond appropriately to environmental stressors, but instead reacts in an unpredictable or erratic manner (e.g., an underactive or overactive response, Sannes et al., 2016; Seeman et al., 2001). Such a process may be particularly prevalent among at-risk populations, such as the elderly, when normative age-related changes in allostatic load and/or dysregulated HPA axis activity could have downstream implications for the regulation of inflammation (Heffner, 2011; McEwen, 2007; Straub et al., 2000). This possibility would parallel research with other at-risk populations, linking intra-individual cortisol variability to psychopathology (Havermans et al., 2011; Sannes et al., 2016). It is also consistent with research on neural circuits and other physiological systems (e.g., those regulating circadian rhythms, learning, and memory), documenting that variability in the performance of these systems can forecast subsequent declines in functioning (Dinstein et al., 2015; Castanon-Cervantes et al., 2010; Sephton and Spiegel, 2003).

The results of this study further revealed two significant interaction effects. First, short-term cortisol variability was related to higher CRP levels only among older adults who exhibited high, but not low, mean baseline levels of cortisol. Second, the effect of long-term cortisol variability on CRP became increasingly stronger over time among older adults who secreted high mean levels of cortisol across waves, but increasingly weaker among their counterparts who secreted comparably lower mean levels of cortisol. Together, these interaction effects indicate that cortisol variability may predict enhanced inflammatory processes particularly when mean levels of cortisol remain elevated despite significant intra-individual variability. By contrast, the effect of cortisol variability on CRP may be absent or even reversed if older adults exhibit low mean levels of cortisol. In this regard, it is possible that adverse effects of cortisol variability emerge if high levels of cortisol reflect an HPA axis that becomes more dysregulated and erratic over time (e.g., if the HPA axis already fails in reducing cortisol output after the experience of specific stressors). In this way, those parts of the obtained interactions that are related to high mean levels of cortisol could capture aspects of cortisol variability that do not relate to adaptive adjustment, but instead are associated with an HPA axis that does not appropriately respond to occurring stressors and further deteriorates over time, thereby forecasting higher mean levels of, and increases in, CRP.

In the context of generally low cortisol levels, by contrast, variability in cortisol could reflect entirely different processes. Here, variability in cortisol levels over time may not contribute to pathology, but instead could, at least at times, serve adaptive function if it is associated with effective adjustment to changing environmental stressors (e.g., as a reaction to a specific threat, Adam et al., 2006). Such a process would be consistent with research indicating that physiological variability can also be indicative of adaptive functioning (e.g., heart-rate variability; Thayer et al., 2012), and thus may explain the non-significant or reversed effects of cortisol variability on levels of, and changes in, CRP among older adults who secreted relatively low levels of cortisol.

Note that our data can not provide much empirical support for the latter conclusion, since there were no significant within-person associations between stress and cortisol in our data (see methods section), and levels of, or variability in, perceived stress were not associated with the observed effects. However, the stress measure used in this study was based on only two self-report items, and future research should incorporate a more comprehensive stress

assessment and associated individual responses and outcomes to shed light on the processes underlying maladaptive and adaptive effects of cortisol variability. In addition, we acknowledge that the findings concerning short-term cortisol variability were based on only three days of cortisol collection, and therefore the reliability of our indicator of short-term cortisol variability (see Wang et al., 2012) should be considered with caution (but also see Almeida et al., 2009; Ice et al., 2004; Peeters et al., 2004; Sannes et al., 2016). Research should address this issue by conducting studies that include a larger number of assessment days to provide guidelines for the assessment of cortisol variability and to substantiate the conclusions derived from our study.

There may also be other explanations for an enhanced effect of cortisol variability on CRP in the context of high levels of cortisol. For example, it could be possible that intra-individual cortisol variability affects immune cells' ability to register cortisol signals and habituate to stressors. Considering that it is an adaptive reaction for an organism to habituate to chronic stress (Nesse et al., 2007), a reliable and strong cortisol response could enable individuals to effectively manage stressful experiences and allow tissues to habituate over time to the hormonal consequences of a stressor. In the context of high, but erratic, cortisol output; however, such a habituation process could be compromised, potentially modulating inflammatory processes. Note that although plausible, these explanations for the occurrence of the observed interaction effects are preliminary and need to be substantiated in future research.

We further acknowledge that the main effect (but not the interaction) of long-term variability in cortisol on CRP was rendered non-significant if the data were detrended, that is if the variance associated with changes in mean cortisol levels across waves was partialled out (see Footnote 3). This finding suggests that the relatively strong main effect of intra-individual cortisol variability, explaining 11.4% of the variance in CRP levels, may be more likely to occur over longer, as compared to shorter, periods of time. A potential explanation for this pattern may be found in research, documenting that cortisol variability can increase with age (see research on CAR, Almeida et al., 2009). Thus, among older adults, the health-relevant implications of intra-individual cortisol variability may be more likely observed over longer periods of time, as such variability could reflect an age-related dysregulation of the HPA axis.

Overall, the reported findings have important implications for theory and research on cortisol secretion and physical health. Stress-related modulation of cortisol secretion has long been suspected to play a significant role in the development of disease-related processes (e.g., Cohen et al., 2007; Lupien et al., 2009; McEwen, 2007). Although research examining this possibility still remains inconclusive, our findings point to a more complex picture by suggesting that intra-individual variability in cortisol secretion across days represents an additional important phenomenon that has been largely neglected in research on physical health. To this end, the reported study builds on the literature of cortisol variability and mental health (Sannes et al., 2016) and demonstrates that intra-individual cortisol variability can predict, independently and in interaction with cortisol level, a biomarker of risk for diseases associated with aging (i.e., CRP, Libby et al., 2002). Thus, in addition to other more commonly studied inter-individual aspects of cortisol secretion, intra-individual cortisol variability merits attention in future research on physical health.

In addition, the study's findings may have some implications for the assessment of intra-individual cortisol variability. Although both indicators of cortisol variability significantly contributed to individual differences in CRP, short-term cortisol variability only exhibited an effect in the context of high cortisol level, while long-term cortisol variability exerted a stronger main effect on CRP regardless of cortisol levels. The latter effect may reflect a developmental process of aging and may further imply that health-relevant intra-individual variability in cortisol can be more reliably assessed through multiple assessments over a longer period of time. In support of this possibility, the intra-class coefficients suggested that intra- as compared to inter-individual variability was substantially greater for the obtained indicator of long-term, as compared to short-term, cortisol variability.

Finally, the reported results inform theories and research on successful aging. The term "inflammaging" has been coined to represent the characteristic low-grade chronic inflammation among elderly individuals, which is indicative of a significant risk factor for morbidity and mortality (Franceschi, 2007). Our findings support previous theories, which suggest that age-related dysregulation of cortisol may have implications for this process (Heffner, 2011; Straub et al., 2000). Thus, a dysregulation of the HPA axis, as indicated by greater intra-individual cortisol variability, is one pathway through which age-related chronic inflammation could emerge and increase older adults' risk for developing physical health problems.

There are limitations to the present study that need to be addressed in future research. First, our study did not include a baseline measure of CRP, which leaves unexamined the possibility that some participants may already had high levels of CRP at baseline. Second, although the reported findings are based on longitudinal data, the long-term indicator of cortisol variability partially overlapped with the measurement of CRP, which makes it difficult to draw directional conclusions about the relation between cortisol and CRP. Third, our sample was modest in size and restricted to a normative sample of community-dwelling older adults. Fourth, the reported study included only one indicator of inflammatory processes (i.e., CRP), and did not consist of a more comprehensive battery of pro- and anti-inflammatory markers (e.g., IL-6 or IL-10). Finally, our analyses did not examine whether high levels of CRP observed in the later waves of the study contribute over longer periods of time to patterns of morbidity and mortality. To address these issues and substantiate our conclusions, future research should conduct fine-grained analyses in long-term longitudinal and experimental studies with a variety of normative and at-risk populations across the lifespan to predict a greater variety of biological processes and long-term outcomes of physical health. We feel that research along these lines is warranted as it may identify the mechanisms underlying an association between cortisol secretion, inflammatory processes, and physical health.

Conflict of interest

None of the authors have a conflict of interest to declare.

Contributors

Heather Herriot conducted the literature review and statistical analyses and wrote the first draft of the manuscript. Dr. Carsten Wrosch designed the study, conducted analyses, and helped with revising the draft manuscript. Dr. Gregory E. Miller and Dr. Jean-Philippe Gouin were also involved in designing the study, providing comments, and contributing to the discussion of the study's findings. All authors contributed to and have approved the final manuscript.

Funding

Acknowledgements

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