Day-to-day dynamics of experience–cortisol associations in a population-based sample of older adults

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In 156 older adults, day-to-day variations in cortisol diurnal rhythms were predicted from both prior-day and same-day experiences, to examine the temporal ordering of experience–cortisol associations in naturalistic environments. Diary reports of daily psychosocial, emotional, and physical states were completed at bedtime on each of three consecutive days. Salivary cortisol levels were measured at wakeup, 30 min after awakening, and at bedtime each day. Multilevel growth curve modeling was used to estimate diurnal cortisol profiles for each person each day. The parameters defining those profiles (wakeup level, diurnal slope, and cortisol awakening response) were predicted simultaneously from day-before and same-day experiences. Prior-day feelings of loneliness, sadness, threat, and lack of control were associated with a higher cortisol awakening response the next day, but morning awakening responses did not predict experiences of these states later the same day. Same-day, but not prior-day, feelings of tension and anger were associated with flatter diurnal cortisol rhythms, primarily because of their association with higher same-day evening cortisol levels. Although wakeup cortisol levels were not predicted by prior-day levels of fatigue and physical symptoms, low wakeup cortisol predicted higher levels of fatigue and physical symptoms later that day. Results are consistent with a dynamic and transactional function of cortisol as both a transducer of psychosocial and emotional experience into physiological activation and an influence on feelings of energy and physical well-being.

loneliness | psychological stress

A convincing nonhuman animal literature documents the effects of stress exposure on the physiology and neurobiology of the hypothalamic–pituitary–adrenal (HPA) axis and shows that changes in glucocorticoid levels affect a wide range of related physiological processes and health outcomes (1–3). As a result, considerable attention is being given to the possibility that emotional and physical health disorders in humans may emerge because of extreme or chronic stress exposure and frequent or prolonged HPA axis activation (4, 5).

Practical and ethical limitations, however, do not allow human researchers to conduct research with the same degree of causal rigor as in the animal literature; it is obviously not feasible to experimentally impose extreme or chronic stress. Consequently, most research on the associations between stress, HPA axis activity, and physical and emotional well-being in humans has relied on correlational data. Much of this research is also cross-sectional, showing point-in-time rather than longitudinal associations (5, 6).

Understanding the role of the HPA axis in human disease processes will ultimately require the longitudinal examination of changes in stress exposure, HPA axis functioning, and disease over the course of months and years. In the present study, however, we show that even examining the day-to-day dynamics of experience–cortisol associations can reveal important information about the likely causal ordering of associations between social and emotional experience, cortisol, and well-being.

Levels of circulating cortisol reflect the activity of central and peripheral pathways that are responsive to experiential input (e.g., social, emotional, and physical experiences) (7, 8). Cortisol is released from the adrenal cortex into circulation following a cascade of signals from the limbic system to the hypothalamus and pituitary, and levels are maintained within bounds by negative feedback to multiple brain regions including the hippocampus, hypothalamus, and pituitary (9). A strong basal diurnal rhythm exists: levels are typically high in the morning upon waking, increase 50–60% in the first 30–45 min after awakening (the cortisol awakening response, or CAR), drop rapidly over the first few hours after waking, and then decline more slowly across the day to reach a low point around midnight (8, 10). Most (60–70%) of the variation in cortisol levels across the waking day is explained by time of day (11, 12).

There is, however, significant variability between people in the shape of their diurnal cortisol rhythms (11, 13). In modeling these differences, the elevation and the slope of the diurnal cortisol curve, and the size of the CAR are frequently examined parameters (6, 14). Research has focused on identifying the differing ways these parameters interact with experience, and the significance of each for human functioning and health. Cross-sectional studies have shown that participants with higher current chronic stress show a larger CAR (15–17). Others have found low cortisol levels and/or flatter diurnal cortisol rhythms to be associated with a history of exposure to stressful social experiences (11, 18) and the presence of disorders such as chronic fatigue, fibromyalgia, and rheumatoid arthritis (19–23). It is often concluded that stressful experiences are responsible for alterations in diurnal cortisol activity and that variations in cortisol levels or rhythms contribute to physical symptoms, fatigue, and disease processes, but, because of the cross-sectional nature of most existing human data, these causal directions are not yet firmly established.

Several studies have examined within-person covariation over time between cortisol levels and experiences, finding associations between stress exposure (24) or negative affect (25–28) and cortisol levels measured shortly thereafter. Although more compelling than between-person analyses, such studies are still subject to the reverse causality interpretation that cortisol levels may influence one's experiences of one's social environment, a reasonable argument given prior evidence of the effects of exogenous glucocorticoid administration on mood and anxiety (29–31).

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Abbreviations: CAR, cortisol awakening response; HPA, hypothalamic–pituitary–adrenal; n.s., not significant.

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Table 1. Multilevel growth-curve model of associations between average experience variables and diurnal cortisol parameters

Fixed effect	Coefficient	SE	*t*	*P*	Interpretation
Model for wakeup cortisol level, $\pi 0$					
Average wakeup cortisol level, $\beta 00$					
Intercept, $\gamma 000$	−1.812	0.038	−47.301	0.000	$\hat{Y} = 0.16$ μg/dl*
Age, $\gamma 001$	−0.003	0.008	−0.407	0.684	n.s.
Male, $\gamma 002$	0.045	0.077	0.579	0.563	n.s.
White, $\gamma 003$	0.189	0.080	2.373	0.019	+21% if White†
Average symptoms/tired, $\gamma 004$	−0.106	0.049	−2.171	0.031	−10% per +1 SD
Noncompliance with wakeup sampling, $\beta 01$					
Intercept, $\gamma 010$	−0.095	0.059	−1.602	0.109	−9% if noncompliant
Model for time since waking, $\pi 1$					
Average effect of time since waking, $\beta 10$					
Intercept, $\gamma 100$	−0.165	0.029	−5.629	0.000	−15% per hour at wakeup
Age, $\gamma 101$	0.002	0.001	1.867	0.063	0.2% flatter
Male, $\gamma 102$	0.014	0.008	1.809	0.072	1.4% flatter
White, $\gamma 103$	−0.017	0.008	−2.135	0.034	1.6% steeper
Average tense/angry, $\gamma 104$	0.011	0.004	3.021	0.003	1.1% flatter
Model for time since waking squared, $\pi 2$					
Intercept, $\beta 20$					
Intercept, $\gamma 200$	0.005	0.002	2.878	0.005	+0.5% per hr^2
Model for cortisol awakening response, $\pi 3$					
Average size of awakening response, $\beta 30$					
Intercept, $\gamma 300$	0.404	0.033	12.328	0.000	+50% if CAR
Psychiatric illness, $\gamma 301$	0.225	0.070	3.244	0.002	+25% if present
Age, $\gamma 302$	0.003	0.007	0.472	0.637	n.s.
Male, $\gamma 303$	−0.037	0.064	−0.584	0.560	n.s.
White, $\gamma 304$	0.023	0.066	0.358	0.721	n.s.
Alcohol use, $\gamma 305$	0.068	0.015	4.576	0.000	+7%/drink/day
Average lonely/sad/overwhelmed, $\gamma 306$	0.120	0.034	3.573	0.001	+13% per +1 SD
Average symptoms/tired, $\gamma 307$	−0.133	0.046	−2.861	0.005	−12% per +1 SD
Wakeup time, $\beta 31$					
Intercept, $\gamma 310$	−0.034	0.018	−1.922	0.055	−3% per hour later
Noncompliance with CAR sampling, $\beta 32$					
Intercept, $\gamma 320$	−0.207	0.094	−2.196	0.028	−19% if noncompliant

All Level 1 predictors are uncentered; Level 2 and Level 3 variables are grand mean centered. Day-level predictors of wakeup values, slopes, and CARs were fixed at Level 2 and Level 3, and Time of day squared was fixed at Level 3; all other coefficients are set as random. Random effects for the Level 3 intercept and for time since waking were significant at $P < 0.05$ in the full model [$u00 = 0.157$, $\chi^2(147) = 502.665$, $P = 0.000$ and $u10 = 0.001$, $\chi^2(147) = 368.578$, $P = 0.000$, respectively], whereas the random effect for the cortisol awakening response was not [$u30 = 0.017$, $\chi^2(144) = 170.530$, $P = 0.065$].

*Because cortisol values were natural-log transformed, the inverse of that transformation (the exponential function) was applied to return the intercept to the original scale of measurement.

†In interpreting effect sizes, use of a logarithmic outcome allows coefficients to be interpreted as percentage change in the outcome per unit change in the independent variable, after applying the following transformation: $B_{\%change} = [\exp(B_{raw})] - 1$.

Laboratory experimental studies of cortisol reactivity to acute stressors do address issues of causal order, identifying the types of laboratory-based exposures that produce higher cortisol (32). Nonetheless, to identify pathways to disease, it is necessary to identify the situations and emotions actually encountered in daily life that are associated with alterations in cortisol levels (6, 25). To better understand cortisol–experience dynamics in naturalistic settings, researchers need to employ causally sensitive research designs and analytic approaches that take advantage of variations in the relative timing of measures of experiences, cortisol, and well-being.

In this study, we examine naturalistic associations between social and emotional experience, cortisol, and perceived physical well-being in a community sample of older adults, with attention to the potential causal ordering of effects built into our design and analytic plan. Salivary cortisol levels were measured at wakeup, 30 min after waking and at bedtime each day for 3 days, and multilevel growth-curve modeling was used to estimate latent diurnal cortisol profiles for each person. The parameters defining those profiles (wakeup cortisol levels, slope from wakeup to bedtime, and size of the CAR) were predicted from diary reports of experiences averaged across the days of testing. Next, in an analysis examining how day-to-day variations in experience are related to day-to-day variations in cortisol, cortisol parameters estimated separately for each day were simultaneously predicted from experiences the day before and the same day as cortisol sampling. Analyses control for the effects of demographic variables, health problems, and health behaviors. By using electronic monitoring data available for half of the participants, the impact of noncompliance with the cortisol sampling protocol is also examined and statistically controlled.

Results

As shown in Table 1, on average, cortisol values followed the expected diurnal pattern in this sample of older adults, with high values upon awakening ($\gamma 000 = -1.812 = 0.16$ μg/dl), a 50% increase in levels in the first 30 min after awakening ($\gamma 300 = 0.404$), and a decline in cortisol levels across the rest of the day ($\gamma 100 = -0.165$, or a decrease of 15% per hour at wakeup).¶ These cortisol parameters are all moderately intercorrelated, with wakeup values being negatively associated with the size of the CAR ($r = -0.250$, $P < 0.001$), higher morning values predicting steeper diurnal slopes ($r = -0.553$, $P < 0.001$), and a higher CAR predicting steeper

¶Because of the inclusion of a quadratic term for time of day, the linear time coefficient reflects the slope at Time = 0, which is time at wakeup in this model. Examination of the quadratic term reveals that the rate of decline in cortisol is approximately half as rapid at midday (8 hours later) and is close to zero by the end of the day (16 hours later).

Table 2. Multilevel growth-curve model of associations between prior-day and same-day experience variables and diurnal cortisol parameters

Fixed effect	Coefficient	SE	*t*	*P*	Interpretation
Model for wakeup cortisol level, π_0					
Same-day symptoms/tired, β_{01}, γ_{010}	−0.082	0.030	−2.735	0.007	−8% per +1 SD
Prior-day symptoms/tired, β_{02}, γ_{020}	−0.016	0.028	−0.568	0.570	n.s.
Model for linear effect of time of day, π_1					
Same-day tense/angry, β_{11}, γ_{110}	0.007	0.003	2.090	0.037	1% flatter per SD
Prior-day tense/angry, β_{12}, γ_{120}	0.003	0.003	0.922	0.357	n.s.
Model for cortisol awakening response, π_3					
Same-day symptoms/tired, β_{31}, γ_{310}	−0.028	0.033	−0.840	0.401	n.s.
Prior-day symptoms/tired, β_{32}, γ_{320}	−0.062	0.039	−1.577	0.115	n.s.
Same-day lonely/sad/overwhelmed, β_{33}, γ_{330}	−0.017	0.038	−0.435	0.663	n.s.
Prior-day lonely/sad/overwhelmed, β_{34}, γ_{340}	0.114	0.042	2.694	0.008	+12% per +1 SD

The quadratic effect of time of day on cortisol and the same set of control variables as in Table 1 were also included in this analysis, and the same centering and fixing strategies were used. Results for these variables are omitted to conserve space because they were very similar to those presented in Table 1.

slopes ($r = -0.147$, $P < 0.001$). Several control variables were significantly associated with basal cortisol rhythms. Non-Hispanic Whites had significantly higher wakeup cortisol levels (21% higher, $\gamma002 = +0.189$), and steeper diurnal cortisol slopes (1.6% steeper; $\gamma103 = -0.017$). In contrast, increasing age and male gender had marginally significant associations with flatter cortisol slopes ($\gamma101 = +0.002$ and $\gamma102 = +0.014$, both $P < 0.10$). Individuals with a diagnosed psychiatric illness had a significantly larger CAR (25% larger; $\gamma301 = +0.225$). Higher reported alcohol consumption was also strongly positively associated with the size of the CAR ($\gamma305 = +0.068$; 7% higher for each additional alcoholic drink per day). Finally, although there were no effects of self-reported hours of sleep, there was a trend for the CAR to be smaller for those who woke up later ($\gamma310 = -0.034$; $P = 0.06$; 3% smaller for each hour later wake time).

Turning to our daily experience variables, when average experiences across the days of testing are examined, three factors showed significant associations with average diurnal cortisol parameters: feeling lonely/sad/overwhelmed, tense/angry, and physical symptoms/fatigue. These factors were strongly intercorrelated (*r* values between 0.55 and 0.61) but nonetheless showed distinct associations with cortisol. Two positive experience factors, feeling active/effective and feeling confident, and also a confused/forgetful factor did not show significant associations with cortisol.

As shown in Table 1, higher levels of physical symptoms and fatigue were associated with lower cortisol levels at wakeup ($\gamma004 = -0.106$), with wakeup cortisol being 10% lower for every SD higher on this factor. Higher levels of tension/anger were associated with flatter diurnal cortisol slopes ($\gamma104 = 0.011$; 1.1% flatter at wakeup for every SD higher tension/anger).[‖] Higher average levels of feeling lonely/sad/overwhelmed across the days of testing were associated with a significantly higher awakening response ($\gamma306 = +0.120$), with the CAR being 13% greater for every SD higher on this factor. By contrast, higher levels of fatigue/physical symptoms were associated with a smaller CAR ($\gamma307 = -0.133$), 12% lower for every SD higher on this factor.

These effects were robust to the impact of noncompliance with sample timings on cortisol estimates. Wakeup samples taken >10 min before or after the reported waketime each day were 9% lower than those taken within 10 min of reported waketimes ($\gamma010 = -0.095$). In addition, individuals who took their 30 min after awakening sample >10 min early or late had 19% smaller awakening responses ($\gamma320 = -0.207$). In general, associations between experience and cortisol were stronger when controls for noncompliance were included in models.

To get better purchase on the causal direction of these associations, our next analyses took advantage of day-to-day variability in experiences and cortisol levels. Prior-day and same-day states were entered simultaneously, predicting day-to-day variation in diurnal cortisol rhythms, to see whether diurnal cortisol parameters each day were more strongly associated with experiences reported the day before or experiences later the same day.

As shown in Table 2, prior-day feelings of being lonely/sad/overwhelmed were associated with a significantly greater CAR the next day (12% per each SD; $\gamma340 = +0.114$), whereas CAR levels each morning did not predict feeling lonely/sad/overwhelmed later that day ($\gamma330 = -0.017$, n.s.). In contrast, wakeup cortisol levels were not predicted by fatigue/physical symptoms the day before ($\gamma020 = -0.016$, n.s.), but were strongly predictive of fatigue and physical symptoms for the rest of the day ($\gamma010 = -0.082$; for every 8% lower wakeup cortisol, fatigue/physical symptoms were 1 SD higher). The fact that lower wakeup cortisol levels predict fatigue later that day, net of any effect of prior-day fatigue on next-day wakeup cortisol, supports the possibility that low wakeup cortisol contributes to fatigue and physical symptoms. Additional analyses showed that these associations remain significant if control variables for number of hours of sleep and perceived sleep quality the night before are included; thus, results are not attributable to insufficient sleep hours or quality influencing both cortisol levels and fatigue the next day.

In predicting cortisol diurnal slopes, tension/anger was significantly associated with flatter same-day cortisol slopes ($\gamma110 = +0.007$), whereas tension/anger the day before did not predict next-day slopes ($\gamma120 = +0.003$, n.s.). Follow-up analyses suggested that the association between tension/anger and flatter same-day cortisol slopes was because of an association between tension/anger and higher evening ($\gamma = 0.047$, $t = 1.924$, $P = 0.05$), but not lower morning ($\gamma = 0.043$, n.s.) cortisol levels. Evening levels were 5% higher for every 1 SD higher tension/anger that day.

In a final set of analyses, our experience factors were broken down into theoretically defined subscales and substituted one by one for their larger factor in the model, to see whether particular elements of each factor were driving the effects. For lonely/sad/overwhelmed, each of the following was related to a higher next day CAR: loneliness (loneliness, isolation; $t = 2.052$, $P = 0.04$); threat (intimidated, threatened; $t = 2.651$, $P = 0.01$); dysphoria (sad, discouraged; $t = 1.87$, $P = 0.06$), and overwhelmed (helpless, unable to control things, difficulties piling up; $t = 2.41$, $P = 0.02$). For tension/anger, tension was marginally associated (tense, uneasy; $t = 1.71$, $P = 0.09$), and anger was significantly associated (angry, annoyed; $t = 2.11$, $P = 0.04$) with flatter same-day diurnal cortisol slopes. For the fatigue/physical symptom factor, both fatigue ($t =$

[‖]This analysis was repeated with time centered at 8 hours after awakening, such that the linear slope coefficient would reflect slope at midday. Similar effects of tension/anger were found for midday slopes ($\gamma104 = 0.01$, $P = 0.05$).

-2.46, $P = 0.014$) and physical symptoms ($t = -2.62$, $P = 0.009$) were significant. When related subscales were entered together, effects became nonsignificant because of their high degree of intercorrelation, suggesting that it is not the unique contributions of each but their shared variance that is important.

Discussion

The results of this study illustrate the additional information that can be gained by modeling the day-to-day dynamics of associations between everyday experiences and diurnal cortisol activity. In prior research, day-to-day variations in cortisol measures have often been treated as noise. Our results suggest that some of this day-to-day variability reflects not error but systematic changes in cortisol in response to changing daily social and emotional experiences. We also find that some daily subjective experiences (such as fatigue and physical symptoms) appear to be influenced by day-to-day variations in cortisol levels.

Looking first at the impact of psychosocial and emotional experiences on cortisol, we provided evidence that levels of tension and anger each day were related to flatter diurnal cortisol slopes the same day (controlling for levels of these states the previous day), primarily through the influence of tension and anger on higher evening cortisol levels. Prior studies have shown that negative emotional states including anger are associated with higher cortisol levels a few minutes later in naturalistic settings (12, 25–28, 33). The current study suggests that these momentary effects may cumulate across the day to result in higher bedtime cortisol and a flatter diurnal cortisol slope, a sort of biological signature of a "bad day." The fact that diurnal cortisol slopes change in systematic ways on a day-to-day basis has several potential implications. First, some of what has been considered stable or trait differences in cortisol slopes in prior studies may in fact be due to differences in experiences on the days of cortisol testing. Slopes measured on any particular day or set of days likely reflect a combination of trait variation and state variation associated with daily experiences, and these sources of variation need to be separated. Second, it seems possible that, over time, repeated daily alterations in cortisol slopes could become entrained, resulting in a persistent alteration of the baseline or trait pattern in ways that have consequences on health and functioning. This is an important hypothesis to test in future research.

A particularly robust effect was found for the effects of prior-day psychosocial and emotional experience on next-day cortisol awakening responses. A factor comprised of loneliness, sadness, and feeling threatened and overwhelmed was associated with a higher next-day CAR, whereas CAR levels measured in the morning did not predict experiences of this factor across the ensuing day. Prior studies have found cross-sectional associations between chronic stress and higher CAR levels (15–17) and between depressive symptoms and loneliness and higher CAR levels (34, 35), but the question of causal order has remained unanswered. Although there is a trait or stable component to CAR levels, including a strong genetic contribution (17), our results suggest that systematic changes in awakening responses do occur in response to psychosocial experience and that they can do so on a relatively rapid time scale (from day to day).

The interpretation of individual differences in CAR levels is still under debate; because of associations with chronic stress (16, 17), a heightened CAR could be regarded as maladaptive, yet a low CAR has also been associated with problematic conditions such as burnout (15). We suggest a functional interpretation that may help reconcile these apparent contradictions: that the CAR is an adaptive response designed to provide the individual with the "boost" needed to meet the anticipated demands of the upcoming day, an evaluation that is influenced, at least in part, by the experiences of the prior day. The observation that a higher CAR is found on weekdays than on weekends (36, 37) and in healthy individuals rather than those with chronic health problems (38) and, in our own data, with lower average fatigue across the days of testing, lends some weight to this interpretation. By extension, it seems plausible that repeated or extreme use of this typically adaptive mechanism, such as may occur in the case of chronic stress or loneliness, could have long-term physiological costs, helping to explain associations between chronic stress, loneliness, and disease (39, 40). Perhaps burnout is a case in which this mechanism has been exhausted over time, such that cortisol awakening responses are no longer effectively modulated by anticipated daily demands.

In our day-to-day analysis, fatigue and physical symptom levels across the day were associated with wakeup cortisol levels that morning (but prior-day fatigue did not predict next-day wakeup cortisol levels). This result suggests that our "boost hypothesis," that higher morning cortisol levels are adaptive in that they contribute resources to help us meet the perceived demands of the day, may extend beyond the CAR to include wakeup cortisol levels. In terms of the mechanism for the fatigue/symptom effects, one possibility is that higher morning cortisol levels, through their influence on metabolic processes, contribute relatively directly to increased energy/lower fatigue. Another possibility is that low morning cortisol levels allow greater activation of immune and inflammatory factors that are typically constrained by corticosteroids and known to produce higher fatigue and malaise (41–43). Research has reported cross-sectional associations between low basal cortisol and burnout (15) as well as serious fatigue, pain, and inflammatory conditions including chronic fatigue, fibromyalgia, and rheumatoid arthritis (19–23, 44). Our results provide evidence, at least for subclinical changes on a day-to-day basis, that the likely causal direction is from low cortisol levels to greater fatigue and symptom experience. Whether this extends to clinical levels of fatigue or pain requires further research, but, in support of this possibility, several clinical trials have found evidence for short-term alleviation of fatigue symptoms in chronic fatigue patients when glucocorticoids are administered (45, 46).

It is of some interest that all of our obtained associations between daily experience and cortisol were for factors containing negative dimensions of experience such as sadness, loneliness, anger, and fatigue; it appears that daily cortisol activity is most tightly linked to variations in our daily environment relevant to potential harm. Two factors reflecting positive psychological states, such as feeling confident or effective, were not significantly related to any of our cortisol indices. One possibility that remains to be tested, however, is whether positive experience may serve to buffer associations between daily negative experience and cortisol activity.

Given how strongly intercorrelated our daily experience factors were, it is notable that they show distinct associations with our different cortisol indices. Future research should continue to examine multiple aspects of diurnal cortisol activity; not only is it likely that various diurnal cortisol parameters differ somewhat in their underlying physiologic regulation (14), our results suggest that they differ in the dynamics of their interplay with everyday emotional, psychosocial, and physical experience.

When examining HPA axis activity in everyday life, thorough measurement and control of confounding variables is necessary, and failure to control for these variables can produce spurious results and obscure true associations. Of our control variable effects, it is worth noting that non-Hispanic Whites had higher wakeup cortisol levels and steeper diurnal cortisol slopes. Other recent studies have reported similar effects, finding African Americans, in particular, to have flatter diurnal cortisol rhythms, after controlling for a wide range of psychosocial and health behavior measures (47). In addition, there were trends for diurnal cortisol slopes to be flatter in older individuals and in males; the extent to which these changes relate to age- and gender-related declines in health remain to be determined. Self-reported psychiatric illness and greater alcohol use were associated with a higher CAR; these effects should be investigated further in future research. CAR levels were also lower ($P < 0.10$) for noncompliant participants and those

who arose later in the morning. These findings are in accord with prior research (38, 48), and speak to the importance of assessing and controlling for the effects of noncompliance and sleep timing.

In summary, with the use of careful methodology and appropriate statistical techniques, the dynamic interplay between subjective experience and HPA axis activity, as indexed by salivary cortisol levels, can be observed and understood in naturalistic settings. Our findings draw attention to the dual function of cortisol as both a reflection of social and emotional experience and a contributor to the energetic and behavioral state of the individual. In comparison with cross-sectional approaches, the use of longitudinal data and analysis methods (to examine change across moments, days, weeks, or years) provides better insight into the causal direction of associations among experience, cortisol, and health. Such approaches will bring us closer to understanding the dynamic and adaptive role of cortisol in helping us respond to environmental experience, and the circumstances under which this function may go awry and play a role in disease processes.

Methods

Participants. Participants were drawn from the first year of data of the Chicago Health, Aging, and Social Relations Study, a longitudinal, population-based study of African-American, Hispanic, and Caucasian individuals born between 1935 and 1952 living in Cook County, IL. The sampling protocol for this study is described in detail in ref. 49. Participants needed to be sufficiently ambulatory to come to the University of Chicago for a day-long laboratory visit.

Of the 229 individuals who participated in year 1 of the Chicago Health, Aging, and Social Relations Study, 170 provided salivary cortisol data. Of these, 5 were eliminated because they provided insufficient cortisol data (<50% of the requested samples), 1 was eliminated because of an unusual schedule/shift work, and 8 were eliminated because of their use of steroid-based medication, leaving a final sample size of 156. The 73 participants excluded from our analyses were not significantly different from the included group in terms of gender, marital status, age, education, or income. Excluded individuals were marginally more likely to be Hispanic (37% vs. 25%; t (227) = 1.9, P = 0.06) and to have higher levels of depressive symptoms (0.26 SD higher; t (221) = 1.8, P = 0.07).

The final sample was composed of approximately equal numbers of men and women (52% men, 48% women) and included 36% non-Hispanic White, 38% African-American, and 25% Hispanic participants. Participants ranged in age from 50 to 68, with a mean age of 57 years (SD 4.5). Fifty-eight percent were married, 3% lived with a nonmarital partner, 4% were separated, 20% were divorced, 11% were widowed, and 4% never married. Education and income levels varied greatly: 12% of participants had less than a high school education, 32% had a high school diploma or GED, 23% had some college, 16% had a college degree, and 17% had attended graduate school. The median annual household income was $53,750, with 10% of households earning less than $15,000 and 4% earning more than $200,000.

Procedures. At the end of a day-long laboratory visit, participants were instructed regarding the at-home procedures used in this study. Materials completed at home were returned in a postage-paid envelope. Participants were paid $36 US for the at-home portion of the study.

Saliva sampling. Participants were given materials and verbal and written instructions to take saliva samples immediately before bed, upon awakening (before getting out of bed), and 30 min after waking on each of 3 study days, beginning with a Sunday and ending on Tuesday. Participants were instructed not to brush their teeth, smoke, eat, or drink beverages containing alcohol, caffeine, or fruit juice during the 30 min before each sample. Saliva was collected by means of an absorbent cotton roll (Salivette; SARSTEDT, Nümbrecht, Germany). The time each sample was taken was recorded on an accompanying form.

Electronic monitoring of compliance. For 52% of participants, compliance with the timing of their cortisol sampling was assessed by having them withdraw the cotton roll used to collect saliva from a small plastic vial that was capped with a lid containing a microchip that recorded each opening of the vial (MEMS Track Cap; Aardex, Denver, CO). Participants were unaware that bottle openings were being monitored. Time stamps for each bottle opening were later compared with participants' self-reports of saliva collection times. Noncompliance has been shown to influence estimates of diurnal cortisol parameters (50).

Cortisol assay procedures. Saliva samples were assayed for cortisol at the Labor für Stress-Monitoring at the University of Göttingen, Germany, by using an RIA protocol. Lower and upper limits of detection were 0.15 and 25 ng/ml. Five identical control samples were included in each assay to test inter- and intraassay consistency. Intraassay coefficients of variation (CV) ranged from 2.8% to 8.4% (mean 4.6%); the average interassay CV was 3.4%. To correct a strong positive skew in the data, cortisol values were natural-log transformed before use in analysis.

Daily experience diaries. Each evening of the 3 days of testing, just before going to bed, participants completed diary reports of the psychosocial, emotional, and physical states they had experienced that day. Participants were asked, "Overall today, to what extent did you feel. . . " each of 22 adjectives (such as sad, lonely, tense, energetic, angry) and responded on five-point scales ranging from "not at all" to "very much." They were also asked, "to what extent did you feel. . . " "physical symptoms (e.g., headache, stomachache, cough);" "unable to control important things in your life;" "confident in your ability to handle your personal problems;" "that things were going your way;" and "that difficulties were piling up so high that you could not overcome them."

Six scales emerged from a principal components analysis (with varimax rotation) of these 26 items: (*i*) lonely/sad/overwhelmed: lonely, isolated, sad, discouraged, helpless, intimidated, threatened, unable to control things, difficulties piling up (α = 0.89); (*ii*) active/effective: lively, energetic, good about self, effective, goal directed, purposeful (α = 0.88); (*iii*) tense/angry: tense, uneasy, angry, annoyed (α = 0.83); (*iv*) confident: felt confident in ability, things going my way (α = 0.71); (*v*) symptomatic/fatigued: felt physical symptoms, fatigued, exhausted (α = 0.73); (*vi*) confused/forgetful: confused, forgetful (α = 0.71). Three versions of each scale were constructed by unit-weighting and averaging relevant items: average levels across all days of testing, levels on the day before each day of cortisol testing, and levels on the same day as cortisol testing.

Demographic and Health Covariates. To help ensure that differences in cortisol were not attributable to demographic characteristics or to health and health behaviors, questionnaire reports of these variables were obtained. Demographic variables included age, gender, education (years completed), marital status (married/partnered vs. not), and race–ethnicity. Health and health behavior variables included: presence of current physical or psychiatric illness;** self-reported depressive symptoms; typical levels of daily caffeine, alcohol, and nicotine use; medications used (coded as steroid-based or nonsteroid based); body mass index (BMI); and typical wakeup times, bedtimes, hours of sleep, and perceived sleep quality. Use of corticosteroid-based medications was grounds for exclusion. The effects of other covariates on cortisol, where present, were controlled statistically in an attempt to isolate the impact of

**A variety of health conditions were reported, including high blood pressure (41% of participants); a history of heart attack (4%), heart failure (4%), stroke (7%), cancer (9%); the presence of emphysema (4%), diabetes (18%), asthma (6%), rheumatoid arthritis (3%), ulcers (4%), kidney problems (3%), liver problems (2%); HIV^+ status (1%); or psychiatric problems (details of diagnoses not specified) (12%). Only the presence of a psychiatric condition was significantly associated with our cortisol parameters; this variable was retained as a covariate.

the primary variables of interest: day-to-day variations in psychosocial, emotional and physical states.

Data Analysis. A three-level multilevel growth-curve analysis (51, 52) was used because moments (cortisol samples within a day) are nested within days of testing, which are nested within persons. Use of a multilevel model adjusts for the nonindependence of observations associated with nesting and allows us to model diurnal cortisol rhythms and to examine both day-level and person-level factors predicting differences in these rhythms. Variables that change with each cortisol sample, such as time of day, are Level 1 or moment-level variables; day-to-day variations in experiences are Level 2 or day-level variables; and trait, typical, or average experiences across all of the days of testing are Level 3 or person-level variables. For analyses at the day level, a lag model was used to examine whether cortisol parameters each day were more strongly associated with experiences the day before, or the day concurrent with cortisol testing. Because prior-day mood data are not available for the first day of cortisol data collection, all 3 days of mood data but only the second and third days of cortisol data are used.

The analysis proceeded as follows. First, a Level 1 model was fit to provide latent estimates of the parameters defining each person's diurnal cortisol rhythm:

(1) Level 1: Cortisol = $\pi_0 + \pi_1 \times$ Time Since Waking + $\pi_2 \times$ Time Since Waking^2 + $\pi_3 \times$ CAR + e

Cortisol values were predicted by the time of each sample, scaled as hours since waking each day, such that the Level 1 intercept (π_0) reflects a latent estimate of each person's average wakeup cortisol level across the days of testing. Both linear (hours since waking) and quadratic (hours since waking squared) terms for time of day were included to account for curvilinearity, with the coefficient on the linear time variable (π_1) reflecting a latent estimate of the slope of each person's diurnal cortisol rhythm at wakeup. The 30-min after awakening cortisol sample was indicated with a dummy variable (0, 1), with the coefficient on that variable (π_3) reflecting a latent estimate of the size of each person's CAR.

The influence of the demographic, health behavior, and health condition variables on diurnal cortisol rhythms were then tested by conducting exploratory analyses of the association between these variables and each of the Level 1 coefficients. Variables significantly associated with cortisol parameters were included at either Level 2 (for day-varying factors such as time of waking) or Level 3 (for person-level factors not varying from day-to-day). In addition, age, gender, and race/ethnicity (a dummy variable for non-Hispanic White) were retained in all models.

(2) Level 2: π_0 to $\pi_3 = \beta_{i0} + \beta_{ij} \times$ Day Level Controls + r_{ij}

(3) Level 3: β_{i0} to $\beta_{ij} = \gamma_{ij0} + \gamma_{ijk} \times$ Person Level Controls + u_{ijk}

Next, we examined the effects of the average diary reports of psychosocial, emotional, and physical experiences by adding these person-level predictors to the Level 3 model predicting each person's average wakeup cortisol value, slope, and cortisol awakening response:

(4) Level 3: β_{i0} to $\beta_{ij} = \gamma_{ij0} + \gamma_{ijk} \times$ Person Level Controls + $\gamma_{ijk} \times$ Average Daily Experiences + u_{ijk}

Finally, the effects of day-to-day variations in experiences on day-to-day variations in diurnal cortisol parameters were examined by adding the experience variables at Level 2 rather than Level 3 and simultaneously entering both prior-day and same-day experiences:

(5) Level 2: π_0 to $\pi_3 = \beta_{i0} + \beta_{ij} \times$ Prior Day Experiences + $\beta_{ij} \times$ Same Day Experiences + $\beta_{ij} \times$ Day Level Controls + r_{ij}

In each set of models, dummy variables were also entered for whether the participant was compliant (0) or noncompliant (1) with the requested timing of sampling.[††] When compliance data were not available, participants were given a 0 on these variables. Although it would be ideal to have compliance data on the full sample, inclusion of this information for half the sample provides some control for and an indication of the extent to which results are robust to the effects of noncompliance.

[††]For the wakeup sample, participants were considered noncompliant if the track cap reading showed that they took the wakeup sample more than 10 min earlier or later than their self-reported wakeup time. For the CAR sample, participants were considered noncompliant if the time between the track cap readings for the wakeup and CAR samples deviated by >10 min from the requested 30-min interval.

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