

# Compliance with Ambulatory Saliva Sampling in the Chicago Health, Aging, and Social Relations Study and Associations with Social Support

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## ABSTRACT

**Background:** Noncompliance with instructed saliva sampling times in ambulatory settings can compromise resulting cortisol findings. **Purpose and Methods:** Here, the impact of noncompliance on the cortisol awakening response (CAR), an established marker for hypothalamic-pituitary-adrenal axis activity, was examined over 3 sampling days in middle- and older-age participants in the Chicago Health, Aging, and Social Relations Study. **Results:** Noncompliant participants had a significantly lower cortisol rise after awakening (assessed by an awakening sample and a 30-min after awakening sample) on 2 of the 3 sampling days (Day 1, *ns*; Days 2 & 3,  $p < .02$ ). Furthermore, social support measured by the Interpersonal Support Evaluation List correlated negatively with the number of “noncompliant” samples ( $r = -.19$ ,  $p < .05$ ), indicating that participants reporting more social support had more “compliant” samples. **Conclusion:** The results confirm that nonadherence to saliva sampling in ambulatory settings can exert a significant impact on the resulting CAR. Furthermore, the data raise the idea that the extent of nonadherence might be systematically associated with psychosocial factors like social support. For future studies on the relationship between CAR and psychological factors, we therefore recommend controlling for saliva sampling adherence because noncompliance might be systematically associated with the phenomenon being investigated.

(Ann Behav Med 2007, 34(2):209–216)

Funding was provided by the National Institute of Aging Grant No. PO1 AG18911 and the John Templeton Foundation. Brigitte M. Kudielka was supported by grants from the German Research Foundation (DFG grant KU 1401/3-1 and KU 140/4-1).

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## INTRODUCTION

Compliance with saliva sampling procedures, especially the timing of sampling, is crucial in the assessment of salivary cortisol levels in ambulatory settings. Electronic monitoring devices record the date and time of each sample collection and thus allow the researcher to monitor participants' adherence with a given sampling protocol. In the first study to use electronic monitoring devices to monitor compliance (1), the cortisol awakening response (CAR) was seen to differ significantly between compliant and noncompliant participants with a robust increase in cortisol from baseline to 30 min after awakening in compliant participants compared to only minimal changes in noncompliant individuals. In a subsequent study in female fibromyalgia patients and sex- and age-matched healthy controls, noncompliance with salivary cortisol sampling during a “7-days and 5-samples-a-day” protocol resulted in flatter slopes (2). In that study, patients adhered more closely to the sampling protocol than controls. In both studies, self-reported (subjective) compliance was substantially overestimated compared to actual (objective) compliance if participants were unaware of electronic monitoring. In another study, Jacobs and coworkers (3) applied a random rather than fixed time sampling protocol in female twin pairs and their sisters and reported that inclusion of “noncompliant” samples did not distort the resulting cortisol day profile. However, their protocol did not include the CAR, a parameter that is most strongly affected by nonadherence (1).

To date, the CAR<sup>1</sup> has been established as a useful and easy-to-measure marker for hypothalamic-pituitary-adrenal axis activity (for a review see 5,6). Recently, in a highly controlled study under sleep laboratory conditions,

<sup>1</sup>In the literature, two synonymously used abbreviations can be found for this marker: CAR (cortisol awakening response) as first introduced by Federenko et al. (4) and ACR (awakening cortisol response) as used by Clow and colleagues (5).

the CAR was shown to be a genuine response to awakening, and the transition from sleep to wake was shown to be essential for the cortisol morning rise to occur (7). After awakening, salivary cortisol levels increase about 50 to 75% within 30 to 45 min. This rise can be observed in about 75% of healthy participants and shows reasonably high stability over time (8). Its magnitude and time course varies, for example, with gender and age (6,8–10) and is in part determined by genetic factors (11,12). It is of interest that there are several studies reporting that the CAR is associated with psychological states (13) and psychopathology, such as perceived chronic stress (8,9,14,15), present or former depressive symptomatology (16–18), chronic fatigue syndrome (19), neuroticism (20), and burnout symptomatology (14,21).

Another line of research has generated evidence that patient adherence with medical treatments or prescription regimens is affected by psychological factors, especially social support (for a meta analysis see 22,23) and depressive symptomatology (23–27). Such evidence from medical science indicates that patient adherence with medical treatments or prescription regimens are especially affected by social support. Other psychological factors, like self-efficacy or different personality factors, have also been suggested to be relevant for specific treatments or patient groups (28,29). As outlined by DiMatteo (22), social support might be implicated in promoting patient adherence by enhancing optimism and self-esteem, buffering the stress of being ill, reducing patient depression, improving sick-role behavior, and giving practical assistance. This raises the possibility that besides patient adherence with medical treatment, social support might also be relevant for compliance with saliva sampling procedures in ambulatory settings in healthy study volunteers.

Therefore, the aim of our study was twofold. First, we analyzed the potential impact of noncompliance on the CAR in a population-based sample of older adults to corroborate our earlier findings in predominantly younger adults. Second, we examined associations between ambulatory saliva collection compliance and social support.

## METHODS

### Study Sample

Participants were drawn from the 1st year of the Chicago Health, Aging, and Social Relations Study (CHASRS), a longitudinal, population-based study of White, Black, and Hispanic persons 50 to 67 years of age. The multistage sample selection procedure is detailed elsewhere (30). During a laboratory visit, participants provided self-reported demographic and medical information and psychological questionnaires, including a measure of social support (see next).

Of the 229 individuals who agreed to participate in Year 1, 170 provided ambulatory salivary cortisol data (see next). For 119 participants, objective compliance with

the timing of their cortisol sampling was available for at least one of the six morning samples (two morning samples per day). Sampling compliance was measured by an electronic monitoring device (MEMS<sup>®</sup> Track Cap; AARDEX, Ltd., Zug, Switzerland) as introduced earlier (1,2).

### Assessment of Social Support

The Interpersonal Support Evaluation List (ISEL) consists of 12 statements (including three subscales with four items in each) to which participants responded on a 4-point Likert scale ranging from 1 (*definitely false*) to 4 (*definitely true*). Cohen and Hoberman (31) and Cohen et al. (32) provided discussions of scale design and psychometric properties (see <http://www.psy.cmu.edu/~scohen> for item content and scoring instructions). In our sample, Cronbach's alpha across the 12 items was .87. In this self-report questionnaire, participants are asked to rate how accurately each item reflects their own feelings. After reverse scoring appropriate items, subscale scores are calculated for appraisal support (e.g., "There is someone I can turn to for advice about handling problems with my family"), belonging support (e.g., "If I wanted to have lunch with someone, I could easily find someone to join me"), and tangible support (e.g., "If I were sick, I could easily find someone to help me with my daily chores"). For the purposes of this study, an overall social support score (range = 4–16) was computed by averaging the subscale scores.

### Study Protocol

*Ambulatory saliva sampling procedure.* At the end of the lab day, participants received saliva sampling materials and both spoken and written instructions to take a first sample upon awakening (before getting out of bed) and a second sample 30 min after waking on each of 3 study days, beginning with a Sunday and ending on a Tuesday. These sampling days were selected to include leisure and work days and thereby enhance the representativeness of measurements in the daily lives of participants in this longitudinal study (see e.g., 13).

Participants were required not to brush their teeth, smoke, eat, or drink beverages containing alcohol, caffeine, or fruit juice during the 30 min preceding each saliva collection. Saliva was collected by means of an absorbent cotton roll (Salivette<sup>®</sup>, Sarstedt, Inc., Nümbrecht, Germany). To obtain the participants' subjective adherence to the given sampling protocol, time of awakening as well as the time of the saliva sample was recorded by participants on an accompanying form. Completed materials were returned to the laboratory in a postage-paid envelope. Participants received US \$36 for the at-home component of the study.

### Cortisol Analysis

Free cortisol was assayed at the Labor für Stress-Monitoring (Göttingen, Germany), using a scintillation

proximity radioimmunoassay with tritium labeled cortisol (Amersham Buchler, Braunschweig, Germany). The cortisol antibody was purchased from Biogenesis (Poole, England). Lower and upper limits of detection were .5 and 86.7 nmol/l. Five identical control samples were included in each assay to test inter- and intra-assay consistency. Intra-assay coefficients of variation (CV) ranged between 2.8% and 8.4% (average 4.6%), and the average interassay CV was 3.4%.

### Electronic Monitoring of Compliance

Objective compliance with the timing of the cortisol collection was assessed by means of an electronic monitoring device (MEMS<sup>®</sup> Track Cap; AARDEX, Ltd., Zug, Switzerland) which time-stamped saliva collections via bottle openings. Participants were instructed to withdraw an absorbent cotton roll at each designated sampling time from a small plastic bottle that was capped with a lid containing a microchip that time-stamped each opening of the bottle. Participants were told that the unused cotton rolls must remain in the bottle to ensure valid hormone analysis in the laboratory and were unaware that bottle openings were being monitored. After collecting a saliva sample, participants were instructed to store the saliva-soaked roll in a pre-labeled and color-coded plastic Salivette tube provided by the experimenter.

A special interface and the software program Power-View (provided by AARDEX) were used to transfer data from the electronic MEMS monitor to a PC. Time stamps for each bottle opening were compared with participants' self-reports of saliva collection times. The degree of discrepancy between objective and subjective saliva collection times represented the criterion for the determination of compliance. Due to the rapid change in cortisol in the first hour after awakening, and for consistency with our earlier work (1,13), a  $\pm 10$ -min compliance window was established to render a dummy coding "compliant versus noncompliant participant." For the waking sample, participants were considered noncompliant if the track cap data indicated the sample was collected more than 10 min before or after the reported awakening time. For the second sample (30 min after awakening), participants were considered noncompliant (a) if the track cap reading revealed that they took the second sample more than 10 min earlier or later than their self-reported sampling time or (b) if the time between the track cap readings for the first and second sample deviated by more than 10 min from the requested 30-min interval between these two samples. Participants were considered noncompliant for that day if they met one or more of these noncompliance criteria.

To assess the association between social support and the degree of noncompliance, noncompliant samples were expressed as a proportion of the total number of provided morning samples (maximum of six samples over 3 days).

### Statistical Analysis

Statistics were performed using SPSS (Version 13.0; SPSS, Chicago, IL). The significance level was set at  $\alpha = .05$  (two-tailed testing). Results in the text and Table 1 are given as mean  $\pm$  standard deviation; data in the figures are presented as mean  $\pm$  standard error of mean.

For cortisol analysis, two shift workers (7) and six participants on steroid-based medication were excluded (10). In addition, particular sampling days could only be included for participants when they provided both the wake-up and the wake +30 min sample required to calculate the CAR that day. This led to a final sample of 59 valid cases on Day 1, 70 on Day 2, and 65 on Day 3. Of included participants, 50 (60%) provided data on all 3 days, 21 (25%) on 2 days, and 12 (14%) on only 1 sampling day, with 83 different participants contributing data on at least 1 day.

To test for possible differences between included and excluded subsamples, we performed Student's *t* tests and chi-squared tests (Fisher's exact test). With reference to all 229 individuals participating in any part of CHASRS in Year 1, excluded participants did not differ significantly from the included groups each day in terms of age, marital status, years of education, or income. Excluded individuals were however more likely to be male for Sampling Day 2 ( $p < .03$ ; at Day 3,  $p = .06$ ) and African American at Sampling Day 1 and 2 ( $p < .03$  and  $p < .04$ ; at Day 3,  $p = .07$ ). Analyses were conducted within sampling day to avoid excessive loss of cases that would have resulted from listwise exclusion across sampling days had a repeated-measures approach been attempted. For a detailed description of the final study sample, including demographics and health conditions, see Table 1.

To assess the impact of sampling accuracy on the CAR at each of the 3 collection days, two-way analyses of variance (ANOVA) were conducted with the repeated factor saliva sample (wake-up sample, +30 min sample), and the dummy coded grouping factor "compliant vs. noncompliant." Because gender, age, smoking, time of awakening, general health status, and day of week have been discussed as important possible sources of between-subject variation in the CAR (4) (for review see 5,6,8–10,33), the impact of gender, age, smoking (number of cigarettes per day), and time of awakening on cortisol levels was first examined by ANOVA, respectively. In case of a significant effect, the respective factor was entered as covariate in subsequent analyses. Day of week was held constant (see Procedure). Skewness and kurtosis of cortisol data did not exceed  $-1$  to  $1$  and  $-3$  to  $3$ , respectively. Accordingly, Kolmogorov-Smirnov tests for nonnormal distributions yielded non-significant results (all  $p < .21$ ). Therefore, raw unlogged cortisol levels were entered into analysis. Finally, Pearson correlations were computed to assess the association between social support and the number of provided "noncompliant" samples (expressed in percentage). For this noncompliance measure, a high score means lower

TABLE 1  
Description of Total Study Sample Contributing Data on at Least One Day

<i>Characteristics</i>	
Gender	
Male	32
Female	51
Ethnicity	
Non-Hispanic White	35 (42%)
African American	24 (29%)
Hispanic	24 (29%)
Age (years)	
Mean ( <i>SD</i> )	58 (4.4)
Range	50–68
Marital status	
Married	50 (60%)
Living with a nonmarital partner	1 (1%)
Separated	2 (2%)
Divorced	16 (19%)
Widowed	9 (11%)
Never married	5 (6%)
Education	
Less than a high school education	10 (12%)
High school diploma or GED	24 (29%)
Some college	21 (25%)
College degree	12 (15%)
Graduate school	16 (19%)
Income	
Mean annual household income ( <i>SD</i> )	\$63,731 (\$51,265)
Range	\$1,045–\$280,612
Smoking status	
Nonsmoking	76
Smoking	7
Awakening time	
Mean ( <i>SD</i> )	Day 1 <sup>a</sup> : 7:16 h (0:11)
Range	3:22–11:17
	Day 2 <sup>b</sup> : 6:48 h (0:10)
	4:12–12:42
	Day 3 <sup>c</sup> : 6:48 h (0:12)
	3:50–11:37
Health condition	
High blood pressure	28 (32%)
History of heart attack	3 (4%)
History of heart failure	3 (4%)
History of stroke	5 (6%)
History of cancer	7 (8%)
Presence of emphysema	2 (2%)
Presence of diabetes	11 (13%)
Presence of asthma	7 (8%)
Presence of rheumatoid arthritis	4 (5%)
Presence of ulcers	3 (4%)
Presence of kidney problems	0
Presence of liver problems	0
Presence of HIV+ status	1 (1%)
Presence of psychiatric problems <sup>d</sup>	14 (17%)
Presence of lupus erythematosus	0
Presence of Alzheimer's disease	0

Note.  $N = 83$ . Numbers may vary somewhat between day-specific subsamples.

<sup>a</sup> $n = 59$ . <sup>b</sup> $n = 70$ . <sup>c</sup> $n = 65$ . <sup>d</sup>Details of diagnoses not specified.

compliance and a low score means greater compliance. Correlational analyses were based on all participants with available cortisol samples, track cap, and questionnaire data ( $n = 116$ ).

## RESULTS

### Extent of Compliance/Noncompliance

Of the 83 participants who provided CAR samples for at least 1 of the 3 sampling days, 36 (43%) were noncompliant on 1 sampling day, 11 (13%) were noncompliant on 2, and 3 (4%) were noncompliant on all 3 sampling days. Fifty of the 83 participants provided CAR samples for all 3 sampling days, and of these, 21 were compliant on all 3 days, 18 were noncompliant on 1 day, 8 were noncompliant on 2, and 3 were noncompliant on 3 sampling days. Of the 21 participants who provided CAR samples for 2 sampling days, 10 were compliant on both days, 3 were noncompliant on both days, and 8 were noncompliant on 1 day. Finally, of the 12 participants who provided CAR samples on only 1 sampling day, 10 were noncompliant and only 2 were compliant.

Applying the compliance criteria to each of the sampling days, Day 1 had 36 (61%) compliant and 23 (39%) noncompliant participants, Day 2 had 46 (66%) compliant and 24 (34%) noncompliant participants, and Day 3 had 49 (75%) compliant and 16 (25%) noncompliant participants.

### Cortisol Covariates

On Day 1 only, gender showed a marginally significant effect on morning cortisol levels ( $p = .10$ ; marginally higher cortisol levels in men), and an interaction with sample ( $p = .07$ ) that showed a tendency toward a smaller cortisol awakening increase in men, whereas age, smoking, and time of awakening were not significantly associated with morning cortisol levels on all 3 sampling days. Gender was therefore included as a covariate in the subsequent analyses.

### Impact of Compliance/Noncompliance on Cortisol

The two-way repeated measures ANCOVA with the grouping factor compliance vs. noncompliance revealed a significant main effect of sample,  $F(1, 56) = 4.5$ ,  $p < .04$ , whereas the main effect of group,  $F(1, 56) = .9$ ,  $p = .35$ , as well as the interaction of sample by group,  $F(1, 56) = .008$ ,  $p = .93$ , were nonsignificant (Figure 1A). On Day 2, the two-way analysis of covariance (ANCOVA) revealed a significant main effect of sample,  $F(1, 68) = 25.1$ ,  $p < .0001$ , and a significant Sample  $\times$  Group interaction,  $F(1, 68) = 6.5$ ,  $p < .02$ . Noncompliant participants showed a smaller CAR than compliant participants (Figure 1B). The main effect of compliance group was not significant,  $F(1, 68) = 1.8$ ,  $p = .18$ . On Day 3, the two-way ANCOVA revealed a significant main effect of sample,  $F(1, 63) = 13.9$ ,  $p < .0001$ , and a significant Sample  $\times$  Group interaction,

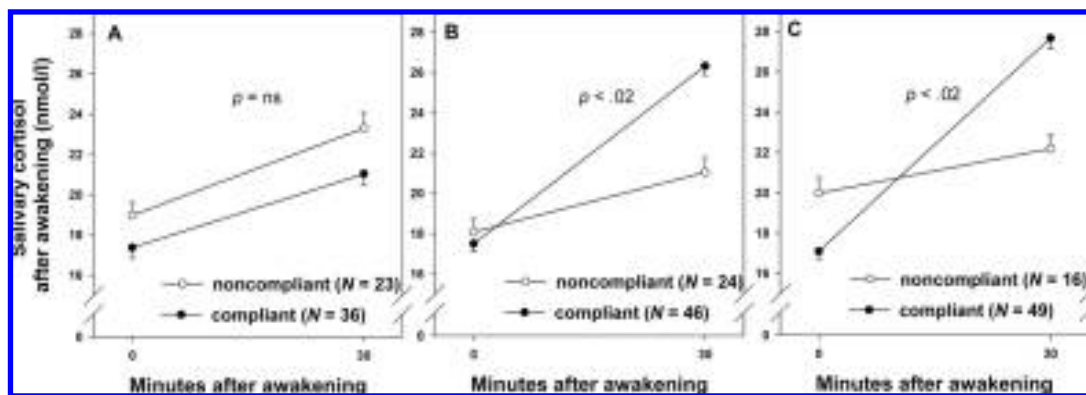


FIGURE 1 The cortisol awakening response (nmol/l) at Day 1 (A), Day 2 (B), and Day 3 (C) in compliant versus noncompliant participants.

$F(1, 63) = 6.4, p < .02$ , whereas the main effect of group,  $F(1, 63) = .35, p = .56$ , was nonsignificant. Again, noncompliant participants showed a smaller CAR than compliant participants (Figure 1C).

With respect to health conditions, chi-squared tests (Fisher's exact test) showed that none of the health conditions differed significantly between compliance groups, except presence of diabetes on Day 2 ( $p < .002$ ). Only presence of high blood pressure, diabetes, psychiatric problems, or history of cancer was indicated by at least 10% ( $n \approx 6$ ) of participants. In additional ANCOVA analyses we included these variables as additional covariates; these analyses rendered the same results.

Analysis of cortisol responder rates, with responders defined as participants who showed cortisol morning increases of at least 2.5 nmol/l from the waking baseline (8,34), revealed that 53% of compliant and 43% of noncompliant participants responded on Day 1, 78% of compliant and 54% of noncompliant participants responded on Day 2, and 76% of compliant and 56% of noncompliant participants responded on Day 3.

### Compliance and Social Support

Correlation analysis revealed that mean social support is negatively associated with the proportion of "noncompliant" samples ( $n = 116, r = -.19, p < .05$ ), indicating that participants reporting more social support had a greater percentage of "compliant" samples. Each subscale of the social support measure appeared to contribute to this general effect (tangible support,  $r = -.18, p = .05$ ; appraisal support,  $r = -.16, p = .09$ ; belonging support,  $r = -.15, p = .11$ ), though the combined effect was stronger than any individual subscale effect.

## DISCUSSION

The present analysis in a population-based ethnically diverse sample of middle- and older-age participants

confirms and replicates prior findings showing that noncompliance with saliva sampling procedures in ambulatory settings is an important issue affecting the accuracy of cortisol measurement in field settings (1,2). Objective compliance with saliva collection times for CAR assessment was tracked by means of an electronic monitor (MEMS Track Cap). Electronic control devices have also been introduced in other areas of psychology. For example, electronic diaries are used to track and eventually improve participants' compliance with diary entries (35,36). In our study, participants were kept uninformed about the real nature of the monitoring device because the purpose of the study was to assess compliance in an ambulatory setting.

We observed that a significant number of participants did not comply with the requested sampling times. Across the 3 sampling days, rates of noncompliance, defined as deviations of more than 10 min from the prescribed sampling times, ranged from 25% (16 of 65 participants on Day 3) to 39% (23 of 59 participants on Day 1). Based on the 83 participants who supplied CAR samples for at least 1 of the sampling days, 3 (4%) were noncompliant at all sampling days, 11 (13%) were noncompliant at 2, and 36 (43%) were noncompliant at 1 sampling day. This indicates that people are not consistent in their noncompliance, as it was rare to be noncompliant across all 3 days. In accordance with our earlier findings in uninformed participants (1,2), our sample of middle-aged and older adults appeared to show a low compliance rate, and it might be hypothesized that even lower compliance would be observed with a higher collection load (more days, more samples) as recently recommended for ambulatory cortisol assessments (37). Second, we analyzed the impact of nonadherence on the CAR and found that participants with noncompliant samples had a significantly lower cortisol rise after awakening, an effect that was found on 2 of the 3 sampling days. The smaller CAR among noncompliant participants could be attributable to either a late-awakening sample, in which cortisol levels were already rising from awakening levels toward the CAR

peak, or an early or late 30-min postwaking sample that failed to capture peak cortisol levels. Consequently, we found a much higher responder rate in compliant versus noncompliant participants; responders defined as participants who showed cortisol morning increases of at least 2.5 nmol/l from the waking baseline. A similar responder rate has been reported earlier by Wüst and coworkers (8). The responder rate in compliant versus noncompliant participants differed much more on Day 2 and 3 than on Day 1. We can think of several possible reasons why this may be the case, and why CAR differences between compliant and noncompliant participants were larger on Days 2 and 3 of our study. First, slightly different subsamples are included on the different sampling days. Alternatively, because Day 1 was a Sunday, and it has been hypothesized that the CAR is a response to the anticipated demands of the day (13), it could be that on a low-demand day (i.e., a Sunday), there is less range in the CAR, hence it is harder to find effects of compliance. Earlier evidence in younger as well as older adults shows that (a) there is a clear weekend-weekday difference in the cortisol response to awakening and (b) this difference is associated with chronic work overload and worry (9,33). Schlotz et al. (9) further reported that independent of weekend-weekday differences, participants with higher levels of chronic work overload and worrying showed a stronger increase and higher mean levels of cortisol after awakening on weekdays but not on weekend days. It might further be speculated that on the 1st day of data collection, when the procedures were new to participants (in this case, the Sundays), either the compliant participants were less accurate (within the range of what was considered “compliant”) or the noncompliant participants were trying slightly harder to be accurate (within the range of what was considered “noncompliant”). These speculations do not rule out the possibility that noncompliance on Day 1 truly did not impact on cortisol results on Day 1 or that other reasons might have been responsible for an absence of a compliance effect at Day 1.

For the most part, however, our observations are in accordance with earlier evidence in predominantly younger adults (1) and in middle-aged female fibromyalgia patients and their healthy controls (2), confirming that nonadherence strongly influences resulting cortisol data. As discussed in more detail there, such an awakening profile might be misinterpreted as a flattened CAR (cf. 38,39). Without information on compliance, the CAR profiles of our noncompliant participants might have been identified as “blunted” or indicative of a sluggish adrenocortical responsiveness. Likewise, had one searched for individuals with a “flat” morning profile, many noncompliant participants in our study might have been selected for subsequent in-depth analysis. In the last years, the investigation of blunted morning cortisol profiles as well as flat circadian rhythms (slopes) have garnered increasing attention, especially in the area of chronic stress and burnout (for an overview, see 21). Consequently, superimposing effects

of noncompliance on measured cortisol levels make it difficult (or even impossible) to distinguish between falsely labelled flat awakening responses and true blunted responses.

As indicated by our analysis of the responder rate, we did find responders as well as nonresponders in both groups, showing that nonresponses can only in part be ascribed to sampling noncompliance. However, such effects of nonadherence might gain high relevance because noncompliance can have important consequences for the ability to detect cortisol-behavior relationships. The error induced by noncompliance may significantly diminish the ability to reveal existing relationships between cortisol and psychological or behavioral parameters.

Third, we showed, for the first time, that sampling noncompliance with an ambulatory saliva collection protocol is negatively associated with social support as is adherence to medical treatments or prescription regimens in patients (for a meta analysis, see 22). Our analysis revealed that social support was significantly negatively associated with the extent of noncompliance, with participants reporting more social support having more compliant samples. In his meta-analysis, DiMatteo (22) hypothesized that social support in patients may (a) lead directly and straightforwardly to increased adherence with medication intake and (b) improve adherence via improved cognitive functioning, self-efficacy, intrinsic motivation, personal control, confidence, self-esteem, and mood. Such mechanisms might also be assumed for compliance with saliva sampling protocols.

To the extent that compliance is systematically associated with the psychological or behavioral phenomenon being investigated, true relationships could be obscured or incorrect relationships could emerge. The latter problem is especially tricky, because such a systematic error cannot be eliminated by increasing the statistical power via enlarged study samples and/or an increased number of samples. Based on our results and this reasoning, we therefore recommend statistical control for noncompliance, as applied in a recent study of day-to-day variations in cortisol diurnal rhythms that examined the temporal ordering of experience-cortisol associations in the CHASRS sample (13).

An important limitation of our analysis is that the data presented here are correlational and do not allow for causal inferences. It is more likely that social support influenced saliva sampling adherence than adherence influenced the earlier self-report of social support, but the direction of the effect cannot be confirmed, and both variables might be determined by other factors. Furthermore, several inherent aspects of the sample may restrict the generalizability of the reported results. First, our participants were 50 and older, and it is unknown whether social support is associated with salivary sampling compliance in a younger sample. Furthermore, this was an observational study, thus participants' motivation may have been lower than in any

intervention or treatment study. Participants received additional compensation for the home component, so it is conceivable that the salivary collection protocol was, at least in part, motivated by additional monetary gain. Although the CHASRS sample is a representative population-based sample gained by a thorough multistage selection procedure (for details, see 13,30), generalizability of our results is constrained by the fact that excluded individuals were more likely to be male and African American, at least on 1 of the 3 sampling days. Another important limitation is that MEMS Track Caps cannot provide a verification of wake-up times. For this information, researchers still rely on self-report to a certain extent. Recently, Kupper et al. (40) suggested verifying awakening times via heart rate and body movement recordings because self-reported (subjective) times in ambulatory assessments might not reliably reflect actual (objective) time points. Also, electronic sleep monitoring via Nightcap that captures sleep stages (e.g., REM sleep) and body movements could be useful in identifying actual wake times in ambulatory settings. A strength of the present data, however, is that 3 sampling days were available for analysis. Furthermore, we were able to check and, where required, control for various potential effects of covariates.

In sum, our results replicate and extend our previous work. First, the data confirm the finding that nonadherence can potentially invalidate resulting cortisol data and, second, support the idea that the extent of nonadherence with saliva sampling protocols might be systematically associated with psychosocial factors. Therefore, for future studies on the relationship between CAR and psychological factors, we recommend controlling for saliva sampling adherence (controlling rather than excluding helps to preserve both the size and representativeness of the sample) because noncompliance might be systematically associated with the phenomenon being investigated.

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