Studies show age-related changes in the pattern of cortisol release over the day (1,2). Cortisol is the final peripheral output of the hypothalamic–pituitary–adrenal axis, a major stress response system, and together with the sympathetic nervous system is responsible for changes in metabolic and immune function in response to internal or environmental stressors. Cortisol follows a circadian rhythm regulated by the biological clock in the suprachiasmatic nucleus of the hypothalamus. Normally, cortisol levels peak in the morning just after habitual awakening time and then gradually decrease throughout the day to a nadir at night. Cortisol diurnal rhythms allow healthy adaptation to the environment and proper daytime functioning. With age, the ability to synchronize internal circadian rhythms with the external environment decreases. This may be due to a physiological decline in suprachiasmatic nucleus function that potentially affects the regulation of behavioral and biological circadian processes (3–6), decreased daytime engagement in vocational and social activities, and/or increased medical burden related to aging. Although aging has been associated with higher basal cortisol levels (1,2,7), a raised cortisol awakening response (CAR [7]), and flatter diurnal slopes (7), it has been associated with decreased reactivity of cortisol levels to an experimental stress task (1). Increases in cortisol levels following a stressor are adaptive and help mobilize the individual to adequately cope with the stressor, whereas chronically low levels of cortisol (ie, hypocortisolism) have been associated with an inability to mobilize energetically to face a stressor and are common in exhaustion states and chronic fatigue syndrome (8). On the other hand, chronically elevated levels are maladaptive and deleterious to health and have been associated with poor sleep.
and a variety of psychiatric conditions (eg, depression [9]) and medical conditions (eg, obesity, diabetes, cardiovascular disease [10,11]).

To our knowledge, there are no studies of the relationship between cortisol levels and health outcomes among older adults undergoing inpatient rehabilitation following an injury or illness. Patients in post-acute rehabilitation settings have generally been exposed to a long-term stressor that may alter basal cortisol levels (ie, the event that led to their need for rehabilitation, such as recovery from hip fracture). In addition, rehabilitation therapies may represent an acute stressor (eg, physical therapy, occupational therapy) that may lead to a temporary adaptive increase in cortisol levels. We examined the relationship between diurnal patterns of cortisol levels and functional outcomes in a subsample (n = 32) of participants in the larger study.

The primary objective of the present study was to investigate whether there was a relationship between daytime cortisol levels and change in functional status between admission and discharge from post-acute rehabilitation. We also explored feasibility and methodological considerations associated with measuring salivary cortisol levels in this patient group.

**METHODS**

**Participants and Setting**

The larger study of sleep and functional recovery among older adults undergoing inpatient post-acute rehabilitation has been previously described (12). Briefly, in the larger study, 245 adults aged ≥65 years were enrolled from inpatient post-acute rehabilitation sites [one community and one Veterans Administration (VA) site] into a prospective, observational cohort study where measures of sleep and functional recovery were collected. Participants enrolled in this larger study at the VA site between December 2003 and May 2004 were invited to provide saliva samples while taking part in the larger study. All patients over age 65 admitted to the study site were approached for screening as soon as possible after admission. Potential participants were excluded if they were admitted for a reason other than rehabilitation (eg, hospice, intravenous antibiotics); resided in a nursing home prior to admission; transferred, died, discharged, or were not identified within 1 week of admission to rehabilitation; or were judged unable to participate due to a severe medical illness or psychiatric disorder.

For the current study, 32 participants (mean age 78 years, 84% male) at the VA site provided up to six saliva samples on 1 day. Written informed consent was obtained from all participants. For individuals who were unable to provide self-consent, written informed consent was obtained from their responsible party, with the assent of the participant. All study procedures were approved by the VA Greater Los Angeles Healthcare System’s Institutional Review Board.

**Procedure**

After enrollment, participants completed a 1-week baseline assessment plus a battery of self-report clinical and descriptive questionnaires (described later). A structured medical record review was completed to abstract information regarding admission diagnoses, characteristics of the rehabilitation stay, medications, and comorbidity information. On the eighth day, six saliva samples were collected from each participant. All data were collected by trained research personnel, and inter-rater agreement was monitored throughout the study.

**Demographic and Clinical Measures**

Basic demographic information was recorded, including age, gender, and ethnicity (see Table 1). Reason for admission to post-acute rehabilitation was recorded from medical records. Hours of rehabilitation therapy (ie, physical therapy,
occupational therapy, kinesiotherapy) received were abstracted from therapists’ detailed documentation in the medical record of minutes of therapy received. All medications received and transfers to acute care wards during the rehabilitation stay were recorded.

The Cumulative Illness Rating Scale for Geriatrics (CIRS-G [13]) was used to assess baseline illness severity and comorbidity. The CIRS-G was completed by a highly trained research registered nurse after a structured medical record review and a brief physical examination by a study physician.

Admission and discharge physical function were measured by the motor component of the Functional Independence Measure (mFIM [14]). The mFIM is a commonly used measure of functional abilities in rehabilitation settings and indicates the level of independence of an individual in performing 13 activities of daily living (eg, dressing, toileting, ambulating). Higher mFIM total scores indicate greater need for assistance with such activities (score range = 13–91). In this study, admission and discharge mFIM scores were abstracted from medical record documentation completed by therapists independent of study personnel. The mFIM change (discharge minus admission mFIM score) was then calculated for each participant as the measure of functional improvement during rehabilitation.

Salivary Cortisol Measurements

On the eighth day, after other assessments were complete, we attempted to collect six saliva samples to measure cortisol levels from each of the 32 participants. Research staff observed collection of each sample and assisted the patient as needed. Patients were asked to insert a small cotton swab similar to a dental pledget into the mouth, chew the pledget for at least 1 minute, remove it when saturated with saliva (about 1 minute of chewing), and place it in a small plastic tube, a Salivette (Sardsedt Inc., Newton, NC).

Samples were collected as close as possible to morning wake time (on average 6:56 AM; SD = 11 minutes; range = 6:37 AM to 7:33 AM), 45 minutes after the morning wake time sample, 11:30 AM, 2 PM, 4:30 PM, and bedtime (on average, 8:22 PM; SD = 46 minutes; range = 7:07 PM to 8:50 PM). To increase the likelihood that we would collect the first sample as close to wake time as possible, a researcher began checking on patients at 6 AM, returning every 20–30 minutes, and collecting a sample on the first occasion the patient was observed awake. The 11:30 AM, 2 PM, and 4:30 PM sample times were selected based on the ward routines (ie, meals at 7 AM, 11:45 AM, and 5 PM; physical therapy typically provided 8–11:30 AM; and patient/team meetings at 3 PM) and procedures used in other diurnal cortisol pattern studies (15,16). This enabled participants to be seated or reclined for a period of at least 15 minutes before each sample was collected. After each sample collection, the Salivettes (Sardsedt Inc.) were centrifuged at 3,000 rpm. Aliquots of 100 ml were transferred to cryovials and frozen at −70°C until assayed.

Data Analyses

Of the 192 scheduled saliva collection occasions, 11 (6% of scheduled samples) could not be collected because the participant was not available, was asleep, or was not on the rehabilitation unit at the designated time. More commonly, an attempt was made to collect the sample, but the samples could not be analyzed due to insufficient saliva (17) in the Salivette [Sardsedt Inc. (33 samples; 17%)].

One participant’s cortisol levels were atypically high at all time points (ie, wake time = 34.0 nmol/L, wake time + 45 min = 277.3 nmol/L, 11:30 AM = 109.3 nmol/L, 2 PM = 926.9 nmol/L, 4:30 PM = 30.0 nmol/L, bedtime = missing). Active substance use immediately prior to admission and receiving antibiotics during the study were documented in the patient’s medical record and may explain the high cortisol levels. As a result, these data were excluded from cortisol analyses. Therefore, 142 cortisol samples, or 74% of the 192 possible samples, were included in the final data set. For the 11:30 AM, 2 PM, 4:30 PM, and bedtime sampling times, analyses were done with and without imputing missing values. Missing values for these four time points (but not for the two morning samples) were imputed based on a linear regression model that included sampling time and the available cortisol values for that individual. For example, we used imputation if a sample was missing at 4:30 PM only if samples were available at the other time points. Imputation could not be performed for the first two samples (waking and waking + 45 minutes) because the individuals who were missing data at those time points did not have sufficient additional available data to run the regression model. We report our analyses both with and without imputation. Cortisol levels (nanomoles per liter) were assayed using time-resolved immunoassay with fluorometric detection (DELFIA). Since log transformation did not significantly reduce the skewness of the cortisol distribution, raw cortisol values were used in all analyses. We computed the mean cortisol level across all available measures in each participant and the change in CAR (wake time to wake time + 45 minutes).

Area under the curve with respect to ground (AUCg) was calculated for the second through sixth time points (ie, from wake time + 45 through bedtime) and third through sixth time points (ie, from 11:30 AM through bedtime) based on the trapezoidal formula that included the cortisol values and sampling times for each of those time points. Calculating AUCg is a well-established method to estimate total cortisol excretion over a particular time period and is often used in cortisol studies among ambulatory individuals (18,19). We used descriptive statistics, correlations, multiple regression models, t test, and area under the curve analysis to analyze
the data. Given the exploratory nature of our study, we considered findings statistically significant if $p < .05$.

**RESULTS**

**Feasibility of Salivary Cortisol Measurement in Inpatient Settings with Older Patients**

A total of 148 samples were successfully collected using this six-sample protocol with 32 participants (total of 192 samples attempted). Across the day, the morning waking sample was the hardest to collect [20/32 (63%) of scheduled samples collected], most often due to insufficient saliva. The next most difficult samples to collect were the bedtime samples [24/32 (75%) of scheduled samples collected], most often because the participant was already asleep when the attempt was made to collect the sample. As previously mentioned, data from one participant were excluded due to acute infection and recent substance abuse resulting in out-of-range values for cortisol levels at all time points (range = 34.02–926.86 nmol/L).

**Correlations and Multiple Regression Models**

Bivariate correlation analyses revealed that mFIM change was negatively associated with comorbidity, (CIRS score), $r(31) = −.49$, $p = .01$, such that participants with fewer comorbid conditions showed greater improvement in function during rehabilitation. The mFIM change was not associated with gender, race, age, pain, cognitive functioning, symptoms of depression, sleep measures, or minutes of rehabilitation therapy received. The mFIM change was, however, associated with several cortisol indices, including cortisol level at 2 PM, 4:30 PM, and BT without imputation, and at 11:30 AM, 2 PM, and 4:30 PM with imputation. Correlations between cortisol levels and mFIM measures and CIRS-G are shown in Table 2.

**Between-Groups Comparisons**

There was a significant difference in mFIM change between participants with a rising CAR (mFIM = 29.8, SD = 7.1) compared with those with a negative CAR (mFIM = 17.8, SD = 9.7, $t = −2.6$, $p = .02$).

**Area Under the Curve**

The mFIM change was associated with AUCg for samples two through six and three through six with imputation, and samples three through six without imputation (Table 2). All associations between cortisol and mFIM were positive, suggesting that higher cortisol, specifically afternoon cortisol, was associated with greater functional improvement during the inpatient stay.

In a follow-up analysis, we entered the two predictors that were most strongly linked with functional improvement in a multiple regression analyses: CIRS-G total score
(comorbidity) and AUCg for cortisol samples three to six, with imputation. Cortisol AUCg was significantly positively associated with mFIM change, $\beta = .47$, $t(21) = 2.75$, $p = .012$, whereas comorbidity was marginally negatively associated with mFIM change, $\beta = -.35$, $t(21) = -2.07$, $p = .051$. In other words, less comorbidity and higher cortisol levels predicted greater functional recovery. This model including both CIRS-G and AUCg explained 44% of the variance in mFIM change, regression model ($R$) = .66, $F(2,21) = 8.30$, $p = .002$. There were no significant relationships between cortisol measures and gender, race, age, minutes in rehabilitation therapy received, sleep measures, cognitive functioning (Mini-Mental State Examination), depression (Geriatric Depression Scale), or pain (geriatric pain measure).

**DISCUSSION**

We were successfully able to measure cortisol levels among older adults undergoing inpatient post-acute rehabilitation. Prior research has gathered samples from older adults living in the community (20) and from older adults who were long-stay nursing home residents (21). This study confirms that similar protocols can be used in an inpatient hospital setting. It was most difficult to gather the morning wake-up sample and the bedtime samples; the morning samples due to insufficient saliva and the bedtime samples because patients frequently were sleeping much earlier than they had planned.

Given the relatively small sample in this study, we chose to use multiple methods for analysis. Importantly, each approach confirmed our initial correlational finding that higher cortisol levels in the afternoon hours were associated with greater functional improvements. Since the majority of physical and occupational therapy activities take place in the mid-morning hours (typically between 9 AM and 11 AM), this relationship may reflect a more adaptive stress response among those with higher cortisol levels at the afternoon sampling times. Also, when comorbidities were accounted for in statistical models, cortisol area under the curve remained a significant independent predictor of functional improvement.

In addition, the presence of a morning cortisol rise predicted more functional improvement in rehabilitation. This compliments other studies that found an association between increased frailty burden and blunted diurnal variation of cortisol in older frail women (22). This also suggests that, in this setting, those with a stronger circadian awakening response (ie, cortisol typically rises in the morning near wake time) benefited most from the rehabilitation therapies they received.

This study has several important limitations that must be considered. First, the sample size was relatively small, and there is a considerable amount of missing data. One of our objectives was to evaluate the feasibility of measuring salivary cortisol levels, and we found that insufficient saliva for assay lead to the majority of missing data. Methods of assay requiring a smaller sample volume are therefore desirable. Second, cortisol samples were collected on only 1 day, which limits our ability to impute data for missing samples. We elected to impute values only for data points in the afternoon hours when adjacent time points were available. Importantly, the pattern of results was consistent across analytic approaches, suggesting that the imputation did not skew or significantly alter the results, but did enable us to enhance the size of the sample available for analyses. Although collecting samples on multiple days would have been desirable, the participant burden associated with the study made this challenging. The use of a predominantly male Veteran sample limits the generalizability of our findings, and the study should be replicated with older women. In the future, studies should also include measures of psychological stress as we cannot differentiate between the effects of psychological versus physiological stress in this study.

Taken together, our results suggest that, in older adults, cortisol levels may be linked with rehabilitation outcomes in a post-acute rehabilitation setting. In addition, measurement of cortisol in saliva may be a useful biological marker for identification of patients who are “at risk” of lower benefits and who may require additional assistance or intervention during their post-acute rehabilitation stay. As with all correlational studies, causality may not be determined from this study. If replicated, however, these findings may spur studies that shed light on the factors that affect cortisol levels in an inpatient setting and may ultimately lead to interventions to improve outcomes for patients in post-acute rehabilitation.

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