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Latent state trait modeling of children's cortisol at two points of the diurnal cycle

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Summary One challenge in examining stable individual differences in basal activity of the HPA axis is controlling for internally or externally based situational factors that lead to day-to-day variation in ambulatory cortisol. Disturbed basal activity is of particular interest in studies with children, for whom a dysregulated HPA axis may play an etiologic role in emotional or health outcomes. The purpose of this study was to determine whether trait vs. situationally specific sources of variation can be identified at different points of the diurnal cycle in children and if so, whether state and trait components vary according to time of measurement. Early morning and late evening salivary cortisol was collected from 164 children aged 7 to 11 years. Samples were collected 30 min after wakeup and 30 min before bedtime on 3 weekdays. State, trait, and error components of cortisol levels were assessed using a latent state trait model. Possible influences of sampling day and outlier treatment on parameter estimates were examined. The results showed that a latent trait factor superimposed on state residuals and measurement error was identified for both early morning and late evening cortisol. Model fit was excellent and criteria for invariance tests were met. Trait factors accounted for 41% and 57% of the variance in morning and evening cortisol, respectively. These findings suggest cortisol attributed to trait factors can be identified and are of substantial magnitude at both the peak and nadir of the diurnal cycle. Latent state trait modeling is a potentially useful tool in understanding the role of stable individual differences in cortisol levels for development and health.

1. Introduction

Activity of the hypothalamic—pituitary—adrenocortical (HPA) system is driven by both endogenous and exogenous influences. Circulating levels of cortisol, the end product of the HPA axis in humans, follows a diurnal rhythm typically characterized by high levels in the early morning, including a burst of activity characterized by a rapid rise and decline known as the awakening response, and a nadir near sleep onset (Pruessner et al., 1997; Tsigos and Chrousos, 2002). Although there are differences among individuals' cortisol levels sampled repeatedly at the same point of the diurnal rhythm, some degree of intraindividual stability across days is observed. In addition to endogenously regulated basal

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activity, systemic stressors, digestion, exercise, and psychosocial stress impact circulating cortisol levels on a momentary basis (Charmandari et al., 2005). Even under baseline conditions in which aspects of a person's environment or activities are tightly controlled, variation in situations and the ways individuals perceive them will impact estimates of cortisol. In addition to these influences, commonly used immunoassay techniques introduce a small amount of variation. This less than perfect reliability is due to differences in antibody lots, the dynamic nature of competitive binding of cortisol to its antibody, and human error. Thus, variance in measured cortisol is likely due to a combination of stable person factors, situational influences or person by situation interactions, and measurement error. Traditional methods of analyzing cortisol data (e.g., using a simple mean average; area under the curve) that average across sampling days cannot distinguish between these sources of variance.

Distinguishing between trait and state components in assessing cortisol is important for understanding the link between cortisol and developmental outcomes. Altered basal cortisol production is associated with a variety of physical and psychological distress and disorders, and life experiences are known to impact circulating cortisol (De Kloet et al., 1998; Heim et al., 2000). A disturbed diurnal rhythm during development is hypothesized to play an etiologic role in some poor outcomes (Lupien et al., 2009). Specific findings linking altered basal cortisol levels to health and behavior are often inconsistent, however, which may be due in part to the confluence of person and situational factors impacting cortisol levels sampled at any given moment. At the same time, emotional experiences vary systematically with within-person day-to-day fluctuations in cortisol (Doane and Adam, 2010; Adam, 2006). Thus, both stable person differences and within-person variation in responses to daily life experiences may be important for emotional and health outcomes. Disentangling the trait, state, and measurement error components has the potential to improve the assessment of relations between HPA axis activities with developmental outcomes.

It has become increasingly common to use multilevel modeling/hierarchical modeling to separate within and between person variation regarding cortisol (e.g. Hruschka et al., 2005). Although these particular models have much promise for studying within-person day-to-day fluctuations in cortisol, they may be less well suited to handle longitudinal multi-method/multi-informant designs. The latent state trait model — for analyzing state and trait components of cortisol — can be easily integrated in the statistical framework of latent variable models (Bollen, 2002) that have wide application in behavioral science. In contrast to hierarchical linear models, latent variable models are particularly well suited to model constructs that are not directly observed while formally modeling measurement error. Many constructs in the behavioral sciences are not directly observed, and generally include systematic sources of measurement error. The latent state trait model we propose in this paper can be easily extended to predict constructs within latent variable modeling applications such as a structural equation modeling, latent growth curve modeling, or growth mixture modeling framework.

Steyer and Majcen (Steyer et al., 1989; Kenny and Zautra, 2001) proposed a stochastic model termed the latent state trait model to estimate the degree to which stable person-based sources and situational factors contribute to the variance of a measured variable. The latent state trait model requires at least two measures on each of two occasions in order for state and trait components to be identified. The model estimates reliability, specificity, and consistency coefficients to distinguish variation accounted for by stable, trait-like factors, by situationally influenced state factors or person × situation interaction, and by measurement error.

Latent state trait modeling has long been used with questionnaire data (e.g., Duncan et al., 1998; Steyer et al., 1989). Few reports to date have examined latent state and trait factors in cortisol data. Kirschbaum et al. (1990) reported that afternoon cortisol levels in healthy adults were primarily accounted for by state factors; trait factors accounted for considerably less of the variation. In a second sample of new mothers, morning salivary cortisol was primarily accounted for by trait factors. Hellhammer et al. (2007) reported primarily state sources of variation for the cortisol awakening response. Only one study has been conducted with children and adolescents in which variation in morning cortisol levels could be primarily accounted for by state sources (Shirtcliff et al., 2005). In that study, variance in morning cortisol that could be attributed to trait factors was associated with externalizing behavioral problems in boys.

These findings point to the potential utility of latent state trait modeling in research on the adrenocortical system. We do not know, however, whether trait cortisol can be identified from samples taken near the nadir of the diurnal rhythm or whether the variation in cortisol that can be attributed to trait factors varies by time of day. In the few studies using a latent state trait model to date, only morning cortisol is reported (Hellhammer et al., 2007; Shirtcliff et al., 2005) or a latent state trait model could not be identified for evening samples (Kirschbaum et al., 1990). There are several reasons why time of cortisol sampling may be an important consideration for identifying state and trait components. As cortisol approaches its nadir, a floor effect can occur in which the distribution of evening values is more skewed than one that is closer to the peak of the rhythm. Another concern is that bioassays are less sensitive to extremely low values, which is also likely to impact the ability to model latent state and trait components. Events that occur during sleep and in the early morning hours, particularly when sampled on weekdays, tend to be more predicable than events that occur later in the day. Mineralocorticoid and glucocorticoid receptors are differentially occupied at the peak and nadir of the diurnal cycle and so exogenous stressors differentially impact receptor occupation depending on when in diurnal cycle they occur. If momentary state or situational factors differentially override trait factors at different points of the diurnal cycle, it may impact estimation of latent state and trait parameters. Thus, the primary goal of this study was to determine whether a latent state trait model can be identified at different points of the diurnal cycle and if so, whether state and trait components vary according to time of day. To address this, a latent state trait model was tested in cortisol samples collected from children at home in the early morning and late evening over three days. Because of the growing interest in the developmental psychobiology of stress (Lupien et al., 2009), we utilized data from children enrolled in a study

examining long-term effects of early life adversity on activity of the adrenocortical system.

This study had two secondary objectives to address other factors that may influence parameter estimates in a latent state trait model of cortisol. In addition to differences by time of day, the novelty of sampling saliva on the first day might impact estimates of trait cortisol. Thus in this study we tested invariance across sampling days in a measurement model. Another issue is that outliers are commonly observed at the positive end of the distribution that cannot be attributed to known biological or psychological events. As with very low values, reduced sensitivity of bioassays to very high values is a concern. Thus, we examined whether outlier treatment — deleting, winsorizing, or retaining — impacts parameter estimates.

2. Method

2.1. Participants

Participants were 164 families with children aged 7—11 (45% male, for a detailed description see Kertes et al., 2008). Briefly, participants were recruited from a registry of 3000+ families who had adopted a child internationally between birth and 8 years of age (median age 13 months). Based on potential influence of medications on the HPA axis, children on asthma, hormonal or psychotropic medications were not included in the study.

2.2. Procedure

Cortisol was assessed in the early morning hours 30 min after wakeup and in the evenings before bedtime (hereafter referred to as AM and PM cortisol) over three days. The 30-min post-waking and pre-bedtime samples were collected to most closely approximate the peak and trough of the diurnal cycle of cortisol (Fries et al., 2009; Pruessner et al., 1997). School days were selected on which children were at home or in the neighborhood by 5 pm to reduce variability in wake-up and bedtime schedules. Based on previous data indicating that structured evening activities impact children's bedtime salivary cortisol (Kertes and Gunnar, 2004), sampling was restricted to days on which children did not participate in sports. Caffeine consumption was precluded 2 h prior to sampling. Parents recorded the time of saliva sampling and completed daily diaries documenting their children's daytime activities and health.

Salivary cortisol was measured in small samples of saliva using methods developed by Schwartz et al. (1998). After rinsing the mouth, children chewed a piece of Trident original flavor gum for 1 min and spit through a straw into a plastic vial. Samples were stored in the family's refrigerator. Upon return of the samples to the laboratory, they were stored at −20 °C until assayed. Samples were assayed in duplicate for cortisol concentration using a time-resolved fluorescence immunoassay (DELFIA). All samples from a participant were assayed in the same batch. Eight percent of samples assayed were blind controls from pooled saliva. Intra- and inter-assay coefficients of variation based on these blind controls were 5.4% and 8.1%, respectively.

2.3. Data analysis

Daily dairies and cortisol values were inspected for quality control. Individual samples were dropped only when diaries indicated severe illness or departure from the normal routine. Following standard conventions, cortisol values deemed biologically implausible (>4.0 μg/dl) and thus likely due to artifact were excluded (1 saliva sample each from 3 participants, <1% of data). Latent state trait modeling for cortisol measures was conducted using maximum likelihood estima-

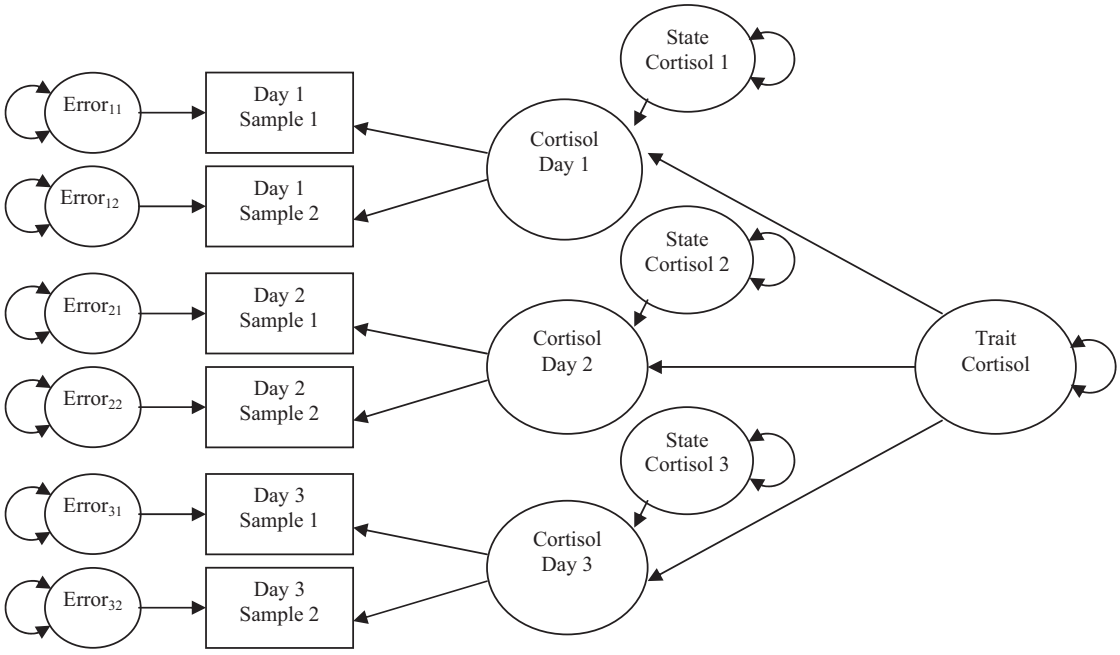

Figure 1 The latent state trait model.

tion in AMOS 5.0.1. The rate of missing data was 2% and met criteria for being missing at random.

Latent state trait parameters were computed to quantify the variance components of the observed variables. As shown in Fig. 1, the latent state trait model includes latent factors that reflect cortisol sampled each day, where both of the technical replicates are used as observed variables. The second-order latent factor reflects the variance in cortisol due to day-to-day fluctuation (state cortisol) among samples collected at the same time of day and variance common across the sampling days (trait cortisol) at the same time of day. We imposed constraints on parameter estimates for three levels of factorial invariance, described by Bontempo and Hofer (2007) as weak, strong, and strict factorial invariance. As applied to a latent state trait model, weak invariance was tested by equating factor loadings from the latent cortisol constructs to each of the observed variables. In other words, the weak invariance assumption tests whether each cortisol assessment contributes similarly to understanding individual differences in cortisol. For strong invariance, factor loadings were equated from the second-order trait factor to the three latent cortisol factors. In other words, within the latent state trait model the strong invariance assumption tests whether the cortisol assessments across different days contribute similarly to the cortisol trait component. Because we were interested in examining the potential of a novelty effect on the first sampling day, this step was repeated 3 times, each time equating a different pair of loadings. For strict invariance, variances of the state factors were equated. In other words, within a latent trait state framework the strict invariance assumption tests whether the state component of cortisol is similar across the various days of measurement. For both the base and invariance models, model fit was determined using chi square, the comparative fit index (CFI) and the root mean square error of approximation (RMSEA). Based on results of simulation studies (e.g. Hu and Benter, 1999) the criterion of acceptable fit was $\geq$.95 for the CFI, $\leq$.06 for the RMSEA, and a non-significant χ^2 value.

As described by Steyer et al. (1999) three coefficients were computed to index the proportion of variance attributed to trait, state, and error components. The *coefficient of consistency* indicates the portion of variance of the observed variables that is due to the stable individual differences across situations and occasions of measurement. In other words, it reflects the proportion of variance due to the latent trait. The *coefficient of specificity* indicates the proportion of variance of the observed variables due to effects of the situation or person by situation interactions. In other words, it reflects the proportion of variance due to the latent state. The *coefficient of reliability* indexes the proportion of variance due to all error-free latent components. In other words, it reflects the proportion of variance due to consistency (trait) and specificity (state).

3. Results

3.1. Descriptives

On all three sampling days, AM and PM cortisol means were within typical morning and evening ranges, with substantial variability (AM M = 0.54—0.66 μg/dl, SD = 0.26—0.33, PM M = 0.10—0.13 μg/dl, SD = 0.11—0.26). Positive skew was observed (1.1—2.0 for AM cortisol, 3.2—6.3 for PM cortisol), thus a conventional $\log_{10}$ transformation was applied to the data (Tabachnick and Fidell, 2007).

Correlational analyses were performed for each pair of saliva sample duplicates (i.e., technical replicates). The duplicate saliva samples gave highly similar steroid concentrations (AM and PM rs = .98—.99), suggesting that measurement error would likely contribute little to the total variance. Correlations across sampling days was considerably lower (AM rs = .38—.52; PM rs = .37—.61) indicating estimation of state and trait components was warranted.

3.2. The latent state trait model

Two latent state trait models were constructed, using the AM and PM data, respectively. For each model, latent cortisol factors were created for each of the three sampling days

Table 1 Fit statistics for the latent state trait model.

Model	X^2	df	p	CFI	RMSEA	ΔX^2	Δdf	Δp
AM base	9.71	9	0.37	1.00	0.02			
AM weak FI	14.50	11	0.20	1.00	0.04	4.79	2	0.09
AM strong FI model 1	14.96	12	0.24	1.00	0.03	0.45	1	0.50
AM strong FI model 2	14.52	12	0.26	1.00	0.03	0.02	1	0.88
AM strong FI model 3	15.17	12	0.23	1.00	0.04	0.66	1	0.83
AM strict FI model 1	16.43	14	0.28	1.00	0.03	0.35	2	0.83
AM strict FI model 2	15.16	14	0.36	1.00	0.02	0.46	2	0.79
AM strict FI model 3	15.73	14	0.33	1.00	0.02	0.40	2	0.81
PM base	7.18	9	0.61	1.00	0.00			
PM weak FI	7.28	11	0.77	1.00	0.00	0.10	2	0.95
PM strong FI model 1	7.30	12	0.83	1.00	0.00	0.02	1	0.88
PM strong FI model 2	8.96	12	0.70	1.00	0.00	1.67	1	0.19
PM strong FI model 3	9.09	12	0.69	1.00	0.00	1.80	1	0.17
PM strict FI model 1	7.94	14	0.89	1.00	0.00	1.55	2	0.46
PM strict FI model 2	11.28	14	0.66	1.00	0.00	1.51	2	0.47
PM strict FI model 3	12.88	14	0.53	1.00	0.00	1.41	2	0.49

Note: FI, factorial invariance.

(see Fig. 1). Assay duplicates were used as the indicators of the latent cortisol factors. Three state factors were modeled, one for each sampling day, with one trait factor estimated as a second-order factor. The base latent state trait model was an excellent fit to the data for both AM (RMSEA = .02; CFI = 1.00) and PM cortisol (RMSEA $<$ .01; CFI = 1.00; see Table 1).

3.3. Factorial invariance

Invariance tests were conducted on the base model described above. The weak, strong, and strict factorial invariance models were treated as nested in model comparison tests. Chi square values for model comparison tests are shown in Table 1. Both the AM and PM latent state trait models met criteria for all three invariance tests. Invariance tests demonstrated a number of constraints could be reasonably imposed on cortisol data in a latent state trait model. This allows for substantial flexibility to maximize degrees of freedom. Thus, further analyses were conducted on the model assuming strict invariance.

3.4. Parameter estimates of state and trait factors

Reliability, specificity, and consistency coefficients were computed according to standard formulae (Steyer et al., 1999). The coefficients indexed the proportion of error free, situational (or person $\times$ situation interaction), and stable trait variance, respectively. Coefficients for the salivary cortisol measures on each sampling day are presented in Table 2. Cortisol measures appeared to be highly reliable, with somewhat higher error for samples collected on day 2. While the majority of the variance was accounted for by state factors, a substantial portion of variance in cortisol was also due to trait factors. Notably, the relative proportion of variance due to state, trait and error components were not substantially different for AM and PM samples, with trait factors accounting for slightly more than half the variance in PM cortisol.

3.5. Analysis of outliers

Differences in parameter estimates were examined following two commonly used methods of dealing with statistical outliers in cortisol research: deletion and winsorization. Statistical outliers were defined as values greater than 3SD above the mean (1.55 μg/dl for AM, 0.90 μg/dl for PM) but did not include data points excluded during quality control checks. Consistency, specificity, and reliability coefficients following both methods are shown in Table 2. Compared to retaining the outliers, winsorizing or deleting outliers had little effect on parameter estimates.

4. Discussion

The goal of this project was to determine whether a latent state trait model can be applied to cortisol data at different points of the diurnal cycle and, if so, to identify the proportion of variance in cortisol attributed to trait vs. situationally specific sources at different times of the day. We were successful in modeling a latent trait factor superimposed on state residuals and measurement error for both early morning and late evening cortisol. Criteria for invariance tests were met. Weak factorial invariance showed that for both AM and PM cortisol, average cortisol was comparable across sampling days. Strong factorial invariance suggested that factor loadings from the two assays within sampling days were comparable across sampling days. Strict factorial invariance indicated that state variances were comparable across sampling days. For all models tested, fit indices were excellent.

We further examined whether a novelty effect exists for cortisol sampling on the first day, and tested the impact of outlier treatment on estimates of trait and state cortisol. With regards to the potential novelty effect, invariance tests conducted on the measurement model indicated samples taken on the first day did not differ from those taken on other days in terms of its contribution to the trait factor. Results of the outlier analysis indicated that the method selected to deal with statistical outliers had very little effect on the proportion of variance attributed to state, trait, and error components.

There are several implications of these results for cortisol data analysis. First, even when fairly strict sampling restrictions are imposed, as they were in this study, measures of ambulatory cortisol levels are substantially influenced by state factors, that is, situational factors or person by

Table 2 Reliability, Stability, and Consistency Coefficients.

	Day 1 AM	Day 2 AM	Day 3 AM	Day 1 PM	Day 2 PM	Day 3 PM
Original data						
Consistency	0.43	0.38	0.43	0.56	0.57	0.57
Specificity	0.52	0.46	0.52	0.39	0.40	0.40
Reliability	0.95	0.84	0.95	0.95	0.97	0.97
Outliers winsorized						
Consistency	0.42	0.39	0.42	0.54	0.54	0.54
Specificity	0.53	0.50	0.53	0.41	0.42	0.42
Reliability	0.95	0.89	0.95	0.95	0.96	0.96
Outliers deleted						
Consistency	0.42	0.37	0.42	0.53	0.54	0.54
Specificity	0.53	0.47	0.53	0.42	0.42	0.42
Reliability	0.95	0.84	0.95	0.95	0.96	0.96

situation interactions that vary from day to day. Second, despite substantial state fluctuations, trait factors can be identified. Moreover, the magnitude of trait variance is not dramatically different for cortisol sampled in the AM and PM. Third, the estimates of variation due to error were small. This suggests that with good laboratory techniques, the convention of averaging across assay duplicates introduces a reasonably small amount of noise in cortisol data. A structural model that estimates the error component, such as the latent state trait model, may be especially useful to partial out this source of variance when coefficients of variation are less than optimal or to facilitate detection of cortisol—behavior relations when associations are expected to be small.

Comparing our study to the only other published study of children's cortisol using a latent state trait model (using waking cortisol only; Shirtcliff et al., 2005), we note differences in results with respect to factorial invariance. Imposing invariance constraints worsened model fit in the Shirtcliff et al. (2005) study. Notably, the two state factors in that paper were measured one year apart whereas in our study they were typically one day apart. Thus, it is possible that imposing invariance constraints is a more reasonable assumption when cortisol is collected closer in time. In fact, in the Shirtcliff et al. (2005) study variance across days within years was equal, suggesting that variances of state factors (adding the strict variance assumption) is reasonable as long as cortisol assays are taken within days.

There has been some speculation that since the experiences of individuals are more similar early in the morning (e.g. sleep and getting ready in the morning) compared to late in the day, that state variance might account for a greater proportion of the variance in the evening (Kirschbaum et al., 1990). Constraints we imposed on sampling day selection may have facilitated the identification of a trait factor for evening cortisol in our study. In fact, by controlling sample day selection, the trait factor for late evening cortisol was even higher than samples collected in the early morning.

In addition to the novel findings regarding evening cortisol, our findings regarding early morning cortisol along with prior reports paint a remarkably consistent picture of the magnitude of the variation in early morning cortisol that can be attributed to trait factors. Our 30-min post-waking sample is embedded within, and typically at the peak of, the cortisol awakening response (Pruessner et al., 1997). The awakening response is a period of the diurnal cycle when cortisol values are typically high and rapidly changing (i.e., a steep rise in the first 30—40 min followed by decline), a point which is relevant to understanding stability across studies of the trait and state contributions. State factors influencing the awakening response include time of waking, sleep duration and quality, and perceived stress the prior day (Fries et al., 2009; Vgontzas et al., 2004; Williams et al., 2005). Our trait estimates accounted for 43% of the variance in cortisol on two days (slightly lower on a third day when error variance was higher). This is highly consistent with trait variance of the cortisol awakening response in adults using the latent state trait model (46% on average; Hellhammer et al., 2007); and of variance in early morning cortisol attributed to basal (trait-like) factors in an independent sample of adopted children using HLM methods (47%, Fries et al., 2008). Thus there is converging evidence regarding the proportion of variation in early morning cortisol due to stable, trait factors within this highly dynamic period of the diurnal cycle. This consistency provides confidence in the accuracy of our estimates of state and trait components of evening cortisol.

This study was conducted on a sample of adopted children. In our experience, adoptive families are highly interested in contributing to research on adopted children's development and thus tend to be highly compliant with study protocols. In addition to the sampling constraints we imposed, this may have facilitated our ability to detect trait components at both points of the diurnal cycle. Parameter estimates should be interpreted in light of the high-risk nature of the sample, although the applicability of this method should not be impacted by the origin of the sample. The observation that the magnitude of state and trait components in AM cortisol did not substantially differ from prior reports, coupled with the high comparability of evening cortisol levels with birth children of the same age (Kertes and Gunnar, 2004), lends confidence that the results are generalizable to other populations. Moreover, detecting state and trait components in high-risk or clinical populations is important because it is among these populations that questions about disturbances in the stable, trait-like component of (or situational fluctuations in) an individual's HPA axis activity may be most relevant.

With these considerations in mind, the results clearly demonstrate the utility of the latent state trait model and provide two novel findings. First, a latent trait component can be identified in children's cortisol both near the peak and nadir of the diurnal cycle. Second, variance in cortisol due to trait and state sources of variation do not dramatically differ across different times of day. Parameter estimates are not affected by either sampling day or the method selected to handle outliers.

Articulating between situationally specific and stable person-based sources of variation may enable us to more accurately detect associations between variables of interest with cortisol attributed to stable/trait and situationally influenced fluctuations distinctly. The application in this paper illustrates how cortisol can be modeled within a statistical framework long known for parsing different sources of measurement error, namely latent variable modeling. A logical next step for psychoneuroendocrinological studies of development is to apply this model within a more comprehensive latent variable modeling framework, for example by including latent predictors and outcomes, and by incorporating the latent state trait model within latent growth curve and growth mixture/latent class growth modeling techniques to study behavioral and biological change across time.

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Conflicts of interest

All authors declare that they have no conflicts of interest.

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