Identifying atypical cortisol patterns in young children: The benefits of group-based trajectory modeling

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Summary The introduction of growth curve modeling into the field of neuroendocrinology has enabled researchers to examine mean patterns of change in unbalanced and/or incomplete repeated measures data. However, growth curve modeling assumes population homogeneity, or that all individuals follow roughly the same pattern of change, with differences expressed as deviation around the mean curve. Group-based trajectory modeling, in contrast, is designed for heterogeneous populations and as a result is able to identify atypical patterns of change over time that may exist within a population. To illustrate the strengths and weaknesses of each technique, we apply both to a sample of diurnal cortisol data measured at home in young children (\(N = 106, 46\) male, \(M\) age = 3.81 years, S.D. = 0.24). We find three distinct trajectories of cortisol and demonstrate that the members of these trajectories are measurably different in terms of cortisol levels across context and time and in terms of the relationship between behavioral problems and parenting. At the same time, our growth curve analysis finds differential response patterns for high vs. low internalizing children with high vs. low parenting quality. We discuss these results in terms of their implications for the proper application of each method.

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1. Identifying atypical cortisol patterns in young children: the benefits of group-based trajectory modeling

Cortisol displays a strong diurnal profile which emerges as early as 6–12 postnatal weeks and is prominent by 3–4 months after birth (Gunnar and Vazquez, 2006). Among adults, a small fraction of individuals fail to show a clear diurnal pattern over the daytime hours (Smyth et al. (1997)). While this may reflect a failure to adhere to sampling protocols, it may also reflect disturbances in the diurnal rhythm (Kudielka et al., 2003). Indeed, a major goal of much of the research on the HPA axis is to identify individual differences in patterns of hormone activity and, having done so, to understand their antecedents and consequences. This includes both the diurnal pattern of cortisol production as
well as cortisol responses to stressors. Theories of early life stress (e.g., Sanchez et al., 2001; Heim and Nemeroff, 2001; Meaney and Szyf, 2005) and allostatic load (McEwen, 1998) argue that differences in HPA functioning will not merely involve varying levels of activity, but will also involve different patterns of change. Thus, researchers are not only interested in whether some individuals exhibit higher or lower levels of cortisol activity but whether changes in cortisol levels over time are typical or atypical, with atypical patterns potentially reflecting dysregulation of the HPA axis.

Based on work with adults and animals, dysregulated change patterns may reflect hyper- or hypo-cortisolism (McEwen, 1998; Heim et al., 2000; Gunnar and Vazquez, 2001). Patterns of hyper-activity include high and relatively unvarying diurnal activity and/or exaggerated and prolonged response to stressors (e.g., Southwick et al., 2005); patterns of hypo-cortisolism include the opposite (e.g., Heim et al., 2000). Because atypical or dysregulated patterns of cortisol activity involve not only differences in levels but also in patterns of change, it is difficult to identify these patterns using statistical methods that assume all individuals in the population will follow the same response pattern over time.

In the following paper, we consider the diurnal cortisol rhythm measured over the daytime hours at home in 3- to 5-year-olds. These data come from a larger study of the impact of child care on children’s stress and emotional development; thus, all the children were in full-day child care (Gunnar et al., submitted for publication). We use two methods to analyze patterns of change and examine how each method links home and child care cortisol measures and how each identifies (or fails to identify) associations with measures of parenting quality and child behavior problems.

1.1. Examination of HPA axis response

A review of the literature reveals that a wide variety of techniques have been used to describe how the HPA axis behaves over time. Area Under the Curve (AUC) continues to be a popular approach as it permits examination of the integrated cortisol response (Pruessner et al., 2003). Because AUC does not permit researchers to describe the nature of change, other techniques also have been applied (e.g., RM-ANOVA or RM-MANOVA). These techniques also have weaknesses, among which are the inability to handle missing data and unbalanced designs (Francis et al., 1991). Pooled within-person regression can create individual estimates of change but treats all data points as independent and thus does not take into account the autocorrelation (i.e., within-person dependence) found in repeated-measures data (Schwartz and Stone, 1998).

A number of more recent neuroendocrine studies have employed a technique known broadly as growth curve modeling (van Eck et al., 1996; Kiecolt-Glaser et al., 1997; Smyth et al., 1998; Adam and Gunnar, 2001; Adam et al., 2006). Growth curve modeling (also known as hierarchical linear modeling, mixed modeling, and random-effects modeling) enables researchers to explicitly model the nature of change over time through the creation of a mean growth curve, and deviation from this curve can then be examined using individual-level predictors (Raudenbush and Bryk, 2002; Fitzmaurice et al., 2004). Most implementations of growth curve modeling make use of maximum likelihood (ML) estimation, which can handle missing data and unbalanced designs. Growth curve modeling also provides a great deal of flexibility in the modeling of covariance structures and thus can accommodate autocorrelation and heterogeneity of variance across time.

The one noteworthy limitation to growth curve modeling is the assumption of population homogeneity, or that all individuals exhibit more or less the same response pattern and differ only in levels of expression around the mean pattern (Nagin, 2005). As a result, it can be difficult to identify atypical patterns of change within a population when using growth curve modeling. Indeed, for populations that contain a substantial number of atypical or dysregulated response trajectories, the use of a single growth curve may obscure the true nature of change in the population. Further, if distinct subgroups do exist that possess qualitatively different patterns of response, then conclusions drawn from research that aggregates across the subgroups may be misleading (von Eye and Bogat, 2006). This is not to say that growth curves are never appropriate—on the contrary, if the assumption of population homogeneity is defensible, then growth curve modeling is entirely appropriate. However, if atypical or dysregulated patterns of change are anticipated, then other techniques may be more suitable.

1.2. Group-based trajectory modeling

The potential for population heterogeneity can be addressed with a technique known as group-based trajectory modeling, a form of finite mixture modeling whose aim is to identify and describe the distinct trajectories or patterns of change that exist within a population (Nagin, 2005). Each unique trajectory is assumed to belong to a latent group, with the members of each group following a given response pattern. Group-based modeling was originally introduced to examine patterns of change over many years (e.g., Lacourse et al., 2003; Mustillo et al., 2003), but these procedures can also be applied in neuroendocrine analysis, in which change is examined over the course of a day or in response to a stressor task. When shorter time frames are examined, we will consider the data to be "time-based" rather than "longitudinal" as is more common in the group-based modeling literature.

Nagin (2005) emphasizes that the latent groups identified by group-based modeling should not be considered literally distinct from one another but rather as a statistical approximation, with some population members clearly belonging to a certain group and others being more difficult to classify. As a result, group-based modeling does not simply assign individuals to a latent group; rather, individuals are assigned probabilistically to each group (i.e., the posterior probabilities of group membership), with these probabilities for each individual adding to 1.0. In a well-defined model, each individual will have a high probability of belonging to a certain latent group and a low probability of belonging to each of the others.

When examining individual covariates of latent group membership (known as "risk factor analysis" in the parlance of group-based modeling), the posterior probabilities enable the derivation of the log odds of the impact of each predictor on the likelihood of membership in each latent group relative
to a designated baseline or comparison group (see Nagin, 2005, Chapter 6). The results from this analysis can be considered a set of binary logistic regression models that predict group membership in the comparison group vs. each other group in the model. A positive coefficient for a predictor implies that higher levels of the predictor increase the probability of group membership in the specified group relative to the comparison group, while a negative coefficient implies the opposite. Thus, when atypical or dysregulated response patterns are identified, “risk factor” analysis can help discern potential contributors to the expression of such patterns. Further, since each latent group is analyzed separately, the problems posed by aggregational analysis (see von Eye and Bogat, 2006) can be avoided. When considering the types of variables often used in neuroendocrine research (e.g., age, height, weight), however, it may not be appropriate to consider them as “risk factors”, so we will simply refer to “predictors” of group membership.

A key step in a predictor analysis is the designation of the baseline or comparison group. The choice of comparison group will always relate to the nature of the research question. For example, if a researcher is interested in examining the correlates of atypical response patterns, the most “typical” pattern is often chosen as the comparison group. If a research question is related to the magnitude of a response, then the group with the lowest or highest response trajectory may be selected as the comparison group. In each case, the research question is defined before the comparison group is selected.

Latent group membership can also be used to examine differences in outcomes, in which it is assumed that a given outcome is a function of group membership. In this analysis, the posterior probabilities are used as weights for each individual’s outcome value, and the resulting values are summed for each latent group to arrive at the group’s average outcome value. As discussed in Nagin (2005, Chapter 6), these outcome values can be compared using a Wald test, which is based upon a chi-squared distribution with degrees of freedom equal to the number of equality constraints being tested. A significant Wald test indicates that the outcome values being compared are significantly different from one another. Thus, the impact of an atypical or dysregulated response pattern on later outcomes can be compared to outcomes from groups possessing more typical response trajectories.

Finally, we note that group-based modeling is conceptually similar to K-means cluster analysis, which was used in a recent study to identify two distinct cortisol response patterns (Lasikiewicz et al., 2008). However, the K-means clustering technique requires the a priori specification of the number of groups to be extracted, and oftentimes competing models are not compared to establish the optimal number of groups; further, unlike group-based modeling, we are not aware of any widely accepted manner with which to assess model adequacy or model fit. Thus, we conclude that the group-based technique is methodologically more rigorous.

1.3. This study

To illustrate some of the differences between group-based trajectory modeling and growth curve modeling, we analyzed a set of cortisol data using the group-based technique along-side a traditional implementation of growth curve modeling known as linear mixed modeling (LMM; Fitzmaurice et al., 2004). These analyses focused on cortisol data obtained in the home across the day in a group of preschoolers, all of whom attended full-day, out-of-home family-based child care. We used LMM to identify a mean growth curve or daytime cortisol rhythm for the overall population and applied group-based trajectory modeling to identify unique daytime cortisol trajectories corresponding to distinct latent groups within the population.

In general, these analyses can be seen as a comparison between the “variable-oriented approach” (i.e., LMM) and the “person-oriented approach” (i.e., group-based modeling) as applied to neuroendocrine data. As noted above, the “variable-oriented approach” has been established in the literature and is likely quite familiar to most readers. However, in taking a “person-oriented approach”, we must be careful to: (1) interpret the latent groups based upon existing theory and (2) use related data to establish the validity of the latent groups (von Eye and Bogat, 2006). Thus, in conducting the group-based analysis, we anticipated that two types of atypical patterns might be found in the home cortisol data, even though we were dealing with a relatively low-risk, typically developing population of children. Given models of allostatic load, we hypothesized that we might find a small percentage of children exhibiting a hyper-cortisol pattern involving high and relatively unvarying cortisol production over the day and another group exhibiting a more hypo-cortisol pattern of very low and relatively unvarying levels. In other words, if we found more than one latent group, we anticipated that there would be three groups in total: normative, hypo-, and hyper-cortisol.

We also examined covariates in each model in an attempt to both validate the group-based model as well as to explore how each technique (i.e., group-based modeling and LMM) incorporates covariates into the model. Our analysis centered around two questions:

1. Can the two methods identify differences in cortisol production in another setting (i.e., child care) and across time (i.e., within a month and 6 months after home sampling)?
2. Can these methods reveal relationships between cortisol, parenting and child behavior patterns? And, if so, are the same associations obtained for both methods?

In posing the first question, we wished to examine the ways in which LMM and group-based modeling could use covariates to identify and/or confirm atypical response patterns using data from other settings. In LMM, covariates are used to predict deviation from the mean growth curve, and theory regarding the impact of these covariates on the “average” response pattern can be tested. In our case, we wished to examine whether children who were higher or lower in child care tended to be above or below the mean growth curve at home. We were also interested in whether child care cortisol data could predict enough deviation from the mean growth curve such that atypical response patterns would be evident.

With regards to the group-based modeling, we wished to use the child care cortisol data to test the validity of the model. As discussed above, group-based modeling can ana-
lyze covariates as either predictors or outcomes of group membership. In general, if such predictor or outcome analysis can be used to demonstrate that the latent groups are genuinely different from one another in theoretically meaningful ways, then the reader can be more confident that the groups are a useful representation of the underlying reality. In our case, if group-based modeling did help us to identify groups of children whose cortisol levels were consistently higher or lower across settings (i.e., home and child care) and across time (i.e., within 1 month and 6 months later), it would support the argument that our results were not context-specific or capitalizing on chance and give the reader more confidence in our solution.

In posing the second question, we noted that a hyper-pattern of daytime cortisol activity has been found in adults with depression (e.g., Southwick et al., 2005). Theoretically, chronic activity of the HPA axis is expected to shape heightened activity in the neural systems underlying anxiety and depression (Sanchez et al., 2001; Heim and Nemeroff, 2001). Among young children, higher scores on measures of internalizing behavior problems may be a developmental risk factor for the development of such disorders. Thus, in the context of the group-based model, we planned to examine whether higher scores on internalizing behavior would predict membership in a hyper-cortisol trajectory, if such a trajectory were found. In the context of the LMM, we planned to examine whether internalizing behavior would predict significant positive deviation from the mean growth curve.

Antisocial behavior and conduct problems, on the other hand, have been associated with low and relatively unresponsive patterns of cortisol production (McBurnett et al., 2000; van Goozen et al., 2000). In young children, higher scores on externalizing problems might thus be expected to predict membership in a hypo-cortisol trajectory, if one were to be found. We might also expect externalizing behavior to predict significant negative deviation from the mean growth curve.

Finally, research on early life stress indicates that parenting quality shapes activity of the HPA axis (Sanchez et al., 2001; Meaney and Szyf, 2005). In rat models, maternal licking and grooming has been shown to be important in the development of HPA axis regulation, and we have argued that the human equivalent would be qualities of maternal care associated with secure attachment relationships (Gunnar and Donzella, 2002). These qualities include sensitivity and responsiveness but not intrusiveness or hostility, as well as behaviors that foster a structured and predictable environment for the child (Bowlby, 1969; Ainsworth et al., 1978).

While both parenting and child behavior problems might independently be associated with patterns of HPA activity, research on parenting has shown that the interaction of child behavioral propensities and parenting behavior is often more instructive (e.g., Kochanska et al., 2007; Propper et al., 2007). If high levels of cortisol can sensitize neural systems underlying fear and anxiety, then we might expect that internalizing problems might be particularly sensitive to variations in parenting among children with a hyper-cortisol trajectory (Kochanska et al., 2007). Conversely, if low levels of cortisol support more externalizing problems behavior, this may be because these children are less responsive to mild parental corrections and thus might take greater advantage of a parent who expresses more sensitive and responsive parenting (Kochanska et al., 2007; Propper et al., 2007). As a result, we might expect that externalizing problems would be particularly insensitive to variations in parenting among children who belong to a hypo-cortisol trajectory. Thus, in the context of group-based modeling, we examined whether the interaction between internalizing problems and parenting could predict membership in a hyper-cortisol trajectory, and likewise whether the interaction between externalizing problems and parenting could predict membership in a hypo-cortisol trajectory. With regards to the LMM, we might expect the interaction between internalizing/externalizing problems and parenting to predict significant deviation upwards or downwards from the mean growth curve.

2. Methods

2.1. Participants

The participants were a subset of 186 children who took part in a study of cortisol levels at child care. Parents were asked to provide home cortisol samples and 75.8% complied. Of these, the 106 (46 males) children who were full-term and not on any form of steroid medication were selected for analysis. The selected children ranged in age from 3.48 to 4.79 years (M age = 3.81 years, S.D. = 0.24) and were an average of 1.6 months younger than the children who were not included in this analysis [M age = 3.95 years, S.D. = 0.42, t(184) = 2.86, p < 0.01]. Otherwise, there were no differences between the children in this analysis and those who were not included. Eighty-nine percent were Caucasian American, 7% were African American, 3% were Hispanic American, and 1% was Asian American. Forty-four percent of the children’s parent(s) had a bachelor’s degree or higher, while less than 8% had not continued education beyond high school. Most (85%) had family incomes of $51,000 or more, while fewer than 4% had incomes under $25,000 per year.

2.2. Procedures

All the children in this study were first observed in their family child care settings (data not presented) and their child care providers were trained to take saliva for cortisol determination (97% complied). Cortisol data collected at this time were designed Time 1. Within the month, the children and their mothers were seen in the laboratory. Part of the laboratory assessment involved a 30-min video-taped parent—child interaction assessment. This period was segmented into 8 min of free-play, 2-min of clean-up, 10 min of structured activity (making a snow-cone) that required the parent to read directions and direct the child in completing the task, and 10 min during which the parent was asked to help the child complete a developmentally appropriate puzzle. During the laboratory assessment, the parents were trained to collect saliva samples from the child and were sent home with a home saliva collection kit. The parents also completed demographic and child behavior questionnaires.

Six months later, a second child care observation and salivary cortisol assessment took place for children who were still in the same child care setting. Of the 106 children in this report, N = 88 (83%) took part in this assessment. Of those children lost to follow-up, only 4 were lost due to the parent...
declining further participation. All but 1 of the 88 children had 6-month follow-up child care cortisol data. Cortisol data collected at this time are designated Time 2.

2.3. Measures

2.3.1. Salivary cortisol
Saliva was obtained for cortisol determination by having the children dip a 1.5-in. cotton dental roll into approximately 0.025 g of cherry flavored Kool-Aid™ mix and mouth the cotton to obtain the sweet taste. This small amount of Kool-Aid™ has not been found to significantly affect the cortisol assay (Taige et al., 2005). Once the cotton roll was saturated, it was placed in a needleless syringe and the saliva was expressed into a 1.5-ml Eppendorf Safe-Lock microtube and sealed. At child care, samples were collected by the child care provider on 2 days between 10:00 a.m. and 11:00 a.m. and 3:00 p.m. and 4:00 p.m. Providers were asked to avoid sampling immediately before a meal, to not give the child caffeinated drinks or dairy products within an hour of sampling, and to wait until 30 min after the child got up from a nap to sample. At home, parents were trained to follow the same sampling and feeding guidelines. They collected samples on 2 non-child care days: 30 min after wake-up, mid-morning (10:00 a.m. and 11:00 a.m.), mid-afternoon (3:00 p.m. and 4:00 p.m.) and within 30 min of bedtime. For the home saliva collection, the cotton dental rolls were in a container with MEMS IV Track Caps (Aardex, Zug, Switzerland) which automatically recorded the time when the container was opened. Use of such devices allows verification of compliance with sampling protocols and also increases compliance (Kudielka and Kirschbaum, 2003). Parents also kept a diary of sampling times and the child’s sleeping and eating times on the days of sampling. Timing of sampling was based on the Track Cap data, unless it was obvious that those times were wrong (i.e., the cap had been left off all day). Once collected at child care or home, the vials were stored in the home refrigerator and then mailed to the laboratory, procedures shown not to affect cortisol data (Clements and Parker, 1998). Once in the laboratory, samples were frozen at −20°C until assay. Assays were conducted in duplicate using a time-resolved fluorescence immunoassay (DELFIA). Intra- and inter-assay coefficients of variation were at or less than 6.7 and 9.0%, respectively, and duplicates correlated highly, \( r = 0.997, p < 0.001 \). Samples were averaged over days within time periods and contexts and values were log_{10} transformed prior to analysis.

2.3.2. Parenting quality
This was evaluated from the 30-min videotaped parent–child interaction using the Emotional Availability scales (Biringen et al., 1998). Four parent scales were scored: sensitivity, structuring, non-intrusiveness, and non-hostility. Parental sensitivity (9-point scale) is a global measure of the parent’s affect and ability to share pleasure in activities with the child, along with appropriate responsiveness to the child’s communications, awareness of timing during interactions and transitions, flexibility, acceptance, clarity of perceptions, and appropriate handling of conflict situations. Parental structuring (5-point scale) measures the degree to which the parent appropriately organizes the child’s play by providing a supportive framework for interaction without diminishing the child’s autonomy. Parental non-intrusiveness (5-point scale) assesses the degree to which the parent is available and supportive to the child without being over-protective, overstimulating, overdirective, or interfering. Finally, parental non-hostility (5-point scale) measures both overt and covert hostility directed towards the child. Specifically, this scale assesses the degree to which a parent is able to engage with his or her child in a way that is positive in nature and not antagonistic, abrasive, impatient or rejecting. Each of the three 10 min segments were coded separately after which an overall score, which took into account the entire 30 min session, was assigned for each scale and subsequently used for analysis. Mothers made up 92% (\( N = 97 \)) of the parent–child dyads. There were no significant differences between mother’s and father’s ratings; therefore, they were not analyzed separately. The 4-scales were highly intercorrelated (\( r’s \) from 0.33 to 0.76, \( p’s < 0.001 \)). Principal axis factor analysis was used to create a single-score reflecting the quality of parenting behavior, with higher scores indicating greater quality (Cronbach’s \( \alpha = 0.79 \)).

2.3.3. Internalizing and externalizing behavior problems
These were measured using the Child Behavior Checklist for 1.5- to 5-year-olds; at the second assessment both parent and child care workers rated the children over the last 2 months (Achenbach and Rescorla, 2000). These instruments have 99 items; 82 of the items are similar, and 17 are specific to home vs. child care. Both require ratings of 0 (not true), 1 (sometimes or somewhat true), 2 (often or always true). The items were combined into syndrome scales which were further combined into two broad-band scales representing internalizing and externalizing problems. The internalizing scale included the syndrome scales of emotionally reactive, anxious/depressed, somatic complaints, and withdrawn, while the externalizing scale included scores on aggressive behavior and attention problems. To provide multi-informant indices of behavior problems, parent and child care provider measures of internalizing and externalizing were standardized and averaged. Scores were obtained from 89 (84%) of the children’s parents and 87 (82%) of the children’s child care providers, with averaging yielding data for 85% \( (N = 90) \) of the sample. The joint-informant measures of internalizing and externalizing were correlated, \( r = 0.59, p < 0.001 \), suggesting significant co-morbidity as if often found in child samples.

2.4. Analysis plan

2.4.1. Model fitting
First we defined a mean population growth curve using LMM. For children at this age, previous research on the daytime rhythm has found a cubic curve, with an initial decrease in the morning, a leveling off from the late-morning to late-afternoon, and a secondary decrease from the late-afternoon to evening (Watamura et al., 2004). Thus, we initially fit a cubic curve to our data. Time of sampling was entered as time since wake-up.

Although from theory we expected to find three latent groups (hyper-, hypo-, and normative cortisol patterns), we
used the empirical methods of group-based trajectory modeling to identify the most appropriate number of groups and their associated trajectories. Model fit was evaluated using the Bayesian Information Criterion (BIC), which can approximate a Bayes factor measuring the odds of one model being correct when compared to another. In line with Nagin (2005), a Bayes factor of 10 is considered strong evidence for one model over another. Thus, starting with one latent group, we added groups until the Bayes factor for the additional group was less than 10, at which point the last group was removed.

Unlike more complex models such as Growth Mixture Modeling (GMM; see Muthén, 2004), group-based modeling only requires the specification of the order of the polynomial equation for the trajectory associated with each latent group (Nagin, 2005). In this case, we initially specified each equation to be cubic, given that we had four data points. Once the optimal number of groups was determined, non-significant higher-order terms were removed from each trajectory’s polynomial equation until the highest-order term was statistically significant (Nagin, 2005). This step serves to limit the overall number of parameters to be estimated in the model.

2.4.2. Model adequacy
As described in Nagin (2005), we used three criteria to assess model adequacy. First, we calculated the average posterior probability for each trajectory group $j$ (AvePP$_j$). Each individual was assigned to a latent group based upon the largest posterior probability (known as the “maximum posterior probability classification rule”, Nagin, 2005, p. 80) and an average posterior probability was calculated for all the individuals in that group. Nagin (2005) argues that the average for each group (AvePP$_j$) should be greater than 0.7. The second criterion was the Odds of Correct Classification for each group $j$ (OCC$_j$). The numerator of this odds ratio represents the odds of correct classification using the model’s grouping scheme, while the denominator represents the odds of correct classification using random assignment. According to Nagin (2005), a well-fitting model has an OCC$_j$ of at least 5.0 for each group. The final criterion is the difference between the probability of group assignment and the actual proportion of individuals assigned to each group using the “maximum posterior probability classification rule”. While Nagin (2005) provides no rule of thumb beyond a “reasonably close correspondence” (p. 89), he does point to a difference of 50% as being uncomfortably large. Thus, for each group, we expect: (1) an AvePP$_j$ greater than 0.7, (2) an OCC$_j$ of at least 5.0, and (3) a close correspondence between the probability of assignment and the proportion actually assigned to each group.

2.4.3. Cross-context and over-time associations
Next we examined how each technique could address research questions related to cortisol levels obtained in a different context (i.e., child care) and over time (i.e., within 1 month and 6 months later). For LMM, we wished to determine whether cortisol as measured in child care was associated with any deviation from the mean growth curve at home and whether this deviation was significant enough to produce atypical response patterns. Following the method outlined in Fitzmaurice et al. (2004), we inserted interaction terms for each coefficient in the LMM’s polynomial equation (i.e., child care cortisol by intercept, child care cortisol by linear term, etc.). We then ran the model and examined the highest-order interaction term; if not significant, we removed this term, re-ran the model, and examined the next-order interaction term. This process continued until either a significant interaction was found or all interactions between model terms and child care cortisol had been removed. We performed this analysis first for the concurrent child care measures and then for the measures from 6 months later. Following this, we used very high and very low values from the child care data and examined whether these would produce atypical home response patterns when inserted into the LMM.

With regards to the group-based modeling, we used the child care measures as outcome variables and examined whether group membership predicted differences in cortisol levels concurrently and six months later. As discussed above, each individual’s posterior probabilities of group membership were used as weights to calculate the child care cortisol levels. If the outcomes of the latent groups were significantly different, then we could be more confident that the latent groups were, in fact, composed of children whose cortisol was predictably different across time and setting.

2.4.4. Behavior problems and parenting quality
The final set of analyses examined the interactions between cortisol levels, behavioral problems, and parenting quality. For LMM, we used the technique described above to enter behavioral problems and parenting into the model separately and then together with their interaction term (i.e., behavioral problems by parenting) to determine whether each predicted significant deviation from the mean growth curve. For the group-based trajectory model, we examined whether behavior problems and parenting alone and together with their interaction term could predict group membership. In the group-based analysis, the cortisol trajectory that followed the expected pattern and had the largest number of children was used as the baseline or comparison. With both techniques, we conducted two separate analyses: one for internalizing and one for externalizing problems.

For the LMM analyses, we used SAS Proc Mixed (Singer, 1998). For the group-based trajectory models, we used SAS Proc Traj (Jones et al., 2001; Jones and Nagin, 2007). Both Proc Mixed and Proc Traj use maximum likelihood (ML) estimation and thus can handle missing data and unbalanced designs. The time metric used in each analysis is the number of hours elapsed since the child awakened for the day (i.e., Time 0). An alpha level of 0.05 was used for all significance tests.

3. Results
Means and standard deviations for all variables are in Table 1 and the inter-correlations of key measures are in Table 2. As expected, home cortisol levels were highly inter-correlated. Home and child care values were also significantly correlated, particularly those levels obtained at approximately the same time of day in both settings (i.e., 10 a.m. and 4 p.m.). As is typical in developmental research, internalizing and externalizing problems were highly correlated and these variables correlated negatively with parenting quality. The cortisol measures did not show any correlation with the parenting or behavioral problem data.
3.1. Model fitting

As anticipated, the cubic term in the LMM was significant (see Fig. 1 and unconditional model in Table 3). Also as anticipated, the results from the group-based trajectory model indicated a 3-group solution (see Fig. 2). Following the criteria set forth in Nagin (2005), we had a very well-fitting model (see Table 4). Trajectory 2 (approx. 74% of the population) was the expected or typical pattern over the day at this age and the pattern of change was very similar to that found in the LMM analysis. Trajectory 3 (10%) appeared to reflect a hyper-pattern of cortisol production, with an extremely high initial level that did not decline to more normal levels during the course of the day. Indeed, the bedtime levels for Trajectory 3 were above the wake up levels for the normative (i.e., Trajectory 2) children. Finally, Trajectory 1 (16%) appeared to reflect a hypo-pattern of cortisol production, with lower levels all day but especially around wake up and a slower decline during the day. In both the LMM and group-based analysis, log$_{10}$ cortisol values were used for the model fitting, and the model coefficients were re-transformed back to the standard scale for presentation of actual values. Thus, in Figs. 1 and 2, the predicted curves are slightly lower than the actual curves, particularly for the highest trajectory.

(i.e., Trajectory 3); this effect also tended to smooth the estimated curve for this trajectory.

3.2. Cross-context and over-time associations

Using LMM, concurrent child care cortisol demonstrated a significant interaction with the intercept term; the child care cortisol measures from 6 months later demonstrated a significant interaction with the linear term (see Conditional Time 1 and 2, respectively, in Table 3). To assist the reader in visualizing the variation around the mean growth curve that is explained by the child care cortisol measures, we constructed expected value equations using the estimated Time 1 model coefficients and then calculated estimated curves for three individuals: one with very low levels of cortisol in the child care setting at Time 1 (5th percentile), one with more moderate levels (50th percentile), and one with very high levels (95th percentile). We then graphed the estimated curves for these individuals (see Fig. 3). The graph for the Time 2 model was very similar and thus is not presented. Note that this analysis showed that children with higher and lower cortisol levels at child care exhibited higher and lower deflections from the normative growth curve, a result that could have been predicted given the high cross-context correlations in cortisol. However, even identifying the top 5% of the child care distribution did not reveal the pattern of hyper-cortisol production identified by the group-based technique, although the pattern noted for the children with the lowest levels of cortisol at child care did approximate the pattern obtained for Trajectory 1 using the group-based procedures.

Table 1  Means and standard deviations for all variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>M</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home cortisol in µg/dl (wake)</td>
<td>101</td>
<td>0.33</td>
<td>0.21</td>
</tr>
<tr>
<td>Home cortisol in µg/dl (10 a.m.)</td>
<td>106</td>
<td>0.12</td>
<td>0.14</td>
</tr>
<tr>
<td>Home cortisol in µg/dl (4 p.m.)</td>
<td>103</td>
<td>0.12</td>
<td>0.14</td>
</tr>
<tr>
<td>Home cortisol in µg/dl (bedtime)</td>
<td>100</td>
<td>0.06</td>
<td>0.16</td>
</tr>
<tr>
<td>Child care cortisol in µg/dl (T1)</td>
<td>102</td>
<td>0.18</td>
<td>0.16</td>
</tr>
<tr>
<td>Child care cortisol in µg/dl (T2)</td>
<td>87</td>
<td>0.16</td>
<td>0.15</td>
</tr>
<tr>
<td>Parenting quality (1—5)</td>
<td>106</td>
<td>4.22</td>
<td>0.40</td>
</tr>
<tr>
<td>Behavioral problems (internalizing)</td>
<td>86</td>
<td>6.95</td>
<td>4.59</td>
</tr>
<tr>
<td>Behavioral problems (externalizing)</td>
<td>86</td>
<td>11.16</td>
<td>7.40</td>
</tr>
</tbody>
</table>

Note: Standard cortisol data are presented rather than log cortisol, and the parenting and behavioral problem data are presented pre-standardization. For the purpose of this table, parenting quality is a combination of four variables, three of which are measured on a 1—5 scale and the fourth measured on a 1—9 scale. Before these variables were averaged, the 1—9 scale was transformed to a 1—5 scale. In the analyses, the standardized versions of these variables were employed. T1 = Time 1. T2 = Time 2.

Table 2  Bivariate correlations for all variables (N = 106)

<table>
<thead>
<tr>
<th>Variables</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Home cortisol (wake)</td>
<td>0.66***</td>
<td>0.51***</td>
<td>0.37***</td>
<td>0.23*</td>
<td>0.18</td>
<td>-0.05</td>
<td>-0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>2. Home cortisol (10 a.m.)</td>
<td></td>
<td>0.67***</td>
<td>0.59***</td>
<td>0.37***</td>
<td>0.36***</td>
<td>0.01</td>
<td>-0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>3. Home cortisol (4 p.m.)</td>
<td></td>
<td></td>
<td>0.79***</td>
<td>0.22*</td>
<td>0.51***</td>
<td>-0.01</td>
<td>-0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>4. Home cortisol (bedtime)</td>
<td></td>
<td></td>
<td></td>
<td>0.41***</td>
<td>0.71***</td>
<td>-0.03</td>
<td>0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>5. Child care cortisol (T1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.34***</td>
<td>0.05</td>
<td>-0.07</td>
<td>-0.01</td>
</tr>
<tr>
<td>6. Child care cortisol (T2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.06</td>
<td>-0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>7. Parenting quality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.23*</td>
<td>-0.29**</td>
</tr>
<tr>
<td>8. Behavioral problems (int.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.59***</td>
</tr>
<tr>
<td>9. Behavioral problems (ext.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Pairwise deletion was used. T1 = Time 1. T2 = Time 2. *p < 0.05. **p < 0.01. ***p < 0.001.
Using group-based modeling, we found that child care cortisol was highest for Trajectory 3 (see Table 5). A Wald test (Nagin, 2005; Jones and Nagin, 2007) revealed a significant difference between Trajectory 3 and both Trajectory 2 $\chi^2(1) = 5.15, p < 0.05$ and Trajectory 1 $\chi^2(1) = 4.66, p < 0.05$. These results were replicated when we considered the child care cortisol measures from 6 months later $\chi^2(1) = 4.92, p < 0.05$, and $\chi^2(1) = 5.17, p < 0.05$, see Table 5. Child care cortisol for Trajectory 1 did not differ from Trajectory 2 in either model $\chi^2(1) = 0.20, ns$ and $\chi^2(1) = 0.42, ns$, respectively, but at home, cortisol levels at 10 a.m. and 4 p.m. also did not differ much between these two trajectory groups (see Fig. 2).

### 3.3. Behavior problems and parenting quality

In the LMM, neither behavior problems nor parenting quality was significant ($F$'s = 1.08 or less, $p$'s = 0.30 or greater). When the parenting by behavior problem interaction term was added, we obtained significance only when using the measure of internalizing problems ($B = 0.007, F = 4.21, p < 0.05$). To interpret the interaction, we split both parenting quality and internalizing problems at the median and plotted the means (see Fig. 4). Visual inspection suggests that the interaction effect was due to differences over the middle portion of the day (10 a.m. to 4 p.m.), given that cortisol decreased fairly uniformly from wake up to 10 a.m. and from 4 p.m. to bedtime. In the time period from 10 a.m. to 4 p.m., high internalizing children showed a substantial increase in cortisol if the parent scored low in parenting quality while this was not the case if the parent scored high on parenting quality or if the child scored below the median on internalizing problems.

For the group-based analysis, we considered Trajectory 2 to be typical and thus used this group as the baseline. Behavioral problems and parenting alone did not predict trajectory group membership, but the interaction between internalizing problems and parenting predicted membership in Trajectory 1 as compared to Trajectory 2 (see Test 1 in Table 6). For children in Trajectory 2, higher quality parenting was associated with fewer internalizing problems. In contrast, children in Trajectory 1 with more sensitive caregivers exhibited greater levels of internalizing problems (see Fig. 5, upper figure). Note that the comparison between Trajectories 2 and 3 was not significant (see Test 1 in Table 6). We found similar results for the interaction between parenting and externalizing behavior problems (see Test 2 in Table 6 and Fig. 5, lower figure).

### 4. Discussion

Our results highlight the fundamental differences between LMM and group-based modeling. Because LMM is based on the assumption of population homogeneity in patterns of change over time, it identified a single growth curve across the

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**Table 3** LMM fixed effects for home cortisol (unconditional and conditional on child care cortisol)

<table>
<thead>
<tr>
<th>Model Term</th>
<th>Unconditional</th>
<th>Conditional Time 1</th>
<th>Conditional Time 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE B</td>
<td>B</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.405</td>
<td>0.034</td>
<td>-0.156</td>
</tr>
<tr>
<td>Linear</td>
<td>-0.340</td>
<td>0.024</td>
<td>-0.338</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.052</td>
<td>0.004</td>
<td>0.051</td>
</tr>
<tr>
<td>Cubic</td>
<td>-0.002***</td>
<td>&lt;0.001</td>
<td>-0.002</td>
</tr>
<tr>
<td>Intercept $\times$ Cortisol (T1)</td>
<td>n/a</td>
<td>0.292**</td>
<td>0.098</td>
</tr>
<tr>
<td>Intercept $\times$ Cortisol (T2)</td>
<td>n/a</td>
<td>n/a</td>
<td>0.211</td>
</tr>
<tr>
<td>Linear $\times$ Cortisol (T2)</td>
<td>n/a</td>
<td>n/a</td>
<td>0.032**</td>
</tr>
</tbody>
</table>

**Note:** Only the highest-order term in each model is evaluated for significance. All estimates are in log10 metric and presented to three decimal places. T1 = Time 1. T2 = Time 2. n/a = not applicable to this model. *$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

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**Figure 2** Predicted (top graph) and actual (bottom graph) cortisol trajectories for group-based model.
population that appeared to follow the typical diurnal rhythm for children of this age. In contrast, group-based modeling tested empirically for heterogeneity in population change patterns and identified both normative and atypical patterns. The evaluation of the adequacy of the model yielded strong statistical evidence for three groups. Consistent with our expectation from theory, the group-based approach identified a normative pattern of change (Trajectory 2, 74% of the sample) and two atypical patterns: specifically, a hyper-cortisol group (Trajectory 3, 10% of the sample) and a hypo-cortisol group (Trajectory 1, 16% of the sample).

We note that we did not find, nor would we expect to find, response patterns that were drastically different from the expected diurnal decrease. The question, nonetheless, is whether these three groups were truly qualitatively distinct and, more importantly, whether group-based modeling provided a more accurate or effective way of approaching the data. In general, when group-based modeling yields groups that vary markedly in patterns of change (e.g., rising vs. falling patterns), then the answer is clear. When these procedures are applied to analyses of diurnal cortisol activity, on the other hand, drastically different patterns of change are not observed even when the system is functioning atypically (e.g., major depressive disorder or post-traumatic stress disorder). Indeed, both of our atypical groups (Trajectories 1 and 3) exhibited the expected pattern of cortisol decrease over the day, although for Trajectory 3 levels were still highly elevated into the evening hours, and for Trajectory 1, the magnitude of the decrease from wakeup to bedtime was about one-third less than that for Trajectory 2. In cases such as these, where trajectory differences are subtle rather than immediately apparent, it may be necessary to look beyond the empirical methods to decide whether using group-based modeling is justified.

Thus, our next step was to validate the group-based model by examining associations with cortisol activity in another context (i.e., day care) and over time (i.e., within 1 month and 6 months later). Although child care cortisol did predict significant deviation from the mean growth curve when using LMM, the consistency of the latent groups in terms of cortisol levels across time and setting tended to validate the group-based approach. Our results demonstrated that children in Trajectory 3 were measurably different in terms of higher cortisol levels across contexts and across time. Although we were not able to differentiate between Trajectories 1 and 2 in the child care setting, this was not surprising given that the most marked difference between these trajectories was their wake-up levels; cortisol in the child care setting was sampled at 10 a.m. and 4 p.m., and the differences in home cortisol between Trajectories 1 and 2 were small at these times.

We also found that a highly correlated predictor such as cortisol measured in another setting within a month of the

Table 5  Estimated average child care cortisol for each trajectory

<table>
<thead>
<tr>
<th>Trajectory</th>
<th>Time 1</th>
<th></th>
<th>Time 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Est. (log₁₀)</td>
<td>SE</td>
<td>Est. (µg/dl)</td>
<td>SE</td>
</tr>
<tr>
<td>3 (high)</td>
<td>-0.69</td>
<td>0.07</td>
<td>0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 (typical)</td>
<td>-0.87</td>
<td>0.03</td>
<td>0.13</td>
<td>0.12</td>
</tr>
<tr>
<td>1 (low)</td>
<td>-0.90</td>
<td>0.07</td>
<td>0.13</td>
<td>0.11</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significantly different at p < 0.05 from other groups.

Table 6  Factors predicting latent group membership as compared to Trajectory 2

<table>
<thead>
<tr>
<th>Factor</th>
<th>Trajectory 1</th>
<th></th>
<th>Trajectory 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Test 1</td>
<td>B</td>
<td>SE</td>
<td>B</td>
<td>SE</td>
</tr>
<tr>
<td>Internalizing behavior</td>
<td>0.32</td>
<td>0.43</td>
<td>-0.11</td>
<td>0.41</td>
</tr>
<tr>
<td>Parenting quality</td>
<td>0.30</td>
<td>0.50</td>
<td>0.11</td>
<td>0.48</td>
</tr>
<tr>
<td>Internalizing behavior&lt;sup&gt;x&lt;/sup&gt;</td>
<td>1.24&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.63</td>
<td>0.15</td>
<td>0.44</td>
</tr>
</tbody>
</table>

| Test 2             | B  | SE | B  | SE |
| Externalizing behavior | -0.03 | 0.50 | -0.40 | 0.38 |
| Parenting quality  | -0.14 | 0.59 | -0.08 | 0.47 |
| Externalizing behavior<sup>x</sup> | 1.50<sup>*</sup> | 0.62 | -0.09 | 0.46 |

<sup>*</sup> p < .05.
home sampling ($r$ as high as 0.71, see Table 2) did not predict a substantial amount of deviation from the mean growth curve when using LMM—certainly not to the degree that Trajectory 3 differed from Trajectory 2 in the group-based model (see Figs. 2 and 3). The LMM framework dictates that departures from the mean growth curve must be described by predictors in the dataset, and these predictors in turn are generally constrained by the reach of extant theory; thus, assuming extant theory to be incomplete, atypical or dysregulated response patterns can remain hidden from view when using LMM. We also examined the utility of LMM and group-based modeling in relation to parenting quality interactions with child behavior problems. Here the results were more mixed, indicating that both approaches yielded interpretable associations with the interaction of parenting by problem behavior, though the associations and their interpretations were different. Supporting the distinctness of Trajectories 1 and 2 in the group-based approach was evidence that the interaction of parenting quality and behavior problems varied as a function of trajectory. In developmental studies, children’s behavior problems typically are higher when parenting quality is lower. This was observed for the children with typical cortisol patterns (Trajectory 2) and this association was even stronger, though not statistically so, for children with high cortisol levels (Trajectory 3). However, for the children with the low and relatively flat pattern of cortisol production over the day (Trajectory 1), behavior problems were positively correlated with parenting quality. For Trajectory 1 children, more sensitive, supportive parenting was associated with higher, rather than lower, scores on both internalizing and externalizing behavior problems. This finding may be consistent with the results of Bates et al. (1998), who noted that oppositional children needed less sensitive, more firm parenting to prevent the development of behavior problems.

While these findings of differential associations between parenting quality and child behavior problems are interesting, the small sample size argues for caution in their interpretation.

Given these findings from the group-based model, it is instructive to consider the LMM results. We found a relationship between parenting-by-internalizing behaviors and the model’s linear slope, and additional inspection of the data suggested that the interaction reflected different changes in cortisol from 10 a.m. to 4 p.m., with a slight increase for children who were high internalizing/low parenting quality that was not observed for the other children (see Fig. 4). These results are consistent with the argument that anxious, fearful children may be highly sensitive to context, functioning well in supportive contexts and experiencing high stress and poor functioning in unsupportive context (Boyce and Ellis, 2005). However, as above, our small sample argues for caution in interpretation.

### 4.1. Limitations and conclusions

The goal of this manuscript was to provide an introduction to the use of group-based trajectory modeling in the analysis of neuroendocrine data and to contrast it with growth-curve modeling. Clearly the two procedures yield different results and ideally should be used to address different research questions relating to different populations (i.e., LMM for more homogeneous populations and group-based modeling for more heterogeneous populations). In our study, both techniques predicted cortisol activity across contexts and time and both yielded interpretable, albeit different, associations with parenting and child behavior. The nature of our sample (i.e., typically developing, largely middle class, and physically healthy) likely explains why both techniques could be seen as viable alternatives for the analysis of these data. Most of the children (74%) followed the normative trajectory and thus were well-described by a single growth curve. The children on the largest atypical trajectory (Trajectory 1, 16%) differed primarily in the wake-up levels, and thus had a pattern that did largely follow that noted by the LMM analysis. Had we used a higher-risk sample or contrasted a clinical and normative group, the expectation of population heterogeneity would have been stronger and likely the strengths of group-based modeling as compared to LMM would have been more apparent. However, we chose our sample specifically to allow the reader to ponder some of the issues that they might well face when choosing between these two approaches.

In addition to issues related to the nature of our sample, the size of the sample also imposed limitations that should be acknowledged. Group-based modeling is traditionally done with larger samples, and one issue with a small sample is the difficulty in detecting latent groups that represent a very small portion of the population (e.g., 5% or less). An additional issue, which can apply when using any analytical technique, is that a small sample increases the likelihood that the results capitalized on chance variation introduced by a small number of outliers. However, when considering our results, there are several points in our favor. First, as noted above, we identified latent groups that were qualitatively different from one another in important ways: Trajectory 3 was significantly higher in cortisol across multiple settings.

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**Figure 5** Internalizing (top graph) and externalizing (bottom graph) problems by parenting quality for each trajectory group.
and Trajectory 1 demonstrated a unique relationship between child behavior and parenting. Secondly, our group-based model demonstrated very good fit when considering commonly accepted criteria (see Table 4). Finally, researchers have used this and related applications of finite mixture modeling with similarly small samples (e.g., Davis et al., 2004; Mareschal and Tan, 2007).

We also had a substantial amount of missing data on the measures collected at Time 2 (see Table 1) because only the children who remained in the same child care from Time 1 to 2 had behavior problems and Time 2 cortisol data. One solution would be to utilize multiple imputation, which is easily done using the expectation maximization (EM) algorithm (Sinhaary et al., 2001; Schafer and Graham, 2002). We did not take this step, however, since our primary goal was to demonstrate the use of the group-based modeling technique using actual rather than imputed data.

In conclusion, both LMM and group-based modeling yielded results that bear further investigation. The contrast between our two sets of findings highlights the importance of selecting the right method given the nature of the research question and the expectations regarding the population to be studied (i.e., homogeneous vs. heterogeneous). In general, by identifying distinct patterns of response in a population, group-based modeling can open the door to fundamental departures from extant theory regarding the factors that contribute to the expression of dysregulation and its associated outcomes. In contrast, LMM and related growth curve techniques can quantify the relationship between individual-level covariates and deviation from a mean growth curve in a population that follows roughly the same pattern of change. Both techniques have their place in the field and both can contribute when used appropriately. Indeed, these techniques can work hand-in-hand; the application of group-based modeling can lead to a deeper understanding of typical vs. atypical response patterns, which can assist researchers in gathering samples from more homogeneous populations for use with LMM.

With regards to the specifics of our results, we see no reason to interpret the two atypical patterns we found as evidence of dysregulation of the HPA axis; further research is necessary before such a conclusion can be drawn. However, the fact that these two groups could be identified with group-based modeling raises the possibility of studying these children over more extended periods, given that they may be at risk for developing the emotional and behavioral problems that are commonly associated with HPA axis dysregulation.

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Conflict of interest

None declared.

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References


