# Salivary Cortisol Profiles of Children with Hearing Loss

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## Abstract

**Objectives**—It has long been speculated that effortful listening places children with hearing loss at risk for fatigue. School-age children with hearing loss experiencing cumulative stress and listening fatigue on a daily basis might undergo dysregulation of hypothalamic-pituitary-adrenal (HPA) axis activity resulting in elevated or flattened cortisol profiles. The purpose of this study was to examine whether school-age children with hearing loss show different diurnal salivary cortisol patterns than children with normal hearing.

**Design**—Participants included 32 children with mild to moderate hearing loss (14 males; 18 females) and 28 children with normal hearing (19 males; 9 females) ranging in age from six to twelve years. Saliva samples were obtained six times per day on two separate school days. Cortisol levels were measured by mass spectrometric detection after liquid-liquid extraction. Salivary cortisol levels between children with hearing loss and children with no hearing loss over the course of the day were examined with Hierarchical Linear Modeling (HLM) using mixed-model statistical analysis. Between-group comparisons were also computed for the area under the curve (AUC), an analytical approach for calculating overall cortisol secretion throughout the day.

**Results**—Significant differences in the cortisol awakening response (CAR) were observed between children with hearing loss and children with normal hearing; however, no differences were observed between the two groups subsequent to the CAR (60- minutes post-awakening, 10:00 am, 2:00 pm and 8:00 pm). Compared to children with normal hearing, children with hearing loss displayed elevated cortisol levels at awakening and a reduced growth in cortisol secretion from awakening to 30-minutes post- awakening. No significant differences in overall cortisol secretion throughout the day were found between groups (area under the curve). Finally, cortisol levels increased with increasing age for children with hearing loss but not for children with normal hearing.

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**Conclusions—**Results of this preliminary study indicate a possible dysregulation in HPA axis activity in children with hearing loss characterized by elevated salivary cortisol levels at awakening and a diminished increase in cortisol from awakening to 30- minutes post-awakening. The pattern of elevated cortisol levels at awakening is consistent with some studies on adults with burnout, a condition characterized by fatigue, loss of energy and poor coping skills. These findings are consistent with the idea that children with hearing loss may experience increased vigilance and need to mobilize energy promptly in preparation for the new day.

## Introduction

In recent years, there has been an increased interest in the study of cognitive fatigue among individuals with hearing loss. Fatigue resulting from sustained listening demands appears to be a significant concern for some working adults with hearing loss. The additional attention, concentration, and effort needed to overcome auditory deficits associated with hearing loss results in increased reports of stress and fatigue compared to adults with normal hearing. Moreover, the fatigue associated with these sustained listening-demands has a significant negative impact on work performance and quality of life (Hetu, Riverin, Lalande, Getty, & St-Cyr, 1988; Kramer, Kapteyn, & Houtgast, 2006; Nachtegaal et al., 2009). Hornsby and Kipp (in press) report that, compared to normative data, adults seeking help for hearing difficulties are more than twice as likely to report severe fatigue and more than four times as likely to report severe energy deficits.

Children with hearing loss (CHL) may also be at increased risk for hearing-related fatigue (Bess & Hornsby, 2014). It is reasonable to expect that CHL could be physically and mentally "worn out" by the end of the school day from listening to the teacher and other children in noisy and reverberant classroom environments. This intuitive assumption has long been supported by pilot studies, anecdotal reports from parents and teachers, and self-reports from persons with hearing loss (Bess, Dodd-Murphy, & Parker, 1998; Bess & Hornsby, 2014; Griswold, 2015; Hicks & Tharpe, 2002; National Deaf Children's Society, 2015; Noon, 2013; Ross, Reference Note 1). Recent empirical studies offer additional support to the longstanding premise that persons with hearing loss may be at increased risk for fatigue (Hornsby, 2013; Hornsby, Werfel, Camarata, & Bess, 2014).

To date, Hornsby and colleagues (2014) is the only study to examine fatigue in CHL using a standardized fatigue scale. This pilot study examined fatigue in a small group of CHL and age-matched CNH (N=10/group) using the PedsQL Multidimensional Fatigue Scale (PedsQL;Varni, Burwinkle, Katz, Meeske, & Dickinson, 2002), a standardized tool developed for use with children between the ages of 5–18 years. The CHL reported more fatigue than the CNH across all fatigue domains. Surprisingly, the CHL reported more fatigue than children suffering from other chronic illnesses such as cancer, rheumatoid arthritis, diabetes, and obesity.

Fatigue is clearly a subjective experience; subsequently, subjective measures of fatigue are important and possess high face validity. The effects of fatigue, however, are multifaceted, comprised of cognitive and behavioral consequences in addition to subjective effects (see Bess and Hornsby, 2014 for a review). Specifically, cognitive processing abilities such as

attention, processing speed, memory, and executive function may be degraded in a fatigued state (Deluca, 2005; Lieberman, 2007). In some cases, these degradations can be observed by monitoring cognitive processing abilities over time. A decrement in cognitive performance over time can be considered a marker of fatigue (see Hockey, 2013 for a review).

Hornsby (2013) used such a method to examine the effects of hearing aid use on speech-processing related fatigue in adults with hearing loss. Participants completed a cognitively demanding, speech-based, dual-task paradigm over a 50-minute period. Fatigue was quantified behaviorally by monitoring for performance decrements on the dual-task over time. Several measures of performance/processing ability were monitored over the duration of the task, including word recognition, word recall, and response times to a secondary visual stimulus.

Word recognition and recall remained stable over time whether listening in an unaided or aided condition, suggesting resiliency or compensation for fatigue effects in these domains. In contrast, visual processing speed systematically decreased over time when listening unaided—an outcome indicative of fatigue. Aided performance, however, remained stable over time, providing preliminary evidence that hearing aid use may mitigate fatigue effects resulting from sustained listening demands.

While subjective and behavioral assessment methods are important, they provide limited insight into the neurobiological bases of fatigue. To improve our understanding of this multidimensional construct, several neurophysiological approaches have been advocated for measuring fatigue, including event-related potentials (ERP; Murata, Uetake, & Takasawa, 2005), pupillometry (Hopstaken, van der Linden, Bakker, & Kompier, 2014); skin conductance (Segerstrom & Nes, 2007), functional magnetic resonance imaging (Lim et al., 2010), and salivary cortisol (Hicks & Tharpe, 2002). The use of these techniques in combination with other subjective and behavioral measures may offer insight into the underlying variables and mechanisms associated with fatigue in CHL (Lieberman, 2007; O'Connor, 2006). Although the assessment of hearing-related fatigue using physiologic techniques is in its infancy, one potentially useful biomarker may be salivary cortisol.

## Cortisol, Stress, and Fatigue

Cortisol is a hormone secreted by the adrenal cortex. It is a part of the body's response to stress and is regulated by the hypothalamic-pituitary-adrenal (HPA) axis; cortisol is considered a valid indicator of this reactivity. The hypothalamus is activated when a stressful event occurs, producing a chain of events that eventually results in the release of cortisol. The HPA axis is a negative feedback system-—that is, the secretion of cortisol from the adrenal cortex is regulated by a complex system of feedback loops at the hypothalamic and pituitary levels to achieve stable cortisol production (Herman & Cullinan, 1997). Cortisol is a glucocorticoid (steroids that reduce inflammation throughout the body) that increases the sugars available in the blood stream, resulting in a surge of energy—energy that is required to respond to a stressful experience. In normal situations, HPA axis activity follows a diurnal or circadian rhythm, rising quickly in early morning and decreasing steadily throughout the remainder of the day (Pruessner et al., 1997).

The early morning rise in cortisol levels upon awakening is referred to as the cortisol awakening response (CAR)–a well-defined phenomenon in healthy humans that results in roughly a two-fold increase in cortisol release in the first 30–45 minutes following awakening. These cortisol levels following awakening may remain elevated for up to 60 minutes. The CAR is a robust and reproducible neuroendocrine phenomenon and is considered a possible indicator of the reactive capacity of the HPA axis (Brosnan, Turner-Cobb, Munro-Naan, & Jessop, 2009; Schmidt-Reinwald et al., 1999). It is thought to be distinct from the basal diurnal pattern of cortisol secretion. In fact, the CAR itself is considered a unique indicator of HPA function and dysfunction—it is especially appropriate for detecting subtle dysregulation in HPA function given that it is unaffected by such variables as age, sleep duration, time of awakening, sleep quality, physical activity or morning routine (Brosnan et al., 2009; Clow, Thorn, Evans, & Hucklebridge, 2004; Fries, Dettenborn, & Kirschbaum, 2009; Wilhelm, Born, Kudielka, Schlotz, & Wust, 2007). Subtle alterations that occur in the CAR have been associated with perceived stress, worrying about the burdens of the upcoming day, and a variety of chronic health problems (Clow et al., 2004; Fries et al., 2009; Schmidt-Reinwald et al., 1999; Ter Wolbeek, van Doornen, Coffeng, Kavelaars, & Heijnen, 2007; Wust, Federenko, Hellhammer, & Kirschbaum, 2000).

Important to our study, alterations in the CAR and/or the basal diurnal pattern, as reflected by increased or decreased cortisol levels at relevant time periods (e.g., during the first hour after waking), may occur when individuals experience unusual stress or fatigue (Deluca, 2005; Fries et al., 2009; Kumari et al., 2009; Schlotz, Hellhammer, Schulz, & Stone, 2004; Whitehead, Perkins-Porras, Strike, Magid, & Steptoe, 2007). Abnormally low cortisol levels (hypocortisolism) have been observed in individuals with chronic fatigue syndrome (Jerjes, Cleare, Wessely, Wood, & Taylor, 2005; Nijhof et al., 2014; Roberts et al., 2010), a disabling stress-related disease with a primary fatigue symptomatology (Crofford & Demitrack, 1996; Parker, Wessely, & Cleare, 2001). Nijhof and coworkers (2014) compared the CAR of a group of adolescents (N=108) diagnosed with chronic fatigue syndrome and to that of a group of healthy peers (N=38). The adolescents with chronic fatigue syndrome exhibited a significantly lower CAR than their healthy peers. Flattened or decreased cortisol profiles have also been reported in persons with exhaustion, a condition characterized by fatigue and lack of vigor (Lindeberg et al., 2008; Nicolson & van Diest, 2000; Sjogren, Leanderson, Kristenson, & Ernerudh, 2006). Individuals with flattened cortisol responses experience difficulty mobilizing sufficient energy to cope with the challenges of daily life activities.

Persons with stress or fatigue might also exhibit elevated levels of salivary cortisol (hyperactive HPA axis functioning). For instance, heightened cortisol levels have been reported in individuals with burnout, a work-related construct characterized by fatigue, lack of energy, and reduced coping capabilities (De Vente, Olff, Amsterdam, Kamphuis, & Emmelkamp, 2003; Grossi et al., 2005; Kudielka, Bellingrath, & Hellhammer, 2006). High levels of cortisol reflect extended activation of the HPA axis due to longstanding stressful experiences as might be expected in CHL struggling to listen and understand in a noisy classroom. Some investigators have posited that heightened cortisol responses for either the CAR and/or the basal diurnal pattern may be an indicator of the early stages of fatigue-related conditions such as work overload and exhaustion (De Vente et al., 2003; Fries, Hesse, Hellhammer, & Hellhammer, 2005; Kudielka, Hellhammer, & Wust, 2009). The HPA

axis is thought to respond to these high levels by reducing cortisol output over time, resulting in a more flattened profile (Fries et al., 2009; Kudielka et al., 2009; Miller, Chen, & Zhou, 2007). That is, hyperactive HPA functioning results in hypoactive functioning when a severe state of fatigue or exhaustion is reached and the individual is no longer able to cope with an ongoing stressor (Kudielka et al., 2009). Hence, the direction and magnitude of the response appears to be a function of the chronicity of the stressor.

In contrast to the above reports of cortisol dysregulation, it is noteworthy that Ter Wolbeek and colleagues (2007) found no differences in CAR's between a group of adolescent girls from the general population who reported fatigue and a control group. Alterations in cortisol production often occur in tandem with health and psychosocial conditions that feature fatigue (e.g., Chronic Fatigue Syndrome, Multiple Sclerosis, Lupus, Depression); however, abnormal cortisol secretion related to fatigue in the general population is reportedly less frequent (Glass et al., 2004).

To date, Hicks & Tharpe (2002) is the only study to explore the use of salivary cortisol in CHL. These investigators collected salivary cortisol samples twice a day in ten CHL and ten CNH. The first sample was collected near the beginning of the school day (approximately 9:00 a.m.) and the second sample was taken at the end of the school day (approximately 2:00 p.m.). No significant differences in cortisol values were observed between the two groups at either time point. However, the limited number of samples obtained during the day did not allow for assessment of the CAR and provided only a gross measure of the basal diurnal cortisol patterns between groups. Moreover, the sample size may have been too small to detect differences between groups.

We suggest that salivary cortisol may be a potentially useful biomarker for the study of stress, fatigue, and expenditure of energy. Presently, however, we have limited information on the utility of salivary cortisol in CHL. The purpose of this study is to examine the effects of mild to moderate hearing loss on diurnal cortisol patterns in school-age children.

## Methods

This research was part of a more extensive study designed to examine the effects of listening effort and fatigue in school-age CHL. In brief, the larger study employs multiple measures and diverse methodologies (e.g., laboratory and field-based measures of subjective and behavioral fatigue, salivary cortisol, and event-related potentials), to assess whether school-age CHL expend greater listening effort and experience more subsequent fatigue than CNH under adverse listening situations. Here we report findings based on salivary cortisol measures in CHL and CNH.

### Participants

Participants included 32 CHL and 28 CNH. All participants were between the ages of 6–12 years, had no diagnosis of learning disability or cognitive impairment as reported by the parents, and spent at least two hours per day in a general education classroom. Children were excluded from this study based on factors known to affect fatigue. This criterion resulted in the exclusion of (a) children who were bilingual or whose primary language in

the home was not listening and spoken language; (b) children with Autism Spectrum Disorder, (c) children with a linear metabolic or endocrine disorders (e.g., diabetes or hypothyroidism), (d) children with a chronic medical condition, and (e) children who utilized medications that might alter HPA axis responses (e.g., stimulant medications). Participants were recruited from Vanderbilt's pediatric audiology clinics, school systems throughout the middle Tennessee area, advertisements in a local parenting magazine, and through the Vanderbilt Kennedy Center's Study Finder website. Children were paid $150 for their participation in the salivary cortisol study. Informed consent and assent were obtained from all participants according to the Vanderbilt University Institutional Review Board.

Upon entry into the study, all children received an audiological assessment using insert earphones (Etymotic ER 3A) and a clinical audiometer (GSI 61) in a sound-treated booth that met ANSI standards for ambient noise levels (American National Standards Institute, 2008). Tympanometric screening (middle ear analyzer-GSI 33) was completed to confirm normal middle ear status for all children (American Speech-Language-Hearing Association, 1997). CNH received a standard hearing screening at 15 dB HL for octave frequencies ranging from 0.25 to 8.0 kHz, bilaterally. CHL received an audiological examination including air and bone conduction threshold testing bilaterally at 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, and 8.0 kHz (Carhart & Jerger, 1959). CHL exhibited mild to moderate, bilateral sensorineural hearing loss in at least the better-hearing ear. We defined mild hearing loss as a pure tone average (PTA; thresholds at 0.5, 1.0 and 2.0 kHz) between 20 and 40 dB HL or thresholds greater than 25 dB HL at two or more frequencies above 2.0 kHz. Moderate hearing loss was defined as a PTA of 45–70 dB HL in the better ear. Children exhibiting a conductive component were included (n= 3; >15 dB at two or more frequencies) in the data set as long as the sensorineural loss fit the above criterion and the hearing loss was not fluctuating. Children with cochlear implants and children with unilateral hearing loss were not included in the data set. Figure 1 shows a composite audiogram for CHL included in this data set.

All children completed a series of standardized tests as part of the larger study protocol. Language ability was measured using the core language index of the Clinical Evaluation of Language Fundamentals – Fourth Edition (CELF-4; Semel, Wiig, & Secord, 2003). The core language score provided a reliable norm-referenced measure of language performance by age. All children received the Test of Nonverbal Intelligence - Fourth Edition (TONI-4; Brown, Sherbenou, & Johnson, 2010). Demographic, nonverbal intelligence, language information, and parent-reported hearing history (CHL) obtained at study entry are shown in Table 1.

### Procedures for Collection of Salivary Cortisol

Saliva samples for determining cortisol concentration were obtained using a sampling protocol similar to that suggested by the MacArthur Research Network of Socioeconomic Status and Health (Stewart, 2000). In order to compare the diurnal variations between the two groups, samples were taken six times per day on two separate days. The second sample day was typically scheduled within one-to-two weeks of the first sample day. Saliva samples for each child were obtained at (1) the time of awakening, T0; (2) 30-minutes post-

awakening, T1; (3) 60-minutes post-awakening, T2; (4) 10:00 am, T3; (5) 2:00 pm, T4; and (6) 8:00 pm, T5. A trained parent or caretaker conducted the home-based sampling (T0, T1, T2, and T5). Parents received thorough instructions on salivary cortisol collection during their first research visit. Several strategies were employed to enhance protocol adherence for saliva sampling and storage. First, members of our research team provided to parents one-on-one instruction regarding accurate collection and labeling of samples. During this instruction, the parent and child completed the saliva sampling protocol to demonstrate to the research staff that instructions were understood. Parents received an illustrated booklet outlining simple step-by-step instructions for eliciting a saliva sample. Finally, one or two days prior to the scheduled date for saliva sampling, a member of the research team communicated verbally or electronically with the parent to answer any questions regarding the sampling/storage protocol.

A research team member visited the child's school to obtain T3 and T4 samples, during which time the child was excused from the classroom for 10–12 minutes. Parents and research team members took special care to ensure that participants did not eat, drink, or brush their teeth prior to providing a saliva sample. Given that cortisol levels can vary with novel versus familiar situations, saliva sampling was not conducted on days in which the child or teacher reported atypical excitement or stress. For example, care was taken to ensure samples were not obtained on school days when there was a field trip, a party, a fire alarm drill, or any other activity that may deviate from the typical classroom routine.

To obtain the saliva samples, cotton dental rolls were placed in the child's mouth for two to three minutes or until the pad was saturated. Once the pad was saturated it was placed in a plastic tube labeled with the child's code, and the date and time of sampling. All collected samples were stored in a refrigerator and individually packaged over dry ice before shipment to the laboratory for analysis. Cortisol levels in saliva samples are reportedly unaffected by environmental conditions associated with the shipping process (Clements & Parker, 1998; Koss, Hostinar, Donzella, & Gunnar, 2014).

### Salivary Cortisol Analysis

Esoterix Inc. (Endocrine Sciences Laboratory) conducted the cortisol analysis using a validated procedure developed in their laboratories. Cortisol was measured by mass spectrometric detection after liquid-liquid extraction (Keevil, 2013). Analysis was performed using high-pressure liquid chromatography separation with tandem mass spectrometric detection. Stable labeled isotopic cortisol was added as an internal standard to saliva aliquots. Samples were then extracted and the solvent was transferred, evaporated, and reconstituted prior to analysis. A triple quadrupole mass spectrometer (MDS-Sciex API-5000) was used for detection. Quantification of analyte and internal standard was performed in selected reaction monitoring mode. The amount of cortisol in each sample was determined using internal standard ratio from duplicate calibration curves.

### Data Management

The cortisol data received from Esoterix Inc., were first converted from µg/dL to the more traditional unit nmol/L. Cortisol values >3 SD from the mean and unusable data (e.g.,

insufficient saliva) were discarded. Coupled with missing samples (n=35), this resulted in a loss of 13.46% of the 720 data points. Both groups exhibited similar amounts of missing data. Study data were managed using REDCap (Research Electronic Data Capture) electronic data capture tools hosted at Vanderbilt University (Harris et al., 2009). REDCap is a secure, web-based application designed to support data capture for research studies.

### Statistical Analysis

All statistical analyses were completed using SAS version 9.4 (SAS Institute Inc, 2013). The distribution of cortisol levels is positively skewed; therefore, values were log transformed (base 10) prior to analyses (Keene, 1995). Cortisol levels were compared between groups (CHL and CNH), using a longitudinal analysis. For the purposes of this study, longitudinal refers to awakening, morning, afternoon, and evening cortisol levels as a repeated measure. The statistical approach was longitudinal—that is, the raw data were used to generate individual growth curves that were compared across groups (Rogosa & Saner, 1995). The general statistical approach was Hierarchical Linear Modeling (HLM) within the context of a mixed model statistical design between groups (CHL and CNL) and within groups (cortisol level across time; Hruschka, Kohrt, & Worthman, 2005). Effects for age, gender, and language level, were also tested within the HLM. In addition, between-group comparisons were completed for the area under the curve (AUC) to compare group differences in total cortisol production throughout the day (AUC computed using Formula 1; Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). The AUC can be used to compare gross cortisol levels between groups across the entire day whereas the HLM detects differences at particular sampling points within the curve (e.g., awakening response) even when overall daylong cortisol levels are not different.

### Longitudinal Data Structure

The longitudinal analysis of cortisol was completed by creating an individual growth curve (Rogosa & Saner, 1995; Singer & Willett, 2003) from each participant's cortisol samples each day. Growth in this context refers to any change, positive or negative. Cortisol, for example, often increases upon awakening and then decreases during the morning, day, and evening. Longitudinal growth curves were derived to allow for the use of slope as an outcome measure within the HLM.

For cortisol longitudinal growth curves, we defined the time zero intercept as the child's first collection at awakening. The cortisol collections occurred at six time points, beginning with time zero. Each time point corresponds to a cortisol sample occurring at approximate times (e.g., T5 corresponds to the cortisol collection at approximately 8:00 p.m.). The measurements of time were continuous, exact, and centered on time zero as the first cortisol sample of the day. Time zero was dependent on a child's individual awakening time each day.

The approach for computing AUC was applied to day one longitudinal growth curves for CHL and CNH. Conceptually, this analytic approach computes area under the curve by calculating the sum of a series of trapezoidal Riemann surfaces fitted to each cortisol longitudinal growth curve (Pruessner et al., 2003). The overall area derived from each group

was then compared statistically. Missing data points were imputed using a multiple imputation process implemented in SAS (PROC MI).

## Results

A preliminary analysis of variance indicated that cortisol samples within groups were not different between days (F = 0.30, $p$ = .59). Therefore, we used data from each time point on both days in a repeated measures fashion within our HLM model to provide more stable estimates of cortisol changes over time. Doubling the data set in this manner is a common procedure in longitudinal cortisol analyses (Miller, Plessow, Kirschbaum, & Stalder, 2013). CNH and CHL showed no difference in time of awakening (F= 0.83, $p$ = .35), despite some individual variation in time of awakening. These findings allowed for each participant's exact data collection time to be used in the longitudinal growth curve analyses. Subsequent differences in sample collection times were then generated by subtracting the exact time at T0 (awakening) from each subsequent sample collection time. Thus, time values in the analysis were noted in hours subsequent to awakening for each individual participant.

Figure 2 shows individual and group average cortisol levels at each time point for CNH (left panel) and CHL (right panel). Based on a visual analysis of diurnal cortisol fluctuations presented in Figure 2, it was clear that a single linear slope would not fit the longitudinal growth curves for both groups. Although there were up to six data points for each curve, these points were well fitted to growth curves for each group using only three slopes. The first slope (Slope 1) spanned from T0 (awakening) to T1 (30-minutes post- awakening). The second slope (Slope 2) spanned from T1 to T3 (approximately 10:00 a.m.), and the third slope (Slope 3) spanned from T3 to T5 (approximately 8:00 p.m.). These slopes were used to describe the daily, individual growth curves for each participant and were used in subsequent statistical analyses.

First, individual growth curves were inspected to ensure the regression scores were continuous and unbroken for each child's data. Next, a piecewise HLM was used to evaluate changes in slopes over time. Piecewise HLM models enabled the construction of simplified models for describing complex, nonlinear, time-dependent processes. This statistical approach is not new and has been used to model multiple curves of almost any desired form, such as changes in mental health outcome over time (Dykens & Lambert, 2013; Lambert, Wahler, Andrade, & Bickman, 2001). In this case, the piecewise HLM model approach allowed for the analysis of change in cortisol levels throughout the day as three slopes.

The first step in the analysis was to examine the general pattern of cortisol responses across groups. This was done to inspect the trajectory of the cortisol for the entire data set as a prelude to testing for between-group differences in the longitudinal analysis. Therefore, piecewise HLM analyses were initially performed on data collapsed across groups to reveal changes in cortisol levels over time. Table 2 shows the results of these analyses. As indicated by the main effects of Slope 1 and Slope 2, there is a significant rise in cortisol levels within the first 30-minutes after awakening followed by a significant decrease in cortisol levels from 30-minutes post-awakening to 10:00 am. This significant increase in cortisol

represented by Slope 1 corresponds to the CAR. No changes in cortisol levels were measured between 10:00 am and 8:00 pm where the slope is roughly flat.

Prior to the longitudinal analysis of differences in cortisol changes between groups, we examined the effect of age, language, and gender to determine whether these factors were significant covariates. Significant covariate terms would suggest that the covariate's main effect or interaction effect may have influenced cortisol levels and thus must be included in the longitudinal growth curve analyses. For example, if age showed a strong effect on cortisol levels, it would have to be considered in all later analyses. By running the piecewise model, including all terms and covariates, we tested the influence of these covariates. Table 3 shows all significance tests involving the covariates. The results suggested that main effects of age, language, and gender were all non-significant. This non-significance suggested that the covariates do not need to be included in the longitudinal model.

The primary purpose of this study was to examine differences in cortisol patterns between CHL and CNH. The results of the HLM analyses are shown in Table 4. A main effect for group revealed that there were significant differences in cortisol slopes between groups. Specifically, CNH show a sharp increase in cortisol within the first 30-minutes after awakening, the change in cortisol within this time period is smaller for CHL. Thus, Slope 1 (region defined as the CAR) shows a distinct difference between groups, with CHL demonstrating a shallower increase in cortisol compared to CNH (i.e., the Slope 1 by Group interaction is significant- see Table 4). This region of significance in the growth curves can be visualized from T0 (awakening)-T1 (30-minutes post) and is highlighted in Figure 3. There were no significant differences between groups for Slope 2 or Slope 3.

We conducted post hoc, between-group, comparisons of cortisol levels at all six time points to examine if overall levels of cortisol contributed to the significant between-group difference for Slope 1. These one-way analyses of variance (Rosenthal & Rosnow, 1985) indicated that there was a significant difference in mean cortisol levels at awakening between CHL and CNH groups ($F = 5.95$, $p < .02$), with CHL having significantly higher cortisol levels upon awakening. There were no differences in cortisol levels observed between groups for the remaining collection times. This result indicates that the group difference in Slope 1 can be attributed to a combination of elevated cortisol levels at awakening and a smaller increase in cortisol at 30-minutes post-awakening in the CHL group (see Figure 3).

The results of the AUC analysis revealed no significant differences between groups as determined by a one-way analysis of variance ($F = 3.58$, $p > .10$). These findings indicate that overall cortisol production across the entire day for CHL (M=1.42, SD = .53) and CNH (M = 1.24, SD = .46) were very similar. Such a finding is not surprising given that the HLM analysis showed significant differences between CHL and CNH only at awakening and for Slope 1. In contrast, the AUC receives much of its information from data subsequent to awakening (T0) where differences were not significant. Taken together with the results of the HLM analysis, our findings indicate a robust difference in the cortisol awakening response—a difference that does not mediate overall cortisol levels across the day.

## Discussion

The purpose of this research was to determine whether school-age CHL and CNH exhibit differences in their diurnal salivary cortisol patterns. To our knowledge, there have been no previous studies of diurnal salivary cortisol profiles in CHL. Our preliminary results showed significant differences between CHL and CNH for the CAR. Specifically, CHL had higher cortisol levels at awakening and a reduced rise in cortisol upon awakening, compared to our CNH. However, beyond the CAR, the cortisol profiles for both groups were similar and declined throughout the remainder of the day in a typical manner. Our findings obtained at 10:00 am and 2:00 pm were consistent with the only other study that has measured salivary cortisol in CHL. Recall that Hicks and Tharpe (2002) collected cortisol samples in CHL and CNH in the morning and near the end of the school day. In keeping with our results, they observed no significant differences in cortisol levels between groups at these two time points.

Cortisol levels that are elevated at awakening, as seen in the CHL included in this study, may indicate increased vigilance and greater need to mobilize energy promptly in preparation for the new day. Other researchers have suggested that findings similar to ours might be indicative of perceived stress, inability to cope, and worrying about the burdens of the upcoming day (De Vente et al., 2003; Wust, Federenko, et al., 2000; Wust, Wolf, et al., 2000). Interestingly, our findings appear to be similar to several studies on adults experiencing high workload, job strain and burnout symptoms (De Vente et al., 2003; Grossi et al., 2005; Kudielka et al., 2006; Melamed et al., 1999; Schlotz et al., 2004; Schultz, Kirschbaum, Pruessner, & Hellhammer, 1998; Steptoe, Cropley, Griffith, & Kirschbaum, 2000). Some of the primary symptoms associated with burnout include mental exhaustion, fatigue, and a loss of energy and concentration problems. Cortisol studies in burnout patients suggest dysregulation of the HPA axis as exhibited by elevated cortisol values in early morning. For instance, De Vente and coworkers (2003) noted that the most pronounced differences in salivary cortisol levels between burnout patients and controls occurred at the time of awakening. The cortisol levels of participants in De Vente et al. (2003) remained elevated during the first 30 minutes; however, at 60-minutes post-awakening, no differences in cortisol levels were observed between the two groups. According to the authors, these findings suggested that burnout patients might not have recovered fully during the night–a sign of sustained activation of the HPA axis. In another study, Grossi and colleagues (2005) reported elevated morning cortisol levels through the first 60-minutes post-awakening in patients with moderate to high burnout.

It thus appears that CHL may need to mobilize more energy than CNH in the early morning hours simply to prepare for the new day. These early energy requirements before school begins may ultimately place CHL at risk for fatigue. Recall that some researchers have hypothesized that elevated cortisol levels are considered a precursor to fatigue-related conditions; and, the HPA system reacts to these high levels of cortisol by triggering down regulation of the HPA axis, which in turn results in a blunted cortisol profile over time (Fries et al., 2009; Kudielka et al., 2009; Miller et al., 2007). Stated otherwise, as stress continues and coping efforts become more difficult, cortisol levels move from hyperactive functioning to hypoactive functioning. Such a concept is consistent with the longstanding belief that the

constructs of stress and fatigue are highly associated and that fatigue may be a direct outcome to the presence of a sustained stress activity (Hockey, 2013; Kocalevent, Hinz, Brahler, & Klapp, 2011; Magbout-Juratili, Janisse, Schwartz, & Arnetz, 2010; Olson, 2007).

We reasoned that one possible outcome for CHL might be flattened cortisol levels similar to those often seen in individuals with chronic fatigue syndrome, a condition in which fatigue is the primary symptom. As a group, we did not observe flattened profiles in our CHL; instead, we found elevated levels at the time of awakening. The possibility that neuroendocrine changes occur over time (as described above) may explain why a flattened profile was not observed in our CHL. That is, our CHL might be in the early stages of fatigue and a more blunted profile may not occur until the fatigue has persisted for an extended period of time or is more severe (De Vente et al., 2003; Fries et al., 2009; Kudielka et al., 2009; Miller et al., 2007).

Assuming that the cortisol response may reflect the evolution from stress to fatigue one might expect to see an association between age and cortisol response over time. That is, younger CHL may exhibit normal or somewhat elevated cortisol levels in the early stages of stress; however, as CHL become older and the stress and/or fatigue becomes more chronic they may show increased elevation in cortisol or blunted cortisol responses. To our knowledge, no longitudinal studies describing the time course from stress to fatigue in children have been reported in the literature.

Given our finding of increased cortisol levels upon awakening in CHL, and the paucity of research in this area, we conducted an exploratory mixed model regression analyses to examine the effect of age on cortisol responses of CHL and CNH. This group by age interaction analysis for overall cortisol levels, was statistically significant $F_{(1, 611)} = 6.09$, p=0.002). Figure 4 graphically depicts the group by age results and reveals that cortisol levels increase with increasing age for the CHL, but not for the CNH. Such a finding offers preliminary support for the premise that length of exposure to stress as determined by age may well predict atypical patterns of cortisol in CHL—in our case, an increased production in total cortisol secretion as reflected in the elevated cortisol levels. It remains unknown whether this increased production in cortisol over time eventually leads to a blunted pattern in CHL. Future studies are needed to explore longitudinal effects and the distinct contributions that the disease-course and age may have on cortisol secretion.

Important strengths of this study were the multiple samples (six) taken on two separate days from each participant in a naturalistic setting. This study, however, has limitations that call for cautious interpretation of the findings. First, our sample size was small–although the number of participants was comparable to other studies of cortisol levels focused on chronic illnesses, especially in young children (Buske-Kirschbaum et al., 1997; Buske-Kirschbaum et al., 2003; Carvalho Fernando et al., 2012; Corbett, Mendoza, Wegelin, Carmean, & Levine, 2008; Koss, Scott, Irons, Smith, & Ullrich, 2013). Nevertheless, a larger sample would have been preferable—hence, there is a need to replicate this study using a much larger sample. A second limitation is related to the issue of compliance. Sampling compliance (e.g., refrain from eating, brushing teeth, drinking; timing of collection; coding and storage of samples) is essential for the precise measurement of salivary cortisol profiles,

and a lack of compliance is a potential source of variance (Dockray, Bhattacharyya, Molloy, & Steptoe, 2008; Rotenberg & McGrath, 2014). Recall, that a parent or caretaker collected the home-based samples and that special care was taken to insure compliance with the saliva collection protocol. These strategies included one-on-one instruction, "hands-on" practice in the sampling and storage process, and the use of an illustrated booklet detailing the steps for a sample collection. Despite these efforts, there is no guarantee that the parent/caretaker adhered to the sampling and storage instructions. One possible explanation for an abnormal CAR is the lack of compliance–namely, error may have been introduced if parents/caretakers were not diligent in collecting the first sample at the precise time of awakening, as the CAR is dependent on the timing of cortisol collection after waking (Dockray et al., 2008). Importantly, however, we found no differences in waking time between CHL and CNH, which suggests that any effect of non-compliance would have affected data in both groups similarly. A third limitation of this study is that we only examined diurnal cortisol patterns– we did not attempt to correlate HPA axis functioning with subjective or objective measures of fatigue. Our priority for this investigation was to simply determine if cortisol profiles in CHL differed from profiles of CNH. An appropriate next step for future research should include examinations of whether elevated CAR levels are associated with measures of fatigue in CHL. A final limitation of this study pertains to the issue of socioeconomic status (SES) and its possible association to cortisol. Some studies have reported an association between lower SES and higher salivary cortisol levels (Clearfield, Carter-Rodriguez, Merali, & Shober, 2014); many other investigators found no such association, with a few studies reporting blunted cortisol levels in lower SES persons (DeSantis et al., 2007; Dowd, Simanek, & Aiello, 2009). Unfortunately, because we did not obtain a direct measure of SES from our participants, we were unable to examine potential effects of SES in our study. Future research should consider the possible influence of SES on the CAR and basal diurnal patterns of cortisol in CHL.

Based on our experience and the experience of others, salivary cortisol appears to have promise as a biomarker of stress, fatigue and expenditure of energy in children (Doom & Gunnar, 2013; Gunnar, 1992; Gunnar, Bruce, & Hickman, 2001; Kirschbaum & Hellhammer, 1999). Salivary cortisol sampling is simple, quick, noninvasive, and can be collected in a naturalistic setting such as the home, classroom or playground (Gunnar, 1992; Kirschbaum & Hellhammer, 1999). Even infants, toddlers and young children are able to provide salivary cortisol samples suitable for laboratory analysis (Gunnar, 1992; Hanrahan, McCarthy, Kleiber, Lutgendorf, & Tsalikian, 2006; Rotenberg & McGrath, 2014; Turner-Cobb, Rixon, & Jessop, 2008). Moreover, numerous studies have demonstrated the utility of salivary cortisol for the measurement of stress in typically developing children (Gunnar, 1992) and in children with various chronic health conditions such as autism spectrum disorders (Corbett et al., 2008; Corbett et al., 2014), chronic fatigue syndrome (Heim et al., 2009; Nijhof et al., 2014), affective disorders (Dahl et al., 1991; Pervanidou et al., 2013; Russ et al., 2012), asthma (Buske-Kirschbaum et al., 1997), child maltreatment (Tarullo & Gunnar, 2006), and social deprivation or early adversity (Gunnar, Morison, Chisholm, & Schuder, 2001; Koss et al., 2014). These studies demonstrate that chronic health conditions are often associated with characteristic stress response profiles. These profiles contribute to our biological and psychological understanding of how adverse life situations such as stress

and fatigue can impact negatively on quality of life and general health (Doom & Gunnar, 2013; Kudielka et al., 2009).

Although salivary cortisol appears to have potential for assessing stress and fatigue in children, limitations to this approach do exist. Some of the challenges to salivary cortisol measurement include 1) the costs and time required for laboratory analysis; 2) the need to control for multiple factors that can influence cortisol responses (e.g., food or drink, atypical classroom excitement or stress, medications that might alter HPA axis); 3) the potential for contaminated data if sampling protocols are not strictly followed; and, 4) the need for multiple daily measurements to improve reliability.

In conclusion, these preliminary findings indicate a possible dysregulation in HPA axis activity in CHL characterized by elevated salivary cortisol levels at awakening followed by a limited increase in cortisol at 30-minutes post-awakening. These results may suggest that CHL exhibit an increased vigilance and need to mobilize energy promptly in preparation for the new day more so than CNH. This early morning need for increased energy may place CHL at increased risk for fatigue. The cortisol results observed in CHL are similar to what has been reported in several previous studies of individuals with burnout–a condition characterized by fatigue, loss of energy and poor coping skills. In addition, the early morning vigilance may result from enhanced arousal and inability to fully recover during the night (e.g., De Vente et al., 2003). Clearly, more research is needed before we can determine whether salivary cortisol is a useful or reliable biomarker for stress, fatigue, and expenditure of energy in CHL. In addition to replicating the current study with a larger and more diversified sample, it is important to investigate whether cortisol responses are associated with perceived (subjective) fatigue measures and to examine cortisol responses as a function of hearing loss severity and socioeconomic status. An intriguing research area worthy of continued exploration is the potential contribution of age and chronicity in the evolution of cortisol responses in CHL. Also, longitudinal research is necessary to gain insight into the directions of the causal chain between HPA axis functioning, stress, and fatigue. Finally, the exact mechanisms by which these stress-induced changes in HPA function occur require further investigation.

## Acknowledgments

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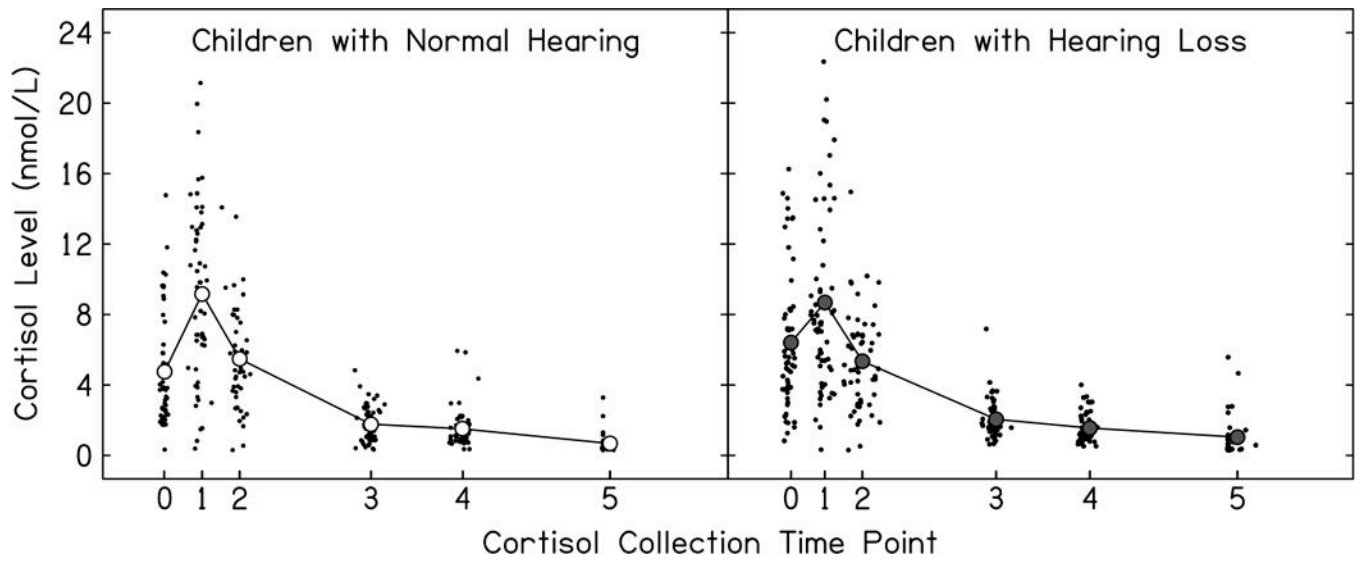## Reference Note

1.

Ross, M. (2012). [Personal communication].
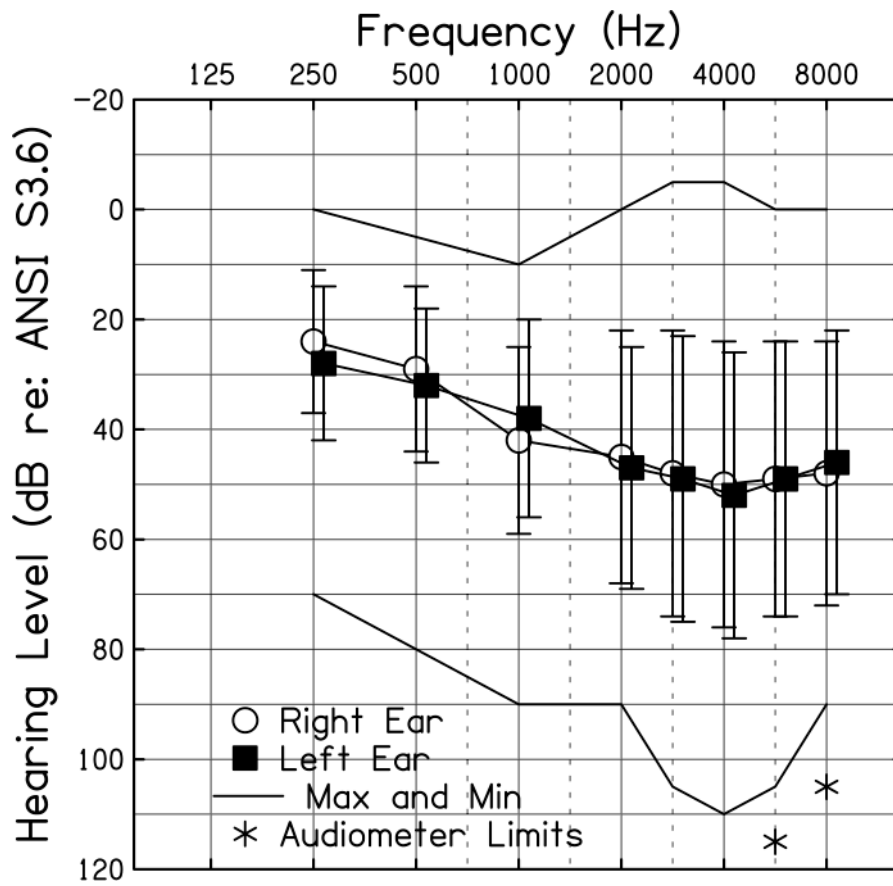
**Figure 1.**
Composite audiogram of study participants with hearing loss
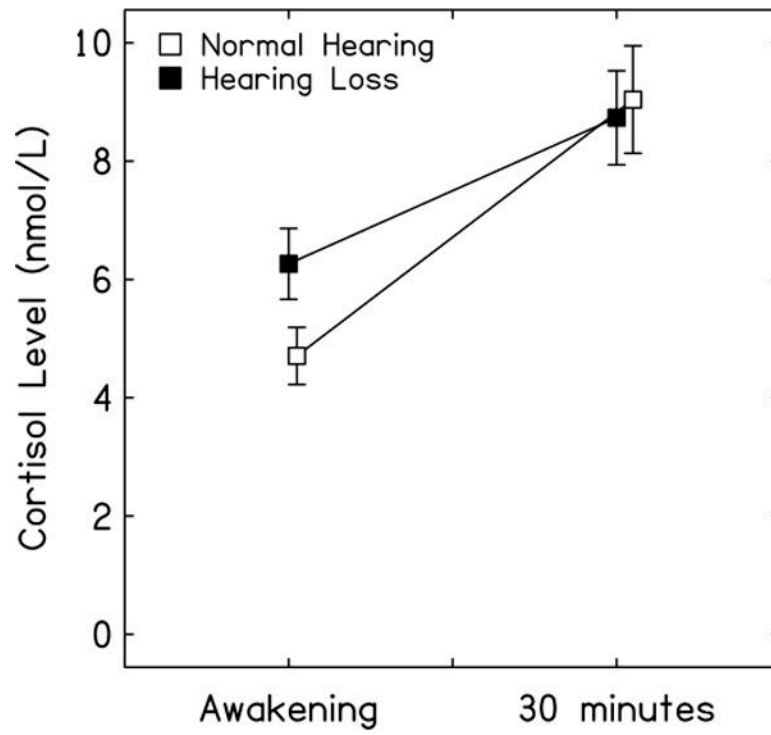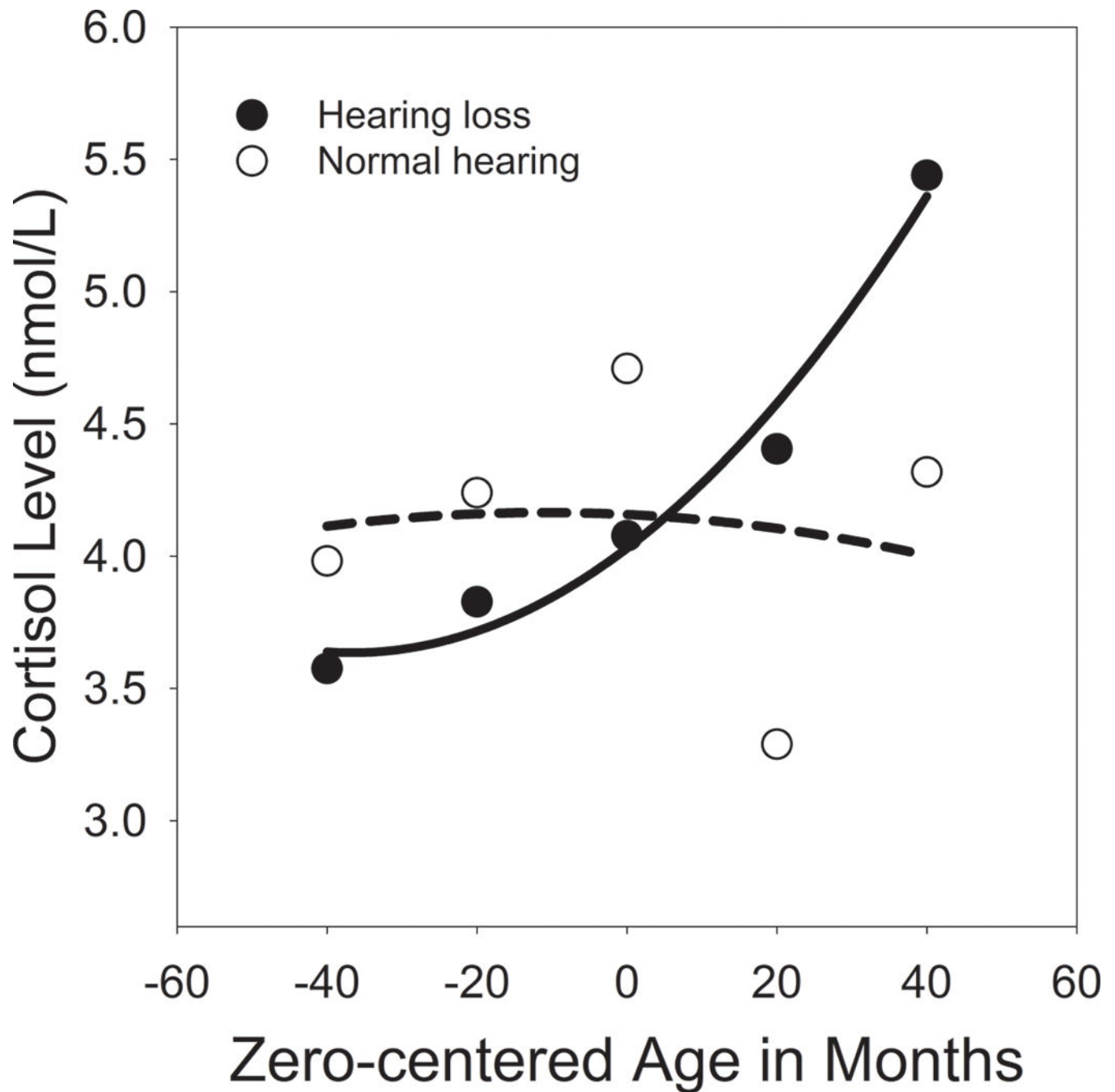
**Figure 2.**
Individual (small black dots) and mean (large open and filled circles) cortisol levels at all six collection times for CNH (left) and CHL (right). Cortisol collection time points 0–5 refer to awakening (T0), awakening post 30 minutes (T1), awakening post 60 minutes (T2), 10 a.m. (T3), 2:00 p.m. (T4) and 8:00 p.m. (T5).

**Figure 3.**
Mean (±1 standard error) cortisol levels for CNH and CHL at awakening (T0) and 30-minutes post-awakening (T1).

**Figure 4.**
Mean cortisol levels averaged across all time points for CHL (solid circles) and CNH (open circles) as a function of normalized age in months (The units for age are z scores with the sample mean age represented by "0". The scores are expressed in SD's around the mean). Curves are simple quadratic fits for reference. The group by age interaction was significant (p=0.002) revealing an increase in overall cortisol with age for CHL but not for CNH.

**Table 1**

Participant characteristics. Mean (±1 standard deviation) age, language, and nonverbal IQ are reported for both groups of children. Parent-reported hearing health history is presented for CHL. Bold text indicates a significant difference (p<.05) between groups.

|  | CNH | CHL | Significance |
|---|---|---|---|
| Participants | 28 | 32 | |
| Males/Females | 19/9 | 14/18 | |
| **Age (years)** | **9.05 (2.30)** | **10.25 (1.20)** | **p =.035** |
| **Language** [*] | **109.43 (10.39)** | **89.38 (23.12)** | **p <.001** |
| Nonverbal IQ [**] | 108.71 (9.06) | 103.28 (14.18) | p = .087 |
| Age at HL Diagnosis (years) | – | 5.45 (3.13) | |
| Age at Initial HA Fitting (years) | – | 6.42 (3.31) | |

HL = hearing loss; HA = hearing aid

[*] Standard score on the core language index of the Comprehensive Evaluation of Language Fundamentals-Fourth Edition (CELF-4)

[**] Standard score on Test of Nonverbal Intelligence-Fourth Edition (TONI-4)

**Table 2**

Results obtained from a piecewise regression analyses on data pooled across groups. Text in bold indicate significant main effects (p< .05) for that parameter.

| Parameter | Estimate (β) | SE | p-value | Interpretation |
|---|---|---|---|---|
| **Intercept** | **4.96** | **0.44** | **<.0001** | **At time zero, average cortisol = 4.96 nmol/L** |
| **Slope 1** | **6.87** | **1.15** | **<.0001** | **Cortisol rises 6.87 nmol/L each hour** |
| **Slope 2** | **−1.78** | **0.16** | **<.0001** | **Cortisol decreases 1.78 nmol/L each hour** |
| Slope 3 | −0.07 | 0.05 | 0.15 | No change in cortisol from time three to time five |

**Table 3**

Covariates considered for inclusion into the mixed model.

| Effect | F | p-value |
|---|---|---|
| Age | 1.57 | 0.21 |
| Language | 0.51 | 0.48 |
| Gender | 0.27 | 0.60 |

**Table 4**

Results of piecewise regression analyses comparing CNH and CHL. Significant parameters (p < .05) indicated with bold text.

| Parameter | Estimate (β) | SE | p-value | Interpretation |
|---|---|---|---|---|
| **Group** | **1.71** | **0.58** | **0.004** | **Differences in cortisol patterns between groups** |
| **Slope 1*Group** | **−4.97** | **1.50** | **.001** | **CHL show less increase in cortisol than CNH** |
| Slope 2*Group | 0.38 | 0.20 | 0.06 | No difference between CHL and CNH |
| Slope 3*Group | −0.03 | 0.07 | 0.69 | No difference between CHL and CNH |