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Sex Differences in the Association Between Cortisol Concentrations and Laboratory Pain Responses in Healthy Children

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Abstract

Background—Research in adult populations has highlighted sex differences in cortisol concentrations and laboratory pain responses, with men exhibiting higher cortisol concentrations and reduced pain responses compared with women. Yet, less is known about the relationship of cortisol concentrations to pain in children.

Objective—This study examined associations between sex, cortisol, and pain responses to laboratory pain tasks in children.

Methods—Salivary cortisol samples from subjects aged 8 to 18 years were obtained at baseline after entering the laboratory (SCb), after the completion of all pain tasks (SC1), and at the end of the session (SC2), 20 minutes later. Blood cortisol samples were also taken after completion of the pain tasks (BC1) and at the end of the session (BC2), 20 minutes later. Subjects completed 3 counterbalanced laboratory pain tasks: pressure, heat, and cold pressor tasks. Pain measures included pain tolerance, and self-reported pain intensity and unpleasantness for all 3 tasks.

Results—The study included 235 healthy children and adolescents (119 boys, 116 girls; mean age, 12.7 years; range, 8–18 years; 109 [46.4%] were in early puberty; 94 [40.0%] white). Salivary and blood cortisol levels were highly correlated with each other. Salivary cortisol levels for the total sample and for boys and girls declined significantly from SCb to SC1 ($P < 0.01$), although there were no significant changes from SC1 to SC2. No significant sex differences in salivary or blood cortisol levels were evident at any assessment point. Separate examination of the cortisol–laboratory pain response relationships by sex (controlling for age and time of day) suggested different sex-specific patterns. Higher cortisol levels were associated with lower pain reactivity (ie, increased pressure tolerance) among boys compared with girls at SC1, SC2, and BC1 (SC1: $r = 0.338$, $P = 0.003$; SC2: $r = 0.271$, $P = 0.020$; and BC1: $r = 0.261$, $P = 0.026$). However, higher cortisol levels were related to higher pain response (ie, increased cold intensity [BC2: $r = 0.229$, $P = 0.048$] and unpleasantness [BC1: $r = 0.237$, $P = 0.041$]) in girls compared with boys.

Conclusions—These findings suggest important sex differences in cortisol–pain relationships in children and adolescents. Cortisol levels were positively associated with increased pain tolerance in boys and increased pain sensitivity in girls.

Keywords

pain; children; cortisol; sex differences

INTRODUCTION

Physiologic consequences of a noxious experience reflect increased arousal in hypothalamic-pituitary-adrenal (HPA) axis–modulated domains, as indicated by cortisol secretion.^{1,2} A review published in 2005 found consistent sex differences in salivary cortisol responses to stress tasks among adults (men > women).³ Men have been reported to have a significantly greater cortisol response to the cold pressor task (CPT), even after adjustment for sex differences in cold pressor tolerance times ($P < 0.05$).⁴ Also, it has been reported that after pain induction, the cortisol response was increased among men compared with women, despite a lack of sex differences in baseline cortisol levels or tolerance time.⁵

Pretask cortisol concentrations have been found to predict lower reported pain during and after the CPT in men but not in women. Yet, a significant positive relationship between cold pain intensity and cortisol concentration, and between pain unpleasantness and cortisol concentration, was found in women only ($P < 0.01$).⁶ Thus, evidence of cortisol–stress sex-specific links has been found in adults.

However, to understand sex differences in cortisol, stress, and pain responses, it is important to discern at what point in the adult life span these sex-differentiated findings begin to emerge. Determining this possible “set point” may have substantial clinical implications. For example, past research has implicated chronic activation of the HPA axis in a number of health-related conditions, such as diabetes, hypertension, hyperlipidemia, hypercholesterolemia, and arterial disease in adults.⁷ Prolonged cortisol responses in childhood may be a factor in the etiology of alcohol and drug abuse.⁸ There is also speculation that adult patients with rheumatoid arthritis may have HPA-axis dysfunction, although a 2004 review did not support this claim.⁹ In a population-based study that included 75 adults with chronic fatigue syndrome (CFS) and 110 healthy controls, there was no evidence of differences in cortisol levels in participants with CFS compared with healthy controls.¹⁰ However, sex differences were evident in that women with CFS in that study had significantly attenuated morning cortisol levels compared with healthy women ($P < 0.05$), whereas men with CFS and healthy men exhibited no such difference.

In children, chronic disease may be associated with an attenuated cortisol response during exposure to stress. Exposure to the Trier Social Stress Test for Children (TSST-C), a task that requires the participant to give an unstructured speech or tell a story and do mental arithmetic in front of an audience, has been associated with a significantly blunted cortisol response after exposure to the stressor in children with allergic asthma (not taking steroids) compared with healthy children ($P < 0.01$).¹¹ A similar pattern was found when cortisol responses were compared between healthy children and children with atopic dermatitis.¹² None of these studies explored sex differences in cortisol responses. However, a significant limitation in studying associations between cortisol and chronic disease is the inability to determine whether HPA-axis dysfunction occurred before or after the onset of the disease. This suggests that healthy children may be an ideal population in which to explore the developmental trajectory of the relationship between sex-specific stress and acute pain. Studying this population also offers the opportunity to gather information about the nature of sex-specific relationships with pain and how they may change during development.

A search of the published literature did not identify any articles examining cortisol and sex-based relationships in children’s responses to pain induced in a controlled laboratory setting.

We were interested in extending the study of HPA–stress relationships—specifically, the determination of the role of sex in these relationships—in a cohort of healthy children. The first objective of this study was to examine cortisol–pain response relationships across 2 methods of assessment (sampling salivary and blood cortisol levels). The second objective was to characterize changes in cortisol levels in response to a series of laboratory pain tasks, as well as potential sex differences in patterns of cortisol reactivity. Finally, we tested for sex differences in the cortisol–pain response relationship. We hypothesized that (1) salivary and blood cortisol levels would be highly correlated; (2) cortisol levels would increase in response to the pain tasks compared with baseline, and cortisol levels would decline during the posttask recovery period (for the total sample, and for boys and girls separately); (3) no sex differences would be evident across salivary or blood cortisol levels; and (4) based on earlier research in adults undergoing laboratory pain tasks,⁵ an increased cortisol response in boys would be associated with decreased pain response, whereas in girls, an increased cortisol response would be associated with greater pain response.

SUBJECTS AND METHODS

Participants

The University of California, Los Angeles Institutional Review Board (IRB) and the IRB recruitment sites approved all recruitment and study procedures. Participants were recruited from the greater Los Angeles, California, area through mass mailings, posted advertisements, and classroom presentations.

Eligible subjects were self-reported healthy children and adolescents, aged 8 to 18 years. In the present study, the term *healthy* referred to children who, by parent and self-report, had no acute or chronic illness such as a heart condition or arthritis, recent surgery, injury to any limb, history of frostbite, history of fainting spells, or developmental delay. Participants were excluded from study participation for the following reasons: (1) acute or chronic illness at the time of study participation; (2) developmental delay or significant anatomic impairment that would preclude understanding of the study procedures (eg, developmental age <8 years) as assessed by parents and principal investigator, or participation in pain-induction procedures (eg, arm immersion in cold water); or (3) daily use of opioid medication. Although data on the use of other medications in children and parents were not formally gathered, use of analgesic medication on the day of study participation was prohibited.

Study Procedures

The data for the present study were drawn from a larger study examining sex and pubertal differences in pain response. Details of the laboratory procedure are provided elsewhere.^{13–15}

In brief, an investigator informed parents and children about the assessment, which was described as a study about how children experience pain. Parents and children signed consent and assent forms, respectively, immediately after arriving at the laboratory, before any assessment or study procedures were performed. Children received a \$30 video store gift certificate and a T-shirt for their participation. On the day of the laboratory session, participants and their parents were greeted by an investigator and escorted to separate rooms. There was no contact between parents and children until the session was finished. After participants completed questionnaires in a quiet room, they were escorted individually to the laboratory. Participants were instructed in the use of the visual analog scale (VAS) for rating pain intensity and unpleasantness.

Participants were then exposed to 3 pain tasks—pressure, heat, and cold pressor—with the order of tasks counterbalanced across participants. For the pressure and heat pain tasks, we used 2 anatomic sites, to avoid local sensitization or habituation, and we used 2 magnitudes of stimulus, to elicit greater variation in pain response. Each pressure and heat pain task included 4 trials presented separately in counterbalanced order (setting and site of exposure) across participants. The experimenter terminated the CPT after 3 minutes, although participants were not aware of this ceiling. Before the start of each pain task trial, participants were informed that they would experience moderate sensation that might eventually be perceived as pain. They were instructed to continue with each task for as long as they could, and to remove their finger or arm from the apparatus at any time during the procedures if it became too uncomfortable or painful. All tasks were extensively pilot-tested with volunteers in the targeted age range.

Laboratory Pain Tasks

Pressure Task—The Ugo Basile 37215 analgesy meter (Ugo Basile Biological Research Apparatus, Comerio, Italy) was used to administer focal pressure to the second dorsal phalanx of the middle finger and index finger of each hand through a transparent plastic point ~1.5 mm in diameter. Four trials, 2 at each of 2 levels of force (322.5g and 465.0g), were conducted with an uninformed ceiling of 3 minutes. A comparable analgesy meter was used in nonclinical and clinical pediatric samples (age range, 5–17 years) without adverse effects.¹⁶

Heat Task—The Ugo Basile 7360 unit (Ugo Basile Biological Research Apparatus) was used to administer a total of 4 trials of 2 infrared stimulus intensities of radiant heat 5.1 cm proximal to the wrist and 7.6 cm distal to the elbow on both volar forearms, with an uninformed ceiling of 20 seconds. Thermal pain tolerance was electronically measured with an accuracy of 0.1 second. A similar task was used without adverse effects in a sample of youths (age range, 6–17 years).¹⁷

Cold Pressor Task—A commercial ice chest measuring 38 cm wide, 71 cm long, and 35 cm deep equipped with a plastic mesh screen to separate crushed ice from a plastic large-hole mesh armrest in cold water was used for the CPT. A pump circulated the chilled water through the ice to prevent local warming adjacent to the hand. For 1 trial, participants were instructed to keep the dominant hand in 10°C water to a depth of 5.1 cm above the wrist for as long as they could, with an uninformed ceiling of 3 minutes.

Assessment of Pubertal Stage—After the pain tasks, pubertal stage was assessed. Each subject was given schematic drawings, including appropriate written descriptions of 5 stages of secondary sexual characteristics on 2 separate anatomic development markers (female breasts and pubic hair, male genitalia and pubic hair) based on Tanner's Sexual Maturity Scale, enabling them to self-report.^{18,19} Pubertal status was highly correlated with age in the present sample ($r = 0.827$), and our previous research suggested that age may be a stronger predictor of physiologic responses to pain tasks than is pubertal status.¹³ For this reason, in the current study, we used age as a covariate instead of pubertal status.

Laboratory Pain Measures

Pain Tolerance—Pain tolerance was defined as the time elapsed in seconds from the onset of the pain stimulus to participants' withdrawal from the stimulus. Tolerance was measured for all 3 tasks—pressure, heat, and cold.

Pain Intensity and Pain Unpleasantness—Immediately after each trial, pain intensity and pain unpleasantness were assessed using a vertical sliding VAS, with one end signifying

no pain (0) and the other end indicating the worst pain imaginable (10). The VAS is considered to be brief, easily understood, and sensitive to changes in pain. With excellent psychometric properties,²⁰ it is appropriate for children aged 8 to 18 years.²¹ Previous research has used the VAS to rate pain in children in laboratory pain tasks.²²

In addition to numerical anchor points, the scale was designed with color cues, grading from white at the bottom to dark red at the top, as well as a neutral facial expression at the bottom and a negative facial expression at the top. Participants were given these instructions regarding the use of the VAS: “This scale is like a thermometer, only rather than measure your temperature, we will use it to measure your feeling or mood. The white color on the bottom represents the lowest values and the dark red at the very top represents the highest values for a particular feeling. By using this thermometer, you’ll let me know how much pain or discomfort you feel. You will do this by sliding the bar up and down on the colors until you get to the shade that equals how you feel.”

To ensure that participants understood the VAS, 3 practice ratings were completed. During the practice trials, participants were asked to use the VAS to answer the following questions: (1) “How afraid or nervous would you be right before taking an important exam or test?”; (2) “How much would it bother you to eat your favorite dessert?”; and (3) “How afraid, nervous, or worried do you feel right now?” The instructions and practice trials were repeated until participants fully understood the VAS.

Pain Intensity—Immediately after each trial, participants were asked to use the VAS to rate the amount of pain they experienced during the task. Also, they were asked, “At its worst, how much pain did you feel during the task?”

Pain Unpleasantness—Immediately after each trial and after rating their pain intensity, participants were asked to rate the amount of distress or bother they experienced during the task using the same vertical sliding VAS. This time, participants were asked, “At its worst, how much did the task bother you?”

Cortisol Assessment

The timeline for sample collection is illustrated in Figure 1. Salivary cortisol samples were obtained from the subjects after they entered the laboratory ([SCb] baseline), after the completion of all pain tasks (SC1), and at the end of the session ([SC2] 20 minutes after the previous assessment). Blood cortisol samples were also obtained after the completion of the pain tasks (BC1) and at the end of the session (BC2), 20 minutes later, for the assessment of recovery.

Participants rinsed their mouth with water to prevent contamination of the saliva sample by food debris ~30 minutes before the baseline saliva collection procedure. Unstimulated saliva samples were collected by placing #5001 salivettes (Salimetrics, LLC, State College, Pennsylvania) in the mouth against the cheek. Participants were instructed to chew on the dental cotton roll for 3 minutes. The saliva samples were frozen at -80°C and were express shipped under refrigeration to an independent laboratory for quantification of cortisol concentrations (Laboratory for Comparative Human Biology, Emory University, Atlanta, Georgia). Participants were not permitted to eat during the laboratory assessment to prevent contamination of the sample collection.

For blood cortisol, 2 finger-prick, blood spot samples were obtained, stored, and transported according to the procedures described by Worthman and Stallings.²³ Briefly, the fourth finger of the non-dominant hand was warmed, rubbed, cleaned with an alcohol swab, and pricked with a Microtainer Safety Flow lancet (Becton Dickinson and Company, Franklin

Lakes, New Jersey). After 5 circles of blood were collected directly on standardized filter paper #903 (Schleicher & Schuell, Bioscience, Inc., Keene, New Hampshire), the procedures were repeated 20 minutes later on the fourth finger of the dominant hand. Samples were air dried at room temperature and express shipped without refrigeration to an independent laboratory within 14 days of collection. Samples were stored at -30°C until the time of assay.

Laboratory analysis was performed in a Worthman laboratory. Quantitative determination of salivary cortisol was performed using an enzyme-linked immunosorbent assay kit (#1-0102/1-0112, Salimetrics), and blood spot cortisol was determined by radioimmunoassay (Bio-Analysis Inc., Santa Monica, California).

Statistical Analysis—The pain unpleasantness VAS was not administered until after data collection was under way, and thus, the sample size for pain unpleasantness ratings was smaller ($n = 188$) than for the other measures. Therefore, these data were averaged across trials for each task, yielding 1 mean rating for pressure intensity, heat intensity, pressure unpleasantness, heat unpleasantness, pressure tolerance, and heat tolerance. Distributions of variables were examined.

Zero-order correlations were used to examine the relationship between cortisol levels obtained from saliva and blood samples, as well as the relationship between cortisol, the child's age, and the time of day when the laboratory session occurred. Repeated analyses of covariance were conducted to examine sex differences in cortisol concentrations across cortisol assessments, controlling for age and time of day for salivary and blood levels. Partial correlation analyses were performed to examine the association between blood and salivary cortisol levels and laboratory pain responses, controlling for age and time of day for boys and girls separately. A standard α level of 0.05 (2-tailed) was used to evaluate the findings.

A sample size of 235 children was chosen to give adequate power (>0.85) to test for moderate effect size differences ($D = 0.04$) for comparisons across age, sex, and age $\times$ sex interaction, with the assumption of no interactions with task type.¹³

RESULTS

Of 489 individuals screened for eligibility by telephone, 17 children (3.5%) were excluded due to acute or chronic illness, or use of medications that could affect study outcomes. Of the 472 (96.5%) invited to participate, 228 (48.3%) declined, mainly because of parental lack of interest (54.0%) or not enough time (21.0%). Four subjects refused to participate in the study, and cortisol samples were not available for 5 children, leaving a total sample size of 235 subjects. Participants in the study included 116 healthy females (49.4%) (mean [SD] age, 12.7 [2.9] years [range, 8–18 years]). The mean ages of girls and boys (12.8 [3.0] years and 12.5 [2.9] years, respectively) were closely matched. Pubertal status, ethnic composition, and parent's socioeconomic status²⁴ are displayed in Table I.

Preliminary Analyses

Pain intensity ratings for the thermal and pressure tasks were highly correlated across the 4 trials of each task ($r = 0.71\text{--}0.89$, $P < 0.001$). Pain unpleasantness ratings for the thermal and pressure tasks were also highly correlated across the 4 trials of each task ($r = 0.72\text{--}0.88$, $P < 0.001$). Pain tolerances for the thermal and pressure tasks were also highly correlated across the 4 trials of these tasks ($r = 0.55\text{--}0.78$, $P < 0.001$). Salivary cortisol, pressure, and cold tolerance were not normally distributed. Thus, a log transformation was used to normalize the distribution.

Mean cortisol concentrations for boys and girls and for the total sample are presented in Table II. Salivary and blood cortisol sample data were highly correlated (Table III). SC1 and BC1 were assessed at the same time, and their correlations were 0.839, 0.869, and 0.814 for the total sample, in boys, and in girls, respectively (all, $P < 0.001$). SC2 and BC2 were assessed simultaneously, and their correlations were 0.808, 0.867, and 0.757 for the total sample, in boys, and in girls (all, $P < 0.001$). Age was positively correlated with cortisol readings at all assessments, and the time of day was negatively correlated with cortisol measurements at all assessments.

Main Effects of Pain Response and Sex

Repeated analysis of covariance in the total sample, controlled for age and time of day, suggested a significant effect of time. Salivary cortisol levels declined from SCb to SC1 and SC2 ($P < 0.01$), but SC1 and SC2 did not differ significantly from each other. Similar results were obtained when boys and girls were examined separately. With regard to sex differences in cortisol levels, boys had marginally higher salivary cortisol at SCb compared with girls ($P = 0.05$), but no significant sex differences emerged at SC1 and SC2 (Figure 2). There were also no significant sex differences found for BC1 or BC2.

Sex Differences in the Relationship of Cortisol to Pain Response

In partial correlations controlled for age and time of day among boys, there was a significant association between pressure tolerance and SC1 ($r = 0.338$, $P = 0.003$), SC2 ($r = 0.271$, $P = 0.020$), and BC1 ($r = 0.261$, $P = 0.026$), indicating greater pressure pain tolerance (ie, lower pressure pain response was linked with increased salivary and blood cortisol responses) (Table IV). Among girls, a different pattern emerged. Cold intensity was positively associated with BC2 ($r = 0.229$, $P = 0.048$), suggesting that increased cold pain reactivity was related to a higher blood cortisol response. Similarly, among girls, cold unpleasantness was positively correlated with BC1 ($r = 0.237$, $P = 0.041$). In girls, the associations between cold intensity and BC1 and SC1, cold unpleasantness and SC1, and cold unpleasantness and BC2 were not statistically significant.

DISCUSSION

The present study explored HPA-system activation in a sample of healthy children undergoing a series of experimental pain tasks. Although much research has focused on cortisol and stress in adults, no published study was identified that examined the relationship of cortisol and pain responses in healthy children and adolescents after laboratory pain-induction procedures.

As expected, saliva and blood cortisol samples correlated well in our sample, supporting the reliability of our assessments and the use of less-invasive saliva collection rather than blood sampling for cortisol assessment in studies of pain and stress responses in children. We also found that children's age and time of day were significantly correlated with all cortisol readings ($P < 0.001$), and therefore these variables were included as covariates in the analyses.

The findings did not support our hypothesis that increased cortisol levels would be evident in response to the pain tasks. In fact, we found a different pattern. Cortisol levels were highest on arrival at the laboratory; these levels subsequently declined at SC1/BC1 after the pain tasks and did not change at SC2/BC2 (recovery, 20 minutes later). This pattern held true for the total sample and for boys and girls examined separately. These data suggest that children in the present study probably experienced the highest levels of stress before the baseline assessment (ie, before arriving at the laboratory). As the children became more

familiar with the laboratory and pain procedures, the pain-inducing tasks were likely insufficient to produce an increase in cortisol beyond baseline. These findings were consistent with data from studies in adult populations,^{25–27} suggesting that the CPTs may be insufficient to produce a strong cortisol response unless a social/evaluative component (ie, being videotaped) is included. Our laboratory procedures lacked such a component. The most stressful activity may have been anticipation of the laboratory procedures, which may suggest that our study design was unable to capture a true baseline cortisol assessment. This explanation is consistent with previous findings that young boys and girls exhibited increased basal cortisol levels immediately before a stress task (as well as during the task), particularly in a laboratory compared with a naturalistic setting.^{28,29}

Despite the fact that boys' cortisol levels at baseline were ~0.18 nmol/mL and girls' cortisol levels were ~0.13 nmol/mL, these differences were not significant, and no significant sex differences in cortisol levels were evident at any other assessment point (Figure 2). Even though the literature on adults suggests that men have higher cortisol levels in response to stress compared with women,³ our data did not indicate similar sex differences in children. This finding is consistent with several previous studies in children that found no significant sex differences in cortisol responses to stress,^{12,30,31} supporting the possibility that the cortisol–stress relationship differs in children compared with adults.

Separate examination of the cortisol–laboratory pain response relationships by sex (controlling for age and time of day) suggested different sex-specific patterns. Higher cortisol levels were associated with lower pain reactivity (ie, increased pressure tolerance) among boys compared with girls. However, higher cortisol levels were related to a higher pain response (ie, increased cold intensity and unpleasantness) in girls than in boys (Table IV). These findings suggest that a greater pain response to the cold task was associated with increased cortisol responses in girls only, whereas a decreased pain response to the pressure task was related to increased cortisol responses in boys. These data from children mirror adult sex-differentiated cortisol findings. An experimental, laboratory-based study examining cortisol responses in 39 men and 37 women (age range, 19–29 years) before and after 2 trials of a cold water plunge test similar to the CPT found higher pain tolerance times during the second trial in men that were associated with a significantly larger increase in cortisol response, compared with the response in women ($P = 0.05$).⁵ However, no significant sex differences in pain intensity or unpleasantness were found in either of the 2 trials. Another experimental investigation of sex differences in cortisol/pain responses to the CPT in 75 adults also found a negative association between pretask cortisol levels and pain perception in men only.⁶ Our data support these differential relationships between cortisol levels and pain in adult men and women and provide evidence that sex-specific stress–pain relationships may emerge early in development.

Another possibility is that sex-specific cortisol concentrations and pain relationships may differ in children and are more difficult to characterize than in adults. For example, one study found that among a sample of 102 children (43 girls, 59 boys) aged 5 years who participated in a stress task similar to the TSST-C, girls had significantly higher mean (SEM) cortisol levels in response to the stressor compared with boys (54.14 [30.28] vs 35.56 [19.84] $\mu\text{g/dL}$, respectively; $P < 0.001$).³² In addition, the relationship of cortisol to behavioral and emotional problems was sex specific. Based on an interview conducted with each child, boys with higher cortisol levels had increased hyperactivity and impulsivity, and more internalizing problems compared with boys with lower cortisol levels. This relationship was not found in girls. However, girls with higher basal cortisol levels used significantly more positive emotions during a storytelling task compared with girls with lower cortisol levels ($P = 0.050$). This relationship was not evident in boys. In another study, no significant sex differences in cortisol levels were found in response to a carbon dioxide

inhalation challenge in a sample of 98 adolescents (51 boys, 47 girls; age range, 9–17 years).³⁰ Similarly, in a sample of 31 healthy children (16 boys, 15 girls; age range, 9–15 years) who took the TSST-C, no sex differences were observed in either stress (as measured by heart rate) or cortisol responses. The data suggest complex sex-dependent relationships in young populations that may be different in children compared with adults.^{12,31}

We also found sex differences in cortisol–pain relationships related to the method of cortisol assessment. Specifically, significant relationships were found with salivary cortisol in boys and with blood cortisol in girls (both, $P < 0.05$). Although salivary and blood cortisol levels were highly correlated, this may suggest another sex-specific difference in cortisol response. To our knowledge, no other studies have examined cortisol differences across saliva and blood assessments in response to stress in children. A double-blind, counterbalanced study that examined salivary and blood cortisol concentrations in response to thermal and cold pain thresholds and tolerance in 26 adults (15 men, 11 women; age range, 18–40 years) found no significant sex-based differences between saliva and blood cortisol concentrations, despite women reporting significantly greater pain during a thermal heat task and the CPT ($P < 0.001$ and $P < 0.01$, respectively).³³ Future studies should include multiple methods of cortisol assessment to continue exploring potential sex-based relationships between blood and salivary cortisol concentrations.

Findings from the present study highlighted sex differences in the cortisol–pain response relationship among children that resemble those found in adults. The current data support the hypothesis that HPA–stress set points may be established during childhood, and that there may be sex differences in these relationships. However, we found no evidence of consistently higher levels of cortisol in boys than in girls, as has been reported in adults. The study provides support for continued evaluation of sex differences in cortisol and pain across the developmental continuum, including longitudinally, and for evaluation of factors that are moderators and mediators of these relationships in males and females.

There are a number of limitations to the present study. Our data suggest we were unable to capture a true baseline cortisol reading before the pain tasks. SCb assessments were obtained as soon as subjects arrived at the laboratory, which did not permit sufficient habituation to the laboratory environment. The SCb reading was the highest compared with the posttask and recovery cortisol readings, indicating that the most significant stressor may have been anticipation of coming to the laboratory rather than the pain tasks. Although we could not obtain a true baseline reading, the data nonetheless suggest a similar pattern of cortisol response and recovery in boys and girls who are anticipating a stressor.

Possibly the study design (ie, presenting 3 pain tasks in 1 session) did not permit sufficient habituation of the central nervous system and pain pathways, thereby unintentionally creating a cumulative effect of increased pain sensitivity during the trials. However, we attempted to control for this possibility by counterbalancing the presentation of pain tasks, and we did not find any order effects during the laboratory session. Therefore, if a pattern of increased pain sensitivity emerged regardless of pain stimuli, we would have expected to see a nonspecific response across pain tasks. However, we found specific relationships for each task in boys and girls, suggesting robust findings.

Another potential limitation of the study was that the pain tasks and the stressor of the finger sticks for blood sampling may not have been sufficiently stressful to produce a reliable increase in cortisol activity. Despite self-reported data indicating that the pain tasks and finger sticks were at least moderately unpleasant and painful, the cortisol data generally did not support a strong HPA response to these tasks. As previously mentioned, it is possible that the children had become habituated to the laboratory and the procedures by the SC1/

BC1 posttask assessment, and that the levels of cortisol decreased accordingly. Another explanation is that healthy children may not respond strongly to stress in the current procedures. In this healthy sample, cortisol represented only one aspect of the stress–pain response, and it is possible that a clinical population of children might exhibit a different pattern. In addition, although tasks (eg, the CPT) were relatively reliable predictors of stress responses, a number of studies have found only minimal to modest increases in cortisol after the CPT.^{3,26,34,35} Tasks that include a social-evaluative stress response are generally better predictors of HPA activity and stress, at least in adults.^{25,27} The pain tasks in the present study did not include a social stressor, as in the TSST-C.³⁶ It is also possible that the timing of the salivary cortisol assessments did not capture the true peak in cortisol occurring 20 to 30 minutes after the first pain task.³⁷ SC1 generally occurred 1 hour after SCb, a time lapse that may have obscured the initial rise in cortisol concentrations in response to the first pain task. However, given the high baseline values at SCb, it is unlikely that any subsequent stress task would have produced cortisol levels in excess of baseline. The biggest stressor may have been the anticipation of the procedures and the novelty inherent in arrival at the laboratory.

The nature of the study precluded obtaining a true random sample of participants, which prevents generalization of the study findings to all healthy children. Because of the study inclusion and exclusion criteria, these relationships may be different in the general population. In addition, sex-dependent relationships between pain responses and cortisol may differ in children with chronic pain.

CONCLUSIONS

These data from healthy children and adolescents who participated in a laboratory pain study partially support the findings in the adult literature, specifically the sex-dependent relationships of cortisol levels and pain. In boys, cortisol was positively associated with increased pain tolerance, but in girls, cortisol was positively associated with hyperalgesia. Despite these significant findings, the present data may not be generalizable to the total population of healthy children, and the relationships may be different in children with chronic pain. In addition, the lack of a true baseline assessment and small sample size may have obscured other sex-dependent cortisol and pain relationships, and our findings must be interpreted with caution. However, sex differences in HPA activation in response to noxious stimuli support a role for the assessment of cortisol in understanding sex differences in pain responses. The study findings highlight the importance of continuing to examine sex-specific cortisol–pain relationships in children, including determining the developmental trajectory of these sex-related findings.

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Figure 1.

Timeline of cortisol assessments. SCb = baseline salivary cortisol; SC1 = salivary cortisol after completion of tasks; BC1 = blood cortisol after completion of tasks; SC2 = salivary cortisol at end of the session; BC2 = blood cortisol at end of the session.

Figure 2. Changes in salivary cortisol across laboratory sessions for boys and girls. SCb = baseline salivary cortisol; SC1 = salivary cortisol after completion of tasks; SC2 = salivary cortisol at end of the session.

Table I

Demographic information for study participants (N = 235).

Characteristic	Value
Male, no. (%)	119 (50.6)
Age, mean (SD), y	12.7 (2.9)
Pubertal status, no. (%) [*]	
Early puberty	109 (47.0)
Late puberty	123 (53.0)
Ethnicity, no. (%)	
White	94 (40.0)
Hispanic	56 (23.8)
African American	33 (14.0)
Asian American	22 (9.4)
Other	30 (12.8)
Parent socioeconomic status, no. (%) [†]	
Menial service work	2 (0.5)
Unskilled work	45 (11.3)
Skilled work	17 (4.3)
Clerical/sales	32 (8.1)
Semiprofessional	53 (13.4)
Minor professional	92 (23.2)
Administrators/lesser professional	70 (17.6)
Executive/major professional	86 (21.6)

^{*} Data are missing for 3 subjects.

[†] N = 397 parents (202 mothers, 195 fathers).

Table II

Cortisol concentrations in male and female children and adolescents.

Gender	Baseline	Salivary Cortisol, nmol/L		Blood Cortisol, nmol/L	
		Completion of Pain Tasks	End of Session *	Completion of Pain Tasks	End of Session *
Males					
No.	119	116	117	112	99
Mean (SD)	0.17 (0.18)	0.10 [†] (0.06)	0.12 [†] (0.13)	10.48 (4.21)	11.31 (5.62)
Females					
No.	116	116	116	111	97
Mean (SD)	0.13 (0.09)	0.09 [†] (0.06)	0.11 [†] (0.13)	10.67 (4.45)	11.47 (6.29)
Total					
No.	235	232	233	223	196
Mean (SD)	0.15 (0.14)	0.09 [†] (0.06)	0.11 [†] (0.13)	10.57 (4.32)	11.39 (5.95)

* 20 Minutes later.

[†] $P < 0.001$ (2-tailed) versus baseline.

Table III

Pearson correlations among cortisol readings.*

Assessment	Salivary Cortisol, nmol/L			Blood Cortisol, nmol/L		
	Baseline	Completion of Pain Tasks	End of Session [†]	Completion of Pain Tasks	End of Session [†]	End of Session [†]
Time of salivary cortisol measurement						
Completion of pain tasks	0.730					
20 Minutes later (end of session)	0.658	0.790				
Time of blood cortisol measurement						
Completion of pain tasks	0.578	0.839	0.675			
20 Minutes later (end of session)	0.497	0.622	0.808	0.732		
Child age	0.333	0.377	0.280	0.350	0.248	
Time of day	-0.383	-0.490	-0.440	-0.380	-0.308	

* $P < 0.001$ (2-tailed) versus baseline.[†] 20 Minutes later.

Table IV

Partial correlations between cortisol concentrations and laboratory pain responses in boys and girls, controlled for age and time of day.

Pain Measure	Boys*						Girls†					
	Salivary Cortisol			Blood Cortisol			Salivary Cortisol			Blood Cortisol		
	Baseline	Completion of Pain Tasks	End of Session	Completion of Pain Tasks	End of Session	Baseline	Completion of Pain Tasks	End of Session	Completion of Pain Tasks	End of Session	Completion of Pain Tasks	End of Session
Pressure tolerance												
<i>r</i>	0.068	0.338 [‡]	0.271 [§]	0.261 [§]	0.150	0.060	0.086	-0.131	0.082	-0.076		
<i>P</i>	0.569	0.003	0.020	0.026	0.205	0.612	0.463	0.263	0.484	0.518		
Heat tolerance												
<i>r</i>	-0.066	-0.014	-0.048	0.001	-0.070	0.048	0.175	0.014	0.127	-0.031		
<i>P</i>	0.578	0.910	0.689	0.992	0.556	0.683	0.134	0.907	0.278	0.789		
Cold tolerance												
<i>r</i>	-0.036	0.213	0.191	0.182	0.125	-0.093	-0.061	-0.121	-0.041	-0.059		
<i>P</i>	0.762	0.071	0.106	0.123	0.294	0.43	0.600	0.301	0.724	0.616		
Pressure intensity												
<i>r</i>	0.048	-0.027	-0.122	0.088	-0.126	-0.082	-0.003	0.069	0.026	0.081		
<i>P</i>	0.689	0.818	0.304	0.457	0.288	0.483	0.978	0.557	0.822	0.490		
Heat intensity												
<i>r</i>	0.135	0.101	0.075	0.145	0.016	-0.062	0.054	0.121	0.101	0.164		
<i>P</i>	0.255	0.394	0.528	0.219	0.892	0.597	0.647	0.300	0.386	0.161		
Cold intensity												
<i>r</i>	0.021	0.025	-0.003	0.158	-0.062	0.013	0.210	0.177	0.224	0.229 [§]		
<i>P</i>	0.859	0.833	0.977	0.182	0.603	0.910	0.071	0.128	0.053	0.048		
Pressure unpleasantness												
<i>r</i>	-0.045	0.006	-0.045	0.023	-0.081	-0.089	0.085	0.072	0.142	0.014		
<i>P</i>	0.707	0.959	0.705	0.846	0.494	0.448	0.469	0.538	0.225	0.226		
Heat unpleasantness												
<i>r</i>	0.060	0.111	0.121	0.073	0.003	-0.052	0.164	0.117	0.186	0.174		
<i>P</i>	0.615	0.350	0.309	0.542	0.978	0.656	0.160	0.318	0.109	0.134		
Cold unpleasantness												

\$watermark-text

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		Boys*				Girls†			
		Salivary Cortisol		Blood Cortisol		Salivary Cortisol		Blood Cortisol	
Pain Measure	Baseline	Completion of Pain Tasks	End of Session	Completion of Pain Tasks	End of Session	Completion of Pain Tasks	End of Session	Completion of Pain Tasks	End of Session
<i>r</i>	0.110	0.087	0.079	0.156	0.029	0.100	0.183	0.237§	0.207
<i>P</i>	0.355	0.465	0.508	0.187	0.811	0.392	0.117	0.041	0.075

End of session = 20 minutes.

* All, *df*= 71 for boys.

† All, *df*= 73 for girls.

‡ *P*<0.001 (2-tailed).

§ *P*<0.05 (2-tailed).