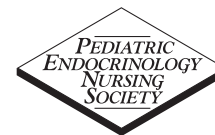


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Salivary Cortisol: A Tool for Biobehavioral Research in Children

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The measurement of salivary cortisol has been widely used in pediatric research for more than 20 years as a biomarker of hypothalamic pituitary adrenal (HPA) axis activity during normal activity and in response to stress. McCarthy et al. (2010) recently reported cognitive-behavioral interventions that were effective in reducing pain and distress in children undergoing intravenous needle insertion, and salivary cortisol measurement was a useful marker of biological response to compare the effect of interventions in conjunction with other physiological and/or behavioral outcome measures. The inclusion of salivary cortisol as an outcome measure will provide a useful biological marker with future research to investigate the following: stress in children undergoing painful procedures, weight loss, sleep problems, and caregiver stress. The use of salivary cortisol in pediatric biobehavioral research has revealed important information about the pattern of cortisol secretion during childhood, the response to stressors in a nonclinical environment, the response to therapeutic interventions, and the identification of dysfunctional patterns of secretion in children. Salivary cortisol is a reliable noninvasive method to assess HPA function; however, collection and measurement of specimens with infants and children require special consideration. This article will summarize pertinent issues related to salivary cortisol collection to encourage “broader employment” of this method in pediatric biobehavioral research.

Various psychological and physical stressors can activate the HPA axis such that corticotrophin-releasing hormone and adrenocorticotropin hormones are released with subsequent rise in cortisol levels. Once the HPA axis is activated, it takes approximately 15–30 minutes for the cortisol levels to peak.

Cortisol secretion has a circadian pattern, peaking in the early morning (approximately 20–30 minutes after awakening) with nadir around midnight (Stratakis, Gold, & Chrousos, 1995) and short bursts of secretion over the course of the day. The diurnal pattern of cortisol secretion is typically established in early infancy (Price, Close, & Fielding, 1983). HPA axis function and regulation have been shown to have a significant impact on growth and development. Poor regulation of the cortisol response to emotional stress correlates with detrimental effects on cognitive and emotional functioning (Lupien, McEwen, Gunnar, & Heim, 2009).

Salivary cortisol is a measure of unbound or “free” cortisol that is biologically relevant with the ease of sampling in clinical and field settings. Saliva sampling is a reliable, noninvasive method to measure biologically active, unbound plasma of cortisol in infants and children. Salivary testing offers advantages over venipuncture because it is noninvasive and less likely to confound results. In addition, multiple samples can be obtained without increasing ethical concerns. This method is extensively used in psychoneuroendocrinological research (Kirschbaum & Hellhammer, 1994). For example, studies of naturalistic life events that are considered stressful, such as starting school for a child, have incorporated salivary cortisol as an outcome measure to investigate normal versus maladaptive responses (Boyce et al., 1995). In addition, studies of diurnal salivary cortisol patterns provide insight regarding the role of early environmental factors on cortisol regulation, cognition, and growth (Gutman & Nemeroff, 2003). In addition, salivary cortisol is a useful diagnostic test in the evaluation of suspected cortisol dysregulation (Gafni, Papanicolaou, & Nieman, 2000). For excellent reviews, see Hanarhan, McCarthy, Kleiber, Lutgendorf, and Tsalikian (2006); Jessop and

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Turner-Cobb (2006); Kirschbaum and Hellhammer (1994); and Schmidt (1997).

Methodological Issues

Time of Collection

Because there are a number of factors that affect HPA axis function and subsequent cortisol secretion, there are several important issues to be aware of when measuring salivary cortisol in children. As mentioned above, because cortisol is secreted in a diurnal pattern, it is imperative to consider time of day in reference to sample collection and to have consistency across subjects. Timing of collection is also relevant depending on whether one intends to measure differences in diurnal pattern or response to a stressor. Individual variations including typical sleep and or napping, activity, eating, and recent illness need to be considered when planning to use salivary cortisol as a measure of stress (Jessop & Turner-Cobb, 2008). In addition to the circadian pattern of cortisol secretion, there is also a cortisol awakening response, which refers to the rapid increase in cortisol 20 to 30 minutes after awakening in the morning. Therefore, instructions for saliva collection should provide specific information regarding timing of first morning collection for data consistency.

Saliva Collection Devices

A major advantage of salivary cortisol is its noninvasive aspect of sampling. The age and developmental level of the child are the major determinants of selection of the sampling device to collect saliva in a safe manner with minimal stress (Schwartz, Granger, Susman, Gunnar, Laird, 1998).

- Whole saliva sampling (spitting in a tube or passive drool with a straw) is appropriate for school-aged children and adolescents. This technique is not feasible with infants or young children, who often lack the coordination and/or cooperation to perform this reliably.
- Braided cotton dental rope has been extensively used with young children to collect saliva. A small section is placed in the child's mouth while the other end is held by the adult collecting the sample. A drawback of this device may be insufficient test volume (from the absorbent cotton) and interference with immunoassays for cortisol (Shirtcliff, Granger, Schwartz, & Curran, 2001).
- Polymer rolls are typically used with infants and toddlers to collect saliva. The Salivette (Sarstedt Inc., Rommelsdorf, Germany), which is widely used by clinicians and researchers, consists of a small polymer roll that fits into a standard centrifugation tube. Limitations with this methodology include safety concerns for use in young children or infants (potential

choking hazard), difficulty in assessing if sufficient saliva was obtained, unwillingness of children to comply with request to chew long enough to obtain sufficient saliva, and some children may find the taste of the roll unpalatable. Longer length Salivettes that allow the swab to be held by the adult assisting with collection are available for infants and young children.

- Small pipettes or disposable mucous extractors (Riad-Fahmy, Read, & Walker, 1982) or modified feeding bottles containing absorption materials inside the sucker (Kirschbaum & Hellhammer, 1994) have also been used for sampling with infants.
- Modified eye sponges (hydrocellulose micro sponge on a plastic shaft) provide a useful alternative for sampling infants and young children. These sponges offer the advantage of safety, comfort, and efficiency of collecting specimens (Visispear, Becton Dickenson, Walton, MA).

Storage of Samples

A major advantage of salivary samples is the ability to perform the collection at home or in the field (e.g., schools, day care). Cortisol concentrations remain stable in salivary samples (unfrozen) for approximately 7 days, so samples may be returned by mail (Aardal & Holm, 1995; Clements & Parker, 1998). Salivary samples may also be stored in the freezer (-20°C) prior to shipping. Thawing of samples may contribute to evaporation but does not significantly alter the specimens; however, refreezing may alter cortisol levels (Aardal & Holm, 1995).

Interfering Substances

Certain medications may affect cortisol secretion or interfere with cortisol measurement, so a careful review of medication history is important. Drugs that may affect cortisol levels include corticosteroids (commonly prescribed to treat respiratory and dermatologic conditions), caffeine, sex hormones, amphetamine, phenytoin, lithium, and spironolactone (Ambrogio, Giraldo, & Cavagnini, 2008). The use of salivary stimulants, such as chewing gum or sugar-free flavor crystals, is controversial because some types have been shown to alter the pH level of samples, which can affect assay results (Harmon, Hible, Romyantseva, & Granger, 2007). Food, beverages, and brushing teeth should be avoided for 30 minutes prior to salivary sampling to avoid interference with collection and/or assay.

Assays

Selection of the laboratory and assay for analysis of cortisol requires careful research to identify the most appropriate choice. Available assays include radioimmunoassay, ELISA, and immunofluorescence. Assays differ in sensitivity, intra- and interassay variation, cost, unit of measure, and amount

of specimen required. With the introduction of ultrasensitive assays, the volume of saliva required has dramatically decreased, with 25 μ l sufficient for some assays. A reference for available cortisol assays is listed at <http://www.endotext.org/protocols/protocols1/protocols1.htm>.

Procedures

To facilitate compliance with sample collection and enhance reliability of results, it is important to provide explicit instructions. Educational material provided to the person responsible for collecting the saliva should include the following: choosing a typical day for the child (i.e., no illness, no change in routine schedule), no food or milk 30 minutes prior to collection, information specific to collection device provided, writing time of day of collection on bag or tube, refrigerate (or freeze) samples until ready to ship, and mailing instructions (no special handling required). It is helpful to include a questionnaire regarding the child's activity, food consumption, and sleep (for sample questionnaire, see Hanarhan et al., 2006).

In summary, salivary sampling of cortisol is a safe, reliable, nonpainful method that permits multiple sampling and extends clinical and research sampling beyond the clinical setting. In pediatrics, numerous applications exist for research with salivary cortisol as an outcome measure to investigate relationships between biology, behavior, environment, and health, including social behavior, response to stress, response to therapeutic/behavioral interventions (e.g., medication, diet, exercise), behavioral disorders, and normal development.

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References

Aardal, E., & Holm, A. C. (1995). Cortisol in saliva—Reference ranges and relation to cortisol in serum. *European Journal of Clinical Chemistry and Clinical Biochemistry*, 33, 927–932.

- Ambrogio, A. G., Giraldo, F. P., & Cavagnini, F. (2008). Drugs and HPA axis. *Pituitary*, 11, 219–229.
- Boyce, W. T., Adams, S., Tschann, J. M., Cohen, F., Wara, D., & Gunnar, M. R. (1995). Adrenocortical and behavioral predictors of immune response to starting school. *Pediatric Research*, 38, 1009–1017.
- Clements, A. D., & Parker, C. R. (1998). The relationship between salivary cortisol concentrations in frozen versus mailed samples. *Psychoneuroendocrinology*, 23, 613–616.
- Gafni, R. I., Papanicolaou, D. A., & Nieman, L. K. (2000). Nighttime salivary cortisol measurement as a simple, noninvasive, outpatient screening test for Cushing's syndrome in children and adolescents. *Journal of Pediatrics*, 137, 30–35.
- Gutman, D. A., & Nemeroff, C. B. (2003). Persistent central nervous system effects of an adverse early environment: Clinical and preclinical studies. *Physiology and Behavior*, 79, 471–478.
- Hanarhan, K., McCarthy, A. M., Kleiber, C., Lutgendorf, S., & Tsalikian, E. (2006). Strategies for salivary cortisol collection and analysis in research with children. *Applied Nursing Research*, 19, 95–101.
- Harmon, A. G., Hible, L. C., Rumyantseva, O., & Granger, D. A. (2007). Measuring salivary cortisol in studies of child development: Watch out—What goes in may not come out of saliva collection devices. *Developmental Psychobiology*, 49, 495–500.
- Jessop, D. S., & Turner-Cobb, J. M. (2008). Measurement and meaning of salivary cortisol: A focus on health and disease in children. *Stress*, 1, 1–14.
- Kirschbaum, C., & Hellhammer, D. H. (1994). Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrinology*, 19, 313–333.
- Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour, and cognition. *Nature Reviews Neuroscience*, 10, 434–445.
- McCarthy, A. M., Kleiber, C., Hanrahan, K., Zimmerman, M. B., Westhus, N., & Allen, S. (2010). Factors explaining children's responses to intravenous needle insertions. *Nursing Research*, 59, 407–416.
- Price, D. A., Close, G. C., & Fielding, B. A. (1983). Age of appearance of circadian rhythm in salivary cortisol values in infancy. *Archives of Disease in Childhood*, 58, 454–456.
- Riad-Fahmy, D., Read, G. F., & Walker, R. F. (1982). Salivary cortisol assays for assessing pituitary–adrenal reserve. *Clinical Endocrinology (Oxford)*, 17, 583–592.
- Schmidt, N. A. (1997). Salivary cortisol testing in children. *Issues in Comprehensive Pediatric Nursing*, 20, 183–190.
- Schwartz, E. B., Granger, D. A., Susman, E. J., Gunnar, M. R., & Laird, B. (1998). Assessing salivary cortisol in studies of child development. *Child Development*, 69, 1503–1513.
- Shirtcliff, E. A., Granger, D. A., Schwartz, E., & Curran, M. J. (2001). Use of biomarkers in biobehavioral research: Cotton-based sample collection methods can interfere with salivary immunoassay results. *Psychoneuroendocrinology*, 26, 165–173.
- Stratakis, C. A., Gold, P. W., & Chrousos, G. P. (1995). Neuroendocrinology of stress: Implications for growth and development. *Hormone Research*, 43, 162–176.