

Steroid analysis in saliva: a noninvasive tool for pediatric research and clinical practice

Wieland Kiess, Roland Pfaeffle*

Saliva is a readily available, noninvasive fluid that can be used to monitor the presence and concentration of a wide variety of drugs, hormones, antibodies and other molecules. Saliva-based diagnostic tests are now frequently used in endocrine practice, pediatric research, in clinical studies in psychology and psychiatry, as well as in stress research. In particular, the use of saliva as a vehicle for the determination of plasma steroid hormone levels in children and adolescents has increased dramatically. In this issue of *Jornal de Pediatria*, Silva et al. report on their use of salivary cortisol to assess the hypothalamic-pituitary-adrenal axis in healthy children aged 45 days to 36 months.¹ The authors established reference values for salivary cortisol using their in-house radioimmunoassay employing an antibody directed against cortisol-3-oxime conjugated to bovine albumin. These data are precious in that they can be used for reference in both pediatric day-to-day routine practice as well as in pediatric research. They also offer insight into the development of circadian rhythms and the physiology of the endocrine system in the human infant.

It is important to note that during recent years, saliva collection devices using for example cotton or plastic swabs have been developed (e.g.: Salivette®, Sarstedt, Germany). Each laboratory should test for cross-reacting substances in these premanufactured devices since in some instances cross-reactivity of the immunoassays with substances contained in

the coating of the swabs were encountered (personal observation). In addition, a number of agents that can provoke increased saliva flow such as 1 or 5% citric acid are being increasingly used when saliva samples are collected. In newborns and very young children, aspiration with a tube and syringe after only gentle stimulation of saliva flow without using chemical irritants is the most practical and less invasive method to use. In older children and adolescents, saliva collecting tubes

which provide swabs and ready to use two-chamber devices that can be centrifuged to remove detritus and also used to freeze-store samples prior to analysis are recommended. Highly sensitive immunoassays employed as radioimmunoassays, enzyme-based, or time-resolved fluorescent immunoassays are widely available. However, it is important to establish reference values over the life span with all such methods separately and at each center, since specificity, mainly determined by cross-reactivity of the first antibodies with different steroid metabolites, as well as sensitivity of the assays differ widely. For example, morning cortisol saliva concentrations reported by Silva et al. average 557.86 nmol/L and range between 76.88 and 1,620.08 nmol/L,¹ while Tornhage & Alfvén report on morning levels of 8.8 nmol/L in older children² and our own reference values for morning cortisol levels in saliva range from less than 2 nmol/L to 100 nmol/L in infants, children and adolescents.³ In adults, morning salivary cortisol levels in healthy volunteers are above 12 nmol/L, while late-night concentrations were between 1.0 and 8.3 nmol/L.⁴

Silva et al. found in their recent paper that the difference between morning and afternoon cortisol levels in saliva become more apparent with age.¹ Indeed, increased morning cortisol levels become apparent within the first three months

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* Hospital for Children and Adolescents, University of Leipzig, Leipzig, Germany.

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of life^{2,3,5,6} while in neonates no circadian rhythm of cortisol saliva concentrations is present.^{5,7} Also, Custodio et al. showed that the mean age of emergence of salivary cortisol circadian rhythm was similar in monozygotic and dizygotic twins (7.8 vs. 7.4 weeks). Seven twin pairs showed coincidence of the emergence of cortisol rhythm, while 10 pairs were not coincident: these data suggest less genetic than environmental impact on the age of onset of cortisol circadian rhythm.⁸

Recent clinical studies using morning salivary cortisol measurements revealed that there is a weak negative association of cortisol levels with length of gestation, but no relation to birth weight or to systolic or diastolic blood pressure.⁹ In a study enrolling 68 boys and 72 girls aged 7-9 years, birth weight in boys was inversely related to salivary cortisol responses to stress, while in girls morning peak cortisol was inversely related to birth weight.¹⁰ Salivary cortisol measurements can be used in association with the overnight 1 mg dexamethasone suppression test: salivary cortisol was suppressed to less than 100 ng/dL after 1 mg dexamethasone in both control and obese patients in one study.⁶ A negative correlation of morning salivary cortisol concentrations and total cortisol concentration with body mass index was found in children with recurrent abdominal pain of psychosomatic origin in another study.² These authors also found in their cohort of 159 healthy girls and 147 boys that salivary cortisol concentration was dependent on the time of sampling, age and menarche. In our sample of 138 children and 14 adults, cortisol levels were also age-dependent and also positively associated with weight and body mass index while we did not find any degree of sex difference (Kiess et al.). In a cohort of 119 healthy neonates, body mass index, arterial cord blood pH and time of saliva sampling after birth influenced salivary cortisol levels.⁷

At the present time saliva sampling and steroid hormone measurements are still competing with urinary collection and measurement and plasma and serum measurement for bound and free steroid hormones. This is surprising, since there are a number of studies that have already shown superiority of salivary hormone measurements.^{11,12} However, if salivary cortisol collection and analysis is to be used more widely, the following considerations should be taken into account:

- 1) The time of sampling and collection of saliva needs to be standardized.
- 2) Food intake, medications and basal conditions such as sleep, stress, fasting/feeding have to be standardized or documented.
- 3) Appropriate highly specific (immuno)assays have to be employed and reference values for these specific assays have to be made available by each laboratory.

- 4) Quality controls and standard operational procedures (SOPs) have to be established at each center.

While cortisol measurements in saliva are of particular interest for researchers studying stress responses and the hypothalamic-pituitary-adrenal axis, the determination of other steroid hormones in saliva can be used to monitor treatment in endocrine disorders: one of the prime examples of the use of steroid hormone measurements in saliva in this respect is the determination of salivary 17-hydroxyprogesterone, the use of which greatly facilitates treatment of children and adolescents with congenital adrenal hyperplasia.¹³ In summary, the paper by Silva et al. is timely, important and very useful for the practitioner as well as for the researcher in all pediatric subspecialties.

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Breastfeeding and Baby-Friendly Hospital Initiative: more important and with more evidence than ever

Miriam H. Labbok*

Breastfeeding is a primary element in child health and survival, and has been so recognized for millennia. With the commercialization of formula in the 20th century, an unparalleled experiment was about to be visited upon mothers and children. In her treatise on "Milk and murder" in 1939, Dr. Cicely Williams outlined the dangers of disrupting breastfeeding: "If your lives were embittered as mine is, by seeing day after day this massacre of the innocents by unsuitable feeding, then I believe you would feel as I do that misguided propaganda on infant feeding should be punished as the most criminal form of sedition, and that those deaths should be regarded as murder." (Many years later, I was privileged to study with Dr. Williams, which certainly contributed to my understanding of the need for breastfeeding and my dedication to the mother/child dyad.)

Nonetheless, commercial formula manufacture became a successful industry, aggressively marketing to the public, health care providers, nurses and pediatricians, alike. As

women in industrialized settings began to enter the labor market in larger numbers, the market for time-saving commercial food products increased. Formula became known as 'modern', and risks associated with the lack of breastfeeding were compensated for by isolating infants and early weaning. Young women did not grow up seeing breastfeeding, nor learning of it from others. As a result, by mid-century, the maternal skills associated with initiation and maintenance of breastfeeding were in danger of being lost in many settings around the world.

The Baby-Friendly Hospital Initiative (BFHI) came about as the result of several concurrent health and health policy events. Following the endorsement of the International Code of Marketing of Breast-Milk Substitutes in the early 1980s, there was increasing recognition that, after nearly 50 years of heavy formula marketing, health care providers and, often, women themselves no longer retained the skills associated with successful breastfeeding. Mr. James Grant, Director of UNICEF, initiated a tightly directed campaign to reduce child mortality: GOBI – Growth monitoring, oral rehydration, breastfeeding and immunization. Subsequently, the Ten Steps to Successful Breastfeeding were developed by the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) in a very collaborative and comprehensive manner in order to provide a simplified outline for

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* MD, MPH, FACPM, IBCLC, FABM, Professor and Director, Center for Infant and Young Child Feeding and Care, Department of Maternal and Child Health, School of Public Health, University of North Carolina, Chapel Hill, NC, USA.

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