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Authors' reply

We want to thank Dr. Silva Segundo for his interest in our recent paper and for the pertinent review on the utility of atopy patch tests (APT) for the diagnosis of IgE-mediated food allergies. Indeed, there is evidence that APT can be helpful in predicting outcomes of double-blind placebo-controlled food challenges. However, most of such studies have been carried out in children with atopic dermatitis or allergic gastroenteropathies such as food-sensitive eosinophilic esophagitis. The majority of patients seen by pediatric gastroenterologists have food-sensitive enteropathies that are not associated with atopic dermatitis or eosinophilic enteropathies.

Moreover, several limitations exist with APT, even in carefully selected patients, as reviewed recently by the European Academy of Allergy and Clinical Immunology.[1] First and foremost are the limited and highly variable sensitivity and specificity of the APT. In the diagnosis of cow's milk allergy, the mean sensitivity (0.51) and specificity (0.86) of the APT are similar to those of skin prick tests. However, the sensitivity of the APT has been reported to vary enormously, from 0.18 to 0.89.[1]

Numerous factors may explain these limited results with APT. These include the potential lack of standardization of the test conditions: allergen source and concentration, vehicle employed, control material, duration of and material used for occlusion, and size of the chamber. Finally, even though the results of APT may correlate with the outcome of properly conducted food challenges, studies still need be carried out to show that the test results predict the outcome of food elimination diet on gastrointestinal symptoms.

References

1. Turjanmaa K, Darsow U, Niggemann B, Rance F, Vanto T, Werfel T. EAACI/GA2LEN position paper: present status of the atopy patch test. Allergy. 2006;61:1377-84.

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Salivary cortisol to assess the hypothalamic-pituitary-adrenal axis in healthy children under 3 years old

Dear Editor,

In the recent publication by Silva et al.,[1] a mean morning cortisol level of 558 nmol/L (range 77-1,620 nmol/L) in children was reported. This is remarkably higher than any of the previously published ranges for salivary cortisol in children. Although Silva et al. did not report the corresponding serum cortisol levels, their data contradict the notion that only the free cortisol component can pass into saliva, and therefore, under basal conditions, salivary cortisol equates to < 10% of total serum cortisol. Most institutions report morning cortisol levels of a much smaller magnitude, with mean morning salivary cortisol levels of < 30 nmol/L (Table 1).

The authors state that they were unable to locate reference salivary cortisol levels for children in the literature. For readers' information, we have included a summary of the previously published pediatric salivary cortisol literature (Table 1). Our group recently published salivary cortisol reference ranges for healthy children.[8] The range for morning cortisol was 0-25 nmol/L.[8]

Silva et al. used an in-house cortisol radioimmunoassay (RIA), using antibodies to cortisol-3-oxime conjugated with bovine albumin, quoting cross reactivity of 8.5% for cortisone and 7.9% for 11-deoxycortisol. These cross reactivities are higher than in commercially available RIAs. For example, the Orion Diagnostica Spectra Cortisol Coated Tube RIA product information quotes cross reactivity of 0.9% for cortisone and 0.3% for 11-deoxycortisol. However, the increased cross reactivity for cortisone and 11-deoxycortisol cannot explain why the results of Silva et al. are so discrepant from previously published data. This highlights the importance of establishing reference values for all methods and at each institution, as mentioned in the Editorial in the same edition of the journal.[9] However, to avoid misleading clinicians, particularly those who are unfamiliar with salivary cortisol, the au-

Table 1 - Studies of salivary cortisol: normal ranges in infants and children[2-7]

Study	Population	Collection method *(assay used)*	Salivary cortisol *(nmol/L)*	Comments
Maguire et al.[8]	22 healthy controls	Sarsted Salivette & swab *(RIA)*	Range 8 a.m.: 0-25 12 midday: 0-10 8 p.m.: 0-4	Salivary & plasma cortisol were correlated (rho = 0.79, p < 0.0005)
Gröschl et al.[4]	252 children Age 4 days-15 years	Sarsted Salivette & swab *(RIA)*	Mean (SD) 7 am: 24.7 (8.5) 1 pm: 8.0 (4.0) 7 pm: 1.7 (1.4)	No gender differences Age difference in < 1 compared with > 1 year old
Tornhage[7]	386 children Age 7-15 years	Sarsted Salivette & swab *(RIA)*	Boys 8 am: median 8.6 (range 1.5-53.9) Girls 8 am: median 8.8 (range 1.0-33.2)	No gender difference Age and pubertal stage differences noted
Calixto et al.[3]	48 preterm infants Age 26-33 weeks gestation	Citric acid stimulation, saliva aspiration from oral cavity *(RIA)*	Mean, 8-10 am (SD) 27.6 (22.1)	Significant rise in salivary cortisol after ACTH stimulation (peak = 71.8±27.6 nmol/L)
Bettendorf et al.[2]	10 term neonates 10 preterm neonates	Citric acid stimulation, saliva aspiration from oral cavity*(RIA)*	10th, 50th & 90th percentiles Full term =1.9, 6.5, 26.7 Preterm =1.9, 5.5, 13.8	Healthy full terms had higher salivary cortisol than healthy preterm infants
Shimada et al.[6]	35 children age 2.5 years (20 ex-preterms, 15 ex-terms)	Sputum from floor of mouth collected directly into serum tube or plastic pipette. *(ELISA and RIA)*	8 am: 28 4 pm: 11 12 MN: 5.5	Circadian rhythm exhibited Ex preterms develop circadian rhythm in same manner as ex-terms
Kiess & Pfaeffle[9]	138 infants children & adolescents	Sarsted Salivette & swab *(fluorescent immunoassay)*	Age: 8-18 years 8 am: 10.9±5.4 1 pm: 5.0±6.2 6 pm: 3.1±3.2	Circadian rhythm seen after 9 months No sex difference > 6 years old, cortisol varied with BMI & pubertal stage

RIA = radioimmunoassay.

thors could have elaborated on this in their discussion. Would the authors care to comment on the degree of discrepancy between their reported salivary cortisol levels and those previously reported and summarized in Table 1?

References

1. Silva ML, Mallozi MC, Ferrari GF. Salivary cortisol to assess the hypothalamic-pituitary-adrenal axis in healthy children under 3 years old. J Pediatr (Rio J). 2007;83:121-6.
2. Bettendorf M, Albers N, Bauer J, Heinrich UE, Linderkamp O, Maser-Gluth C. Longitudinal evaluation of salivary cortisol levels in full-term and preterm neonates. Horm Res. 1998;50:303-8.
3. Calixto C, Martinez FE, Jorge SM, Moreira AC, Martinelli CE. Correlation between plasma and salivary cortisol levels in preterm infants. J Pediatr. 2002;140:116-8.
4. Gröschl M, Rauh M, Dörr HG. Circadian rhythm of salivary cortisol, 17alpha-hydroxyprogesterone, and progesterone in healthy children. Clin Chem. 2003;49:1688-91.
5. Kiess W, Meidert A, Dressendorfer RA, Schriever K, Kessler U, Konig A, et al. Salivary cortisol levels throughout childhood and adolescence: relation with age, pubertal stage, and weight. Pediatr Res. 1995;37(4 Pt 1):502-6.
6. Shimada M, Takahashi K, Ohkawa T, Segawa M, Higurashi M. Determination of salivary cortisol by ELISA and its application to the assessment of the circadian rhythm in children. Horm Res. 1995;44:213-7.

7. Tornhage CJ. Reference values for morning salivary cortisol concentrations in healthy school-aged children. J Pediatr Endocrinol Metab. 2003;15:197-204.

8. Maguire AM, Ambler GR, Moore B, Waite K, McLean M, Cowell CT. The clinical utility of alternative, less invasive sampling techniques in the assessment of oral hydrocortisone therapy in children and adolescents with hypopituitarism. Eur J Endocrinol. 2007;156:471-6.

9. Kiess W, Pfaeffle R. Steroid analysis in saliva: a noninvasive tool for pediatric research and clinical practice. J Pediatr (Rio J). 2007;83:97-9.

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Authors' reply

The quantitation of salivary cortisol requires adrenal assessment, which can be performed in a practical and unstressful way, thus being suitable for use in pediatric patients.

Salivary cortisol levels can be determined by various highly sensitive and specific methods. In our study, we used the radioimmunoassay (RIA) method without extraction.[1] In this method, after the centrifugation of the saliva samples, the rabbit antibody to cortisol-3-oxime conjugated with bovine albumin produced by Prof. Dr. José Gilberto Vieira (Fleury laboratory) was used, having yielded a sensitivity of up to 5 ng/dL[2]. The analysis was run in duplicate and the efficacy of this method was based on a coefficient of variation below 12 to 15%.[2]

Age plays an important role in the determination of salivary cortisol levels, and several studies have shown discrepant results in this respect.[3,4]

The commitment of authors towards establishing reference values for salivary cortisol levels in healthy children aged less than 3 years arose from the necessity to fill the gap for studies with an adequate number of samples performed on a restricted age range and that require careful sampling.

In this regard, we read with interest the letter from Dr. Ann M. Maguire and Prof. Christopher T. Cowel from The Children's Hospital at Westmead, Sydney and the University of Sydney, Australia, in which they address some aspects related to our paper entitled "Salivary cortisol to assess the hypothalamic-pituitary-adrenal axis in healthy children under 3 years old." Their comments give us the opportunity to broaden the discussion and also clarify some issues that could lead to misinterpretations.

Among the studies mentioned in Table 1 in the letter to the editor written by Dr. Maguire and by Prof. Cowell, we turn our attention to the study by Gröschl et al.,[5] in which 252 healthy and well-nourished children were assessed. By analyzing the sample size related to each of the age ranges (< 1 month, n = 13; 1 to 12 months, n = 17; 1 to 2 years, n = 10; 2 to 15 years, n = 212), we obtained a less-than-80% power among infants, with an alpha error greater than 0.05. In another study, by Kiess et al.,[3] of 138 patients, 10 were aged between 0 and 1 year and 17 were aged between 1 and 4 years.

In our study, for a variance of 95% and error of 1%, the sample size was calculated to be 71, which is a smaller number than that which was used (n = 91).

With regard to the list of studies on salivary cortisol mentioned by Dr. Maguire and by Prof. Cowell, we would like to add studies that used a similar method to ours, such as Fogaça et al.,[6] who assessed 11 children aged 4 to 6 months, and Few et al.[7] In the latter study, the authors assessed 106 children aged 1 to 12 months, with cortisol measurements at the following times of the day: morning = 9:30 to 12 hrs and afternoon = 14 to 16 hrs. These intervals are so close that they certainly hamper the analysis of the circadian rhythm. Another important aspect regarding the time of measurement refers to possible differences in morning and afternoon salivary cortisol levels between infants aged less than 1 year and children aged over 1 year.[3,5] Thus, the study by Törnhage,[8] which only took into consideration the morning cortisol measurement, does not allow assessing any differences across age ranges.

The discrepancy between the values obtained in our study and those mentioned by Dr. Maguire and Prof. Cowell was revised. The results were issued by Fleury Laboratory in ng/dL, and the conversion to nmol/L was based on the value recommended by the Reference Manual for Exams of the Radioimmunoassay Center of São Paulo.[9] We requested that the laboratory provide an explanation, and they informed us that the conversion value stipulated in the manual is incorrect.[10] The measurements were recalculated, and the mean values (±standard error) expected for the morning levels were 5.32±0.36 nmol/L, with a range of 0.73 to 15.46 nmol/L (2.5th to 97.5th percentiles), and 3.30±0.22 nmol/L, with a range of 0.47 to 11.16 nmol/L (2.5th to 97.5th percentiles) for the afternoon levels.

The corrected table and Figures 1 and 2, including the percentile values, appear below.

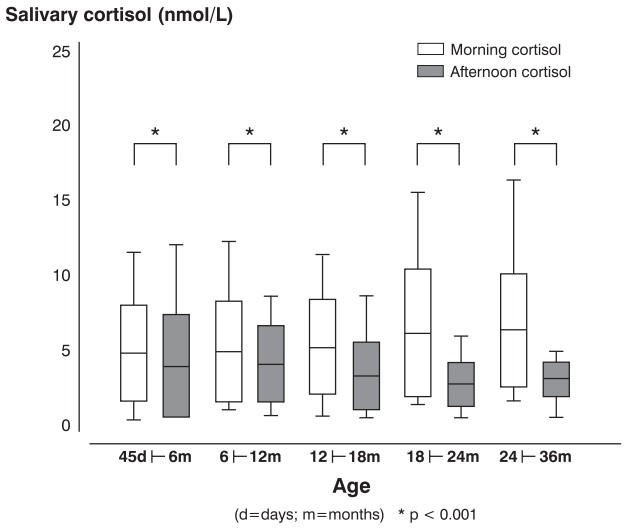

Figure 1 - Morning and afternoon salivary cortisol levels according to age (n = 91) in quartiles

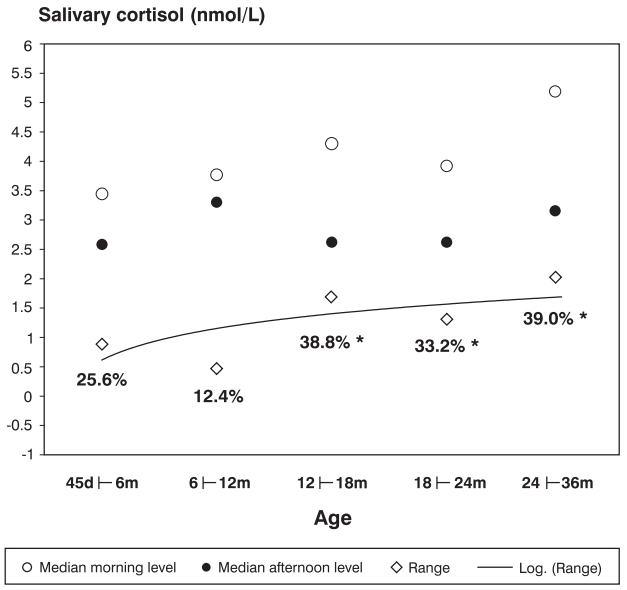

Figure 2 - Percentage variation and tendency in the range between morning and afternoon salivary cortisol levels according to age (n = 91)

Table 1 - Percentile distribution of morning and afternoon salivary cortisol levels in children aged up to 36 months (n = 91)*

Percentiles	Morning salivary cortisol nmol/L	Afternoon salivary cortisol nmol/L
2.5	0.73	0.47
5	1.44	0.63
10	1.95	1.13
15	2.16	1.37
20	2.43	1.67
25	2.75	1.91
30	2.96	2.03
35	3.19	2.39
40	3.80	2.56
45	3.93	2.63
50	4.41	2.79
55	4.95	3.08
60	5.24	3.32
65	5.93	3.56
70	6.40	4.07
75	7.94	4.41
80	9.02	4.82
85	9.96	5.43
90	10.83	6.62
95	12.68	8.59
97.5	15.46	11.16

Mean (± standard error): morning cortisol level: 5.32±0.36 nmol/L; afternoon cortisol level: 3.30±0.22 nmol/L. * Method used: competitive radioimmunoassay using antibodies to cortisol-3-oxime conjugated with bovine albumin.

References

1. Vieira JGH, Noguti KO, Hidal JT, Russo EMK, Maciel RMB. Ensaio do cortisol na saliva como um método para avaliação da fração livre sérica. Arq Bras Endocrinol Metab. 1984;28:8-10.
2. Hanrahan K, McCarthy AM, Kleiber C, Lutgendorf S, Tsalikian E. Strategies for salivary cortisol collection and analysis in research with children. Appl Nurs Res. 2006;19:95-101.
3. Kiess W, Meidert A, Dressendörfer RA, Schriever K, Kessler U, König A, et al. Salivary cortisol levels throughout childhood and adolescence: relation with age, pubertal stage, and weight. Pediatr Res. 1995;37(4 Pt 1):502-6.
4. Törnhage CJ, Alfvén G. Diurnal salivary cortisol concentrations in school-age children: increased morning cortisol concentration and total cortisol concentration negatively correlated to body mass index in children with recurrent abdominal pain of psychosomatic origin. J Pediatr Endocrinol Metab. 2006;19:843-54.
5. Gröschl M, Rauh M, Dorr HG. Circadian rhythm of salivary cortisol, 17 alpha-hydroxyprogesterone, and progesterone in healthy children. Clin Chem. 2003;49:1688-91.
6. Fogaça MC, Carvalho WB, Peres CA, Lora MI, Hayashi LF, Verreschi ITN. Cortisol salivar como indicador da função adrenal em lactentes sadios com massagem terapêutica. Sao Paulo Med J. 2005;123:215-8.
7. Few JD, Mangat TK, Oppe TE, James VH. Saliva aldosterone concentration in healthy infants. Arch Dis Child. 1986;61:508-9.
8. Törnhage CJ. Reference values for morning salivary cortisol concentrations in healthy school-aged children. J Pediatr Endocrinol Metab. 2002;15:197-204.
9. Di Dio R, Barbério JC, Pradal MG, Menezes MA. Procedimentos hormonais: Central de Radioimunoensaio de São Paulo. 3ª ed. São Paulo: CPD CRIESP; 1995.
10. Olesen H, Ibsen I, Bruunshuus I, Kenny D, Dybkaer R, Fuentes-Arderiu X, et al. Properties and Units in the Clinical Laboratory Sciences. Part XII. Properties and Units in General Clinical Chemistry. (IUPAC Technical Report). Pure Appl. Chem. 2000;72:747-972.

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Gamma-hydroxybutyrate for sedation in children

Dear Editor,

We read with interest the article by Mencia et al. on analgesia and sedation in children.[1] In addition to the plethora of drugs discussed by the authors, we would like to add our experience on the use of gamma-hydroxybutyrate (GHB) for sedation in children.[2] GHB was first introduced into clinical anesthesia in 1960. Although it reliably induces sedation without significantly depressing respiratory or cardiocirculatory parameters, it has been unpopular because of its prolonged duration of action. Recent clinical studies suggest a revaluation of its use in critical care medicine and general anesthesia.[3] Clinical trials with GHB-induced sedation in children have shown good results, but so far only limited data are available.[2,4]

In our prospective randomized trial, we showed that GHB induces deep sedation (Ramsay score 5) in children undergoing MRI studies. GHB was associated with vomiting despite the prior administration of an antiemetic. This may in part be attributable to the fact that GHB sedation was used in pediatric cancer patients, making them more prone to this side effect because of concurrent chemo- and radiotherapy. Although none of our GHB-sedated patients aspirated during the study, the physician should be aware of this possibility. Moreover, none of our patients required administration of physostigmine, a short-acting anticholinesterase agent, to treat prolonged sedation.

We conclude that GHB sedation is a reasonable alternative for children undergoing noninvasive diagnostic procedures. Pediatricians that are not familiar with potent short-acting sedative drugs (propofol, remifentanyl, etc.) may consider it for deep sedation in pediatric patients.

References

1. Mencia SB, Lopez-Herce JC, Freddi N. Analgesia and sedation in children: practical approach for the most frequent situations. J Pediatr (Rio J). 2007;83(2 Suppl):S71-82.
2. Meyer S, Gottschling S, Georg T, Lothschutz D, Graf N, Sitzmann FC. Gamma-hydroxybutyrate versus chlorprothixene/phenobarbital sedation in children undergoing MRI studies. Klin Padiatr. 2003;215:69-73.
3. Kleinschmidt S, Schellhase C, Mertzlufft F. Continuous sedation during spinal anaesthesia: gamma-hydroxybutyrate vs. propofol. Eur J Anaesthesiol. 1999;16:23-30.
4. Poschl J, Kolker S, Bast T, Brussau J, Ruef P, Linderkamp O, et al. [Gamma-hydroxybutyric acid sedation in neonates and children undergoing MR Imaging]. Klin Padiatr. 2006;[Epub ahead of print].

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Authors' reply

We read with interest the comments made by Dr. S. Meyer et al.[1] about the use of gamma-hydroxybutyrate (GHB) for sedation in children. We have not had any experience with this sedative drug in children. In the medical literature, there are few references other than these authors to the use of GHB in children. The use of GHB is not included in sedation guidelines for children.[2] It has been unpopular because it induces deep sedation, has prolonged duration of action and is associated with vomiting.

Pediatric sedation practice involves a large number of pediatric subspecialists using a variety of sedation strategies and tools. Most employed drugs are still propofol, midazolam and ketamine, although there are new strategies coming up.[3] The effectiveness and safety of this practice needs careful scrutiny. Recent studies concerning depth of sedation have suggested reconsidering systems that employ moderate sedation for painful procedures in children.

Dexmedetomidine sedation delivered by pediatricians is rapidly increasing and has provided adequate sedation in most children. Dexmedetomidine could be an alternative reliable sedative drug in selected patients because it causes fewer cardiorespiratory effects.[4] Similarly, nitrous oxide for pediatric sedation, while promising, will require careful study