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Circadian rhythm of salivary cortisol in infants with congenital heart disease

Giovanna Capriolo, MD¹, Nancy S. Ghanayem, MD^{1,4}, Kathy Murkowski, BS, RRT, CCRC¹, Melodee L. Nugent, MA², Pippa M. Simpson, PhD², and Hershel Raff, PhD³

¹Department of Pediatrics, Divisions of Critical Care at Medical College of Wisconsin, Milwaukee, Wisconsin

²Department of Pediatrics, Divisions of Quantitative Health Sciences at Medical College of Wisconsin, Milwaukee, Wisconsin

³Department of Medicine, Surgery and Physiology at Medical College of Wisconsin; Children's Hospital of Wisconsin, Endocrine Research Laboratory, Aurora St. Luke's Medical Center, Milwaukee, Wisconsin

⁴Herma Heart Center, Milwaukee, Wisconsin

Abstract

Children with congenital heart disease (CHD) have associated extracardiac co-morbidities at the time of surgery and during ongoing growth and development. Perioperative events include disrupted glucose homeostasis, capillary leak, and fluid retention. The hypothalamic-pituitary-adrenal (HPA) axis has an important role in homeostasis in that the secretion of cortisol contributes to the response to stress, glucose regulation, blood volume control and immune regulation. We investigated the diurnal rhythm of the HPA axis in infants with CHD by measuring salivary cortisol in the morning (0600–0900 hr – circadian peak) and evening (2100–2400 hr – circadian nadir). Twenty-nine infants aged 12 weeks to 1 year were included: 16 with acyanotic disease (SpO₂ ≥90%) and 13 with cyanotic disease (SpO₂<90%). Morning salivary cortisol was similar between the two groups [acyanotic 7.0 nmol/L (1.8–23.1); cyanotic 9.7 nmol/L (0.9–15.6); $p=0.68$]. Evening salivary cortisol was similar between the two groups [acyanotic 0.9 nmol/L (0.2–8.5); cyanotic 1.4 nmol/L (0.5–14.9); $p=0.32$]. Both cyanotic and acyanotic groups demonstrated an intact diurnal rhythm. In conclusion, chronic hypoxia secondary to cyanotic CHD does not affect the circadian rhythm of the HPA axis. By twelve weeks of age, infants with hypoxia secondary to cyanotic CHD have a normal cortisol diurnal rhythm.

Corresponding author: Giovanna Capriolo MD, gcapriolo@sjumed.edu, Telephone (217) 757-6655, Fax: (217) 757-6654, Academic address: Division of Pediatric Critical Care, 414 N. 9th St. Suite 4W64, Springfield, IL. 62769.
G. Capriolo, N. Ghanayem, K. Murkowski, Division of Pediatric Critical Care, Medical College of Wisconsin, 9000 W. Wisconsin Ave., Milwaukee, WI 53226, USA
H. Raff, Aurora St. Luke's Medical Center, Endocrine Research Laboratory, 2801 W KK River Pky Suite 245, Milwaukee, 53215, USA
P. Simpson, M. Nugent, Section of Quantitative Health Science, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, 53226, USA

Keywords

Cortisol; cyanotic; acyanotic; congenital heart disease; hypothalamic-pituitary-adrenal axis; circadian rhythm

Introduction

The incidence of cyanotic congenital heart defects is approximately 1,400 per million live births [1]. Postoperative events following congenital heart surgery with cardiopulmonary bypass can include disrupted glucose homeostasis, capillary leak, fluid retention and infection [2]. With advances in surgical and medical management, the number of infants surviving through childhood has improved [3]. However, these children may develop morbidities including growth failure and cognitive delays, which are both more pronounced in children with cyanotic congenital heart disease [4, 5, 6, 7].

The hypothalamic-pituitary-adrenal (HPA) axis has a critical role in homeostasis. With cortisol as its principal end product, the HPA axis participates in the response to stress, glucose regulation, blood volume and pressure control, and immune regulation. An adequate response of the HPA axis is important in the adaptation to hypoxia [8].

Sustained increases in cortisol levels can affect multiple organ systems. Hypertension is common in individuals with hypercortisolism, and may lead to progressive heart failure. Increased cortisol can suppress immune function and increase susceptibility to infections. In children with increased cortisol, growth hormone secretion and linear growth are impaired, leading to short stature. Other effects can include hyperglycemia and osteoporosis [9].

An increase in endogenous glucocorticoid production from the adrenal cortex has been found during hypoxia in animal models of cyanotic heart disease [10, 11, 12, 13, 14, 15]. Understanding the effect that hypoxia has on the HPA axis may lead to a better understanding of the response of cyanotic children to stressors like surgery and illness, and may also suggest ways to improve therapy.

In healthy infants, the circadian rhythm of the HPA axis, as assessed by measuring salivary cortisol in the morning and evening, is established and maintained as early as 8 weeks of age [16]. The measurement of salivary cortisol has been extensively validated as a highly reliable and reproducible surrogate for biologically active serum free cortisol [17, 18, 19]. Therefore, the current study measured salivary cortisol at the circadian nadir and peak in infants between 3 months and 1 year of age with hypoxia secondary to cyanotic heart disease compared to a group with acyanotic heart disease. We hypothesized that hypoxia disrupts the normal rhythm of the HPA axis and causes an increase in salivary cortisol levels.

Material and Methods

This is a prospective observational study of patients with congenital heart disease treated at Children's Hospital of Wisconsin. The Institutional Review Board approved this study and

written informed parental consent was obtained. Infants between 12 weeks and 1 year of age with acyanotic and cyanotic congenital heart disease were eligible for entry into this study. Cyanosis was defined as arterial saturation measured by pulse oxymetry (SpO₂) less than 90%; acyanotic patients had SpO₂ greater than or equal to 90%. Infants were excluded if they had known endocrine dysfunction, if they received any type of steroid therapy from two weeks prior to obtaining informed consent to the day of sampling, if the primary cardiologist advised that patient not to be approached, or if parents were non-English speaking due to inability to obtain informed consent.

To minimize exogenous stress, saliva samples were collected by parents at home and returned for analysis by postal service. This method of obtaining samples has been shown to be highly reliable with no loss of cortisol in the sample [20]. Saliva was sampled just before feeding or not less than 1 hour after the previous feeding. Saliva samples were collected from the floor of the mouth using Sorbettes (WECK-CEL, Waltham, MA). Two samples were collected from each subject: one between 0600–0900 hr. (circadian peak), and another between 2100–2400 hr. (circadian nadir). Salivary cortisol concentration was measured using an enzyme immunoassay [21].

Clinical data was collected through chart review and included age, sex, weight, length, arterial saturations measured by pulse oximetry, cardiac diagnosis, current and previous medications and surgical history.

Statistical analysis was performed with Fisher exact test to compare categorical variables. Mann-Whitney test was performed to compare continuous variables. A *p*-value of <0.05 was considered significant.

Results

Forty-eight patients qualified for inclusion in this study (Figure 1). Five patients declined participation and three were excluded at the suggestion of the primary cardiologist who deemed the patient a poor candidate for study due to complicated medical care. After obtaining informed consent, 40 subjects were enrolled (acyanotic *n*=24; cyanotic *n*=16). Samples from 11 subjects (8 acyanotic and 3 cyanotic) were not analyzed. Of the 8 acyanotic subjects, 5 did not return saliva samples, 1 had oral aversion and did not tolerate the Sorbettes in his mouth, 1 sample did not contain enough saliva to analyze and 1 sample was contaminated with steroid cream (mother's topical medication). Three cyanotic subjects did not return samples. The return rate of saliva samples from consented subjects was 29/40 (73%): 16 acyanotic (9 male/7 female) and 13 cyanotic (8 male/5 female).

Patient demographics and clinical characteristics are shown in Table 1. The age at the time of sampling, weight percentile, and height percentile were similar between the two groups. The median RACHS (Risk Adjustment for Congenital Heart Surgery) score was 2 for both groups [22].

Salivary cortisol (SalCort) levels are shown in Figure 2. The AM SalCort levels were similar between groups: acyanotic 7.0 nmol/L (1.8–23.1 nmol/L) compared to cyanotic 9.7 nmol/L (0.9–15.6 nmol/L) *p*=.68. The PM SalCort levels were also similar between groups:

acyanotic 0.9 nmol/L (0.2–8.5 nmol/L) compared to cyanotic 1.4 nmol/L (0.5–14.9 nmol/L); $p=.32$. There were outliers and extreme values in both the acyanotic and cyanotic groups. SalCort levels considered to be outliers and extreme values did not correlate with the degree of hypoxia. In the cyanotic group, the extreme SalCort level of 14.9 nmol/L occurred in a subject with an SpO₂ of 86%, and the SalCort level of 5.3 nmol/L was in a subject with an SpO₂ of 78%. In the acyanotic group, the extreme value was observed in a subject with an SpO₂ of 98%. Both cyanotic and acyanotic groups demonstrated higher AM SalCort compared to PM SalCort indicating an intact HPA axis circadian rhythm. The ratio of AM:PM salivary cortisol, an index of intact diurnal rhythmicity [23], was not different between groups: 5.0 (3.0–15.4) in the acyanotic group and 6.7 (1.2–9.8) in the cyanotic group ($P=0.64$). Two acyanotic and four cyanotic subjects had an abnormal salivary cortisol diurnal rhythm (AM:PM ratio <2 ; $P=0.36$). Pharmacologic therapies for study subjects at the time of sample collection are listed in Table 2. More subjects in the cyanotic group were treated with ACE inhibitors ($p=0.03$). Subjects in the cyanotic group tended to have congenital heart surgery more frequently (69% versus 38%) prior to sampling, but this was not statistically significant ($p=0.13$).

Discussion

We did not demonstrate a difference in salivary cortisol levels between subjects with cyanotic and acyanotic CHD. By 12 weeks of age, both groups demonstrated an AM salivary cortisol level that was appropriately higher than the PM salivary cortisol level, indicating that chronic hypoxia secondary to CHD did not affect the development of an intact circadian rhythm.

Hospitalization and cardiac surgery can disrupt cortisol levels [24, 25]. Many subjects enrolled in our study underwent surgical repair or palliation prior to enrollment. In order to minimize the effect of hospitalization and surgery as a potential cause for disrupted salivary cortisol levels, we waited at least 3 weeks after the last surgical intervention before obtaining saliva samples. Santiago and colleagues demonstrated healthy infants as young as 2 weeks of age develop a cortisol circadian rhythm; cortisol circadian rhythm emerged in the group as a whole at a mean age of 8 weeks [16].

To our knowledge, this is the first study that evaluated salivary cortisol levels in infants with chronic hypoxia. Zayour et al. placed newborn Sprague-Dawley rat pups in a normobaric hypoxic environment with oxygen levels maintained at 10% for up to 8 weeks of age as a way to mimic cyanotic heart disease, and found that both 4 and 8 week old rats had increased corticosterone [15].

Our original hypothesis was that hypoxia would lead to a disrupted HPA axis and cause increased salivary cortisol levels, particularly in the evening at the circadian nadir. Infants with chronic hypoxia secondary to cyanotic CHD did not have a disrupted HPA axis as has been observed in animal models. Possibly the degree of hypoxia observed in this cohort was not as severe as in animal studies. Raff and colleagues studied the interaction between graded acute hypoxia and corticosteroids in non-surgically stressed, pentobarbital-anesthetized, galamine-paralyzed ventilated dogs and found that only severe hypoxia (PaO₂:

27 torr, SaO₂: 55%) produced a large increase in corticosteroids, and moderate hypoxia (PaO₂: 38 torr, SaO₂: 70%) produced a small increase in corticosteroids [26]. This level of hypoxia has been observed in infants with cyanotic heart disease; however, their PaO₂ is most commonly reported between 40–50 mmHg [27, 28].

An additional mechanism for the apparently normal HPA axis in our study cohort is potential acclimatization to hypoxia in infants with cyanotic CHD, similar to changes observed after acclimatization to high altitude hypoxia studied previously. Cortisol secretion rates were the same in high altitude natives when compared to men that lived at sea level [29]. Moncloa and colleagues measured cortisol secretion in 10 young adult males exposed to 4300 meters (PaO₂: 47; SaO₂: 82%) of altitude for 2 weeks. Cortisol levels were increased during the first few days of exposure to high altitude; however, by the 14th day, cortisol level returned to baseline levels [30]. Larsen and colleagues measured cortisol levels in 8 men that were taken from sea level to an altitude of 4559 m above sea level. For this group of subjects, cortisol tended to increase in response to hypoxia (day 2), but after prolonged (day 7) hypoxia, cortisol concentrations were not different from sea level values [31].

Limitations for our study included a small sample size, which may have contributed to the lack of a difference in salivary cortisol levels between the two groups. Depending on the assumptions made, a minimum of 60 patients (PM levels) would be needed to observe a statistically significant difference between groups.

We conclude that chronic hypoxia secondary to cyanotic CHD does not affect diurnal cortisol levels or the circadian rhythm of the HPA axis in 3–12 month old infants. By twelve weeks of age, infants with hypoxia secondary to cyanotic CHD have a normal HPA axis diurnal rhythm.

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References

1. Hoffman JI, Kaplan S. The incidence of congenital heart disease. *J Am Coll Cardiol*. 2002 Jun 19; 39(12):1890–900. [PubMed: 12084585]
2. Hamada Y, Kawachi K, Tsunooka N, Nakamura Y, Takano S, Imagawa H. Capillary leakage in cardiac surgery with cardiopulmonary bypass. *Asian Cardiovasc Thorac Ann*. 2004 Sep; 12(3):193–7. [PubMed: 15353454]
3. Boneva RS, Botto LD, Moore CA, Yang Q, Correa A, Erickson JD. Mortality associated with congenital heart defects in the United States: trends and racial disparities. *Circulation*. 2001 May 15; 103(19):2376–81. [PubMed: 11352887]
4. Feldt RH, Ewert JC, Stickler GB, Weidman WH. Children with congenital heart disease. Motor development and intelligence. *Am J Dis Child*. 1969 Mar; 117(3):281–7. [PubMed: 5765145]
5. Silbert A, Wolff PH, Mayer B, Rosenthal A, Nadas AS. Cyanotic heart disease and psychological development. *Pediatrics*. 1969 Feb; 43(2):192–200. [PubMed: 5763857]

6. Aisenberg RB, Rosenthal A, Nadas AS, Wolff PH. Developmental delay in infants with congenital heart disease. Correlation with hypoxemia and congestive heart failure. *Pediatr Cardiol.* 1982; 3(2): 133–7. [PubMed: 6185932]
7. Samango-Sprouse C, Suddaby EC. Developmental concerns in children with congenital heart disease. *Curr Opin Cardiol.* 1997 Jan; 12(1):91–8. [PubMed: 9132087]
8. Kodama T, Shimizu N, Yoshikawa N, et al. Role of the glucocorticoid receptor for regulation of hypoxia-dependent gene expression. *J Biol Chem.* 2003 Aug 29; 278(35):33384–91. Epub 2003 Jun 16. [PubMed: 12810720]
9. Kliegman, et al. *Nelson Textbook of pediatrics.* 18th. Saunders Elsevier; 2007. p. 2352–2353.
10. Bruder ED, Taylor JK, Kamer KJ, Raff H. Development of the ACTH and corticosterone response to acute hypoxia in the neonatal rat. *Am J Physiol Regul Integr Comp Physiol.* 2008 Oct; 295(4):R1195–203. Epub 2008 Aug 13. [PubMed: 18703410]
11. Raff H, Lee JJ, Widmaier EP, Oaks MK, Engeland WC. Basal and adrenocorticotropin-stimulated corticosterone in the neonatal rat exposed to hypoxia from birth: modulation by chemical sympathectomy. *Endocrinology.* 2004 Jan; 145(1):79–86. Epub 2003 Oct 2. [PubMed: 14525916]
12. Fujimori K, Takanashi A, Endo C, Sato A. Stress hormone responses during 24-hour hypoxemia in preterm goat fetus. *Tohoku J Exp Med.* 2008 Jun; 215(2):189–97. [PubMed: 18577848]
13. Raff H, Jacobson L, Cullinan WE. Augmented hypothalamic corticotrophin-releasing hormone mRNA and corticosterone responses to stress in adult rats exposed to perinatal hypoxia. *J Neuroendocrinol.* 2007 Nov; 19(11):907–12. [PubMed: 17927669]
14. Raff H, Jacobson L, Cullinan WE. Elevated corticosterone and inhibition of ACTH responses to CRH and ether in the neonatal rat: effect of hypoxia from birth. *Am J Physiol Regul Integr Comp Physiol.* 2003 Nov; 285(5):R1224–30. Epub 2003 Jul 10. [PubMed: 12855418]
15. Zayour D, Azar ST, Azar N, et al. Endocrine changes in a rat model of chronic hypoxia mimicking cyanotic heart disease. *Endocr Res.* 2003 May; 29(2):191–200. [PubMed: 12856806]
16. Santiago LB, Jorge SM, Moreira AC. Longitudinal evaluation of the development of salivary cortisol circadian rhythm in infancy. *Clin Endocrinol (Oxf).* 1996 Feb; 44(2):157–61. [PubMed: 8849569]
17. Gozansky WS, Lynn JS, Laudenslager ML, Kohrt WM. Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic–pituitary–adrenal axis activity. *Clin Endocrinol (Oxf).* 2005 Sep; 63(3):336–41. [PubMed: 16117823]
18. Raff H. Salivary cortisol and the diagnosis of Cushing’s syndrome: a coming of age. *Endocrine.* 2012 Jun; 41(3):353–4. [PubMed: 22457047]
19. Belaya ZE, Iljin AV, Melnichenko GA, et al. Diagnostic performance of late-night salivary cortisol measured by automated electrochemiluminescence immunoassay in obese and overweight patients referred to exclude Cushing’s syndrome. *Endocrine.* 2012 Jun; 41(3):494–500. Epub 2012 Mar 25. [PubMed: 22447310]
20. de Weerth C, Jansen J, Vos MH, Maitimu I, Lentjes EG. A new device for collecting saliva for cortisol determination. *Psychoneuroendocrinology.* 2007 Sep–Nov; 32(8–10):1144–8. Epub 2007 Sep 11. [PubMed: 17850983]
21. Raff H, Homar PJ, Skoner DP. New enzyme immunoassay for salivary cortisol. *Clin Chem.* 2003 Jan; 49(1):203–4. [PubMed: 12507989]
22. Jenkins KJ, Gauvreau K, Newburger JW, Spray TL, Moller JH, Iezzoni LI. Consensus-based method for risk adjustment for surgery for congenital heart disease. *J Thorac Cardiovasc Surg.* 2002 Jan; 123(1):110–8. [PubMed: 11782764]
23. Raff H, Raff JL, Findling JW. Late-night salivary cortisol as a screening test for Cushing’s syndrome. *J Clin Endocrinol Metab.* 1998 Aug; 83(8):2681–6. [PubMed: 9709931]
24. Scheer FA, Van Paassen B, Van Montfrans GA, et al. Human basal cortisol levels are increased in hospital compared to home setting. *Neurosci Lett.* 2002 Nov 22; 333(2):79–82. [PubMed: 12419485]
25. Plumpton KR, Anderson BJ, Beca J. Thyroid hormone and cortisol concentrations after congenital heart surgery in infants younger than 3 months of age. *Intensive Care Med.* 2010 Feb; 36(2):321–8. Epub 2009 Oct 7. [PubMed: 19809804]

26. Raff H, Shinsako J, Keil LC, Dallman MF. Vasopressin, ACTH, and corticosteroids during hypercapnia and graded hypoxia in dogs. *Am J Physiol.* 1983 May; 244(5):E453–8. [PubMed: 6303130]
27. Jones RW, Baumer JH, Joseph MC, Shinebourne EA. Arterial oxygen tension and response to oxygen breathing in differential diagnosis of congenital heart disease in infancy. *Arch Dis Child.* 1976 Sep; 51(9):667–673. [PubMed: 999325]
28. Schmitt HJ, Schuetz WH, Proeschel PA, Jaklin C. Accuracy of pulse oximetry in children with cyanotic congenital heart disease. *J Cardiothorac Vasc Anesth.* 1993 Feb; 7(1):61–5. [PubMed: 8431577]
29. Moncloa F, Pretell E. Cortisol secretion rate, ACTH and methopyrapone tests in high altitude native residents. *J Clin Endocrinol Metab.* 1964 Sep; 24:915–8. [PubMed: 14217911]
30. Moncloa F, Donayre J, Sobrevilla LA, Guerra-García R. Endocrine studies at high altitude. II. Adrenal cortical function in sea level natives exposed to high altitudes (4300 meters for two weeks). *J Clin Endocrinol Metab.* 1965 Dec; 25(12):1640–2. [PubMed: 5845863]
31. Larsen JJ, Hansen JM, Olsen NV, Galbo H, Dela F. The effect of altitude hypoxia on glucose homeostasis in men. *J Physiol.* 1997 Oct 1; 504(Pt 1):241–9. [PubMed: 9350634]

Figure 1.
Flowchart outlining the inclusion of patients in this study

Figure 2.
Salivary cortisol levels

Table 1

Subject demographics

	Acyanotic (n=16)	Cyanotic (n=13)	p- value
Age in months	6.3 (3.8–9.5)	7.0 (3.5–12.0)	0.8
Weight percentile	32 (0–94)	22 (1–90)	0.2
Length percentile	54 (0–93)	59 (6–95)	0.7
RASCH score	2 (1–6)	2 (2–6)	0.9

Data presented as Median with Range

Table 2

Treatment medications

	Acyanotic (n=16)	Cyanotic (n=13)	<i>p</i> - value
ACE Inhibitor	3 (18.8)	8 (61.5)	0.027
Beta Blocker	2 (12.5)	2 (15.4)	0.99
Digoxin	1 (6.2)	4 (30.8)	0.14
Calcium Channel Blocker	0 (0)	2 (15.4)	0.19
Diuretic	3 (18.8)	3 (23.1)	0.99
Clonidine	0 (0)	1 (7.7)	0.44
Anticoagulant	5 (31.2)	8 (61.5)	0.14
Proton pump inhibitor	0 (0)	2 (15.4)	0.19
Histamine 2 Blocker	2 (12.5)	4 (30.8)	0.36

Values are presented in n (% of subjects taking medication)