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Cortisol in deciduous tooth tissues: A potential metric for assessing stress exposure in archaeological and living populations

Leslie Quade [a,*], Miroslav Králík [a], Petra Bencúrová [b], Erin C. Dunn [c]

[a] Department of Anthropology, Faculty of Science, Masaryk University, Kotlářská 267/2, Brno 61137, Czech Republic
[b] Central European Institute of Technology (CEITEC), Masaryk University, Žerotínovo nám. 617/9, Brno 60177, Czech Republic
[c] Massachusetts General Hospital and Harvard Medical School, 55 Fruit St, Boston, MA 02114, United States

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ABSTRACT

Objective: Cortisol is a glucocorticoid hormone produced by the hypothalamic-pituitary-adrenal axis that is regularly assessed in modern human and non-human populations in saliva, blood, and hair as a measure of stress exposure and stress reactivity. While recent research has detected cortisol concentrations in modern and archaeological permanent dental tissues, the present study assessed human primary (deciduous) teeth for cortisol concentrations.
Materials and Methods: Fifty-one dentine and enamel samples from nine modern and 10 archaeological deciduous teeth were analyzed for cortisol concentrations via enzyme-linked immunosorbent assay (ELISA).
Results: Detectable concentrations of cortisol were identified in 15 (of 32) dentine and 8 (of 19) enamel samples coming from modern and archaeological deciduous teeth.
Conclusions: This study is the first known analysis of cortisol from deciduous dental tissues, demonstrating the potential to identify measurable concentrations.
Significance: The ability to analyze deciduous teeth is integral to developing dental cortisol methods with multiple potential future applications, including research on the biological embedding of stress in the skeleton. This study marks a key step in a larger research program to study stress in primary dentition from living and archaeological populations.
Limitations: Multiple samples generated cortisol values that were not detectable with ELISA. Minimum quantities of tissue may be required to generate detectable levels of cortisol.
Suggestions for Further Research: Future research should include larger sample sizes and consideration of intrinsic biological and extrinsic preservation factors on dental cortisol. Further method validation and alternative methods for assessing dental cortisol are needed.

1. Introduction

The influence of stress on health and well-being is an integral research theme in palaeopathology, as well as clinical, biological, and health sciences, often forming the foundation of investigations into the detrimental impact of social inequalities (Ford et al., 2016; Goodman et al., 1984; Goodman and Leatherman, 1998; Larsen, 1997; McDade, 2002; Reitsema and McIlvaine, 2014; Schreier and Evans, 2003; Temple and Goodman, 2014). Cortisol is a key biomarker of stress, being one of the primary hormones produced in response to psychosocial, physiological, and environmental stressors (Charmandari et al., 2005). Cortisol concentrations from bodily fluids (e.g., saliva, blood) and hair are regularly tested in modern human and animal populations as a measure of stress levels (Bozovic et al., 2013; Fischer et al., 2017; Gow et al., 2010; Kambalimath et al., 2010; Lee et al., 2015; Novak et al., 2013; Preis et al., 2019; Šušoliaková et al., 2018; Van Uum et al., 2008). However, studies of stress in past populations and across time have been limited by methodological constraints. For example, in archaeological settings, often only skeletonized individuals are available for analysis. In living populations, retrospective analysis of cortisol is restricted to hair, which may be limited by sample availability and length (Ford et al., 2016; Romero-Gonzalez et al., 2021).

Recently, cortisol concentrations were obtained from permanent tooth structures from modern (Nejad et al., 2016) and archaeological

* Corresponding author.
E-mail addresses: lesliequade9@gmail.com, quade@sci.muni.cz (L. Quade).

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contexts (Quade et al., 2021). However, further investigation is necessary to develop this new methodology and appropriately interpret dental cortisol concentrations. Although as yet untested, there are many potential benefits to examining cortisol in deciduous teeth. Deciduous dental cortisol (DDC) from archaeological contexts could provide productive comparisons with other skeletal stress markers (e.g., dental enamel hypoplasia), or reveal differences in stress experience and frailty not currently accessible through non-destructive methods (Aucott et al., 2008; Baylis et al., 2013; Temple and Goodman, 2014; Wood et al., 1992). Because free cortisol in the bloodstream is hypothesized to be incorporated into tooth structures during tissue development (Balíková, 2005; Camann et al., 2013; Cattaneo et al., 2003; Cippitelli et al., 2018; Gow et al., 2010; Sharpley et al., 2012), DDC could reflect stress exposure during the intrauterine period and early infancy, a critical and sensitive period in development (AlQahtani et al., 2010; Brickley et al., 2020; Camann et al., 2013; Davis et al., 2020; Dunn et al., 2022). Further, DDC has the potential to link paleopathology with studies of stress in living populations, providing new ways to understand the skeletal embodiment of stress. Deciduous teeth from living populations are comparatively abundant and can be ethically sourced (Buikstra et al., 2022; Squires et al., 2022, 2019) from willing and informed donors. Access to larger numbers of teeth permits further testing and validation of the method, which are crucial for advancing the technique. Additionally, living donors can provide relevant demographic and contextual information, including histories of stressful life events.

In this pilot study, we assess human deciduous teeth from living and archaeological populations for cortisol concentrations for the first time, using methods previously applied to permanent teeth (Quade et al., 2021). Our primary aim is to identify if it is possible to detect cortisol concentrations from deciduous teeth, establishing the groundwork for future research.

2. Materials and methods

2.1. Materials

Six living individuals from the Czech Republic donated nine deciduous teeth. Three individuals provided two teeth each, which were used to test intraindividual differences in DDC concentrations (Table 1).

Archaeological teeth came from the 11th-12th-century cemetery known as 'Brno-Vídeňská Street' (modern Czech Republic) (Černá and Sedláčková, 2016) because of the relatively high number of non-adults with deciduous teeth available for analysis. Ten deciduous teeth were selected from nine non-adults. Skeletal sex was not estimated. In one individual (5813), two teeth were selected for analysis. For individual 4890, the extracted enamel was divided into two samples as a preliminary test for consistency in detection methods.

This research was conducted in accordance with the Helsinki Declaration. Living participants provided written informed consent for their teeth to be used in these analyses and all data were stored in compliance with General Data Protection Regulation standards (Masaryk University Research Ethics committee reference number EKV-2021–103).

2.2. Methods

2.2.1. Tooth sampling

Dentine (D) and enamel (E) samples were extracted from 19 teeth, coming from 15 individuals (Table 1). Circumpulpal dentine (CD) (dentine immediately surrounding the pulp chamber) (Montgomery, 2002) was also extracted and tested for cortisol concentrations (13 samples). It was not always possible to test all tissue types from each tooth due to insufficient or unsuitable sample quality. Each tooth was assessed macroscopically for stage of mineralization (AlQahtani et al., 2010) and signs of pathology (Brothwell, 1981; Hillson, 1996). Only teeth free from visible pathological lesions or wear that exposed the dentine were selected. The entire crown of each selected tooth was utilized, yielding the maximum possible tissue mass, and ensuring the highest likelihood of generating detectable levels of cortisol. The utilization of tissues from different tooth types resulted in variable sample masses, ranging from 2.3 to 297.2 milligrams. Results are reported as initial calculated cortisol concentrations (μg/dL) and as concentrations divided by the respective sample mass to account for these differences, rendering the data comparable.

Selected teeth were photographed (BABAO Working Group for Ethics and Practice, 2019) (Fig. 1), chemically and mechanically cleaned before bisection from crown to root apices. Dental tissues were sequentially removed using a micromotor drill, beginning with circumpulpal dentine, represented by a layer of approximately 0.5 mm of dentine surrounding the crown pulp chamber, followed by dentine and enamel. All tools and surfaces were cleaned between sample preparation to prevent cross contamination (Quade et al., 2021). Each sampled tissue was ground into a powder and placed in 1 mL of methanol for 24 h to extract the cortisol. After extraction, the samples were dehydrated and frozen until the day of analysis via ELISA.

2.2.2. Cortisol analysis

Two competitive ELISA salivary cortisol kits by Salimetrics (USA) were used to quantify the cortisol concentrations in the tooth dentine and enamel. Although no kit has been manufactured to test cortisol in mineralized tissues, salivary kits have been successfully used in modern and archaeological hair and permanent teeth. Future research is needed to investigate any potential error this may introduce. The ELISA kit was run according to the manufacturer's instructions and wells were read in a Tecan Sunrise™ ELISA plate reader at 450 nm with a secondary filter correction at 490 nm. A standard curve was generated for each kit based on standards and controls, and fourth order polynomial curve fit regressions were produced to define the cortisol concentrations within each sample.

3. Results

Detectable concentrations of cortisol were identified in 23 out of 51 samples (45%), with measurable results coming from both modern and archaeological deciduous tooth tissues (Figs. 2–3; Tables 2–3). The remaining 28 samples generated cortisol values below the ELISA kit's minimum detection threshold (0.007 μg/dL), meaning they could not be quantified within the parameters of the assay.

A larger percentage of archaeological samples generated results with detectable levels of cortisol than modern samples (63% versus 25%) (Fig. 2). When detectable in modern samples, cortisol concentrations were more variable, especially in relation to sample mass (Fig. 3).

Cortisol was detected in 17 archaeological samples (CD-5; D-5; E-7), coming from different individuals and teeth. Six modern samples (CD-2; D-3; E-1) generated results detectable within the assay (Table 2).[1] Differences between circumpulpal dentine, dentine, and enamel cortisol concentrations were not formally evaluated due to small sample sizes.

In individual 4890, whose enamel was divided into two separately tested samples, there was consistency in the calculated cortisol concentrations (0.00026; 0.00023 μg/dL/Sample Mass) (Table 3). Four of the detectable modern samples came from individual 3937 in multiple tissues, where cortisol concentrations ranged from 0.00028 (D) to 0.00709 (E).

[1] In archaeological samples, the lowest sample masses to yield detectable cortisol concentrations were CD= 15.7 mg, D= 80.5 mg; E = 29 mg. In modern teeth, samples as low as CD= 6.1 mg, D= 12.5 mg; E = 13.2 mg yielded detectable cortisol concentrations.

Table 1
Number of individuals, teeth, and tissue samples included in this analysis.

Site	Dating	Individuals	Teeth	Circumpulpal Dentine Samples	Dentine Samples	Enamel Samples	Total Samples
Brno-Vídeňská Street	11th-12th c.	9	10	7	10	10	27
Modern	-	6	9	6	9	9	24
Total	-	15	19	13	19	19	51

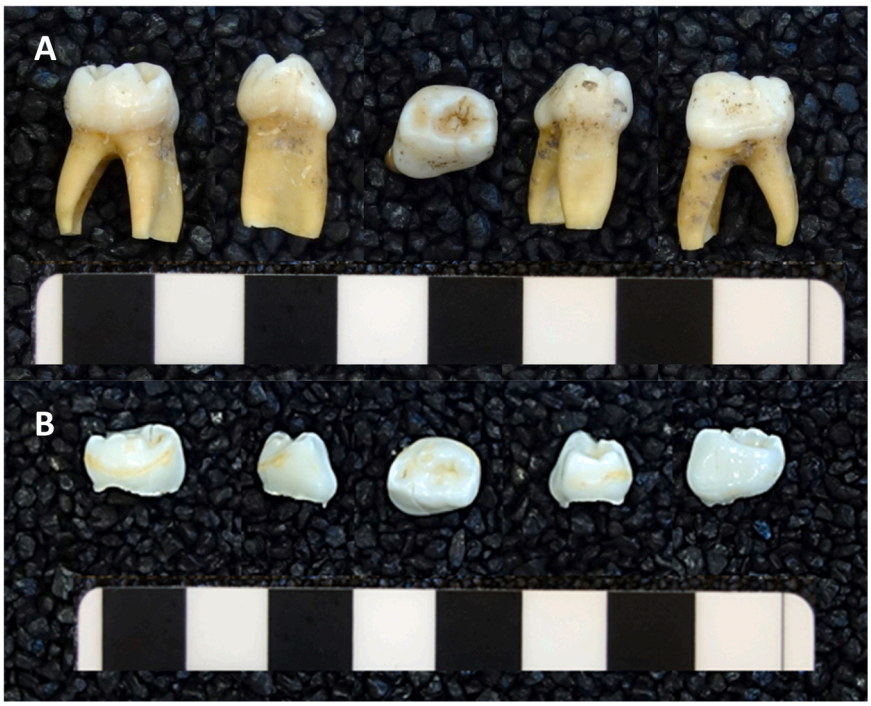

Fig. 1. A. Individual 4813, Brno-Vídeňská Street. Five surfaces of the 1st left mandibular deciduous molar; B. Individual 9738, Modern. Five surfaces of the 1st left mandibular deciduous molar.

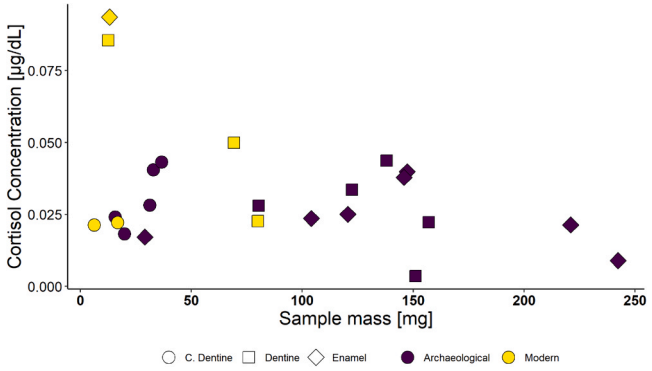

Fig. 2. Samples with detectable levels of cortisol.

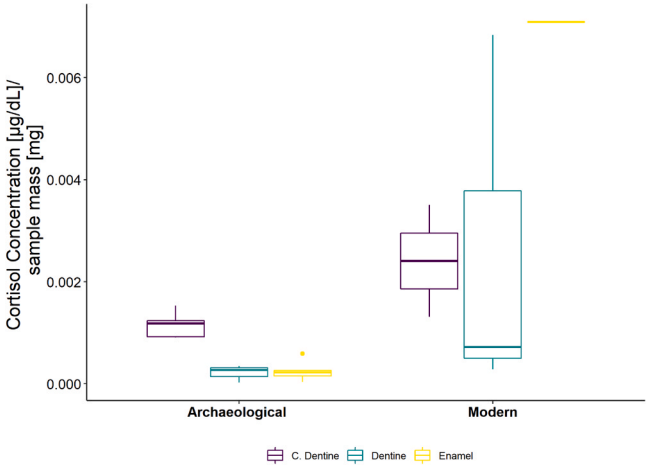

Fig. 3. Box and whisker plot of samples with detectable levels of cortisol, adjusting for different sample mass. The line in this plot is the sample median, the box represents the second and third quartiles, and whiskers are the maximum and minimum values, not including outliers. Additional dots are outliers.

4. Discussion

This study demonstrates that it is possible to detect cortisol from deciduous dental tissues in some individuals using methods previously tested in permanent teeth. Archaeological teeth more consistently yielded detectable cortisol results than modern teeth, though cortisol values were higher overall in modern samples. Future studies, particularly with directly comparable datasets and life history measures, can help reveal the full extent to which deciduous teeth record cortisol exposure. To that end, we interpret our results and encourage research in several specific areas.

First, baseline levels of cortisol within dental tissues are unknown. Although cortisol can/should reach every tissue (Beisel et al., 1964; Dallman and Hellhammer, 2011; Kudielka and Kirschbaum, 2005), cortisol may only be detectable in teeth when a person is exposed to a

Table 2
Dental cortisol concentration results.

Site	Ind.	Sex	Tooth	Circumpulpal Dentine (CD)			Dentine (D)			Enamel (E)		
				Smp Mass mg	Cortisol µg/dL	Cortisol µg/dL /Smp Mass	Smp Mass mg	Cortisol µg/dL	Cortisol µg/dL /Smp Mass	Smp Mass mg	Cortisol µg/dL	Cortisol µg/dL /Smp Mass
Brno-Vídeňská	3000		ulm2 (65)	12.5	ND^{+}	-	122.4	0.0336	0.00027	147.4	0.0398	0.00027
Brno-Vídeňská	4813		llm1 (74)	-	-		80.5	0.0281	0.00035	120.6	0.0250	0.00021
Brno-Vídeňská*	4890		urm2 (55)	32.8	0.0405	0.00124	156.9	0.0223	0.00014	145.8	0.0379	0.00026
				-	-		-	-	-	104.0	0.0236	0.00023
Brno-Vídeňská	4892		urm2 (55)	15.7	0.0241	0.00153	168.6	ND	-	173.8	ND	-
Brno-Vídeňská	4895		ulm2 (65)	36.5	0.0432	0.00118	138.1	0.0437	0.00032	242.5	0.0090	0.00004
Brno-Vídeňská	4897		urm2 (55)	19.8	0.0182	0.00092	151.1	0.0035	0.00002	221.1	0.0214	0.00010
Brno-Vídeňská	5802		uli1 (61)	2.3	ND^{+}	-	26.1	ND^{+}	-	-	-	-
Brno-Vídeňská	5804		uli1 (61)	-	-		67.3	ND^{+}	-	45.5	ND^{+}	-
Brno-Vídeňská	5813		urm2 (55)	31.1	0.0282	0.00091	117.7	ND	-	126.4	ND	-
Brno-Vídeňská	5813		uri1 (51)	-	-	-	52.4	ND^{+}	-	29	0.0171	0.00059
Detectable					**5**			**5**			**7**	
Total	**9**		**10**		**7**			**10**			**10**	
Modern	3881	F	lrc (83)	-	-	-	69.1	0.0499	0.00072	48.9	ND^{+}	-
Modern	3937	F	uli1 (61)	6.1	0.0214	0.00350	12.5	0.0855	0.00684	13.2	0.0935	0.00709
Modern	3937	F	uri1 (51)	-	-	-	80.1	0.0227	0.00028	74.3	ND^{+}	-
Modern	7650	M	ulm1 (64)	-	-	-	94	ND^{+}	-	116.1	ND	-
Modern	9024	F	urm2 (55)	16.1	ND^{+}	-	134.7	ND	-	288.4	ND	-
Modern	9024	F	lrm2 (85)	19.8	ND^{+}	-	112.3	ND	-	297.2	ND	-
Modern	9117	M	lrm2 (85)	15.4	ND	-	166.3	ND	-	148.6	ND	-
Modern	9117	M	urm2 (55)	31.1	ND	-	118.2	ND	-	204	ND	-
Modern	9748	F	llm1 (74)	16.9	0.0222	0.00131	82.4	ND^{+}	-	120.1	ND	-
Detectable					**2**			**3**			**1**	
Total	**6**		**9**		**6**			**9**			**9**	

Ind= Individual; ND= Not Detectable; Tooth types are abbreviated where the first letter= upper (maxillary) or lower (mandibular), the second letter= side (right or left); the 3rd letter= tooth type (incisor, canine, molar); the 4th character= tooth position number; numbers in parentheses refer to the FDI tooth notation system (FDI, 1971); '-' denotes sample not tested due to insufficient or unsuitable material; * enamel tissue homogenized and split into two samples to test broad consistency; $^{+}$ indicates samples that did not have detectable levels of cortisol, but which also have notably low sample mass

Table 3
Dental cortisol concentrations from intra-individual and intra-tooth analyses. Cortisol concentrations are presented as µg/dL divided by sample mass.

	Brno-Vídeňská				Modern					
Individual	4890		5813		3937		9024		9117	
Tooth	urm2 (55)*	urm2 (55)*	urm2 (55)	uri1 (51)	uli1 (61)	uri1 (51)	urm2 (55)	lrm2 (85)	lrm2 (85)	urm2 (55)
Circumpulpal Dentine	-	-	0.00091	-	0.00350	-	ND^{+}	ND^{+}	ND	ND
Dentine	-	-	ND	ND^{+}	0.00684	0.00028	ND	ND	ND	ND
Enamel	0.00026	0.00023	ND	0.00059	0.00709	ND^{+}	ND	ND	ND	ND

ND = Not Detectable; Tooth types are abbreviated where the first letter= upper (maxillary) or lower (mandibular), the second letter = side (right or left); the 3rd letter = tooth type (incisor, canine, molar); the 4th character = tooth position number; numbers in parentheses refer to the FDI tooth notation system (FDI, 1971); '-' denotes sample not tested due to insufficient or unsuitable material; * enamel tissue homogenized and split into two samples to test broad consistency; $^{+}$ indicates samples that did not have detectable levels of cortisol, but which also have notably low sample mass

certain degree of stress. This hypothesis may explain why multiple modern and archaeological deciduous teeth had sub-detection levels of cortisol in all tested tissues; and could suggest why several modern individuals, presumably exposed to fewer stressors, had no detectable DDC. In analyses of permanent teeth, not all samples yielded detectable results either (Quade et al., 2021). Therefore, determining 'expected' dental cortisol concentrations represents an opportunity for future research.

Second, intrinsic biological factors such as sex or age-related maturational events (Goldstein et al., 2016; Greaves et al., 2014; Kirschbaum et al., 1992; Panagiotakopoulos and Neigh, 2014) may influence DDC. Of the living individuals, only females had detectable DDC, although sample sizes are small, and sex was not estimated for archaeological individuals. Additionally, taphonomic, or diagenetic factors could affect DDC (Cappellini et al., 2018; Hollund et al., 2015; Kendall et al., 2018; Schmidt et al., 2017; Turner-Walker, 2008). Cortisol is thought to be fairly resistant to degradation (Hamel et al., 2011), but can be affected by long-term exposure to temperatures above 37 °C (Khonmee et al., 2020). Although diagenesis in the archaeological teeth was anticipated, those individuals died and were buried with their teeth intact, anchored within the jaw. In contrast, when modern deciduous teeth are exfoliated, they can be conserved in different ways, including boiling or disinfecting teeth. Data related to the storage of modern teeth prior to donation were not collected. This represents a potential problem and future studies will need to explore this hypothesis.[2]

Third, the current methods may need additional optimization to capture cortisol from dental tissues. As one example: although ELISA kits are extensively tested for cross-reactivity, sensitivity and linearity by kit manufacturers and through independent research (Russell et al., 2015; Slominski et al., 2015), some studies have noted problems and inconsistencies with the comparability and quantification processes (Hendy, 2021). Ultimately, other methods and technologies (e.g., mass spectrometry) may be preferable.

Several limitations of the study are noted. Eleven samples had very small quantities of tissue available for analysis; these samples may have been too small to generate detectable results. Additionally, three living

[2] Modern individual 3937 provided two teeth for analysis, where one (uli1) was processed very shortly after receipt and the second (uri1) several months later. DDC differed between the two teeth, possibly indicating that storage of teeth is an important factor

individuals donated multiple teeth, comprising the majority of modern tooth samples (17/24, 71%). Therefore, their teeth could be exerting an undue influence on this analysis.

4.1. Conclusions

This study is the first known analysis of cortisol derived from deciduous dental tissues, acting as a proof of concept for future research. The ability to detect cortisol from deciduous teeth is integral to expanding and developing techniques to assess early life stress, facilitating research on the biological embedding of stress in the skeleton, new methodological approaches, and comparisons with alternative forms of stress evidence from living and archaeological populations.

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CRediT authorship contribution statement

Leslie Quade: Project conception and development; Data collection; Data processing; Data analysis and interpretation; Drafting of manuscript; Editing of manuscript; Funding acquisition. **Miroslav Králík:** Support of application to ethics committee; Permission to access archaeological skeletal materials; Editing of manuscript. **Petra Bencúrová**: Access to machinery and resources; Data processing; Editing of manuscript. **Erin C. Dunn:** Concept development; Data interpretation; Editing of manuscript.

Declaration of Competing Interest

None.

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