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BRIEF REPORT

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The feasibility of salivary sample collection in an international pediatric cohort: The the TEDDY study

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Abstract

Saliva offers a relatively noninvasive method for measuring analytes such as cortisol, holding particular promise for use in pediatric populations on a large scale if a rigorous collection protocol is feasible in diverse settings. The Environmental Determinants of Diabetes in the Young study protocol, conducted in centers in the United States, Sweden, Finland, and Germany, used salivary collection to assess cortisol level as a physiologic marker of stress. Saliva was collected using Sorbettes from subjects at 3.5, 4.5, and 5.5 years of age. Parents collected a morning sample, and staff collected pre- and post-blood draw samples. Feasibility was assessed based on protocol completion, adherence with instructions, factors affecting adherence, and sufficiency of saliva sample for cortisol determination. Collection of saliva samples in a diverse pediatric population is feasible. Establishing non-invasive and acceptable methods for collecting physiological parameters of stress will allow better exploration of determinants of health in this important population.

KEYWORDS
adherence, methodological feasibility, multi-center international, pediatric cohort study, salivary collection, salivary cortisol

1 | INTRODUCTION

The last decade has seen a marked increase in the use of saliva for determination of many analytes including corticosteroids, alpha-amylase, immunoglobulin, as well as DNA (Chiappin, Antonelli, Gatti, & De Palo, 2007; Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007; Rohleder & Nater, 2009). Interest and further understanding of the role stress hormones play in disease risk has prompted an increase in the number of studies using salivary cortisol as a biomarker of stress in many populations (Hellhammer, Wust, & Kudielka, 2009). Furthermore, saliva sampling is an inexpensive and noninvasive method offering advantages over serum testing, particularly in pediatric populations. However, there are no reports in the literature that evaluate the feasibility of saliva collection generally, or salivary cortisol collection specifically, in large-scale pediatric, international cohort studies. The Environmental Determinants of Diabetes in the Young (TEDDY) Study has completed salivary collection on about 4,000 children aged 3.5–5.5 years old, across four countries in six study centers and provides a unique opportunity to assess multiple aspects of this method.

Collecting saliva is convenient for self-sampling in naturalistic environments and has been used with young children (Gunnar & White, 2001; McCarthy et al., 2009; Neu, Goldstein, Gao, & Laudenslager, 2007). Research has used saliva samples to assess general stress levels using the indicator of Cortisol Awakening Response (CAR) (Bäumler, Kirschbaum, Kliegel, Alexander, & Stalder, 2013; Bright, Granger, & Frick, 2012; Bruce, Fisher, Pears, & Levine, 2009; Freitag et al., 2009; Gribbin, Watamura, Cairns, Harsh, & Lebourgeois, 2012; Saridjan et al., 2010; Stalder et al., 2013; Tegethoff, Knierzinger, Meyer, & Meinlschmidt, 2013). Salivary cortisol can also be used to assess the child's response to a particular stressor (e.g., venipuncture, injection, inoculation) (Davis & Granger, 2009; Felt et al., 2000; McCarthy et al., 2009; McCarthy et al., 2011) and other stress evoking procedures (e.g., Trier Social Stress Test for Children) (Gunnar, Talge, & Herrera, 2009; Kirschbaum, Pirke, & Hellhammer, 1993). However, all of these studies employed relatively small samples of children.

Adherence with sampling procedures, especially the timing of sampling, is crucial for accurate assessment of cortisol levels (Gunnar & Talge, 2007; Hall et al., 2011; Hanrahan, McCarthy, Kleiber, Lutgendorf, & Tsalikian, 2006; Jessop & Turner-Cobb, 2008; Kudielka, Hawkley, Adam, & Cacioppo, 2007; Schwartz, Granger, Susman, Gunnar, & Laird, 1998; Smyth, Clow, Thorn, Hucklebridge & Evans, 2013; Stalder, Kirschbaum, Kudielka et al., 2016). Instructions such as preventing the child from eating or drinking something except water, crying or participating in vigorous activities for 30 min prior to collection, as well as managing timing of the child's medication may pose additional challenges (Hanrahan et al., 2006).

The constraints of the sampling protocol in conjunction with child and parent factors (e.g., motivation, ability, work, and family responsibilities) significantly raise the potential for protocol non-adherence. In a small study, Smith and Dougherty (2014) found substantial discrepancy between parent self-report and electronically monitored report of CAR sample collections, with parents reporting higher collection rates (78–85% vs. 56–77% of requested samples). Furthermore, to our knowledge, there are no reports of staff adherence with salivary sampling protocols. These strict protocol requirements of saliva collection for cortisol measurement combined with the diverse cohort in the TEDDY study provided an ideal opportunity to conduct a rigorous examination of feasibility of a saliva collection protocol among parents and study staff.

Another important consideration is whether the study's saliva collection protocol results in sufficient sample volume for laboratory assay requirements. Very few studies report these data. McCarthy et al. (2009) reported 19 out of 1,355 cortisol samples were inadequate, 11 due to questionable sampling.

The aim of this study was to conduct a multifaceted assessment of the feasibility of salivary collection for cortisol determination in a large longitudinal multi-national cohort study of preschoolers. Feasibility was assessed by an examination of protocol acceptance and completion, adherence to instructions by both parents and study staff, factors affecting adherence, and sufficiency of sample for cortisol determination.

2 | METHOD

2.1 | Study design and population

TEDDY is a prospective cohort study funded by the National Institutes of Health (NIH) with the primary goal to identify environmental triggers of type 1 diabetes (T1D) in children genetically at-risk for the disease. It includes six clinical research centers, three in the United States (US)—Colorado, Georgia/Florida, Washington, and three in Europe (EU)—Finland, Germany, and Sweden. Detailed study design and methods have been previously published (The TEDDY Study Group, 2007, 2008). The study was approved by local Institutional Review Boards and is monitored by an External Evaluation Committee formed by NIH. Participants in this study are children determined at birth to have high risk genetic markers for T1D whose parents agreed to participate in a 15-year observational study starting at the age of 3–4.5 months. TEDDY's protocol includes 2–4 visits per year with extensive data collection through interviews and biologic sample collection. The salivary collection protocol was approved in 2008 with the European centers being the first to start followed 2–3 years later by the US centers. The focus of the current report is on the feasibility of collecting salivary samples that would be used to assess cortisol in the morning after awakening and before and after receiving a venous blood draw at TEDDY visits when the child was 42, 54, and 66 months of age.

As of December 2014, 12,690 TEDDY visits had been completed where the saliva cortisol protocol was in place (8,977 EU and 3,713 US TEDDY visits). The experience from these 12,690 study visit saliva collections is the basis for the feasibility assessment.

2.2 | Method and procedures of saliva collection

Each annual collection consisted of three salivary samples: (1) a morning sample about 30 min (time window 25–35 min) after

awakening taken at home by parents; (2) a sample collected before a blood draw at a TEDDY study visit by study staff (<10 min); and (3) a sample collected about 20–30 min (time window 15–35 min) after a blood draw by study staff.

All study staff received in-person training in the collection technique. At the clinic visit prior to the first home collection, research staff demonstrated the saliva collection technique to the parent. Some parents were given the written instructions and the saliva kit to take home while other parents were mailed the kit and instructions prior to the next study visit. The instructions ask parents to collect the saliva the morning of their next study visit, after the child has been awake for about 30 min and before eating, drinking, or brushing the child's teeth.

Each Salimetrics salivary collection kit consisted of three Sorbettes (cotton pads on a stick developed for collection in infants) and a storage tube (Donzella, Talge, Smith, & Gunnar, 2008; Putnam et al., 2012). The Salimetrics kit instructions indicate that up to three Sorbettes should be used in immediate succession to assure sufficient sample volume. A single Sorbette should be placed under the child's tongue until the Sorbette tip is saturated with saliva. Upon removal, the second Sorbette is placed under the child's tongue until saturation, removed, and replaced by a third Sorbette. All three Sorbettes are placed in one storage tube. Upon delivery to the study clinic for processing, the tube is placed in a centrifuge and spun at 1,500*g* for 15 min. After the spinning is complete, the Sorbette are removed with tweezers and discarded. The saliva sample is then stored in a −70°C freezer.

On the morning of the in-home collection, the parent was instructed to document the time the child woke up and the time the first saliva sample was collected. In-home collection was scheduled on the same day as a TEDDY study visit. If a parent failed to complete the morning in-home collection on the same day as the TEDDY study visit, collection within 1 week of the study visit was accepted. Home samples were returned to the child's study site either in person or by standard mail. Cortisol concentrations remain stable for days if samples are mailed before freezing (Clements & Parker, 1998).

At the in-clinic saliva collection, the staff documented the time the child last ate, whether caffeine was consumed since waking up and whether the child had participated in any vigorous activity or had been upset or crying in the prior 30 min. If the child had some food or drink—except water—in the prior 30 min, the collection was either postponed for 30 min or the protocol violation was noted. If the child was on steroid medication the salivary cortisol collection was not performed. The protocol instructions included an initial saliva collection <10 min before the child's blood draw and a second saliva collection 20–30 min after the blood draw. The time of the pre- and post-blood draw saliva collection, the time of the blood draw attempt, and whether it was a venous or capillary blood draw were documented.

2.3 | Center differences in the implementation of the protocol

The multi-center, cross-national, longitudinal structure of TEDDY requires a consistent investment in developing standardized protocols that can be implemented successfully in diverse settings. We attempted to minimize the differences in implementing the salivary cortisol protocol between the clinical sites by use of extensive training and written documentation in the manual of operations.

Although a collection demonstration was given to parents at all TEDDY study sites, there were important center differences in the timing of when the saliva kit was given to the parent. The Swedish and Finnish centers distributed the saliva kits and instructions to the parents at the visit 3–6 months before the collection visit. The other four clinical centers mailed the saliva kit and instructions 2–8 weeks before the sample collection visit.

Centers also differed in their inclusion or exclusion of participants who were on a long-distance protocol (LDP), meaning that they do not come into a study center but do the study protocol (including blood draws) at a remote location. This protocol option was developed in part for US TEDDY centers where there is greater population mobility resulting in a portion of the cohort that has moved too far away to continue to be seen at the study site, but who are committed to continuing with the basic study protocol. Due to the increased burden on the family, the US sites made the decision to exclude LDP participants from the saliva protocol. The Swedish and Finnish study sites do not routinely have subjects on a LDP.

The German site is unique in that 80% of the cohort have their study data collected at their local pediatrician's office and not at a study clinic. Therefore, the staff developed remote sampling collection procedures where children have all of their saliva collections (morning-, pre-, and post-blood draw) completed by parents. This was accomplished by developing instructions that were more detailed and included pictures of the collection procedure since in-person demonstration was not possible. These instructions were reinforced with labels for each collection tube.

2.4 | Saliva cortisol analysis

Ability to consistently obtain a sufficient sample for analysis is an important feasibility criteria for any assay. To assess the sufficiency of the saliva cortisol sample collected, a subset of 5,495 samples collected was sent to the laboratory of the Department of Clinical Chemistry, University Hospital, Linkoping, Sweden. The assay used for determination of cortisol in saliva is a commercial enzyme-linked immunoassay from Salimetrics LLC (Carlsbad, CA) applied on a Tecan Freedom EvoLyzer (Tecan, Männedorf, Switzerland).

3 | RESULTS

3.1 | Protocol acceptance

As of December 31, 2014, TEDDY participants who attended the 42 (N = 4,307), 54 (N = 4,545), or 66 (N = 3,838) months visit were eligible to participate in the salivary cortisol protocol, for a total of 3,713 US and 8,977 EU TEDDY visits. Table 1 describes the participation and refusal rates for each of these visits for all TEDDY participants and for EU and US centers separately. In the 12,690 TEDDY visits, 814 (6.4%) of potential participants were not offered the protocol by study staff

TABLE 1 Saliva sample collection among eligible TEDDY subjects at United States (US) and European (EU) Centers

	42-Month visit		54-Month visit		66-Month visit		Total	
	N	%	*N*	%	*N*	%	*N*	%
Total								
Completed visit	4,307		4,545		3,838		12,690	
Not offered SSP	207	4.8	345	7.6	262	6.8	814	6.4
Total eligible for SSP	4,100	95.2	4,200	92.4	3,576	93.2	11,876	93.6
≥1 sample	3,948	96.3	4,016	95.6	3,426	95.8	11,390	95.9
Refused	152	3.7	184	4.4	150	4.2	486	4.1
US								
Completed visits	1,090		1,402		1,221		3,713	
Not offered SSP	141	12.9	247	17.6	164	13.4	552	14.9
Total eligible for SSP	949	87.1	1,155	82.4	1,057	86.6	3,161	85.1
≥1 sample	935	98.5	1,128	97.7	1,038	98.2	3,101	98.1
Refused	14	1.5	27	2.3	19	1.8	60	1.9
EU								
Completed visits	3,217		3,143		2,617		8,977	
Not offered SSP	66	2.1	98	3.1	98	3.7	262	2.9
Total eligible for SSP	3,151	97.9	3,045	96.9	2,519	96.3	8,715	97.1
≥1 sample	3,013	95.6	2,888	94.8	2,388	94.8	8,289	95.1
Refused	138	4.4	157	5.2	131	5.2	426	4.9

SSP, salivary sample protocol; US, United States; EU, Europe.

for reasons such as clinic preparedness to start the protocol, difficult or missed visits where data collection was completed over the phone rather than in person, saliva collection packets not received, or clinic discretion that this visit/subject was not a good candidate for this protocol. More US (14.9%) compared to EU (2.9%) participants across all visit intervals were not offered the protocol ($\chi^2 = 624.6, p < 0.0001$). The proportion not offered the protocol in the United States ranged from 5.9% to 30.7% and was more common in the Georgia/Florida (30.7%) and Washington (19.3%) centers where a greater percentage of the cohort has challenges completing the core TEDDY visit protocol and staying in the study. For the EU centers the range was 2.7–9.1% (Center specific data not shown) .

Study-wide the rate of completing at least one of three samples on any given saliva collection day was 95.9% among those participants offered the protocol. All centers had rates above 90%, ranging from 91.9% to 98.6%. This included Germany where a majority of participants were on LDP. There was no meaningful difference in the completion rates for US centers (98.1%) compared to the EU centers (95.1%). Very few participants offered the protocol refused to do it.

3.2 | Protocol adherence

The number and percentage of visits that were missing elements of the saliva collection protocol among the 11,390 visits where at least one cortisol sample was obtained is shown in Table 2. Missing samples at each time point (morning, pre-, and post-blood draw) were generally uncommon, ranging from 0.8% to 4.9% across all sites. The morning sample, which relied solely on the parent for collection, was missing more often than saliva samples collected at the study visit ($\chi^2 = 353.1$, $p < 0.0001$). There was considerable site variability in success getting the morning sample, ranging from 1.3% to 19.8% missing (data not shown). Overall the US centers had greater difficulty obtaining the morning cortisol sample compared to the EU centers (11.2% and 2.5% missing, respectively; $\chi^2 = 363.1$, $p < 0.0001$). When samples were collected in clinic there were very few missed collections and no differences between centers.

Some collected samples were excluded for the following reasons: brushed teeth without rinsing, ate or drank less than 30 min before cortisol collection, or were on oral steroids during last 30 days. Exclusion of a sample for any of these reasons was uncommon, suggesting that parents were good at following the instructions. There was limited center variability in proportion of samples excluded (1.0–5.2%), though on average this was not different for US (3.7%) compared to EU (2.5%) sites.

Staff adherence with timing of saliva collection was best for both EU and US centers for the sample collected after blood draw, with 95% and 95.6%, respectively, being collected within the 15–35-min window (data not shown). The pre-blood draw sample had worse adherence, with center differences emerging. Among EU centers, 78.7% of samples were collected in the protocol window of <10 min before blood draw, compared to 60.4% ($\chi^2 = 393.6, p < 0.0001$) in the US centers. Parent adherence with the timing of the morning saliva sample collection (25–35 min after awakening) was 65.8%, with a persistent difference between the EU (70.3%) and US (54.0%) ($\chi^2 = 264.3, p < 0.0001$) centers.

TABLE 2 Completion of salivary sample collection for TEDDY study overall, United States (US) and European (EU) Centers

	42-Month visit		54-Month visit		66-Month visit		Total	
	N	%	*N*	%	*N*	%	*N*	%
Total								
≥1 cortisol samples	3,948		4,016		3,426		11,390	
No morning sample	134	3.4	241	6.0	180	5.3	555	4.9
No clinic sample	21	0.5	32	0.8	33	1.0	86	0.8
Sample excluded[a]	129	3.3	106	2.6	85	2.5	320	2.8
US								
≥1 cortisol samples	935		1,128		1,038		3,101	
No morning sample	87	9.3	142	12.6	117	11.3	346	11.2
No clinic sample	9	1.0	11	1.0	15	1.4	35	1.1
Sample excluded[a]	42	4.5	40	3.5	33	3.2	115	3.7
EU								
≥1 cortisol samples	3,013		2,888		2,388		8,289	
No morning sample	47	1.6	99	3.4	63	2.6	209	2.5
No clinic sample	12	0.4	21	0.7	18	0.8	51	0.6
Sample excluded[a]	87	2.9	66	2.3	52	2.2	205	2.5

US, United States; EU, Europe.

[a]Sample exclusion reasons: child brushed teeth without rinsing, ate or drank less than 30 min before cortisol sample collection or were on oral steroids in last 30 days.

If saliva was collected, a sufficient sample was obtained almost all of the time at all sites, with across site sample sufficiency ranging from 97% to 100%. The one exception to this was Germany where sample sufficiency was lower (79.5%) but improved over time to 90%.

4 | DISCUSSION

Salivary cortisol collection has emerged in pediatric research as a biomarker of stress in both US (Bright et al., 2012; Bruce et al., 2009; Felt et al., 2000; McCarthy et al., 2009, 2011) and European pediatric studies (Bäumler et al., 2013; Freitag et al., 2009; Saridjan et al., 2010; Stalder et al., 2013; Tegethoff et al., 2013) with relatively small sample sizes. However, these reports provide very limited information about collection adherence and quality, information important for any effort to collect saliva samples on a large scale. The TEDDY experience provides an opportunity to examine several aspects of the feasibility of salivary collection in a large multi-center, cross-national longitudinal study of children ages 3.5–5.5 years old that required sample collections from both parents and staff. Following a well developed standardized protocol, the TEDDY experience demonstrates that a salivary cortisol protocol could be implemented successfully in diverse settings. This analysis has also suggested areas where challenges to saliva collection completion and protocol adherence exist and that could be attended to in the protocol design.

Different aspects of adherence were investigated which are associated with saliva collection feasibility. Among all TEDDY children offered the saliva sampling protocol, 95.9% provided one or more saliva samples (US: 98.1%, EU: 95.1%). Among the US and EU parents collecting the morning saliva sample, 95.1% were able to obtain a sample, with a notable range between centers where US parents had lower adherence (88.8%) compared to EU parents (97.5%). Among the staff, 99.2% of samples were collected. These rates are comparable or higher than those reported by studies where adherence was objectively assessed (Broderick, Arnold, Kudielka, & Kirschbaum, 2004; Smith & Dougherty, 2014).

The refusal rate was very low at 4.1% (US: 1.9%, EU: 4.9%). The lower refusal rate as well as the high adherence rate among those offered the protocol in United States stands in stark contrast to the proportion of subjects who were selectively not offered the protocol (US 14.9%) because they were experiencing challenges completing the standard TEDDY protocol compared to those not offered it in the EU centers (2.9%).

Center differences in offering the protocol reflected EU/US differences in retention concerns and participant burden, where retention challenges have been more apparent for the US centers (Baxter et al., 2012; Johnson et al., 2011, 2014, 2016; Lernmark et al., 2016). Retention of study participants in a longitudinal cohort is critical to the overall study design and can be particularly challenging for a pediatric cohort enrolled at the age of 3 months and followed for 15 years.

Overall completion and adherence to the sample timing collection protocol was lower for the morning sample compared to the pre- and post-blood draw samples. Smith and Dougherty (2014) observed 78–85% self-reported adherence in parents who collected the CAR saliva samples. In the present study, with a more diverse population and longitudinal sample collection, higher completion rates were observed (EU: 97.5%; US: 88.8%). The pre–post-blood draw collection rates were even higher, with only 0.8% before and 1.0% after the blood draw samples not collected. These completion rates are

especially striking because although they were collected primarily by staff in the clinic setting, they also included pre- and post-blood draw saliva collections by parents on the German LDP. In addition, very few saliva samples had to be excluded (in total 2.8%, EU: 2.5%, US 3.7%) because of failure to follow the protocol properly.

Saliva can be used to measure many different analytes which may have specific requirements such as time of collection and restrictions on food, drink, and medications (Chiappin et al., 2007; Hellhammer et al., 2009; Nater et al., 2007; Rohleder & Nater, 2009). TEDDY's salivary cortisol collection is useful to assess feasibility as it was done in four countries, by different collectors, in different settings, with detailed instructions regarding timing and restrictions. Though a majority of all samples were collected according to protocol within the timing windows specified, this element had the most variability in compliance across sample collections and centers. The morning collection had the lowest percentage of saliva samples collected in the protocol time window and showed a wide degree of variability across centers. Strict adherence to the instructions may not be as easily accomplished by parents in the child's home environment with its increased demands (child's sleep patterns, morning family schedules, etc.) compared to a clinic setting. Since the timing of the morning saliva collection was provided by parent report, parents may have over-estimated adherence with the instructions. Future studies should examine common barriers to in-home saliva collection protocol adherence, so that strategies to address these barriers could be designed. The use of objective devices to measure the child wake up and saliva collection times could be very useful (Stalder et al., 2016).

Failure to follow the protocol in terms of the timing of the saliva collection was also a problem among TEDDY staff. Of the two saliva samples collected by staff at the TEDDY visit, the pre-blood draw collection time adherence was lower than the post-blood draw time adherence. The logistical variables associated with any TEDDY visit are more complex at the beginning of a visit compared to its end, when the post-blood draw saliva sample is done. It is not always easy to predict when the blood draw will occur relative to other data collection elements, with staff making the best effort possible to determine when the pre-draw saliva collection was to be administered. The differences in adherence for this element between the EU and US centers may reflect differences in how the clinics are organized generally around timing and conduct of the blood draws. It is possible that a stricter visit schedule for those visits that included a saliva sample collection, using an objective electronic device to record sample collection time (Stalder et al., 2016) and improved organization might have increased the time adherence for the pre-blood draw saliva collection.

Among the 5,495 saliva samples sent to the lab for cortisol assay, only 1.6% of samples were excluded due to an insufficient amount of saliva. Bright et al. (2012) reported 34 invalid samples of 256 (14%) mostly due to insufficient sample sizes. The excellent sufficiency results in the TEDDY study also confirmed the suitability of the Sorbette as collection device for toddlers (Donzella et al., 2008; Putnam et al., 2012), although Salimetrics now offers another saliva collection device for children and infants (https://www.salimetrics.com/collection-system/childrens-swab).

The study has several limitations. Smyth, Clow, Thorn, Huklebridge, and Evans (2013) reported that delays of 5–15 min between awakening and the start of saliva sampling matter in assessment of the CAR. Since both the awakening time of the children the saliva collection time was determined by parent report, we cannot be sure of the accuracy of these reports. Although evidence suggests that parents are reasonably reliable reporters of children's awaking times (Tikotzky & Sadeh, 2001), the use of actigraphy would provide a more objective assessment of children's wake times and cap tracks could be used to record the saliva collection (Smith & Dougherty, 2014). Recently published expert consensus guidelines for the collection of morning cortisol for the assessment of the CAR include both the use of various electronic devices and careful attention to participant instructions (Stalder et al., 2016). Adam and Kumari (2009) described benefits and challenges of assessing saliva cortisol in large-scale epidemiological research in comparison to convenience samples. They suggest using electronic monitoring devices to measure timing adherence for at least a subsample of participants.

We acknowledge that the TEDDY population is a highly motivated sample of educated parents who have volunteered to participate in a longitudinal study with a demanding protocol. Such parents might be more motivated than most to be highly adherent with the study saliva collection protocol. Or, they might be more motivated to report greater adherence. In either case, our study findings may not replicate in other populations.

The same limitations hold for the time of saliva collection of the pre- and post-blood draw reported by clinical staff. Sampling time details in the clinic were noted by clinical staff without objective device. Though research staff may feel pressure to report greater adherence, Stalder et al. (2016) conclude that clinical staff are reliable in recording. In this study, the highly trained TEDDY clinical staff reported poorer adherence to some aspects of the pre–post-blood draw saliva collection and there were differences in adherence between centers, suggesting biased or better adherence reporting is not likely a limitation in this case.

Nevertheless, we believe the findings of this study are valuable and provide important information about using salivary collection in an international and cross-cultural context of a longitudinal study. This effort demonstrated that saliva collection by both parent and staff according to a standardized protocol was possible and yielded sufficient samples for laboratory analysis. Also, demonstrated here was the importance of creating a well-documented, standardized protocol that was attentive to center specific conditions and was flexible, within reason, for parents and staff to complete. Establishing non-invasive and acceptable methods for collecting physiological parameters of stress in children will allow better exploration of determinants of health in this important population.

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