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Implementing Self-collection of Biological Specimens With a Diverse Sample

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Abstract

Collecting saliva is the most noninvasive way to detect changing levels of cortisol (Adam & Kumari, 2009; Soo-Quee Koh & Choon-Huat Koh, 2007), a stress hormone of interest to behavioral and health scientists, where there are benefits from multiple samples taken over a period of days. Various self-collection strategies have been employed, ranging from treated cards to cotton swabs and passive drool methods. The current study investigates the effectiveness of a variety of reminder techniques in encouraging adherence with procedures requiring 4 samples per day on 3 separate days of passive drool collection among African American and European American young adults. The findings suggest that direct texts were associated with the greatest level of adherence, while phone reminders were most effective when controlling for total number of contacts. Results indicate that both traditional and novel reminder methods can positively influence adherence, even with challenging populations.

Keywords

saliva collection; diurnal cortisol; biological samples; biomarker

Background

Biological mechanisms can provide a view into the etiology of psychopathology and a potential explanation of individual differences in vulnerability to mental health and substance related disorders. Consequently, social scientists interested in the prevalence and prevention of these disorders are called upon to collect biological specimens that provide valuable measures of possible biological mechanisms (biomarkers). The challenge of effective data collection, especially with sensitive populations, has remained a salient issue for researchers. When data collection involves bodily fluids, the difficulties are compounded. Conventional forms of data collection have proven costly for researchers and inconvenient for participants, leading to higher attrition (Jaszczak et al., 2009). Studies have shown that requiring subjects to travel to a clinic to provide samples of blood or saliva has reduced adherence with the study requirements (Rockett et al., 2004). Researchers have

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studied the effectiveness of self-collection by participants to address these issues. Success has been significant, with most studies reporting improved adherence over clinic collection methods (Jaszczak et al., 2009). Self-collection is effective and efficient for saliva samples, and a wide range of biological measures can be obtained from saliva, including the stress hormone cortisol, which benefits from multiple samples taken over a period of hours or days. Collecting saliva is the most noninvasive way to detect changing levels of cortisol (Adam & Kumari, 2009; Soo-Quee Koh & Choon-Huat Koh, 2007). Because the research focus on cortisol and behavioral outcomes is currently evolving, improvements in strategies for collecting saliva samples are especially valuable.

In the existing research on cortisol collection, various methodological strategies have been employed to ensure valid samples. Self-collection methods have ranged from treated cards that inhibit bacterial growth (Harty et al., 2000), to cotton swabs (Thorn et al., 2006) and passive drool methods (Desantis et al., 2007; Gallagher et al., 2006; Oskis et al., 2009). The use of Salivettes with cotton swabs is common, with researchers reporting increased adherence and ease of use, especially for elderly and disabled participants (Broderick et al., 2004; Kudielka & Kirschbaum, 2003; Kunz-Ebrecht et al., 2004). The use of Salivettes requires the research participant to place an untreated cotton swab in their mouth for 1–2 minutes to collect the saliva. However, research suggests that samples could be compromised by cotton swabs influencing the results of assays (Shirtcliff et al., 2001). Passive drool methods have been used to combat possible contamination of the sample (Oskis et al., 2009). The passive drool method requires subjects to salivate through a straw into a vial, eliminating the use of cotton swabs or mouth rinses that may bias the sample. Poll et al. compared the efficacy of passive drool to Salivettes, finding that data from Salivettes was a better predictor of cortisol levels than passive drool methods (Poll et al., 2007). However, the study sample was small (10 participants), and similar studies have not concurred with such findings (Gallagher et al., 2006). In their review of large-scale epidemiological studies, Adam and Kumari (2009) suggest that passive drool may be the preferred method of saliva collection for studies that are testing for a variety of biomeasures. However, they found that the validity of results hinges on the number of sample collections required. Most studies report increased adherence across multiple collection periods, with the majority of returned samples containing usable biological material (Rockett et al., 2004).

Multiple studies have relied on single collection samples to measure the effect of cortisol on a number of physiological, psychological, and behavioral outcomes. These studies are sufficient for examining biomarker levels at one point in time or when comparing the efficacy of various collection methods (Gallagher et al., 2006; Harty et al., 2000; Rylander-Rudqvist et al., 2006). However, a single sample is not sufficient when research questions concern changes in cortisol levels from morning to night, or comparisons of levels between types of activities, for instance comparing work days to days off. Hellhammer et al. (2007) found that multiple samples isolate the effects of cortisol fluctuations, independent of intervening factors such as the amount of sunlight at waking. The study presented here collected multiple samples: four times per day on 3 separate days, allowing for an evaluation of cortisol levels across various times of day. Recent studies using multiple collection times find more comprehensive and accurate results (Desantis et al., 2007; Kudielka & Kirschbaum, 2003). The assessment of slope patterns is essential for understanding how cortisol is related to behaviors and health outcomes. Research finds that flattened cortisol slopes are associated with greater difficulties in interpersonal relationships and responses to stressful situations, as well as increased risk for cardiovascular disease and diabetes (Adam & Kumari, 2009; Desantis et al., 2007). Adam and Kumari (2009) reviewed epidemiological studies to assess various collection and procedural methods used in salivary cortisol studies. They concluded that multiple measures allow researchers to evaluate the curvilinear rise and fall of cortisol levels throughout the day, especially in response to daily stressors. With only

single measures, researchers are at risk of compromising not only the connection to behavioral and health outcomes, but also the reliability of cortisol measurements (Adam & Kumari, 2009).

The diurnal cortisol rhythm, which is relatively stable across days and years, is typically characterized by a sharp increase within 45 minutes of morning waking, followed by a steep decline by mid-morning and then more gradual decline over the course of the day, with the lowest values at bedtime. The larger aim of the current study is to assess the influence of diurnal patterns of cortisol on behavioral outcomes among young adults. Measures taken at multiple time points allow researchers to examine the relationships between the rate of change in cortisol levels across the day and behaviors such as drug use and violence. In order to capture meaningful diurnal patterns in cortisol, saliva samples must be collected at specific times relative to waking.

Tracking the schedules of young adults in various stages of emerging from adolescence, from age 18 to 22, can be challenging for researchers. Many people in this developmental period are living away from parents for the first time, have less structured sleep and waking cycles, and are less likely to sleep in the same place every night. The validity of our findings depends on adherence to collection procedures and consistent recording of pertinent collection information.

Research projects that require participant collection of bodily fluids are often confronted with difficulties in assuring adherence with collection specifications. Errors in reporting dates, times, and conditions at the time of collection compromise the validity of the samples and the study as a whole. Researchers have begun to use electronic monitoring devices to record incidents of noncompliance for medication regimens and self-collection procedures for saliva (Broderick et al., 2004; Hugen et al., 2002; Kudielka & Kirschbaum, 2003; Malow et al., 1998). The most common method is Medication Event Monitoring System (MEMS) Track Caps (AARDEX Ltd., Switzerland). The MEMS caps contain microchips that record the day and time of the collection once the vial has been opened. Kudielka et al. (2003) tracked adherence of self-collected cortisol samples using MEMS Track Caps and evaluated the possible effect on nonadherence. They found that subjects' self-reports often underreport time delay in saliva collection, with a mean deviation of 13 minutes per sample and a maximum of 264 minutes. Such discrepancies can bias results, especially when evaluating diurnal cortisol patterns. Kudielka et al. report that nonadherent subjects showed a smaller cortisol spike after waking compared to adherent participants. The use of MEMS Track Caps allows researchers to assess the extent of nonadherence with self-collection procedures. However, the addition of MEMS cap technology is often prohibitively expensive for large-scale research studies, and is not sufficient for ensuring adherence with study procedures.

Researchers have taken extra steps to ensure adherence with study specifications, incorporating participant reminders via telephone, mail, cell phone, and email. The difficulty of the collection procedure and maintaining the validity of the sample necessitates consistent and recurring contact. Adherence is largely predicated on regular, positive contact with participants to reinforce study procedures and address any sampling problems (Adam & Kumari, 2009). The widespread decrease in home phone use requires adaptation by research teams. The increased use of email and cell phone text messages allows data collectors to reach certain populations that have traditionally been difficult to contact. In studies where adolescents are the primary sample, these alternative methods have proven effective in bolstering adherence with self-testing procedures (Couper, 2005; Oskis et al., 2009). With the increasing ubiquity of new technology, adolescents' and emerging adults' (ages 18–22) familiarity and comfort with the operation and management of these technologies is

becoming widespread. Increasingly, cell phones, smartphones, and the internet are becoming cornerstones of their working and social lives. Oskis et al. (2009) sent text messages to participants at each specified collection time, requesting return messages that provided time information about their passive drool sample collections. They found that this method was accepted by the adolescent subjects, and was effective in creating rapport between participant and researcher.

The current study integrates a variety of reminder techniques to ensure adherence with proper data collection procedures. Participants were contacted daily by data collection staff, either by phone calls or direct text messages. The current study used an indirect text message website that sent out messages to specified participants at scheduled intervals. Our primary aim was to determine what types of contact with participants were most effective for successful adherence to the saliva sample self-collection protocol.

Methods

Participants

Participants were recruited from a larger longitudinal study that examined the effectiveness of the *Parents Who Care* substance abuse prevention intervention for families (Haggerty et al., 2006; , 2007). Parents of eighth-grade students in the Seattle school district received a letter describing the study and were contacted by phone. Families were included if the teen and one or both parents consented to participate. Eligibility included self-identifying as African American (AA) or European American (EA), speaking English as their primary language, and planning to live in the area for at least 6 months. Recruitment stopped when an adequate number of AA and EA males and females had agreed to participate. Forty-six percent of families who received letters consented (55% of AAs and 40% of EAs). The parents who refused were more likely to be EA, married, and had a higher education on average than those who consented. Other ethnic groups were not recruited.

The sample was stratified by teen race and gender. There were significant differences by race in several demographic variables. EAs reported higher per capita income and parental education, and AAs reported higher prevalence of single parenthood (Table 1). Some teens in each race group self-identified as mixed race (19.6% AA, 12.5% EA), but were included in these analyses. Most primary caregivers were female (> 80%), with 71.6% being the adolescent's biological mother. Caregiver gender and relationship were similar across race with one exception: more African American youth had another female caregiver (e.g., grandmother, aunt) as a primary caregiver than did European American youth [$\chi^2(1) = 13.95, p < .001$]. Data were collected before and after the intervention when the children were in the eighth grade, with follow-up 12 and 24 months later.

At the long-term follow-up we attempted to re-contact all 331 young adults who then ranged in age from 18 to 22 (mean 19.7 years). From the original participant pool, 301 (90.1%) completed self-administered surveys on a laptop, provided a urine sample for drug screening, and were asked to participate in the saliva collection phase of the study. Of these, 67 were AA males, 73 AA females, 82 EA males, and 79 EA females. Most were currently enrolled in school (57.8%); 45.6% were employed at the time of the study; and 18% were neither employed nor attending school regularly.

Collection Procedure

The objective of the current study was to collect 12 saliva samples over 3 days, with corresponding collection dates and times. Participants were given a collection kit complete with 12 vials for each collection, diaries for each of the 3 days, a card with saliva collection

instructions, and freezer packs to keep the saliva samples cool when away from a freezer. The caps for each of the 12 vials were in a pill bottle outfitted with a MEMs cap. Participants were told that the cap recorded the time that the bottle was opened in order to retrieve an accurate record of date and time of each sample. MEMs caps were used to increase the accurate recording of collection dates and times. Their efficacy, however, was restricted due to the expense of the caps and the inability of interviewers to monitor their use. Caps were considered too valuable to risk sending through the mail so were not used when supplies were mailed to participants out of the area; therefore the data from the MEMs caps were limited. Evaluation of their use was also hindered by participants' misuse or loss of the caps over the course of the study.

Saliva was collected using the passive drool method, which requires participants to salivate through a straw into the vial without the use of cotton swabs or mouthwash. A valid sample consisted of 1.8mL of saliva in the vial. Over 90% of samples returned had sufficient volume for assaying. Samples were self-collected four times per day 3 consecutive or nonconsecutive days. The participants were asked to collect the first sample when they woke up and before they were out of bed, and the second sample 30 minutes after the initial sample before brushing their teeth or eating any food. The third was to be taken in the mid afternoon at least 1 hour after eating a meal, and the fourth at bedtime. Participants were urged not to eat, drink, exercise, or brush their teeth for an hour before collection to avoid contaminating the sample. The saliva samples were usually refrigerated or frozen until picked up by study staff, then frozen until assayed. Participants were asked to complete paper diaries after each collection. The diaries required participants to record the date and time each sample was collected and answer a series of questions evaluating social and physiological factors that may have influenced their cortisol levels. The diaries posed a series of questions that assess stress-inducing triggers that participants may have experienced throughout the collection period. The questions centered first on logistical factors such as the participants' surroundings and companions at the time of collection and any physical activity that the participant had engaged in preceding the collection. Physiological determinants of cortisol levels were measured with questions asking about the consumption of alcoholic or caffeinated beverages and the use of prescription and/or illicit substances. Psychological and social measures of anger, happiness, sleep disturbances, and minor hassles during the day were included to evaluate the stressors that may contribute to shifts in cortisol results. The number of questions ranged from 31 for the waking sample to 45 for the bedtime sample.

In the current study, six data collectors trained participants on the research protocols and facilitated collection, from scheduling the first meeting until picking up the saliva samples. Data collectors coordinated reminder schedules with participants to promote adherence with the saliva collection procedures. The type and timing of reminders were specific to each participant, depending on their work and school commitments and communication preferences. Participants were contacted via phone call, direct text (between the interviewer and participants), or automated texts. All participants were contacted on their personal cell phone; if the participant did not have a cell phone at the beginning of the collection period, they were provided with a prepaid cell phone.

All six data collectors were trained at the beginning of the field period for approximately 40 hours. Training topics included a review of previous study waves, how to gain voluntary consent, how to respond to questions about the survey items, protecting the confidentiality of participants, locating participants, and collection procedures for biological specimens. Data collectors received booster training sessions as needed throughout the field period on the following topics: best practices for reminding participants to self-collect samples, procedures to follow when participants missed a sample, retrieving samples, logging all

samples accurately into the tracking database, etc. The team of data collectors varied in skill level. Some team members required more training and retraining than others. Management performed quality control checks continuously on the retrieved diaries and saliva sample location, count, and quality to assure adherence to collection protocol by the data collection staff.

Measures

Adherence—A dichotomous (0/1) measure of adherence was used to evaluate participants at two levels (adherence and full adherence). Participants who provided 12 sealed samples to a data collector were considered to have met the criteria for partial adherence. Participants who completed all samples with corresponding dates and times noted in the diaries achieved full adherence. Full adherence did not require that all samples be collected at the prescribed times. The emphasis for the current study was both adherence in terms of collecting samples at the suggested times (at waking, after getting out of bed, after lunch, and at bedtime) and flexibility given the variability of the participants' schedules. Prescribed time frames were suggested, however, based on the demands of work, school, and other obligations; the exact timing of the saliva collection often varied between participants. This variability added to the subsequent analyses by providing data over a very wide range of times since first waking without demanding that participants take samples every hour all day long. However, this variable protocol made it especially important that accurate times be recorded in the diaries for when the sample was actually collected.

Collection phase—Data were collated separately for two phases of the data collection process. The collection phase was the time between survey completion and the participant completing the saliva self-collection. The pick-up phase was the time from sample completion until samples were collected by research staff. The efficacy of the reminder methods was evaluated for its contribution to adherence overall and for the collection and pick-up phases separately.

Contact types—The four main contact types were autotexts, direct texts, phone calls, and participant-initiated contact in the form of either a text message or phone call. The automated texts were sent from a free website service (textmelater.com) that sent text messages at scheduled intervals to valid cell phone numbers. Direct-text contacts were text messages initiated by data collectors; back-and-forth exchanges were counted as single contacts. Phone contacts were any attempted phone calls initiated by data collectors, including voicemails and missed calls. A voicemail message was recorded as evidence of a reminder. Data collectors attempted to have a minimum of three direct conversations with the participant per week throughout the study period to encourage adherence and the return of the sample materials. Participant-initiated contacts were any of the above that were initiated by the participant rather than a data collector. The type and frequency of contact was logged by data collectors at each phase of data collection. All contact methods were used by data collectors based on the preferences and scheduled responsibilities of each respondent. Hence a single respondent may have received several different types of contacts at either collection or pick-up phase. However, autotexts were not used during the pick-up phase since the point of making contact was to arrange a meeting. The contact totals were summed within each type of contact and the phase in which it occurred. Dichotomous variables were coded to indicate whether a participant received any contacts of that type.

Statistical Analysis

Chi-squared tests of independence and logistic regression were conducted to examine whether receiving each type of contact was related to adherence and full adherence. Logistic

regression was conducted to determine which type of contact was uniquely associated with higher probability of adherence.

Results

Adherence

From the available sample of 301, 275 (93.6%) of the participants returned at least one sample, 231 (76.7%) completed all 12 saliva samples (adherence) and 189 (62.8%) returned all 12 samples with dates and times for each vial (full adherence). Among the partially adherent participants, there were no racial or gender differences. African Americans and European Americans were almost equally successful in returning the saliva samples (76.7% and 77.6%, respectively). More females (80.9%) than males (72.5%) met partial adherence; however, the difference was not statistically significant. For full adherence, the gender effect remained consistent (67.8% female, 57.7% male); however, race differences were significant ($p = 0.02$). Only 55.7% of the African American sample met full adherence, compared to 68.9% of the European American participants.

Full Adherence by Contact Type

Table 1 contains the results from the chi-square analyses, finding that data collector-initiated contacts in the form of auto and direct texts and phone calls were significantly related to full adherence of the study protocols overall. Completion of all 12 samples with corresponding dates and times was required for full adherence. Direct texts resulted in full adherence from 69% of participants compared to 55% full adherence for those who did not receive direct texts. Autotexts were as useful, with 72% of respondents compared to 58% who did not receive autotexts. Phone contacts resulted in full adherence from 70% of subjects, compared with 47% adherence from participants who were not contacted by phone. All results are statistically significant ($p = 0.020$, $p = 0.015$ and $p = 0.000$, respectively). Participant-initiated contacts were not associated with full adherence. Logistic regression analyses were conducted to determine which type of contact was uniquely associated with higher probability of adherence. When controlling for total number of contacts, only phone reminders significantly predicted full adherence, more than doubling its likelihood (odds ratio = 2.5, $p = .001$).

Partial and Full Adherence by Phase

In order to fully evaluate the effect of various forms of contacts on adherence, we considered whether contacts were made during the collection phase or the pick-up phase. For contacts made during the collection phase, autotext and phone contact were the only significant method related to full adherence ($p = .01$ and 0.03 , respectively). Partial adherence was related to receiving autotexts during the collection phase. Both methods were significant at the 0.05 level ($p = 0.04$). Partial and full adherence were predicted by both direct text messages and phone contacts made in the pick-up phase. Direct texts during the pick-up phase predicted both partial and full adherence significantly at the 0.05 level ($p = 0.030$ and $p = 0.031$, respectively), while phone contacts were significant at both levels of adherence at the 0.001 level ($p = 0.000$ for both partial and full adherence). Participant-initiated contacts during the pick-up phase were associated with partial adherence as well ($p = .020$).

Discussion

Advancements in the self-collection of saliva have enabled researchers to reach a more diverse population compared to traditional methods that require participants to come to a lab. Overcoming the challenges of self-collection, especially with young adult participants,

has driven researchers to develop innovative strategies to encourage adherence to study protocols. The difficulties inherent in saliva collection over multiple and specific times of the day called for effective reminder techniques that promote timely saliva sampling and accurate recording of the date and time. The need for continuous reminder methods cannot be underestimated; studies have documented unreliable or biased results due to false reporting of sampling dates and times by subjects (Kudielka & Kirschbaum, 2003). Previous studies have shown that among African American populations, adherence to study protocols is often decreased (Hall et al., 2011). The findings from the current study, which show that African American participants were less likely to achieve full adherence, reflect this challenge. Tailoring reminder methods to certain participant populations may be useful in overcoming such disparities. The results from the current study suggest that consistent and frequent personal contacts by data collectors, in the form of phone contacts and text messages, is the most effective method to promote adherence with saliva collection protocols.

Phone calls, especially during the pick-up phase of the study, were instrumental in ensuring adherence, facilitating the timely return of the completed samples and diaries. Overall, phone contact was the most effective reminder method for encouraging both partial and full adherence throughout the course of the collection period. With detailed self-collection procedures, participants need to have sufficient communication with data collectors to answer questions that arise or to address difficulties. While this form of communication is more labor intensive and time consuming for the data collectors, the direct phone calls seem to yield reliable samples and diary entries with recorded sample dates and times from the majority of participants. This result corresponds with previous research that suggests traditional forms of contact can be as effective, if not more so, than new technological advances (Adam & Kumari, 2009). The personal and immediate nature of a phone call aids in reminding participants about the study protocols and the importance of their thorough participation. Data collectors may establish a rapport that engenders a sense of loyalty to the project and the collection procedure requirements. It allows data collectors the opportunity to identify and prevent potential impediments to adherence throughout the data collection period. While the robust results for phone contacts suggest that this traditional method should not be overlooked, it is perhaps unrealistic to expect research staff to make phone calls every time that self-collection is scheduled, especially for participants on odd or unpredictable wake/sleep cycles. New technologies such as text messages have proven to be viable options to promote adherence.

In the current study, text messages were positively related to adherence, and were tailored more to those participants who are generally difficult to reach using conventional methods. These results indicate that for overall adherence, using the new forms of technology is beneficial for achieving the collection aims of the study. Without steady employment and set schedules, young adults may find it challenging to adhere to strict self-collection procedures. They require prompting to comply fully with study protocols. Incorporating technology, in the form of text messages, into the study can lessen the burden on data collectors. Furthermore, increased familiarity with new technology, especially in the target population, makes these reminder methods more accessible.

The current study provides an innovative framework for organizing and facilitating saliva self-collection from young adults. The use of frequent and consistent contact methods was instrumental in the successful collection and return of the saliva samples. While phone contact resulted in the most robust findings, the use of direct text messaging yielded promising results, especially when made in the collection phase. With a variety of reminder methods at their disposal, the data collectors were able to tailor schedules and reminder strategies to individual participants. Data collectors were also adept at establishing open

lines of communication, allowing for focused and consistent contact. This attention to detail played an integral role in the collection and return of viable samples.

This study was not a randomized trial, which would be a stronger test of the efficacy of particular reminder strategies. It would be advantageous to randomly assign participants to specific reminder types or combinations of reminder types. In the current study, the participants chose their preferred methods of contact, which points to possible selection effects in the findings. This feature of the study design limits the conclusions we can draw about the causal relationship between reminder methods and adherence. Further research is needed to determine which reminder methods are most effective for certain demographic populations. The current study found that African American males were least likely to fully complete study protocols; uncovering efficacious reminder methods for this population may yield more comprehensive results.

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Table 1
Percentages Receiving Each Type of Contact by Full and Partial Adherence and Collection and Pick-up Phase

	Autotext		Direct text		Phone		Participant initiated		Total
	Yes	No	Yes	No	Yes	No	Yes	No	
n	100	201	167	134	210	91	107	194	301
% of total	33.2	66.8	55.5	44.5	69.8	30.2	35.5	64.5	
Full adherence									62.8
Collection	72.3	58.0**	67.6	58.8	69.0	57.2*	59.7	63.6	
Pick up	n/a	n/a	70.4	58.1*	72.8	51.7**	67.1	61.3	
Total	72.0	58.2*	68.9	55.2*	69.5	47.3**	65.4	61.3	
Partial adherence									76.6
Collection	83.2	73.5*	79.4	74.5	88.0	75.7	75.8	77.0	
Pick up	n/a	n/a	83.5	72.6*	86.7	65.7**	84.2	74.2*	
Total	85.0	73.6*	81.4	70.9*	84.3	59.3**	80.4	74.7	