Biomarkers in Medicine

C-reactive protein in saliva and dried blood spot as markers of stress reactivity in healthy African–Americans

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Aim: Noninvasive assessments of C-reactive protein (CRP) in stress contexts have seldom been compared. This study evaluated CRP response to acute social stress as measured in saliva and dried blood spot (DBS). **Materials & methods:** African–Americans (N = 118; mean age = 32 years) participated in a laboratory-based social-evaluative stressor task. Six saliva samples taken before, during and after were assayed for salivary CRP. DBS measurements of CRP were taken alongside saliva at the first and last collection. **Results:** Salivary and DBS CRP were modestly positively associated with one another at baseline, and only salivary CRP increased in response to the stressor task. **Conclusion:** Noninvasive measures of CRP reactivity may be only moderately related to one another in stress reactivity contexts.

Immunological response to chronic stress is primary pathway through which psychosocial factors influence health and illness, including through links to low socioeconomic status, job strain and social isolation [1–3]. In tandem, a growing literature supports that acute immunological responses can result from brief exposure to psychosocial stressors, especially including social-evaluative threat [4]. Aligned with evidence that acute stress responses play a fundamental role in health and illness [5], stress reactivity literature has increasingly highlighted a critical need to understand links between psychosocial factors, disease processes and acute inflammatory responses [6].

One inflammatory marker that has been attended to in psychosocial stress research is CRP. CRP is an acute-phase protein secreted by the liver in response to IL-6 [7]. Among its multiple functions, CRP responses generate the production of proinflammatory cytokines that respond to infection, suggesting that CRP reactivity is an important component of an evolved defense against stress-related trauma [8,9]. Acute changes in CRP can also result from psychosocial stress, similar to reactivity that has been observed in other inflammatory markers [4]. Perhaps most notably, CRP responses to psychosocial stress have been observed as a late-phase increase that occurs during stress recovery [10–12]. Of clinical importance, repeated experiences of psychosocial stress are thought to contribute to dysregulated immune functioning, and to deleterious chronic inflammation [13,14]. In turn, chronically elevated circulating levels of CRP accompany many stress-related illnesses, including cardiovascular disease and depression [15,16].

Although CRP is both clinically important and useful in biobehavioral research, one potential obstacle to measuring CRP in stress studies is the requirement of venous blood [17]. Venipuncture is a relatively invasive, and logistically onerous procedure, requiring a trained phlebotomist and readily accessible facilities where whole blood samples can be promptly processed and stored. Venipuncture may also introduce a stressor that can confound efforts to link acute immunological responses to psychosocial variables [18]. Not surprisingly then, stress researchers have

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increasingly favored the use of noninvasive measures, especially as technical advances have made subtle assessment of a wide array of biological responses possible [19].

At present, two alternatives to venipuncture have gained momentum as a route for noninvasively assessing CRP in biobehavioral research. First, assaying CRP using whole blood dried on filter paper has been suggested as a noninvasive option [17,20]. Compared with whole blood, requirements for collecting, storing and processing dried blood spot (DBS) samples are minimal – a simple finger prick and 50 µl of blood spotted onto standardized filter paper is generally sufficient. Available literature supports that DBS and whole blood measures of CRP are very highly correlated ($r \sim 0.95$ over normal serum range, i.e., <10 mg/l) [20,21], suggesting that DBS CRP may provide a viable alternative to venous blood in biobehavioral stress research [22]. However, little is yet known about the potential to use DBS to measure acute changes CRP that can occur in response to psychosocial stress.

A second noninvasive option is measurement of CRP in saliva. Salivary CRP (sCRP) is similarly easy to collect and store [23,24]. Like DBS, collecting oral fluids also does not require skilled professionals or unique laboratory equipment, and the process is considered stress free and painless for participants. The noninvasive nature of oral fluid measurement is particularly appealing in stress-reactivity research, where repeated assessment can provide an opportunity to consider the trajectory of inflammatory and other biological responses over the course of a stressful experience, including during the event and recovery phases [10]. Although useful in these respects, the utility of salivary markers of inflammation in stress reactivity research continues to be debated [25]. A central issue is that levels of many immune-related analytes are different in oral fluids than in serum/plasma, and serum-saliva associations of many markers of inflammation are typically modest [26]. The core assumption in measuring inflammation via oral fluids is that although local–mucosal secretory and regulatory mechanisms, the status of oral hygiene, injury to the mouth and periodontal disease each contribute to the degree of inflammation in the mouth, and to measured levels in oral fluids, the contribution of the immune system remains highly compartmentalized [27–29]. In support, several studies have shown that salivary and whole blood measures of CRP are indeed significantly and reliably positively associated [30–32].

To our knowledge, research to date has not directly compared the capacity of DBS versus oral fluid measurement of CRP to benchmark an inflammatory response to acute psychosocial stress. This dearth includes comparing their functionality in popular laboratory-based stress paradigms, such as the Trier Social Stress Test (TSST) [33]. Directly comparing DBS and sCRP can further establish the validity of both noninvasive approaches at a time when momentum for use in biobehavioral research continues to build [28]. Moreover, a direct comparison may provide valuable insight into the strengths and weaknesses provided by each noninvasive approach in stress reactivity research. For example, DBS CRP may be preferable to the extent that it is very strongly associated with whole blood CRP. However, the repeated use of a lancet to obtain bloodspots may not be practical in many laboratory-based stressor paradigms. Alternatively, oral fluid may be preferable if repeated assessments are desired, though potential benefits may be offset by a comparatively modest association with whole-blood CRP. More generally, studies are needed to consider tradeoffs that characterize these alternative noninvasive approaches, and to suggest protocols for maximizing their validity, feasibility and utility in stress reactivity paradigms [34].

In the present study, we administered the TSST to a community sample of African–Americans, and we directly compared DBS and salivary measurements of CRP reactivity in response to this social evaluative threat. To perform this comparison, we selected ELISA assay kits for salivary and DBS CRP that are commonly used in biobehavioral stress research. Although the potential for cultural insight was ancillary to our more general interest in comparing noninvasive measurement modalities to one another, the recruitment of an African–American sample provided an opportunity for an additional contribution. Namely, little attention has been given to the fidelity of measuring inflammatory markers in oral fluids across cultures – a practical consideration that may be relevant to working with underserved minorities. For example, African–Americans more often confront barriers to obtaining recommended dental care [35], and resulting oral health disparities could affect measurement of inflammatory markers in saliva.

Materials & methods

Overview

This study was performed as a secondary data analysis in adjunct to alternate considerations of this laboratory-based stress reactivity study. The data were drawn from an overarching program of research concerned with evaluating stress reactivity among healthy African–Americans in response fairness and unfairness [36]. Prior publications of this study relay a strong reactivity response to the current stressor task across a number of biological and self-report stress measures [10,36,37]. The current exploration was undertaken after conducting additional CRP assays on DBS

measurements taken before and after stress induction. Our participant sample, as well as procedures for recruiting participants and implementing the stressor task are largely identical to previous descriptions, excepting changes in sample size due to unique constraints required by assessing CRP. Our focus on African–Americans in the current secondary analysis reflects this source of origin.

Participants

Participants were recruited from the metropolitan Detroit (MI) area via advertisements and snowball sampling. After completing an institutional review board (IRB)-approved online prescreen to determine eligibility, participants were contacted by phone or email and invited to participate. Individuals were eligible to participate if they were over 18, African–American, and if they did not report a pre-existing mental health condition that would prohibit undertaking a mild stress induction, specifically including medically diagnosed anxiety or depression. Individuals were also excluded if they reported poor oral health, any type of endocrine disorder, or if they were using steroid based anti-inflammatory medication or adrenergic agonists or antagonists (i.e., β-blockers). A sample of 118 African–American adults met criteria and enrolled in this research. Participants ranged in age from 18 to 63 years (M = 31.92; SD = 13.89). Three participants self-reported that they were diabetic, and three additional participants reported a thyroid condition. All participants received the modest financial compensation for participating in a single laboratory session that lasted about 3 h. The laboratory protocol was IRB-approved and took place at least 1 week after completing the prescreen measure.

Task procedure

The TSST was used to induce mild psychosocial stress and associated physiological responses [33]. TSST sessions were conducted between 10:00 and 13:00 h (the majority starting between 12:00 and 13:00 h and ending between 14:00 and 15:00 h) using two adjacent rooms in a centrally located campus laboratory. Immediately after providing consent, participants were given 10 min to acclimate, after which the TSST was administered. The TSST protocol entailed a 10-min speech preparation period, a 10-min performance (5-min speech and 5-min arithmetic task), done in front of a two-person panel (one male and one female). After the task, participants were given a 1-h period in which to recover. Participants were then debriefed and thanked for their participation.

DBS collection & preparation

Each participant provided a total of two-finger prick blood samples. The first bloodspot was collected after the initial 10-min acclimation period, alongside the first oral fluid collection. The second and final bloodspot was collected 60 min after the participant completed the TSST, alongside the last oral fluid collection. Finger pricks entailed wiping the middle finger of the participant's nondominant hand with an alcohol wipe, pricking the finger with a lancet, wiping away the first drop of blood, then collecting three to five blood spots dropped onto filter paper. Blood collection commenced after the participant provided the saliva sample. The blood spot collection cards were allowed to dry before being stored at -80°C until they were shipped frozen by overnight delivery.

Saliva collection & preparation

A total of six saliva samples were collected via passive drool from each participant using polypropylene collection tubes [23], the first of which was collected following the 10-min acclimation period. Samples 2 and 3 were collected immediately before and after the TSST performance phase. The remaining three samples were collected during the recovery period at 15, 30 and 60 min. Participants were provided with 2.5 ml of water at least 10 min prior to providing each sample [23]. To ensure quality, participants were asked to abstain from consuming food, caffeine, citric drinks and dairy, and to avoid vigorous exercise or brushing teeth in the 30 min prior to saliva collection and to report adherence to these guidelines [24]. Participants were asked to provide 2 ml whole saliva at each time point. Samples were briefly vortexed before being aliquoted into two equal samples of approximately 1 ml into polypropylene cryogenic vials to minimize the impact of multiple freeze–thaw cycles on the salivary analyte data. Aliquoted samples were stored at -80°C until they were shipped frozen by overnight delivery to Salimetrics Laboratories (PA, USA) where they were again stored at -80°C until assayed.

Oral health status

Participants self-reported oral health with four yes–no items: 'did you brush your teeth today?', 'did your gums bleed today?', 'do you have any mouth bruises?', 'have you had any recent dental work?' These responses were subsequently considered for possible inclusion as covariates.

DBS CRP assay methods

A microtiter plate-based sandwich enzyme immunoassay was used to measure CRP in DBS specimens. The assay, described in full elsewhere [20], uses capture and detection antibodies purchased from Meridian Life Science, Inc. and calibrators purchased from Fitzgerald Industries International, Inc. All specimens, calibrators and controls were assayed in duplicate. Within and between assay coefficients of variation were 6.5 and 5.3%, respectively, for the high (0.0089 mg/l) control, 2.9 and 12.0% for the medium (0.0054 mg/l) control, and 2.1 and 5.3% for the low (0.0034 mg/l) control (n = 15 plates). Analytical sensitivity, defined as the concentration three SD above the zero dose calibrator, is 0.00007 mg/l (n = 20 plates). Functional sensitivity, estimated as the concentration at which the within-assay coefficient of variation is consistently less than 10%, is 0.00015 mg/l (n = 230 duplicate specimens across eight plates). No missing or out of range values were obtained for DBS CRP.

sCRP assay methods

sCRP was assayed at Salimetrics Laboratories using a commercially available immunoassay without modification to the manufacturer's recommended protocol. The kit uses high sensitivity sandwich enzyme-linked immunoassays. The kit standards ranged from 93.75 to 3000 pg/ml with a lower limit of detection of 10 pg/ml. Samples were thawed to room temperature, centrifuged at 3000 rpm for 15 min to remove mucins and diluted with phosphate buffer and preservative to 1:10 prior to assay. All samples were assayed in duplicate, and the average value of each sample was used in subsequent analyses. Interassay and average intra-assay coefficients of variation were less than 4 and 10%, respectively. Sixteen participants were excluded because of missing or out of range values, resulting in a final sample size of 102 participants for sCRP.

Analytic strategy

Prior to considering baseline and reactive CRP responses, CRP data were screened for exceptionally high values that would suggest acute infection or illness that would interfere with considering either chronic or reactive CRP levels. To begin, we excluded cases where DBS CRP levels exceeded 10 mg/l; a clinically relevant cutoff that is commonly used to denote that acute infection or illness is suspected [38]. Because clinical criteria for acute inflammation in salivary CRP are not yet well developed [32], we opted to conservatively exclude participants whose sCRP level exceeded 3.3 standard deviations at the first collection timepoint. These procedures resulted in an effective sample size of N = 79 (see also the Supporting Information). Consistent with much prior research, the remaining DBS and salivary CRP measures were log transformed to correct for significant positive skew. Reactivity of DBS CRP was quantified both as the absolute change (pre- to post-task), and as percentage change. Integrated measures of salivary CRP reactivity were calculated as area under the curve with respect to ground (AUCg) and area under the curve with respect to increase (AUCi) using formulas provided by Pruessner *et al.* [39]. AUCg represents total analyte concentration across the six time points, whereas AUCi represents total increase in the analyte across the six time points.

Prior to DBS and saliva comparison, independent-samples *t*-tests were conducted to determine whether there were any differences in sCRP levels for participants across the six salivary collections based on self-reported oral health variables. There were no differences for self-reported bleeding gums, p's >0.210, mouth bruises, p's >0.364 or for recent dental work, p's >0.496. However, sCRP was significantly higher among participants who brushed their teeth (n = 47) than among those who did not (n = 32) at the first timepoint (M = 3.31, SD = 0.27 vs M = 3.19, SD = 0.32; t(77) = 1.76, p = 0.083); the second timepoint (M = 3.33, SD = 0.28 vs M = 3.18, SD = 0.33; t(77) = 2.20, p = 0.031); and the third timepoint (M = 3.35, SD = 0.28 vs M = 3.20, SD = 0.36; t(77) = 2.03, p = 0.046).

Two sets of analyses were conducted to compare sCRP and DBS CRP to one another. First, we computed bivariate associations. Specifically, we considered links between DBS CRP and sCRP at each collection timepoint, as well as between integrated reactivity measures that were calculated for each measurement modality. For bivariate

Table 1. Associations between nonintegrated and integrated CRP in dried blood spot and saliva (N = 79).								
Integration	**M (SD)**	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**
Nonintegrated								
1. DBS CRP T1	1.84 (2.24)							
2. DBS CRP T2	1.82 (2.21)	0.992[‡]						
3. sCRP T1	2206.41 (1334.77)	0.183	0.196					
4. sCRP T2	2312.08 (1615.16)	0.165	0.180	0.805[§]				
5. sCRP T3	2428.04 (1665.97)	0.158	0.176	0.800[§]	0.958[§]			
6. sCRP T4	2558.19 (2326.03)	0.224[†]	0.254[†]	0.792[§]	0.678[§]	0.735[§]		
7. sCRP T5	2455.53 (2886.70)	0.211[†]	0.233[†]	0.823[§]	0.606[§]	0.642[§]	0.887[§]	
8. sCRP T6	2720.10 (2736.30)	0.262[†]	0.288[‡]	0.706[§]	0.574[§]	0.644[§]	0.908[§]	0.906[§]
Integrated		**1.**	**2.**	**3.**	**4.**			
1. Δ DBS CRP	-0.026(0.38)							
2. %Δ DBS CRP	-0.008(0.14)	0.167						
3. %Δ sCRP	1.915(8.645)	0.161	0.041					
4. AUCg sCRP	347,542.38 (246,302.56)	0.087	0.209[†]	0.123				
5. AUCi sCRP	33,651.78 (160,273.91)	0.169	0.107	0.809[§]	0.236[†]			

Data screening involved first removing cases in which scores exceeded 3.3 standard deviations, then controlling for age and gender and finally controlling for smoking, brushing teeth and dairy consumption (df = 72). DBS is in mg/l. sCRP is in pg/ml. %Δ sCRP = percentage change in sCRP from time 1 to time 6. Significance is one tailed.
[†] p < 0.05.
[‡] p < 0.01.
[§] p < 0.001.
AUCg: Area under the curve with respect to ground; AUCi: Area under the curve with respect to increase; DBS: Dried blood spot; sCRP: Salivary CRP; SD: Standard deviation.

associations, partial correlations were computed, with age and gender entered as control variables.[1] We also entered tooth brushing as a control variable, based on our assessment of oral health variables. Finally, we entered dichotomous variables for smoking (yes = five, no = 74) and dairy consumption (yes = eight, no = 71) based on salivary assay kit manufacturer recommendations. In addition to bivariate associations, we considered the potential for DBS CRP and sCRP to similarly benchmark an acute reactivity response to the stressor task by assessing mean differences between the first and last collection timepoints. For all bivariate associations, we used one-tailed significance tests, given that a strong and positive association between CRP as measured in multiple noninvasive measures could be reasonably expected. Additionally, we used one-tail significance tests to assess mean differences, as an increase in CRP from pre- to post-stressor task could also be reasonably expected [25].

Results
Bivariate associations between sCRP & DBS CRP
Table 1 presents bivariate associations for nonintegrated CRP measurements collected in DBS and saliva at all available timepoints. Pre (T1) and post (T6) measures of DBS CRP were very strongly associated with one another (r = 0.992, p < 0.001), and the analogous association for sCRP was similarly strong (r = 0.706, p < 0.001). Of greater interest, T1 measures of DBS CRP and sCRP were more modestly associated (r = 0.183, p = 0.059). Similarly, T1 DBS CRP was significantly though modestly associated with T6 sCRP (r = 0.262, p = 0.012). T6 DBS and T6 sCRP were also associated (r = 0.288, p = 0.006). However, a Fisher r-to-z transformation revealed that this association was not significantly larger than that observed between T1 DBS CRP and T6 sCRP (z = -0.173, p = 0.862). Overall, while associations within each noninvasive measurement modality were strong, associations between DBS CRP and sCRP were more modest and were only significant when later sCRP responses were considered.

Table 1 also presents bivariate associations for integrated measures of DBS CRP and sCRP reactivity. Absolute and percent change in DBS CRP were only modestly positively associated with one another (r = 0.167, p = 0.077).

[1.] This study included two minor variations to the traditional Trier Social Stress Test (TSST) protocol. One variation led participants to believe that their individual performance during the TSST was judged to be either satisfactory or unsatisfactory by a speech expert. A second variation called for a laboratory assistant to treat participants either politely or slightly impolitely just prior to the post-task recovery portion of the session. These variations were fully crossed and simultaneously implemented 10 min prior to the fourth salivary collection timepoint. To ensure that associations among CRP measures were not affected, correlations were repeated while controlling for both protocol variations. The pattern of associations and corresponding statistical significance was unchanged.

AUCg and AUCi scores for sCRP were similarly modestly positively associated (r = 0.236, p = 0.021). Of greater interest, percent change in DBS CRP was positively associated with AUCg for sCRP (r = 0.209, p = 0.037), though no association was observed for AUCi sCRP (r = 0.107, p = 0.183). Absolute change in DBS CRP was not significantly correlated with either sCRP measure (r's > 0.169). Overall, percentage change in DBS CRP was significantly though modestly positively associated with total activation of sCRP.

Comparing mean differences to assess reactivity of CRP in saliva & DBS

Table 1 also reports mean concentrations of DBS CRP and sCRP. Two separate paired sample t-tests were conducted on log transformed means to assess whether the expected increase in CRP from T1 to T6 was observed for both DBS and saliva. The difference between T1 sCRP and T6 sCRP was significant (t(78) = -1.879, p = 0.032, d = 0.20), indicating that sCRP increased across the session (M_{T1} = 3.26, SD = 0.29; M_{T6} = 3.32, SD = 0.32). However, DBS CRP did not significantly differ between T1 and T6 (M_{T1} = -0.047, SD = 0.55 M_{T6} = -0.055 mg/l, SD = 0.56; t(78) = 1.10, p = 0.138, d = -0.01).

Discussion

Aligned with momentum toward greater use of noninvasive measures in stress research, and with rapidly proliferating interest in immunological response to psychosocial stress [24], the current research compared individual differences in CRP response to social evaluative threat as measured noninvasively in saliva and DBS. Results highlight that noninvasive measures of CRP may be only moderately related to one another in stress reactivity contexts.

To begin, we compared CRP measurements obtained at baseline through DBS and saliva to one another. This comparison is analogous to existing studies that have examined associations between whole blood and oral fluid measures of CRP as chronic markers of inflammation [32,40,41], though the current study is the first to our knowledge to directly compare DBS and oral fluid measures of CRP to one another in this manner. Similar to prior studies that have collected whole blood, we observed only a modest association between T1 DBS CRP and T1 sCRP. This association was not statistically significant, which may be partly attributable to a small sample size as compared with available and larger correlational studies that did not address CRP reactivity, as the magnitude of the association between DBS and salivary CRP was comparable to available whole blood studies. For example, Out *et al.* [32] observed diurnal correlations between commensurately collected measures of sCRP and whole blood CRP ranging from r = 0.20 to 0.61, which seems fairly comparable to presently obtained commensurate associations of r = 0.18 and 0.29 at T1 and T6, respectively.

Of greater interest, our study is also the first to our knowledge to consider associations between DBS and oral fluid measures of CRP in response to social evaluative threat. Similar to T1 associations, reactivity of CRP as measured in DBS and oral fluid were only modestly positively correlated with one another, and this association was significant only for the association between percent change in DBS CRP and total activation of sCRP. Moreover, interpretation of the lone significant association between DBS CRP and sCRP is clouded to the extent that no statistically significant increase in DBS CRP was observed. Thus, a direct comparison of CRP reactivity highlights that noninvasive measurement modalities may be only modestly associated, while also suggesting that CRP reactivity may be captured differently by each measurement modality. This result aligns with a handful of available studies that similarly suggest immunological reactivity as measured in serum and saliva may be unrelated to one another. For example, Minetto *et al.* showed that serum and saliva reactivity measures of IL-6 in response to exercise stress were not significantly associated with one another [28,42].

With an eye toward presently obtained differences between DBS CRP and sCRP, the current results suggest the potential for technical and logistical aspects of noninvasive CRP measurement to influence the assessment of immunological reactivity in response to a stressor task. We consider three potential parameters that researchers might attend to when endeavoring to measure CRP reactivity through noninvasive measurement modalities. First, the lack of strong agreement between CRP measurement modalities might be partly explained by differences in the kinetics of CRP between blood and saliva. Changes in CRP are thought to be observed more rapidly in blood than in saliva, and it could be that reactivity correlations between noninvasive measurement modalities partly reflect that post task DBS CRP was assessed too late to capture a peak response to the social evaluative stressor task, In support, prior stress reactivity studies have shown a more consistent significant rise in serum CRP when measured 10–30 min post-stressor task [12,43], whereas studies that have measured whole blood CRP 120 min post-stressor task have shown mixed results [11,44]. It is possible that an association between T4 or T5 DBS CRP would be more strongly associated with sCRP reactivity than the presently assessed T6 DBS CRP. However, collecting a post

task measurement of DBS earlier could pose challenges to researchers who wish to simultaneously measure stress reactivity in oral fluids. Of note, an earlier post task assessment of DBS CRP could introduce a naturalistic stressor that impedes subsequent measurements of inflammatory and concomitant stress markers in oral fluids.

A second potential source of concern is the stability of DBS samples both at room temperature and in long-term storage. Specifically, Brindle *et al.* reported that DBS CRP quickly degraded both when exposed to ambient temperatures and in long-term storage at 20°C [20]. Of present relevance, our collection and storage protocol was such that pre-task DBS CRP samples were left ambient for a period of approximately 2 h during administration of the stressor paradigm, whereas post-task DBS CRP samples were stored and frozen after approximately 30–45 min. Although speculative, stronger correlations between post-task DBS CRP and sCRP that we observed may have been driven by greater ambient degradation of pre-task than post-task DBS CRP samples. Future research may wish to more formally explore the potential for DBS CRP to demonstrate stronger associations with sCRP if DBS samples are more promptly stored. Although DBS samples were stored at -80°C, this feature was consistent between pre- and post-task DBS assessments. Thus, potential degradation due to long-term storage likely does not explain divergences that we observed between pre- and post-task assessments of DBS CRP.

A third source of consideration centers on the fidelity of oral fluid as a marker of acute inflammation. The potential for oral fluid measurement of CRP to indicate local inflammation is well recognized, and a key assumption is that the contribution of systemic inflammation is compartmentalized [24]. Although acute stress could lead to an increase in CRP in saliva via infiltration of blood (for a review, see [45]), which would suggest linkages to an increase in systemic inflammation (for review, see [46]), acute stress could directly affect oral inflammation in the mouth [47]. At present, the extent to which sCRP reactivity indicates local versus systemic inflammation is not well known. Issues related to local versus systemic inflammation are further confounded in the present research by our use of an African–American sample, as it is conceivable that local inflammation may be more pervasive in African–Americans due to oral health disparities [48].

Four limitations suggest caution as well as direction for future research. First, this study only included African–Americans. This cultural focus was ancillary to our more general interest in comparing noninvasive CRP measurement modalities to one another, though the recruitment of an African–American sample provided an opportunity to highlight additional considerations. Future studies should conduct formal cross-ethnic comparisons of the presently considered noninvasive measurement modalities to gain a better sense of cultural similarities and differences, including the potential for oral health disparities to interfere with salivary measurement in African–Americans [35]. Second, the present research only focused on CRP. Available studies suggest that alternative biomarkers of inflammation such as IL-6 and TNF-α perhaps better portray inflammatory reactivity to psychosocial stress [25]. To our knowledge, however, DBS measures of these markers are not yet widely available. Future research will be needed to compare other inflammatory markers across noninvasive measurement modalities as DBS and other options become more readily available. Third, our effective sample size for this study was significantly reduced by accounting for missing and out of range CRP values, and we did not include a resting control group. Future studies using larger sample sizes can better attend to acute inflammation, out of range values and other measurement issues that can affect whether assessing CRP is practical. Larger sample sizes may also permit including a resting control group. Finally, the present study did not collect whole blood measures of CRP. Although whole blood would provide a definitive yardstick with which to compare noninvasive measurement modalities, several aforementioned tradeoffs are inherent in collecting whole blood in stress reactivity research. Given momentum for use of noninvasive measures of CRP in stress reactivity research, our initial focus was on comparing DBS and oral fluid measurements. Nonetheless, future research may collect and compare CRP reactivity as measured in whole blood to both of the presently considered noninvasive measurement modalities, which could provide insights necessary to resolve many of the presently identified questions. For example, because both are peripheral measures, sCRP and DBS CRP may correlate more strongly with a systemic measure of CRP than with one another. Related, it is possible that peripheral measures of CRP may provide an incrementally useful indicator of inflammation above and beyond more systemic whole blood measurement of CRP.

Conclusion

The current research contributes in important ways to the evolving literature on inflammatory stress responses and noninvasive measurement of inflammatory stress markers. Namely, the present results highlight that noninvasive measures of CRP reactivity may be only moderately related to one another and suggest a critical need to further consider the strengths and challenges of noninvasive measurement modalities. This includes comparing DBS

and oral fluid measures of other inflammatory markers that may help to establish protocols for effective use in psychosocial stress research.

Summary points

- Inflammatory responses to stress contribute to numerous illnesses, including through links to psychosocial stressors.
- CRP is an inflammatory response that may be particularly important in stress and illness.
- Acute social stress may elicit CRP reactivity, suggesting a potentially critical role in chronically dysregulated stress and illness.
- Noninvasive measures of CRP would be particularly useful in biobehavioral stress research.
- Using salivary and dried blood spot measure obtained from healthy African–Americans before, during and after participating in a social stressor task, we examined correlations between two popular noninvasive measures of CRP.
- We found that salivary and dried blood spot CRP were modestly, though not significantly positively associated with one another at baseline. Moreover, CRP demonstrated a significant increase in response to the stressor task only when measured in saliva.
- Findings demonstrate that noninvasive measures of CRP reactivity may be only moderately related to one another in stress reactivity contexts.
- Although noninvasive measures of CRP could serve as a useful biomarker of inflammatory response to acute stress, future research is critical to establishing protocols for effective use in stress reactivity contexts and for deciphering the meaning of reactive change.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Data sharing statement

This manuscript does not report either the original results of a clinical trial, or the secondary analysis of clinical trial data that have been shared with them. As such, there is no data sharing statement.

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