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Blood Substrate Collection and Handling Procedures under Pseudo-Field Conditions: Evaluation of Suitability for Inflammatory Biomarker Measurement

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Routine incorporation of blood-based biomarker measurements in population studies has been hampered by challenges in obtaining samples suitable for biomarker assessment outside of laboratory settings. Here, we assessed the suitability of venous blood left unprocessed for 4, 24, or 48 hours post-collection at either room temperature or 4°C for quantification of two biomarkers, Interleukin-6 (IL-6) and C-reactive protein (CRP). Blood samples were collected in both K_2EDTA tubes and a dedicated plasma-preservation tube, P100. Dried blood spot (DBS) samples from the same subjects were also collected in order to compare delayed-processing plasma performance against a popular alternative collection method. We found that K_2EDTA mean plasma concentrations of both IL-6 and CRP were not significantly different from concentrations in plasma processed immediately; this was observed for tubes stored up to 48 hours pre-processing at either temperature. Concentrations of IL-6 measured in P100 tubes showed significant time-dependent increases when stored at room temperature; otherwise, levels of IL-6 and CRP were similar to those found in samples processed immediately. Levels of

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CRP in DBS were correlated with plasma CRP levels, even when pre-processed blood was stored for up to 48 hours. These data indicate that plasma is suitable for IL-6 and CRP estimation under data collection conditions that involve processing delays.

Introduction

Biomarker measurement is increasingly of great interest as research progressively demonstrates the importance of biomolecules as both predictors and consequences of health outcomes (Crimmins, Kim, and Vasunilashorn 2010). Of particular interest are biomolecules related to inflammation, such as Interleukins and C-reactive protein (CRP), which are measured in isolated blood and their products, including plasma. Historically, the handling and processing requirements of whole blood samples destined for plasma-based analyses have proven a barrier to incorporation of blood collection in large population-based studies. A key concern is that separation of the plasma fraction soon after blood draw is necessary to prevent stimulation and/or degradation of biomarkers. Best practices suggest that blood should be processed into plasma and other blood products within four hours of collection for optimal preservation of biomarker profiles (e.g. (Tuck et al. 2009)). Since many population-based studies are conducted in locations remote from a processing laboratory, it is difficult or impossible to deliver blood samples for processing in a timely fashion. Alternative strategies, such as the use of mobile processing laboratories or blood collection in central clinical facilities, are logistically challenging and prohibitively costly. As such, identification and validation of a blood collection strategy that prolongs this pre-processing time frame could open up the possibility of assessing biomarkers in large, population-representative studies.

Plasma is usually derived from blood collected in blood tubes containing preservatives such as K_2EDTA and citrate. These tubes are popular, as they are widely available and relatively inexpensive. Contrary to the "best-practice" scenario described previously, a variety of research suggests that certain plasma biomarkers derived from these tubes show temporal and thermal stability under conditions outside of the optimal processing time frame (Flower et al. 2000; Skogstrand et al. 2008). However, this finding is not universally applicable across the complete range of available biomarkers, and this inconsistency has prompted researchers to develop alternative strategies for collection.

One strategy is to rely on specialized collection tubes. Currently, there are no commercial products available that can preserve unprocessed blood intended for plasma isolation over prolonged periods. However, there do exist products intended to preserve plasma profiles in processed blood, meaning that blood samples require some immediate post–blood draw processing, usually centrifugation. One such product, the P100 tube available from Becton Dickinson (BD), contains K_2EDTA as well as proprietary additives intended to stabilize plasma biomarker profiles in post-centrifuged blood for up to five days at room temperature. The stability of biomarker profiles in blood left unprocessed for this period of time has not been assessed, but these tubes could be an attractive alternative if demonstrated to preserve profiles comparably.

A second strategy is to collect substrates other than plasma. A popular alternative method of collecting blood under field conditions is via dried blood spots (DBS) (McDade, Williams, and Snodgrass 2007; Danese et al. 2011; Crimmins et al. 2014; Danese et al. 2014). The main advantages of this method are that (a) it is minimally invasive, and (b) DBS are easier to handle than whole blood and purport to preserve biomarker profiles under non-restrictive time and temperature conditions. The main disadvantage is that a smaller sample volume is obtained, which can limit the number of biomarkers that can be analyzed.

We sought to assess the stability of two commonly measured plasma biomarkers, IL-6 and CRP, across a variety of pre-processing blood handling conditions. We aimed to mimic common conditions encountered in a field-based population study, that is, (1) time periods between collection and processing ranging from zero to 48 hours; and (2) sample storage temperatures of either 4°C or room temperature. These conditions were executed in parallel in both K_2EDTA and P100 tubes. In addition, we compared CRP levels in DBS to the levels in plasma samples from the same subjects in order to confirm the applicability of this method as an alternative collection strategy.

Methods

Blood Collection and Processing

Venous blood samples were collected from six volunteers by a trained phlebotomist using standard blood collection apparatus. Blood was collected in either 3-ml K_2EDTA Vacutainer tubes (BD, Franklin Lakes, NJ) or P100 tubes (BD) in series and was anonymized. After the necessary initial inversion, tubes were incubated, without further agitation, according to the experimental protocol outlined in Figure 1. After the appropriate incubation period, tubes were processed by centrifugation at 2,000 g for 10 minutes (K_2EDTA) or 2,500 g for 20 minutes (P100), and the plasma fraction was transferred into 2-ml cryovials. Tubes selected for zero-hour processing (hereafter termed "baseline") were centrifuged immediately upon collection. All plasma was stored at −80°C until analysis.

In addition, DBS samples were collected from each volunteer via the use of Whatman Protein Saver Cards (GE Healthcare, Pittsburgh, PA), as described previously (Danese et al. 2011). Briefly, a lancet was used to pierce the side of the middle fingertip, and a series of five blood spots was collected. After collection, cards were air-dried until dry (a minimum of 30 minutes) before being transferred to an airtight box and placed at −20°C until analysis. The DBS samples were prepared for analysis as follows: one 3-mm punch of the fourth spot on the Protein Saver card was taken per subject; these spots were placed into 250 μl of DBS elution buffer (1 × PBS [Sigma Aldrich, St. Louis, MO], 0.05% Tween 20 [Sigma Aldrich]). Spots were allowed to elute for 18 hours at 4°C. Prior to assaying, eluted DBS samples were placed on a shaker for 1 hour.

Biomarker Measurement

Levels of IL-6 and CRP were determined using human IL-6 and CRP Quantikine ELISA kits (R&D, Minneapolis, MN) following the manufacturer's instructions. Briefly, 100 μL of sample for IL-6 or 50 μL for CRP were used per reaction. For CRP analysis, plasma was diluted at a level of 1 in 100 in calibrator diluent prior to assaying, as recommended by the manufacturer, while eluted DBS samples remained undiluted. For IL-6 analysis, plasma samples were used neat. All ELISA reactions were performed in duplicate. A twofold serial dilution of a manufacturer-provided standard was included, with the lowest being a sample blank. Upon completion of the reactions, Optical Density (OD) readings at 450 nm (correcting for background at 540 nm) were read using a Spectramax-384 spectrophotometer (Molecular Devices, Sunnydale, CA), and data were acquired using SoftMax Pro software (Molecular Devices, Sunnydale, CA). Raw OD units were transformed by subtracting the mean OD value of the sample blank, and a standard curve was calculated using four-parameter polynomial regression. The resulting equation was used to extrapolate sample OD values into concentrations. For plasma CRP, these concentrations were multiplied by

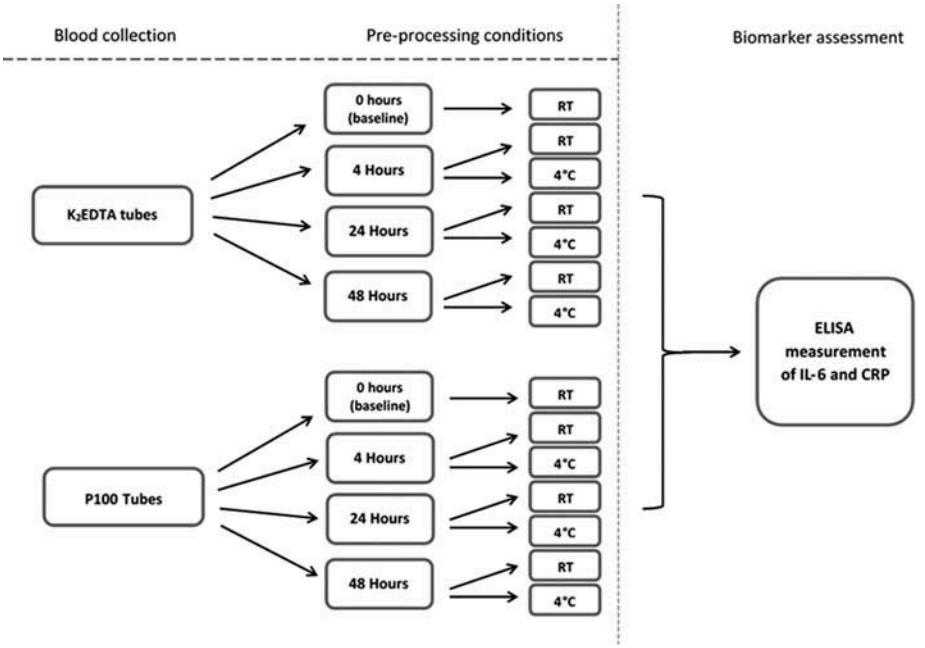

Figure 1. Diagrammatic representation of blood pre-processing conditions employed in the current investigation. Fourteen separate experimental conditions (tubes of whole blood) were collected per subject. Processing consisted of centrifugation of whole blood and collection of plasma fraction for downstream biomarker measurement. Briefly, tubes selected for room temperature (RT) incubation were placed in racks within the laboratory at ambient temperature (22–23°C) for the allotted period of time. Tubes selected for 4°C incubation were placed within a laboratory refrigerator for the allotted time. The designated incubation periods were 4, 24, and 48 hours.

the initial dilution factor (1 in 100) to derive an estimate of plasma concentration. Duplicate values were then averaged to derive a mean concentration per sample. DBS samples were assayed for CRP only as a result of hypothesized levels of circulating IL-6 falling below the limit of detection of our assay. Plasma-equivalent concentrations of CRP in DBS samples were calculated by regressing uncorrected values against the concentration measured in plasma at baseline (Stockl, Dewitte, and Thienpont 1998; McDade 2014). The mean within-assay coefficient of variation (%CV) for IL-6 measurements was 4.13 percent, and for CRP measurements it was 3.34 percent.

Statistical Analysis

The effects of average time to processing, temperature, and tube type on biomarker concentrations were calculated and tested using paired-sample *t*-tests and repeated measures ANOVA in SPSS version 22 (IBM, New York, NY).

Results

Measurement of IL-6 and CRP

In K_2EDTA-derived plasma, baseline concentrations of IL-6 and CRP were 0.99 ± 0.50 pg/mL (mean $\pm$ SD) and 0.87 ± 0.46 mg/L, respectively. In P100-derived

plasma, those values were 1.00 ± 0.47 pg/mL and 0.87 ± 0.44 mg/L. For both analytes, baseline levels were highly correlated between the two tube types ($r = 0.99$, $p < .01$), suggesting no initial differences in concentrations relating to blood collection procedures.

Does Length of Time Between Blood Collection and Plasma Processing Influence IL-6 and CRP Concentrations?

Concentrations of both IL-6 and CRP in plasma derived from K_2EDTA tubes were not significantly affected by time course whether stored at room temperature (RT; 22–23°C) or 4°C (Figure 2A and B). Concentrations of both biomarkers in plasma derived from P100 tubes were not significantly affected by time-course when stored at 4°C. At RT, however, there was a significant rise in IL-6 concentration over the time-course (Figure 2C). This was not observed for CRP (Figure 2D).

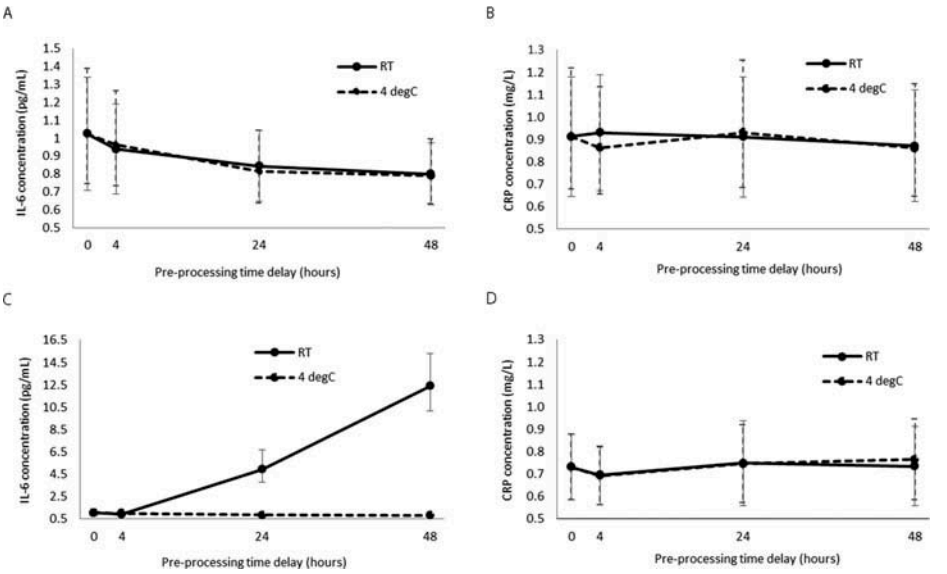

Figure 2. Time course profile of mean IL-6 and CRP concentrations in plasma from blood collected in K_2EDTA (**A** and **B**) and P100 (**C** and **D**) tubes. For blood stored in K_2EDTA tubes, a repeated measures ANOVA (with Greenhouse-Geisser correction where appropriate) showed that time course did not significantly affect mean IL-6 concentrations at either RT ($F(1.01, 3.03) = 1.45$, $p = .32$) or 4°C ($F(1.12, 4.49) = 3.63$, $p = .12$; see **A**). Similarly, mean CRP concentrations were not significantly affected by time course at either RT ($F(3, 9) = 0.75$, $p = .55$) or 4°C ($F(3, 12) = 1.31$, $p = .32$; see **B**). For blood stored in P100 tubes at 4°C, the same analysis showed that time course did not affect IL-6 concentrations ($F(1.92, 9.60) = 1.79$, $p = .22$; see **C**). However, storage at RT resulted in significantly elevated estimates of IL-6 concentrations (note the Y-axis scale of **C**); mean concentrations differed significantly between time points ($F(1.26, 6.32) = 25.77$, $p < .01$). The same analysis determined that for P100 tubes, time course did not affect mean concentrations of CRP at either RT ($F(3, 15) = 0.27$, $p = .85$) or 4°C ($F(3, 12) = 0.99$, $p = .43$; see **D**). Error bars represent Standard Error of the Mean (SEM). Plots are derived from subjects with a full complement of data points for comparison purposes.

Does Storage Temperature Have an Effect on IL-6 and CRP Concentrations?

For this analysis we compared blood stored at RT and blood stored at 4°C, within the same tube type and for the same length of time pre-processing. For blood stored in K_2EDTA tubes, mean differences in IL-6 concentration did not differ significantly between RT and 4°C incubation (Table 1, **Panel A**; Figure 2A). In contrast, IL-6 concentrations in blood stored in P100 tubes were significantly different between incubation temperature conditions (Figure 2C), a finding explained by the time course profile described previously.

Regardless of pre-processing incubation time, there were no significant mean differences in CRP concentration between blood stored at RT and blood stored at 4°C within either tube type (Table 2, **Panel A**). All paired sample comparisons were highly correlated ($p < .01$; Figure 2B and 2D).

Does Collection Tube Type Have an Effect on IL-6 and CRP Concentrations?

For IL-6, we restricted this analysis to blood stored at 4°C, since we had already observed that the profile from RT P100 plasma is too dissimilar from that of RT K_2EDTA plasma to make any meaningful comparison. Regardless of pre-processing incubation time, there were no significant mean differences in concentrations of either biomarker, whether collected in K_2EDTA or P100 tubes (Table 2, **Panel B**).

Are DBS CRP Concentrations Predictive of Plasma CRP Concentration?

First, we compared the concentration of DBS CRP against the baseline plasma concentration of CRP (Stockl, Dewitte, and Thienpont 1998; McDade 2014). The reasoning was that (a) we aimed to mathematically determine the dilution factor for DBS samples, and (b) the baseline plasma concentrations should reflect subjects' CRP levels before the introduction of any bias due to the effects of pre-processing conditions. Correlations between DBS CRP concentrations and both K_2EDTA and P100 CRP concentrations at baseline were high ($r = 0.97$ for both), which allowed us to use the resulting regression equation ($y = 256.18x - 0.0665$) to correct DBS values to estimated plasma-equivalent concentrations. The observation that uncorrected concentrations of CRP from DBS samples are an order of 256 times lower than those measured in neat plasma suggests that elution of one DBS punch dilutes CRP levels approximately 256 times; values measured using 256 μl of DBS elute would approximate those made using 1 μl of neat plasma. This suggests that our single-punch DBS samples have a plasma-equivalent volume of 0.98 μl (250 μl/256).

We then sought to determine whether there were any mean differences between estimated DBS CRP levels and those in K_2EDTA- and P100-derived plasma stored for 48 hours at RT. This analysis would determine whether "worst-case scenario" venous blood collection strategies yielded significantly different values from those derived using DBS. The intra-individual estimates of CRP under each condition can be seen in Figure 3. Within-individual plasma-equivalent DBS values were similar to values measured in plasma from either tube type ($r = 0.98$, $p < 0.01$), suggesting that both methods of blood collection yield good approximations of baseline plasma CRP concentrations.

Discussion

Our data suggest that (1) blood stored in K_2EDTA tubes for up to 48 hours pre-processing is suitable for estimation of plasma IL-6 and CRP levels; (2) for K_2EDTA tubes, profiles of plasma biomarkers are similar for refrigerated and room temperature–stored blood;

Table 1

Comparison of mean IL-6 concentrations between different tube types stored at different temperatures. Concentrations of IL-6 are not significantly different between room temperature (RT) and 4°C incubated K_2EDTA tubes at any time period; however, there is a significant increase in IL-6 in RT incubated P100 tubes compared with 4°C tubes over time (**Panel A**; significant differences are marked in bold). At 4°C, concentrations of IL-6 do not differ significantly between K_2EDTA and P100 tubes (**Panel B**).

	Panel A						Panel B			
IL-6	K_2EDTA			P100			4°C			
Comparison	4 hrs incubation, RT vs. 4°C	24 hrs incubation, RT vs. 4°C	48 hrs incubation, RT vs. 4°C	4 hrs incubation, RT vs. 4°C	24 hrs incubation, RT vs. 4°C	48 hrs incubation, RT vs. 4°C	Baseline, K_2EDTA vs. P100	4 hrs incubation, K_2EDTA vs. P100	24 hrs incubation, K_2EDTA vs. P100	48 hrs incubation, K_2EDTA vs. P100
Mean fold change (SEM)	0.98 (0.02)	1.04 (0.03)	1.01 (0.04)	0.85 (0.05)	4.70 (0.72)	15.19 (1.94)	0.99 (0.05)	0.95 (0.04)	0.95 (0.04)	0.91 (0.05)
Mean difference (SEM)	−0.02 (0.02)	0.03 (0.03)	0.00 (0.04)	−0.17 (0.08)	3.44 (1.08)	11.72 (2.16)	0.00 (0.03)	−0.04 (0.04)	−0.03 (0.02)	−0.06 (0.04)
95% CI of difference (lower, upper)	−0.06, 0.03	−0.07, 0.13	−0.10, 0.10	−0.36, 0.03	0.66, 6.21	6.17, 17.23	−0.09, 0.08	−0.15, 0.07	−0.09, 0.02	−0.18, 0.05
Paired *t* (df)	−0.98 (4)	0.96 (3)	0.70 (4)	−2.22 (5)	**3.18 (5)**	**5.42 (5)**	−0.06 (5)	−1.08 (4)	−1.67 (4)	−1.50 (4)
p	.38	.41	.95	.08	**.03**	**<.01**	.96	.34	.17	.21

Table 2

Comparison of mean CRP concentrations between different tube types stored at different temperatures. Concentrations of CRP are not significantly different between room temperature (RT) and 4°C incubated K_2EDTA or P100 tubes at any time period (**Panel A**). Additionally, concentrations of CRP do not differ significantly between K_2EDTA and P100 tubes whether stored at RT or stored at 4°C (**Panel B**).

	Panel A							Panel B					
CRP	K_2EDTA			P100				RT			4°C		
Comparison	4 hrs incubation, RT vs. 4°C	24 hrs incubation, RT vs. 4°C	48 hrs incubation, RT vs. 4°C	4 hrs incubation, RT vs. 4°C	24 hrs incubation, RT vs. 4°C	48 hrs incubation, RT vs. 4°C	Baseline, K_2EDTA vs. P100	4 hrs incubation, K_2EDTA vs. P100	24 hrs incubation, K_2EDTA vs. P100	48 hrs incubation, K_2EDTA vs. P100	4 hrs incubation, K_2EDTA vs. P100	24 hrs incubation, K_2EDTA vs. P100	48 hrs incubation, K_2EDTA vs. P100
Mean fold change (SEM)	1.06 (0.04)	0.99 (0.02)	1.02 (0.02)	1.01 (0.01)	0.98 (0.04)	0.98 (0.03)	1.01 (0.04)	1.05 (0.04)	1.21 (0.2)	0.93 (0.04)	1.03 (0.03)	0.98 (0.05)	0.90 (0.06)
Mean difference (SEM)	0.06 (0.04)	−0.02 (0.02)	0.01 (0.02)	0.00 (0.01)	−0.02 (0.05)	−0.01 (0.04)	0.01 (0.03)	0.03 (0.06)	0.11 (0.13)	−0.10 (0.05)	0.02 (0.02)	−0.02 (0.05)	−0.12 (0.07)
95% CI of difference (lower, upper)	−0.05, 0.17	−0.10, 0.06	−0.06, 0.08	−0.02, 0.02	−0.15, 0.10	−0.12, 0.09	−0.08, 0.09	−0.12, 0.17	−0.27, 0.48	−0.24, 0.04	−0.05, 0.08	−0.17, 0.13	−0.32, 0.07
Paired *t* (df)	1.56 (4)	−0.77 (3)	0.40 (4)	0.53 (4)	−0.52 (5)	−0.35 (5)	0.16 (5)	0.47 (5)	0.79 (4)	−2.06 (4)	0.82 (3)	−0.32 (4)	−1.74 (4)
p	.19	.50	.71	.63	.62	.74	.88	.66	.48	.11	.47	.77	.16

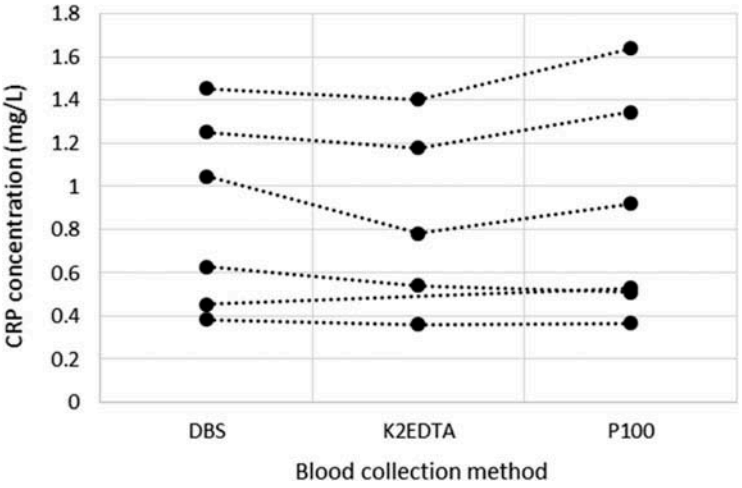

Figure 3. Plots of CRP levels measured in (a) DBS, (b) K_2EDTA plasma from blood stored 48 hours at RT pre-processing, and (c) P100 plasma from blood stored 48 hours at RT. Each point represents an individual subject with lines linking within-individual measures for comparison purposes. DBS CRP levels were scaled to plasma-equivalent values using the regression equation of baseline CRP values vs. uncorrected DBS values. A paired *t*-test analysis determined there were no significant differences between estimated plasma-equivalent DBS concentrations and those of K_2EDTA-derived plasma (mean difference $= -0.10$, $SE = 0.04$, $t(4) = -2.33$, $p = .08$), or P100-derived plasma (mean difference $= 0.02$, $SE = 0.05$, $t(5) = 0.31$, $p = .77$).

(3) the effectiveness of P100 tubes in preservation of plasma biomarkers is temperature and biomarker specific; and (4) DBS can be used as a non-invasive alternative to whole blood for measuring CRP.

These findings reinforce the view that it is possible to collect venous blood intended for biomarker measurement in field collection settings, and that prolonged pre-processing times are not significantly detrimental to their estimation. Furthermore, our findings confirm that, at least for the biomarkers examined, refrigerated pre-processing conditions do not improve this estimation. Our results support those of others demonstrating that IL-6 shows temporal stability in plasma derived from K_2EDTA tubes (De Jongh et al. 1997; Jackman et al. 2011; Riches et al. 1992; Thavasu et al. 1992), although there have also been reports of significant time-dependent increases (Skogstrand et al. 2008) and decreases (Flower et al. 2000). Our study is the first to show stability of CRP in unprocessed K_2EDTA-derived plasma over a 48-hour time frame. Interestingly, neither time, temperature, nor tube type affect CRP plasma profiles, suggesting that CRP is relatively resilient to stimulation or degradation in unprocessed blood. This is a phenomenon observed previously in serum-measured CRP (Aziz et al. 2003) and in plasma stored up to 36 hours pre-processing (Pai et al. 2002). To our knowledge, only one other study has assessed the stability of CRP under the same conditions that we report here, although that study demonstrated significant increases over time (1.3-fold and 1.9-fold under 4°C and RT conditions, respectively; Skogstrand et al. 2008). One difference between the two studies is the method by which the analytes were measured. In our study, CRP levels were comfortably within the working range of the assay, while those reported in Skogstrand et al. were below the limit of detection. Further experiments are clearly needed, and we recommend

that all researchers confirm the feasibility of this method within the framework of their own collection protocols prior to implementation.

Comparison of K_2EDTA tubes and P100 tubes as collection vessels suggests that under RT conditions, P100 tubes are not a suitable product for assessing levels of IL-6. To our knowledge, this is the first report in the literature on the testing of these particular commercially available tubes as an option for collection and preservation of plasma biomarkers with pre-processing delay. Given the relatively stable levels of IL-6 in K_2EDTA tubes under the same conditions, and the stability in P100 tubes at 4°C, our findings suggest that the proprietary preservative employed in the tubes has a stimulatory effect upon IL-6 levels at RT. The reason for this *de novo* production of IL-6 is unknown; given that this response is seen at RT only, it is unlikely to be a direct effect of the preservative per se. Since the effect is not seen for CRP, it is unlikely that this effect is related to a nonspecific biological and/or physical event, such as an acute phase response. Clearly, these tubes are not intended to be used in the way in which we tested them; they are designed to preserve profiles post-processing. Although we can conclude that P100 tubes can be used "off-label" under certain circumstances, they are a more expensive option than K_2EDTA tubes, and any benefit observed in biomarker estimation needs to be weighed against the financial disadvantage related to their use. As we only assayed two biomarkers, we cannot rule out the possibility that P100 tubes perform better than K_2EDTA tubes for other biomarkers.

Finally, we observed that DBS estimation of CRP levels was similar to that obtained by venous blood. Our findings confirm previous observations (McDade, Williams, and Snodgrass 2007; Danese et al. 2011; Crimmins et al. 2014) that DBS can be used as an alternative substrate for estimating levels of certain biomarkers. DBS samples have some advantages over venous blood samples for in-field collections, most notably the fact that biohazard risks during collection and transportation are much lower. The disadvantage is that plasma-equivalent volume in DBS samples is low; in our experiment, we calculated that DBS samples had a plasma-equivalent volume of approximately 1 μL per 3-mm punch, which is consistent with previous observations of serum-equivalent volumes estimated in DBS samples (Mei et al. 2001; Brindle et al. 2010). This fact potentially restricts the range of biomarkers that can be assayed using this method to those that are detectable in small volumes of blood. Here, we chose to assay CRP since normal levels are well above the limit of detection of our assay method (ELISA). Other assay methods might allow greater flexibility because of their increased sensitivity to detect low amounts of circulating biomarkers.

We acknowledge several limitations to this study. First, we assessed only a small number of subjects. Although our findings replicate those found in other reports, validation in larger sample pools would be valuable. Second, we assayed only two out of a large range of biomarkers currently of interest to the research community. Previous findings have demonstrated that stability is highly biomarker dependent (Flower et al. 2000; Skogstrand et al. 2008). Third, levels of IL-6 and CRP within our test subjects were within the normal range for healthy adults; we were not able to determine the effects of pre-processing conditions on samples with high levels of inflammation. Fourth, we tested the stability of samples under room temperature conditions; we did not test the profiles of samples incubated at elevated temperatures. Previous findings have demonstrated that incubation of plasma samples at 35°C for up to 48 hours has no effect on IL-6 concentration, but that CRP shows evidence of induced levels (Skogstrand et al. 2008). Researchers intending to analyze (1) biomolecules other than IL-6 or CRP, (2) samples whose levels of inflammation are anticipated to be elevated, or (3) samples for which transport temperatures might significantly exceed normal room temperature would be advised to independently test the stability profiles prior to

collection. In this regard, we hope that the design of the present study offers a paradigm for conducting systematic pre–data collection evaluations.

In conclusion, we report that plasma collected from blood stored for up to 48 hours pre-processing can be used to estimate normal levels of IL-6 and CRP, and that blood can be transported at RT without significant detriment to biomarker estimations. In addition, we confirm the utility of DBS as a minimally invasive alternative for pre-validated biomarkers. The use of these two methods of blood collection, in complement or in isolation, can facilitate wider incorporation of biomarkers into biodemographic data collection protocols.

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