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# Measuring Pro- and Anti-Inflammatory Biomarkers Among Low-Income Hispanic Adults: A Feasibility and Pilot Assessment

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## Abstract

Using the Orsmond and Cohen feasibility framework, the primary aim of this study was to assess the feasibility of the implementation of recruitment strategies, data collection procedures, and managerial resources needed to assess pro- and anti-inflammatory biomarkers from low-income, younger Hispanic adults. The secondary aim of this study was to describe the relationship between discrimination stress and inflammation as pilot work for future studies. Data were collected in a Houston-area community center from self-identifying Hispanic adults (ages 21–35) (August 2018). Inflammation was evaluated from blood samples, and interviewer-administered surveys in participants' preferred language measured discrimination stress (Hispanic Stress Inventory-2 discrimination subscale). Spearman rank-order correlations evaluated the relationships between discrimination stress and inflammatory biomarkers. The recruitment strategies, data collection strategy, and the associated resources were evaluated and found to be feasible. While 50 participants consented to donate blood, five were too dehydrated for sample collection. Among the 45 participants [*M*age = 28.9 (SD = 4.4), 17.8% U.S.-born, 42.2% 1.5 generation, 40% 1.0 generation], discrimination stress was negatively correlated with proinflammatory cytokine interleukin-8 ($p < 0.01$). This study demonstrated feasibility using established benchmarks. The negative correlation between discrimination stress and interleukin-8 suggests discrimination stress may contribute to inflammatory dysregulation.



## Keywords

Immigrant Health, Inflammation, Inflammatory Biomarkers, Latinos, Minority Health

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## Abstract

Using the Orsmond and Cohen feasibility framework, the primary aim of this study was to assess the feasibility of the implementation of recruitment strategies, data collection procedures, and managerial resources needed to assess pro- and anti-inflammatory biomarkers from low-income, younger Hispanic adults. The secondary aim of this study was to describe the relationship between discrimination stress and inflammation as pilot work for future studies. Data were collected in a Houston-area community center from self-identifying Hispanic adults (ages 21–35) (August 2018). Inflammation was evaluated from blood samples, and interviewer-administered surveys in participants' preferred language measured discrimination stress (Hispanic Stress Inventory-2 discrimination subscale). Spearman rank-order correlations evaluated the relationships between discrimination stress and inflammatory biomarkers. The recruitment strategies, data collection strategy, and the associated resources were evaluated and found to be feasible. While 50 participants consented to donate blood, five were too dehydrated for sample collection. Among the 45 participants [$M_{age}$ = 28.9 (SD = 4.4), 17.8% U.S.-born, 42.2% 1.5 generation, 40% 1.0 generation], discrimination stress was negatively correlated with proinflammatory cytokine interleukin-8 ($p < 0.01$). This study demonstrated feasibility using established benchmarks. The negative correlation between discrimination stress and interleukin-8 suggests discrimination stress may contribute to inflammatory dysregulation.

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## Introduction

Hispanics constitute the largest ethnic minority in the United States (Velasco-Mondragon et al., 2016). Yet, Hispanic representation in research studies is disproportionately low, particularly among those of low socioeconomic status (Sage et al., 2018). Low recruitment and retention of Hispanic participants is a challenge when conducting behavioral and biomedical research. Barriers to research participation among Hispanics include insufficient information on active research studies, privacy concerns exacerbated by political incivility, and logistical barriers to research participation (Ceballos et al., 2014; George et al., 2014; Rhodes et al., 2018; Sage et al.,

2018). The inclusion of biological samples in research provides a rich resource to examine how the social and behavioral context may influence physiology and potentially contribute to chronic disease. Yet, Hispanic under-representation in biomedical research limits the translation of findings needed to inform health promotion strategies.

Recommendations to increase Hispanic research participation include engaging participants in a community setting (versus an university setting), providing childcare, and offering appropriate compensation (George et al., 2014; Rhodes et al., 2018). A previous large-scale study that collected biological samples demonstrated the efficacy of using such approaches within the Greater Houston-area among middle-aged (mean age 40.8±14.2 years) Hispanic participants (Chow et al., 2017). However, it is also important to document the feasibility of recruitment strategies, data collection procedures, and managerial resources needed to assess biological samples among a younger Hispanic adult population (ages 21-35). Documenting the procedures is important so that similar procedures can be applied to future studies that focus on studying younger Hispanic adults prior to disease onset. Therefore, documenting the feasibility of recruiting and collecting blood samples to measure pro- and anti-inflammatory biomarkers among younger Hispanic adults, along with the resources needed to manage recruitment and data collection, are the main objectives of this study.

The secondary aim was to describe the association between self-reported discrimination stress and inflammation, as preliminary work for future studies. Hispanics experience high levels of discrimination that can elicit substantial psychosocial stress (Gonzalez-Barrera & Lopez, 2020; Torres & Wallace, 2013). Chronic psychosocial stress is linked to chronic inflammation and exaggerated acute stress responses, which are known risk factors for chronic disease (Brown et al., 2018; Fagundes et al., 2013; Marsland et al., 2017; Rohleder, 2014). Mechanistically, excessive activation of stress pathways disturbs inflammatory regulation and hampers healthy initiation and resolution of inflammation, thereby accelerating progression towards disease (McEwen, 2005). Accordingly, the psychosocial stress of discrimination may adversely affect inflammatory regulation (Rohleder, 2014; Saban et al., 2018; Stepanikova et al., 2017), contributing to a disproportionate burden of diseases with inflammatory etiology among Hispanics (Shoelson et al., 2006; Velasco-Mondragon et al., 2016).

Using the feasibility assessment framework outlined by Orsmond and Cohn (2015), and data collection methods applied in other studies that recruited Hispanics in the Houston-area (Chow et al., 2017; Hernandez, 2016), we aimed to assess the feasibility of implementing recruitment and data collection procedures among a community-based sample of low-income Hispanic adults. We also assessed the resources needed to manage the study. As an exploratory aim, we described the relationships between discrimination stress and pro- and anti-inflammatory blood biomarkers. These descriptive associations will serve as preliminary pilot findings for future studies focused on health disparities.

## Methods

### Participants and Study Design

Participants were recruited from a community center with high Hispanic representation in Houston, TX. Bilingual undergraduate research assistants (URAs) recruited participants by approaching community center clients attending the

weekly food distribution. The URAs explained the study and provided clients with a brief informational flyer in both English and Spanish. The flyer was also placed around the center. Eligibility criteria for the study included self-identifying Hispanic adults between the ages of 21 and 35. This age span was chosen to encompass a range of younger adults, while limiting the influence of age on inflammatory cytokine levels (Franceschi & Campisi, 2014). Exclusion criteria included: self-identification as having diabetes; being a current smoker; being pregnant; having a disease that affects blood clotting; having an infection or fever in the past six weeks; or currently taking anti-inflammatory drugs, lithium/other anti-psychotics, or statins. Recruitment occurred twice in July 2018 and twice in August 2018.

Data collection occurred in August 2018 on three non-consecutive days coinciding with the weekly food distribution. The day before data collection, bilingual URAs sent recruited adults reminder emails, texts, and/or a phone call. On the day of data collection, potential participants were screened for eligibility by the PI/last author who is bilingual. Eligible participants were provided written informed consent documents in their preferred language by a bilingual URA. The URA that provided the informed consent also conducted an interviewer-administered survey in the participant's language of choice. This approach has previously been successful (Hernandez, 2016).

The current feasibility study includes a subsample of participants ($n$ = 45) from a larger cross-sectional pilot study examining behavioral stress measures and direct assessments of health among low-income Hispanic adults (N = 82). Included participants are those that provided blood samples for determination of circulating pro- and anti-inflammatory biomarkers. Due to funding limitations, the subsample selected to assess the feasibility of collecting blood samples was restricted to 50 participants. Participants were selected based on willingness to donate blood. Blood samples were collected after completing the interviewer-administered survey or before initiating the survey if the room designated for the blood draws was available. Childcare was provided and families were compensated $50 in a gift card to a nationwide discounted department and grocery store. This study was approved by the University of Houston's Institutional Review Board (STUDY00000461).

## Study Variables

**Recruitment capability**. Recruitment capability was assessed through various indicators. The research team documented: whether the target number of participants was met, the number recruited to meet the target number, whether eligibility criteria were clear, the total time dedicated to recruitment and duration of recruitment activities, which strategies were most successful, and obstacles to recruitment and enrollment.

**Participant characteristics**. Age, country of origin, year of immigration (as applicable), generation status, annual household income, education, and employment were determined by self-reported surveys. For foreign-born participants, time in the United States was calculated as the difference between their year of immigration and the year of study participation (i.e., 2018). Generation status was based on when participants immigrated to the U.S.: 1.5 generation = participants who immigrated younger than age 18; 1.0 generation = participants who immigrated at 18 years of age or older; U.S.-born = participants who were born in the United States with immigrant parents (2$^{nd}$ generation) or grandparents (3$^{rd}$ generation) (Gonzales & Chavez, 2012). Annual

household income was assessed in eight levels (< $15,000/year; $15,000 - $29,999; $30,000 - $44,999; $45,000 - $59,999; $60,000 - $74,999; $75,000 - $99,999; $100,000 - $149,000; ≥ $150,000). Education was assessed at five levels (< 8th grade education, 8th grade completion, high school diploma, associate degree, ≥ four-year college degree). Employment status was assessed at three levels (unemployed, part-time employed, full-time employed).

Height was assessed by stadiometer and recorded to the nearest 0.1 cm. Weight was recorded to the nearest 0.1 kg via BIA Tanita TBF-310GS scale (Tanita Corporation, Arlington Heights, IL). Body mass index (BMI) was calculated from height and weight data by standard formula (CDC, 2017). Anthropometric data and participant age were collected as both BMI and age postively correlate with systemic inflammation (Franceschi & Campisi, 2014; Shoelson et al., 2007).

**Data collection procedures.** The total number of data collection days, hours for data collection per day, and minutes required for data collection per participant were recorded. Participants' understanding of the questions and whether they perceived data collection procedures to be burdensome were also recorded.

**Resources.** The research team documented the administrative capacity needed for recruitment, data collection, data entry, the physical space needed to conduct the study, driving time between the community center and the university lab, and the funds required to cover study costs.

**Discrimination stress**. Ten items from the discrimination subscale of the Hispanic Stress Inventory-2 (HSI-2) were used to assess participant self-reported lifetime discrimination stress (Cervantes et al., 2016). The items assess discrimination stress due to immigration status and Hispanic ethnicity using a Likert scale from one ("not at all worried/tense") to five ("extremely worried/ tense"). Items included statements such as, "I have felt unaccepted by others due to my Hispanic culture," and "I have seen friends treated badly because they are Hispanics/ Latinos." Subscale responses were summed with higher item and total scores (out of 50) indicating greater discrimination stress (Cervantes et al., 2016). In this sample the Crohnbach's alpha for the discrimination stress subscale was 0.94.

**Pro-inflammatory and anti-inflammatory biomarkers**. Blood was collected by venipuncture into 10 mL serum separation tubes (Vacutainer Plus plastic serum tubes, BD Vacutainer Systems, Franklin Lakes, NJ) and allowed to clot for two hours prior to processing and cryopreservation for later analysis. Samples were transported to the university lab in batches to ensure processing two hours post-collection. Serum levels of ten pro- and anti-inflammatory cytokines (proinflammatory: interleukin-(IL-)1β, IL-2, IL-6, IL-8, IL-12p70, tumor necrosis factor-(TNF-)α, interferon-(IFN-)γ; anti-inflammatory: IL-4, IL-10, IL-13) and four markers of vascular inflammation (C-reactive protein (CRP), intracellular adhesion molecule-(ICAM-)1, serum amyloid A (SAA), vascular cell adhesion molecule-(VCAM-)1) were assessed by commercially available kits (V-PLEX Plus Proinflammatory Panel 1 and V-PLEX Plus Vascular Injury Panel 2; Meso Scale Diagnostics, LLC, Rockville, MD). These biomarkers are involved in inflammatory signaling and are among those frequently measured to assess the effects of acute and chronic stress (Coussons-Read et al., 2007; Djuric et al., 2008; Heinz et al., 2003; Marsland et al., 2017; Stepanikova et al., 2017). Samples and plates were prepared according to manufacturer instructions and read using a MESO QuickPlex SQ 120 imager. These multiplex kits are validated to yield inter-run and inter-lot coefficients of variation < 10%. Samples were assayed in

duplicate on the same plate. Samples with coefficients of variation > 15% were rerun.

### Statistical Analysis

Data were screened for statistical assumptions prior to analyses. Outliers in the biomarker data were identified and removed using criteria delineated by Hoaglin and Iglewicz (1987). Due to outlier removal and data either outside the range of the standard curve or below the lower limit of detection of the kit [IFN-γ (0.21 pg/mL, N = 3), IL-10 (0.02 pg/mL, N = 1), IL-12p70 (0.02 pg/mL, N = 27), IL-13 (0.03 pg/mL, N = 11), IL-1β (0.01 pg/mL, N = 36), IL-2 (0.01 pg/mL, N = 31), IL-4 (0.01 pg/mL, N = 20), IL-8 (0.03 pg/mL, N = 1)], some biomarkers failed to yield valid data for > 50% of participants. Consequently, IL-1β, IL-2, IL-12p70, and IL-4 were excluded from the current analyses. Limits of detection are technical features of biomarker quantification and failure to acquire values within these ranges is not uncommon (Kleiner et al., 2013; Koelman et al., 2019; U.S. Department of Health and Human Services, 2018). Therefore, exclusion of the above biomarkers does not speak to the feasibility of assessing these biomarkers within this population.

Relationships between discrimination stress total score and blood biomarker levels were assessed by Spearman rank order correlations. Analyses were completed using SPSS 25.0 with the α-level set *a priori* to 0.05.

## Results

### Evaluation of Recruitment Capability and Sample Characteristics

We proposed to collect data from 50 Hispanic adults (ages 21-35 years) within five months from a Houston-area community center with a high Hispanic representation. Prior to data collection, 54 individuals were recruited by bilingual URAs attending two, four-hour food distributions, in addition to a community center volunteer distributing recruitment flyers. An additional 30 interested individuals were recruited after data collection began by URAs attending two more four-hour food distributions and through word-of-mouth, for a total of 84 recruited adults. Four recruited adults exceeded the age limits and one recruited adult declined to participate at the time of screening. Thus, the eligibility criteria appeared to be clear and not too restrictive.

In one month (over three non-consecutive data collection days), 50 Hispanic adults enrolled in the study, and all 50 adults consented to provide a blood sample. In-person recruitment during weekly food distributions provided the greatest number participants for enrollment (N = 27), followed by snowballing techniques, including participants telling others about the study (N = 15). Recruitment strategies with no in-person communication (i.e., flyer displayed in the community center, N = 5; and flyer distributed by a community center volunteer, N = 3) resulted in the fewest enrollees.

Barriers to data collection reminders included disconnected phone lines. Ten participants had a working number at the start of the study but not the following week. These participants could be reached via email. The percentage of recruited adults who attended the data collection visit was 41% in week one, 23% in week two, and 30% in week three.

Among those enrolled, five participants were too dehydrated for blood donation. Sample characteristics did not differ between these five participants and those who could provide a sample. The data reported herein are limited to the 45 participants from whom blood samples could be obtained. These participants were 28.9±4.4 years old, mostly women (N = 43), and were obese (mean

BMI = 30.7±6.9 kg/m$^2$) (Table 1). Most participants were foreign-born (N = 37), predominantly from Mexico (65%), and had lived in the United States for an average of 12.4±6.6 years. The majority (64.4%) of participants were unemployed at the time of data collection, and higher frequency of unemployment was observed among foreign-born participants (70.3%) vs. U.S.-born participants (37.5%). Of the foreign-born participants, 19 were 1.5 generation and 18 were 1.0 generation. Additional descriptors are listed in Table 1.

**Evaluation of Data Collection Procedures**

Data collection occurred over three non-consecutive days. The PI/last author and URAs arrived an hour before data collection to set up and stayed an hour after data collection to clean data collection spaces. There was a four-hour window (9am – 1pm) that participants could arrive to participate in the study. This coincided with a weekly food distribution at the community center. Participants spent between 45 and 60 minutes completing the data collection procedures. After study participation, clients were escorted to the front of the food distribution line. Participants understood study procedures and did not indicate that the data collection process was burdensome.

**Evaluation of Relevant Resources**

Three to four bilingual URAs were involved in recruitment. The day before data collection three to four bilingual URAs sent reminders to recruited adults. On the day of data collection, a combination of four to six monolingual and bilingual URAs assisted with the consent process and conducted surveys. A bilingual phlebotomist from an outside organization was contracted for blood draws. Two monolingual URAs with car access transported blood samples from the community center to the university lab. Two monolingual URAs provided childcare for participants during data collection. Four monolingual and bilingual URAs assisted with data entry.

The community center provided two offices, one conference room, and one classroom for data collection. One office was used for eligibility screening, anthropometric measurements, and gift card compensation, and the second for the blood draws. The conference room and classroom were used for informed consent and survey data collection. Travel between the community center and the university lab took 30 minutes, and an additional 15 minutes was needed to find parking and walk to the lab. The day prior to data collection, the PI/last author notified the lab to ensure they were ready to receive samples the following day. On the day of data collection, the PI/last author notified the lab when the samples were in transit so that the lab could prepare for sample arrival.

Aside from gift card compensation, orange juice and doughnuts were made available to the participants after the blood draw. Coloring books, stickers, and toys were available during childcare. Grant funds associated with the PI/last author covered all study-related costs. (See funding acknowledgement).

**Relationships Between Discrimination Stress and Inflammatory Biomarkers**

Table 2 provides serum levels of pro-and anti-inflammatory biomarkers. Table 3 provides correlations between discrimination stress total score and serum biomarkers. IL-8 negatively correlated with discrimination stress ($r_s(41) = -0.440$, $p < 0.01$). None of the following were significantly correlated with IL-8: age, BMI, time in United States, annual household income, educational attainment, or employment ($p > 0.05$, data not shown).

Findings in Table 2 and 3 were recalculated with the two male participants excluded, and results remained the same (data not shown).

Table 1

*Participant Characteristics for the Full Sample and by Generation Status/US-born*

| | Full Sample[a] | 1.5 Generation | 1.0 Generation | US-born |
|---|---|---|---|---|
| | N = 45 | N = 19 | N = 18 | N = 8 |
| **Measures** | | **Mean ± SD (Range)** | | |
| Discrimination Stress, Total Score[b] | 18.6±9.6 (10-50) | 22.1±12.7 (10-50) | 16.3±6.3 (10-29) | 15.6±4.6 (10-24) |
| Age (years) | 28.9±4.4 (21-35) | 29.2±4.8 (21-35) | 30.1±3.3 (25-35) | 25.8±4.5 (21-32) |
| BMI (kg/m$^2$)[c] | 30.7±6.9 (19.3-46.6) | 32.2±7.6 (21.5-46.6) | 28.5±5.7 (19.3-37.7) | 31.8±7.2 (19.6-40.8) |
| Time in U.S. (years)[d] | 12.4±6.6 (2-24) | 16.9±5.2 (2-24) | 7.5±3.9 (3-14) | -- |
| Country of Origin[e] | | **N (%)** | | |
| Mexico | 24 (65%) | 14 (74%) | 10 (56%) | -- |
| El Salvador | 5 (13%) | 4 (21%) | 1 (6%) | -- |
| Honduras | 5 (13%) | -- | 5 (28%) | -- |
| Guatemala | 3 (8%) | 1 (5%) | 2 (11%) | -- |
| Sex | | **N (%)** | | |
| Male | 2 (4.4%) | 1 (5.3%) | 0 (0.0%) | 1 (12.5%) |
| Female | 43 (95.6%) | 18 (94.7%) | 18 (100.0%) | 7 (87.5%) |
| Annual Household Income | | **N (%)** | | |
| < $15,000 | 19 (42.2%) | 7 (36.8%) | 9 (50.0%) | 3 (37.5%) |
| $15,000-$29,999 | 17 (37.8%) | 7 (36.8%) | 9 (50.0%) | 1 (12.5%) |
| $30,000-$44,999 | 4 (8.8%) | 3 (15.8%) | 0 (0.0%) | 1 (12.5%) |
| $45,000-$59,999 | 2 (4.4%) | 2 (10.5%) | 0 (0.0%) | 0 (0.0%) |
| $60,000-74,999 | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| $75,000-$99,999 | 3 (6.7%) | 0 (0.0%) | 0 (0.0%) | 3 (37.5%) |
| $100,000-$149,000 | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| ≥ $150,000 | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Education | | **N (%)** | | |
| < 8$^{th}$ Grade | 8 (17.8%) | 2 (10.5%) | 6 (33.3%) | 0 (0.0%) |
| 8$^{th}$ Grade | 11 (24.4%) | 4 (21.0%) | 3 (16.7%) | 4 (50.0%) |
| High School Diploma | 22 (48.9%) | 11 (57.9%) | 9 (50%) | 2 (25.0%) |
| Associates Degree | 3 (6.7%) | 1 (5.3%) | 0 (0.0%) | 2 (25.0%) |
| ≥ College | 1 (2.2%) | 1 (5.3%) | 0 (0.0%) | 0 (0.0%) |
| Employment | | **N (%)** | | |
| Unemployed | 29 (64.4%) | 13 (68.4%) | 13 (72.2%) | 3 (37.5%) |
| Part-time Employed | 4 (8.9%) | 0 (0.0%) | 3 (16.7%) | 1 (12.5%) |
| Full-time Employed | 12 (26.7%) | 6 (31.6%) | 2 (11.1%) | 4 (50.0%) |

*Notes.* [a] While 50 participants consented to providing blood samples, participant characteristics are based on the 45 from whom blood samples could be obtained. [b]Discrimination Stress = Total Score out of 50 possible. [c]BMI = Body Mass Index; [d]Time in US = Years since migration among immigrant participants (N = 37). [e]Among immigrant participants (N = 37).

Table 2

*Pro- and Anti-inflammatory Blood Biomarkers*

| Measure (N) | Mean±SD | Range |
|---|---|---|
| **Proinflammatory Biomarkers** | | |
| IL-6 (pg/mL) (N = 42) | 1.10 ±0.64 | 0.13-2.73 |
| IL-8 (pg/mL) (N = 43) | 15.38±9.11 | 0.06-39.22 |
| TNF-α (pg/mL) (N = 42) | 3.01±0.80 | 1.53-4.85 |
| IFN-γ (pg/mL) (N = 41) | 3.11±2.27 | 0.54-8.77 |
| CRP (mg/L) (N =39) | 4.28±4.31 | 0.01-18.24 |
| ICAM-1 (ng/mL) (N = 39) | 501.69±107.06 | 284.49-748.47 |
| SAA (mg/L) (N = 39) | 2.23±1.81 | 0.02-8.47 |
| VCAM-1 (ng/mL) (N = 39) | 583.39±123.62 | 400.24-896.28 |
| **Anti-inflammatory Biomarkers** | | |
| IL-10 (pg/mL) (N = 38) | 0.19±0.10 | 0.07-0.50 |
| IL-13 (pg/mL) (N = 25) | 0.54±0.44 | 0.15-2.03 |

*Note.* Sample numbers reflect number of valid samples (i.e., within assay detectable range, non-outliers) out of a total of 45 collected samples. Valid samples for > 50% of participants were not obtained for the biomarkers: interleukin-1β (N = 11), interleukin-2 (N = 13), interleukin-12p70 (N = 14), and interleukin-4 (N = 21). These biomarkers were excluded from the analysis. Abbreviations: IL-6 = interleukin-6; IL-8 = interleukin-8; TNF-α = tumor necrosis factor alpha; IFN-γ = interferon gamma; CRP = C-reactive protein; ICAM-1 = intracellular adhesion molecule-1; SAA = serum amyloid A; VCAM-1 = vascular cell adhesion molecule-1; IL-10 = interleukin-10; IL-13 = interleukin-13.

## Discussion

### Feasibility Assessment

Using an established feasibility framework (Orsmond & Cohn, 2015), this study demonstrated the feasibility of recruiting and collecting blood samples among a community-based sample of low-income U.S.-born and foreign-born Hispanic adults (ages 21-35). Specifically, participants were willing to provide blood samples, as evidenced by the consent to blood donation among the first 50 participants that enrolled in the study.

The most successful form of recruitment was in-person recruitment by bilingual URAs. In particular, of the 84 recruited individuals, 54 of the individuals were recruited in person. Among the 50 enrolled adults in the study, 27 of the participants were recruited in person. In-person verbal com-

Table 3

*Correlation Matrix Indicating Relationships Between Discrimination Stress Total Score and Pro- and Anti-inflammatory Blood Biomarkers, Data are Presented as Spearman Correlation Coefficients ($r_s$).*

| | 1. | 2. | 3. | 4. | 5. | 6. | 7. | 8. | 9. | 10. | 11. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Discrimination Stress Total Score | 1.0 | | | | | | | | | | |
| 2. IL-6 | -0.034 | 1.0 | | | | | | | | | |
| 3. IL-8 | -0.440** | 0.374* | 1.0 | | | | | | | | |
| 4. TNF-α | -0.077 | 0.067 | 0.069 | 1.0 | | | | | | | |
| 5. IFN-γ | -0.071 | 0.144 | 0.316* | -0.007 | 1.0 | | | | | | |
| 6. CRP | 0.123 | 0.545** | 0.332* | 0.320 | -0.061 | 1.0 | | | | | |
| 7. ICAM | 0.260 | 0.279 | 0.015 | 0.193 | -0.289 | 0.585** | 1.0 | | | | |
| 8. SAA | 0.091 | 0.191 | 0.262 | 0.313 | -0.010 | 0.544** | 0.461** | 1.0 | | | |
| 9. VCAM | 0.243 | -0.113 | -0.349* | 0.144 | -0.025 | -0.105 | 0.074 | 0.072 | 1.0 | | |
| 10. IL-10 | 0.027 | 0.161 | 0.019 | 0.268 | 0.201 | 0.393* | 0.429* | 0.394* | -0.102 | 1.0 | |
| 11. IL-13 | 0.120 | -0.340 | -0.392 | 0.326 | -0.356 | -0.149 | -0.048 | 0.001 | 0.176 | 0.035 | 1.0 |

Note: *$p < 0.05$, **$p < 0.01$. Discrimination stress measured by discrimination subscale of the Hispanic Stress Inventory-2 Survey (Cervantes et al., 2016). Abbreviations: IL-6 = interleukin-6; IL-8 = interleukin-8; TNF-α = tumor necrosis factor alpha; IFN-γ = interferon gamma; CRP = C-reactive protein; ICAM-1 = intracellular adhesion molecule-1; SAA = serum amyloid A; VCAM-1 = vascular cell adhesion molecule-1; IL-10 = interleukin-10; IL-13 = interleukin-13.

munication facilitated real-time dialogue, an advantage over flyers. Verbal and non-verbal cues helped build rapport between research team members and the potential participant and may have increased the likelihood of participation. Recruitment occurred at community center food distribution events, and on some occasions the research staff assisted with the food distribution. Engaging with the community center's staff and clients through a center-sponsored activity helped foster bonds between the research team and the community and may have contributed to successful recruiting of the current sample.

Scheduling data collection to coincide with food distribution helped ensure participants attended the community center for data collection. This approach increased convenience for participants, reduced transportation barriers, and may have minimized fears of engaging in a new environment (e.g., a university setting). Overall, collecting data at a community center with high Hispanic representation provided access to willing participants.

In the current study, participants welcomed the strategy of interviewer-administered surveys, especially Spanish-speaking participants. Participants enjoyed the process and expressed that they felt heard. It is also important to make the data collection the least burdensome for community center staff. Arriving early to set up and staying after data collection for cleaning helped maintain a collaborative environment between the research team and the community center.

Various resources facilitated data collection in the community. First, the PI/last author had an established partnership with the community center. However, an established and ongoing partnership does not necessarily equate to having the required space to conduct the desired research study. Consequently, it was important for the research team to creatively use space provided and be flexible with the order in which data was collected (e.g., survey first, followed by blood draw or vice versa). Second, data collection in the community center was facilitated by monolingual and bilingual URAs. Given the experience in this study, a minimum of four to six bilingual URAs are recommended. Third, community-based research is advantageous when studying a vulnerable population; yet, it is important to consider the distance between the collection site and the processing lab when biological samples are being collected. The site in the current study allowed timely processing of samples (~30 minutes transportation time). For community centers located more than an hour from the processing lab, alternative data collection procedures may need to be considered.

**Pilot Assessment**

We explored correlations between discrimination stress and proinflammatory and anti-inflammatory biomarkers. The results revealed a negative correlation between discrimination stress and the proinflammatory cytokine IL-8. No other significant correlations were observed. These results were unexpected, as psychosocial stress (including discrimination stress) has previously been shown to positively correlate with inflammation (Heinz et al., 2003; Rohleder, 2014; Stepanikova et al., 2017). However, such relationships are not observed uniformly and negative relationships between stress and inflammation have also been demonstrated (Lespérance et al., 2004; Schmidt et al., 2016; Steptoe et al., 2003; Whooley et al., 2007). Interleukin-8 is involved in the acute inflammatory response to injury or stress (Moore & Kunkel, 2019). As an inflammatory mediator, IL-8 is associated with many acute and chronic inflammatory conditions, with higher levels generally indicating more severe pathology

(Moore & Kunkel, 2019; Ye et al., 2009). However, evidence suggests IL-8 is also important for signaling within the central nervous system, wherein higher IL-8 is associated with better mental health (Giovannelli et al., 1998; Janelidze et al., 2015; Puma et al., 2001). Data from a variety of sources have demonstrated a negative association between levels of IL-8 and depression and anxiety (Janelidze et al., 2015; Zou et al., 2018), as well as risk for suicide (Black & Miller, 2015; Keaton et al., 2019). It has been hypothesized that diminished levels of IL-8 following chronic stress may reflect compensatory suppression of acute inflammatory signaling (Zou et al., 2018). Thus, lower levels of IL-8 in this sample may represent immune dysregulation related to the psychosocial stress of discrimination, and may indicate risk for mental illness.

## Limitations

While the study demonstrated feasibility in obtaining biological samples from a vulnerable population, several limitations must be acknowledged. The findings from this study lack generalizability and the statistical power to potentially reach significance; yet, this is partially a function of the objectives of feasibility studies. Feasibility studies are intended to be based on small samples and are not designed to yield adequate power for statistical hypothesis testing. The interpretation of the findings associated with feasibility studies are intended to be mainly descriptive (Arain et al., 2010; Orsmond & Cohn, 2015). Therefore, the results reported herein should be interpreted as demonstrating the viability and potential of our approach given the study conditions, rather than a universal approach or evidence of relationships broadly applicable to the U.S. Hispanic population. The study's results remain to be replicated in a larger cohort and in other environments. Modifications to recruitment, data collection, and/or analysis methods may need to be applied for other regions and populations. Relatedly, the sample was largely female, meaning the results may not equally apply to men. This limitation is not uncommon among studies recruiting from community centers and health fairs (Chow et al., 2017), as well as those that have examined stress and health among Mexican immigrants (Garcini et al., 2018). Because previous literature indicates that discrimination stress may affect men and women differently (Kershaw et al., 2016), greater efforts should be made to recruit Hispanic men in research studies. For example, times that facilitate increased Hispanic male participation (e.g., evenings and weekends) may need to be given greater consideration when designing research studies (U.S. Bureau of Labor Statistics, 2015). Finally, though the discrimination stress survey has shown concurrent validity with several measures of mental health, mental health (e.g., depression, anxiety) was not directly measured in the current project and additional stressors may have contributed to inflammation (Cervantes et al., 2016). A majority of the sample (64.4%) reported being unemployed and foreign-born participants reported unemployment at a higher rate (70.3%). Higher unemployment rates have been observed in the U.S. Hispanic population and unemployment stress may have a negative impact on systemic inflammation (Cervantes et al., 2016; Rohleder, 2014). However, unemployment stress was not the focus of this study and was not analyzed versus inflammation.

## Conclusion

The current study demonstrates the feasibility of recruiting and collecting biological samples from low-income Hispanic adults (ages 21-35) in an urban

community setting, and describes necessary resources. The study also provides a foundation for future work assessing the relationship between discrimination stress and IL-8 among U.S.-born and foreign-born Hispanics. This relationship warrants additional research and emphasizes the need to increase Hispanic participation in biomedical and behavioral studies. Such efforts will improve understanding of the pathways by which psychosocial stressors like discrimination stress influence physiological health.

## Implications for Health Behavior Research

Conducting research on vulnerable populations with limited food and transportation access, who display English-language barriers, and who have been historically underrepresented in research studies is time consuming. However, excluding these individuals from research studies only perpetuates health disparities. Including first-generation, underrepresented undergraduate students as research assistants, such as in the current research study, is a valuable resource when designing and conducting research studies. Some of the students may have similar lived experiences as the potential research participants, helping to reduce study participation hesitancy. In return, many of these students become interested in research and/or graduate school, and "pay it forward" through community service (Hernandez et al., 2019).

## Discussion Questions

1. While the recruitment procedures demonstrated feasibility, what are additional recruitment procedures that could be implemented, that are not coercive, but address immediate needs of low-income, primarily Spanish-speaking immigrant adults?

2. This was a cross-sectional study; thus, retention procedures were not necessary. What retention procedures could be considered when conducting research on low-income, food insecure, primarily Spanish-speaking immigrant adults that reside in a large city? Are these retention procedures applicable to other vulnerable populations?

## Abbreviations

U.S.: United States; URAs: undergraduate research assistants; BMI: body mass index; HSI-2: Hispanic Stress Inventory-2; IL-: interleukin-; TNF-: tumor necrosis factor-; IFN-γ: interferon-gamma; CRP: C-reactive protein; ICAM-1: intracellular adhesion molecule-1; VCAM-1: vascular cell adhesion molecule-1; SAA: serum amyloid A

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The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author. The data are not publicly available due to privacy or ethical restrictions. This study was approved by the Institutional Review Board at the University of Houston (STUDY00000461). Ezemenari M. Obasi is the founder and sole owner of HEALTH Equity Empowerment, LLC; the authors have no additional conflicts of interest to report.

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