Examining the relationships between psychosocial stress exposure, glucocorticoid resistance in immune cells and cardiometabolic risk

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The present study examined the relationships between chronic stress, glucocorticoid resistance in immune cells and cardiometabolic risk factors. 247 adults completed measures of psychological stress and underwent a blood draw and anthropometric assessment. Glucocorticoid resistance was measured as the concentration of dexamethasone required to reduce IL-6 concentration in half after mitogen exposure, and cardiometabolic risk consisted of a composite score of blood pressure, triglycerides, glucose scores, HDL, and waist circumference. Linear regression analyses revealed largely null results. Stress was not found to be associated with glucocorticoid resistance or cardiometabolic risk across 3 of 4 measures of psychological stress. A small, negative association between a novel measure of chronic stress and glucocorticoid resistance was observed. Results suggest need for further research into the conditions under which stress alters immune and cardiometabolic functioning.

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1.0 Introduction

For nearly a century, cardiovascular disease (CVD) has been the leading cause of death in the United States (Centers for Disease Control and Prevention Report, 2000; Ahmad & Anderson, 2021). As such, immense research effort has been devoted to understanding the disease's etiology, revealing that CVD develops from multiple biological and psychosocial risk factors. A well-established cluster of biological risk factors for cardiovascular disease includes hyperlipidemia, hypercholesteremia, hypertension, insulin resistance, and excess adiposity. Concurrent dysregulation across these parameters has been shown to predict the development of type II diabetes and CVD, is known as the "metabolic syndrome" and is often measured as a continuous composite score termed "cardiometabolic risk" (Grundy, 2005; Cardiometabolic Working Group, 2011). Recent research has also begun to consider the role of inflammation in CVD development (Libby 2006, 2012), and cardiometabolic risk (Hotamiligil, 2006; Marsland et al., 2010; Olefsky & Glass, 2010).

With respect to psychosocial risk factors, a strong body of work links psychosocial stress to CVD and cardiometabolic risk (Steptoe & Kivimaki, 2012, 2013; Kivimaki & Kawachi, 2015; Kivimaki & Steptoe, 2018). In this context, stress refers to exposure to challenges that exceed one's coping resources (Lazarus & Folkman, 1984; Gianaros & Cohen, 2016) such as work-related difficulties, relationship conflicts, or threatening life events. Current evidence suggests that stress may be associated with cardiometabolic risk (Wantabe et al., 2018; Tenk et al., 2018; Kuo et al., 2019; Winning et al., 2015; Pedersen et. al., 2016) and a growing body of literature seeks to identify the mechanisms of this relationship. This research has identified hypercortisolemia (Girod & Brotman, 2004; Whitworth et al., 2005; Walker, 2007; Chida & Steptoe, 2009), altered

catecholamine secretion (Barth et al., 2007), and exaggerated inflammation (Hotamisligil, 2006) as putative mechanisms.

However, co-occurrence of dysregulation in these proposed pathways can be challenging to explain given their known counterregulatory relationships. Chiefly, concurrent hypercortisolemia and elevated inflammation is paradoxical because cortisol has profound immunosuppressive effects (Oppong & Cato, 2015). One would intuit that elevated cortisol secretion after stress exposure would result in reduced concentrations of pro-inflammatory cytokines. This is not the case and heightened inflammation has been observed after stress exposure (Segerstrom & Miller, 2004). One possible explanation for this pattern of findings is that prolonged stress exposure results in diminished sensitivity of immune cells to cortisol. This would explain increases in the concentrations of both cortisol and pro-inflammatory cytokines during chronic stress, as well as increased likelihood of developing inflammatory disease (see Miller et al., 2002 for discussion).

Diminished sensitivity of immune cells to cortisol can be measured as the extent to which cortisol or a synthetic glucocorticoid suppresses the production of pro-inflammatory cytokines in peripheral blood mononuclear cells after exposure to a mitogen (Quax et al., 2013). In this context, relative increases in the amount of glucocorticoid required to suppress cytokine production population is termed “glucocorticoid resistance” (GCR). Consistent with the hypothesis that diminished glucocorticoid sensitivity of immune cells may explain the relationship between stress and inflammation, chronic stress exposure has been shown to associate with GCR, prospectively and across species (for review see Walsh, Bovbjerg, & Marsland, 2021). Indeed, GCR has been associated with chronic caregiving of a family member with cancer (e.g. Miller, 2002; Miller et al., 2014; Rohleder et al., 2009; Walsh et al., 2018), social isolation (e.g. Cole 2008), low

socioeconomic status (e.g. Murray et al., 2019), varied stressful life events (Cohen et al., 2012), and bereavement (e.g. Schultze-Florey et al., 2013).

Given that stress-related changes in GCR could potentially explain stress-related increases in cortisol and inflammatory cytokine concentrations (each of which are links between stress and cardiometabolic risk), it has been hypothesized that GCR may partially account for the relationship between chronic stress and cardiometabolic risk (Kuo 2019; Quax, 2013). However, no study to date has directly tested this hypothesis. Indeed, clarifying if stress relates to cardiovascular disease risk through GCR is one important next step in this literature.

The rationale for examining whether GCR predicts cardiometabolic risk lies in the finding that GCR in immune cells is marked by increased production of inflammatory cytokines, and that inflammatory cytokines play a role in the development of cardiometabolic risk. The pathways by which inflammation affects cardiometabolic risk are increasingly well understood. For instance, increased concentrations of local inflammatory cytokines interleukin 1β (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α) are associated with insulin resistance (Olefsky & Glass, 2010). This occurs through activation of Jun N-terminal kinase (JNK) and inhibitor of kβ kinase (IKK) (Hotamisligil, 1996; Weston & Davis, 2007) which in turn contribute to insulin receptor substrate protein serine phosphorylation, resulting in impaired insulin signaling (Aguirre et al., 2000; Paz et al., 1997). This inflammation-initiated insulin resistance, then, can contribute to hyperglycemia, a risk factor for the development of cardiovascular disease (Haffner & Cassells, 2003; Coutinho et al., 1999; Levitan et al., 2004).

As briefly mentioned in the forgoing discussion, the literature that links inflammatory cytokines to cardiovascular disease has commonly examined concentrations of cytokines in local tissues or in circulation. It is critical to note that while *in vitro* operationalizations of GCR measure

cytokine concentrations, this is performed after stimulation with endotoxin—it is not self-evident that the relatively higher amounts of stimulated cytokines in those with GCR correlate with similarly high concentrations of cytokines in circulation. To date, only three human studies have concurrently examined GCR and in-vivo concentrations of pro-inflammatory cytokines, and these have produced mixed results (Rohleder et al., 2009; Walsh et al., 2018; Cohen et al., 2012).

Two of these studies examined GCR in the context of chronic caregiving stress (Rohleder et al., 2009; Walsh et al., 2018). Both studies found the expected relationship between caregiving stress and increased GCR. Rohleder et al., (2009) found that stress was associated with increased GCR and increased concentration of plasma C-reactive protein (CRP), but that stress was not associated with changes in plasma IL-6, nor did they report directly testing whether GCR was associated with CRP. This finding is puzzling given that IL-6 and CRP concentrations are often correlated and that some research links caregiving stress to IL-6 (e.g., Lutgendorf et al., 1999; Keicolt-Glaser et al., 2003; Miller et al., 2008). Walsh et al., (2018) did find increases in plasma IL-6 after chronic caregiving stress, as well as an association between stress and GCR. Furthermore, this study reported that GCR was not statistically associated with the observed changes in IL-6. The third, non-caregiving study examined whether GCR predicted increased likelihood of developing a cold after exposure to a rhinovirus and the subsequent cytokine response. They found that GCR *was* associated with elevations in nasal IL-6 concentrations and increased likelihood of developing a cold (Cohen et al., 2012).

It is difficult to draw conclusions from this constellation of findings. Rohleder et al., 2009 recruited a fairly small sample size (18 caregivers, 19 controls), did not find the expected pattern of mutual increases in CRP and IL-6 after stress exposure, and did not report testing whether GCR was related to CRP concentrations. The study by Walsh and colleagues provides more reliable

evidence in favor of a null relationship between GCR and systemic IL-6 as this study measured IL-6 over three timepoints. The Cohen et al., (2012) paper, conversely, suggests that GCR may be predictive of in-vivo concentrations of IL-6, but this occurred in the context of an immunological challenge and in nasal tissues rather than in circulation. Further research, in non-immunologically challenged individuals, appears necessary to clarify these mixed results.

There are therefore two notable gaps in the literature which the present study aimed to address. The first is whether GCR is associated with increases in cardiometabolic risk and whether this can account for a relationship between stress and cardiometabolic risk. The second is whether GCR is associated with increased systemic concentrations of pro-inflammatory cytokines and whether these explain a relationship between GCR and cardiometabolic risk. The current study sought to address these gaps and examine the relationships between stress, cardiometabolic risk, glucocorticoid resistance, and circulating concentrations of IL-6 in a non-immunologically challenged, community sample of adults. Hypotheses were as follows:

H1: The number of moderate or severe life stressors experienced over the previous year will be positively associated with cardiometabolic risk.

H_2: The number of moderate or severe life stressors will be positively associated with glucocorticoid resistance.

H_3: Glucocorticoid resistance will be positively associated with cardiometabolic risk.

H4: Glucocorticoid resistance will partially mediate the association between life stressor exposure and cardiometabolic risk.

H_5: Glucocorticoid resistance will be positively associated with circulating concentrations of IL-6.

H_6: Circulating concentrations of IL-6 will be associated with cardiometabolic risk.

H7: Circulating concentrations of IL-6 will partially mediate the association between glucocorticoid resistance and cardiometabolic risk.

2.0 Methods

2.1 Overview

Participants of the present study will be drawn from the study of Social Health and Interactions in the Natural Environment (SHINE). This study aimed to better understand the relationships between social well-being and indices of physical and psychological health, including immune functioning, cardiometabolic risk, and other key health outcomes. The final sample of this study included 390 adults from the greater Pittsburgh, PA region. Participants completed an array of measures to assess social functioning and health status, many of which are not relevant to the present study and are described elsewhere (e.g. Chin et al., 2022). Participants underwent a blood draw to facilitate the assessment of cardiometabolic risk factors. The Life Events Assessment Profile, a computerized version of the Life Events and Difficulties Schedule (LEDS: Brown and Harris, 1989), and measures of immune activity, including GCR and inflammatory cytokines, were administered to a subset of the total sample. The LEAP interview was conducted after blood draw assessment, to capture stress exposure occurring up to the assessment of GCR. All comparisons of biomarkers, however, are cross sectional. All procedures were approved by the Institutional Review Board of the University of Pittsburgh and participants were paid $400 for their participation.

2.2 Participants

Of the complete SHINE sample (N=390) the LEAP interview was only administered to 283 individuals. Of these participants, a further 36 were not included in the present study or had missing data including 14 individuals who demonstrated assay results that were incompatible with producing an IC-50 score, 8 who did not complete GCR assessment, 5 individuals who did not have their blood taken, 3 who did not complete the psychiatric epidemiology research interview life events scale[1] (PERI), 3 individuals for whom LEAP data that was incomplete due to technical error, 2 individuals who had missing data for circulating IL-6, and one individual who had missing data for waist circumference. This left a final sample size of 247, using list-wise data deletion[2].

2.3 Procedures

Overview

Data were collected over multiple laboratory visits. At the first visit, participants completed informed consent, demographic and anthropometric measures, as well as one set of blood pressure readings. At the second visit, participants completed a social network interview not pertinent to

[1] The PERI is a widely used checklist of stressful life events. After detecting unexpected results, the PERI was used in exploratory analyses.

[2] To account for the possibility that results might differ as a function of missing data, a second dataset was created using regression-based imputation for outcome variables of interest. This increased the total sample size to N=390 for primary analyses. Primary analyses were repeated in this dataset and the results were not substantively different from the results of the sample which utilized list-wise deletion. For simplicity, only the results of the list-wise deleted dataset are presented.

the present study. At visit three, participants completed a second set of blood pressure readings, and they were instructed in how to perform ambulatory monitoring procedures not pertinent to the present study. At the last visit (Visit 4), participants underwent a blood draw. A 2-hour LEAP interview phone call took place approximately 2 weeks after the last laboratory visit (see "Measures" section below).

Blood draw

Blood draws at Visit 4 were performed by a trained phlebotomist and blood samples were processed immediately after collection (<90 mins after venipuncture). Participants were first screened to ensure that they had not eaten or consumed alcohol in the previous 12 hours, and had not consumed nicotine or caffeine or exercised in the previous 2 hours. Participants were also considered ineligible for the blood draw if they were currently taking antibiotics, or if they had an active or recent infection (past 14 days), or recent cold/flu symptoms of moderate severity. Participants were rescheduled if they had violated any of these requirements. The phlebotomist drew 19 mL of blood into several vacutainers for processing.

The assays for each biomarker used in the present study are described in detail below. Originally, all samples were to be processed at the Heinz Lipid Laboratory (Pittsburgh, PA). Unexpectedly, the laboratory closed near the end of the study, and blood samples from the last 16 participants were sent to the Clinical Laboratory at the University of Pittsburgh Medical Center (UPMC) for processing. The assay methods used by each laboratory were essentially identical and confirmatory reanalysis of previously assayed samples showed very high correlations between readings from the different laboratories (e.g. CRP correlations were $r = .99$, n = 10, $p < .0001$).

2.4 Measures

Chronic stress exposure

Exposure to stressful life events was measured using the Life Events Assessment Profile (LEAP), a computerized stress assessment tool developed in our laboratory (Anderson, Whethington, & Kamarck, 2011). Broadly, the LEAP is an interview-based assessment of life stress exposures occurring in the previous 12 months and directly builds on the Bedford College Life Events and Difficulties Schedule (Brown and Harris, 1989). Both the LEAP and the LEDS take a contextual approach to stress exposure assessment, meaning that these measurements are concerned not with subject's perceptions of stress exposures, but with the characteristics of the stressors to which subjects have been exposed. This is intended to allow raters to determine whether the stressor would challenge an average person. This approach to stress measurement has been validated repeatedly since its inception (for review see Anderson et al., 2011) and has been shown to predict many health outcomes including the development of depression (Kessler et al., 1997), the severity of inflammatory diseases such as MS (Ackerman et al., 2003; Grant et al., 1989), cardiovascular events (Dickens et al., 2004), the likelihood of developing infectious disease (Cohen et al., 1998), and the development of GCR (Cohen et al., 2012).

In the contextual approach to stress measurement, interviewers utilize standardized probing questions to garner detail about potentially stressful life experiences. Only after uncovering the full context of a potentially stressful experience can an experience be deemed a stressful "event" in LEDS parlance. For example, a loss of job may be recorded as an "event" if the respondent has no other source of income and no partner to help support their family, while it may not be rated as an event if the respondent has a second job, a partner's income to rely on, and was expecting the employment to end. This sort of discrimination was developed in response to the poor reliability

of checklist approaches to stressor exposure, which ask whether given experiences occurred over a period of time without determination of the extenuating circumstances (Anderson et al., 2011).

Despite its predictive value, and adequate psychometric properties (Brown and Harris, 1989; Whethington, Brown, & Kessler, 1995), the LEDS is a time-consuming measurement of exposure to stressful life events. Thus, the LEAP was created to retain the specificity and rigor of the LEDS while reducing burden associated with this measure. This was accomplished by computerizing and consolidating the LEDS assessment process. In the LEAP, a trained interviewer speaks over the phone with the research participant while completing the semi-structured interview on the computer. The interviewer first gathers demographic information before proceeding to establish an individual's social network. This is done because common stressors often include negative life events that happen to or with close others. It is also used to help determine severity of the event. For example, it is important to distinguish whether a death or illness in a respondent's social network concerns a close family member as opposed to a work acquaintance. Next, the interviewer proceeds to asking general questions for each of 12 domains and 124 subdomains taken directly from the LEDS. More specifically, respondents are asked if they have experienced a given life event associated with each subdomain in the previous 12 months. If the respondent indicates that they experienced one of the events inquired about, this automatically triggers additional follow-up questions which provide contextual detail. In general, this process is very similar to the LEDS interview, except for the fact that the questions are close ended, the follow-up probes are algorithm driven, and the responses are recorded in a digital format. The digital format used for the response options allows the interviewer to score the interview in an automated fashion. Scoring algorithms weigh the closeness of the individual involved, the specific contextual factors of the exposures, and the duration and intensity of events/difficulties when scoring the interview.

As in the LEDS, LEAP "events" are defined as stressors lasting less than one month, whereas "difficulties" are considered stressors lasting one month or more. Events are given one of two severity ratings: severe or not severe. Difficulties can be given one of three severity ratings in the LEAP: mild, moderate, or severe. The generalized factors that differentiate event and difficulty severity are presented below, but it is critical to note that the pieces of information that alter the severity of a given stressor are domain-specific. This is the case in both the LEDS and the LEAP.

Events were considered severe if they were judged to have posed a high degree of threat to an individual's or close associate's well-being. Difficulties were considered mild if they posed a low degree of threat to one's well-being or if they occurred in a distant associate; moderate if they demonstrated marked threat to well-being for oneself or a close associate; and severe if they lasted longer than 6 months and were initially moderate in severity (e.g. a loss of income for 7 months), if a subsequent, related event occurred that further heightened the difficulty the severity (e.g. experiencing ketoacidosis 2 months after having been diagnosed with diabetes), or if the difficulty was life threatening or severely impairing from the beginning (e.g. undergoing cancer treatment and starting chemotherapy). In previous, convergent validity analyses, the LEAP performed similarly to the LEDS—the LEAP and the LEDS were correlated r =.84 for severe events, r = .74 for non-severe events, and r = .75 for difficulties (Anderson et al., 2011).

Stress exposure was operationalized as the number of moderate or severe life difficulties experienced over the previous year. We chose to examine life difficulties rather than events as most previous work in this literature has examine chronic stress exposure rather than acute stressful event exposure (e.g. Miller et al., 2002; Miller et al., 2014; Rohleder et al., 2009, but see Rohleder et al., 2002). Additionally, as this previous literature has examined life-threatening or very severe stressors (e.g. caring for a family member with cancer), we elected to count only moderate and

severe stressors. In in a previous study examining the relationship between stress and glucocorticoid resistance using the LEDS, Cohen et al., (2012) operationalized stress exposure as a dichotomous measure of whether a subject experienced at least one severe event or difficulty in the previous year. This left unclear whether observed effects of stress could be attributable to discrete "one off" events, multiple short lasting stress exposures, or prolonged stressors. By contrast, the current operationalization is narrower—being limited to difficulties, or chronic stressors lasting one month or more—a standard that we expected to be associated with greater precision.

Correlational analyses were performed comparing the LEAP interview to the perceived stress scale (PSS; Cohen, Kamarck, & Mermelstein, 1994) and the psychiatric epidemiology research interview life events scale (PERI; Dohrenwend et al., 1978), a standard self-report checklist of recent life events. Given previous research comparing interview-based stressor assessment to checklist approaches, we did not expect strong associations between the LEAP and PERI (Anderson et al., 2011). Log transformed total score on the PERI was correlated with the PSS ($r = .26$, $n = 247$, $p < .01$) . Scores on the LEAP interview were not significantly correlated with scores on the PSS($r = .09$, $n = 247$, $p = .18$). The total number of events on the PERI and the LEAP were modestly correlated ($r = .13$, $n = 247$, $p = .04$). These modest associations may be explained by the important conceptual differences between each measure, and were not unexpected. In a different sample, the PERI was also modestly correlated with the LEDS (Anderson et al., 2011) suggesting that this magnitude of this correlation may be due to the conceptual differences between interview and checklist-based approaches, rather than to weakness of the LEAP.

Cardiometabolic Risk

Cardiometabolic risk was measured using a composite score technique consisting of the average of the z scores for the following variables: fasting blood glucose, triglycerides, HDL concentrations (where HDL z scores are reverse scored), blood pressure (where systolic and diastolic blood pressures are first standardized and averaged before contributing to the composite), and waist circumference. The scores for HDL cholesterol and waist circumference were standardized relative to each sex's group mean, given the known sex differences in these health markers. Our research group has previously utilized this composite measure of cardiometabolic risk (Thomas et al., 2020), and similar measures have been used elsewhere as well (Mayne et al., 2019; Merkin et al., 2020).

Glucose. 100 µl of serum were used for assessment of blood glucose levels. This assay was performed using reagents from Beckman Coulter (#OSR6121; Center Valley, PA). The intra- and inter coefficients of variation were 1.0% and 2.1%, respectively.

Triglycerides. 100 µl of serum was used to assess for concentrations of triglycerides. This was done enzymatically using reagents from Beckman Coulter (#OSR61118; Center Valley, PA). The intra- and inter assay coefficients of variation were 1.8% and 3.7%, respectively.

HDL Cholesterol. 3µl of plasma was used to directly assess for concentrations of HDL cholesterol. This was performed via a two-reagent method using materials obtained from Beckman Coulter (#OSR6195; Center Valley, PA). The intra- and inter assay coefficients of variation were 3.5% and 6.7%, respectively.

Blood Pressure. Participants' clinic blood pressure readings were taken using a mercury sphygmomanometer and stethoscope. Two blood pressure readings were taken at each of two visits. At each of these two visits, a research associate placed a brachial artery cuff over the left arm of the participant after 5 minutes of seated rest. The associate then determined the maximum

inflation pressure and took two readings with 2 minutes in between each reading. Participants were required to refrain from vigorous physical activity and food and drug consumption (besides water) for the 2 hours preceding each visit. The average of the four readings (two per BP visit) constitutes the clinic blood pressure reading for the present study.

Waist Circumference. Waist circumference was measured by a research associate using a tape measure. The research associate then took a circumference measurement from the participant's side at the level of the navel. A circumference measure was taken at the level of the widest point of the hips (top, palpable end of the femur). Each participant's z score was computed from the average for their sex, as there are known size differences in adult bodies by sex.

Glucocorticoid Resistance

Glucocorticoid resistance was measured using a stimulated cytokine production paradigm. These assays were conducted at the Behavioral Immunology Lab at the University of Pittsburgh (Pittsburgh, PA). This method assesses the ability of a synthetic glucocorticoid (dexamethasone) to inhibit interleukin-6 production after exposure to an immunological challenge. Whole blood was treated with 150 ul of lipopolysaccharide and allocated to 6 different wells, each with a different concentration of dexamethasone. The concentrations of dexamethasone (DEX) used in the present study were 0nM + NaCI, 10-9nM, 10-8nM, 5x10-8nM, 10-7nM, and 10-6nM, and each well contained 1.5ml of whole blood and a phosphate buffer. The samples were incubated for 24 hours at 37 C with 5% CO2, spun, and the supernatant liquid was stored at -80 C until the assay was performed. ELISA kits were used (BD Biosciences kits, catalog #555220). GCR was quantified as the amount of DEX required to reduce IL-6 concentrations in half (IC-50), per previous work in our group (Lindsay et al., 2021). Inter- and intra-assay coefficients of variability were 7.12% and 4.34%, respectively.

Cytokine Concentrations

IL-6. 200 µl of plasma were used for IL-6 analyses. These assays were performed in duplicate using a commercially available ELISA kit (R&D systems, Minneapolis, MN). Samples were allowed to incubate for 3 hours at room temperature in microplate wells coated with murine monoclonal antibodies against Il-6. The plates were then washed, and 200 µl of conjugate was added and the samples allowed to incubate at room temperature for another 2 hrs. Next, the plates were again washed, and 50 µl of NADPH substrate was added. The samples were then incubated for 60 minutes then 50 µl of alcohol dehydrogenase/diaphorase amplifier was added and samples incubated for another 30 minutes. The reaction was stopped with 50 µl of sulfuric acid. Intra and inter-assay coefficients of variation are 9.1% and 10.2%, respectively.

3.0 Analyses

Analyses were conducted using SAS (9.4) statistical software. Generalized linear models were used to assess the main effect of chronic stress exposure on cardiometabolic risk and GCR, respectively. In all analyses, age, sex, race, and education level attained were included as covariates. Due to the failure to detect expected main effects, across nearly all indirect paths, simple mediation analyses were not performed as intended. Power analyses were performed *a priori* using G-Power Software (3.1.9.7). Across all hypothesized associations, power analyses indicated sufficient statistical power to detect effect sizes previously seen in the literature (which were all in the small to medium effect size range).

Descriptive statistical analyses were used to identify outliers and confirm that the distributions of variables conformed to the assumptions of linear regression analyses, before performing inferential statistical procedures. Transformation procedures were guided by the principle of performing the most conservative operations first (standard log transformation), before attempting less conservative operations (e.g. winsorization). This allowed us to retain all available data and not eliminate observations that were deemed outliers. A given observation was deemed an outlier if the observation was greater than ± 3 interquartile ranges above/below the 1^{st} and 3^{rd} quartiles, respectively. Data were considered to have a non-normal distribution if the skewness statistic was greater than 1 and/or the kurtosis statistic was greater than 3. If a variable had observations that were outliers or the distribution of that variable was non-normal by this definition, then the variable was first transformed using log transformation. If log transformation failed to alter the status of observations as outliers or the distribution remained significantly

skewed, winsorization was then performed. If necessary, data were winsorized to 3 interquartile ranges above the 3rd interquartile range.

The following variables had observations that were deemed outliers: glucose values (n = 6), triglyceride values (n = 3), IL-6 (n =6). The following variables showed non-normal distributions: glucose values, triglyceride values, IL-6, & PERI scores. The following transformations and adjustments were made: PERI scores were log transformed, glucose scores were winsorized, triglyceride scores were log transformed, and IL-6 values were winsorized and log transformed.

Visual analysis of the plots of residuals, descriptive statistics, and Cook's Distance were used to evaluate the possibility that observed effects were due to outliers or influential cases for the models predicting CMR and cardiometabolic risk from LEAP stressors. After the adjustments described above, no obvious outliers were visible in the plots of the residuals for either primary model (see figures 1 and 2). Using the common cutoff criteria of Cook's Distance > 1 as indicative of influential cases, no cases were flagged as influential. Using the more conservative criteria of influential cases being those with Cook's Distance of $D > 4/n$ we noted 13 cases exceeding this criteria for the model predicting cardiometabolic risk and 9 for the model predicting IC50 (see "Sensitivity Analyses" section for further detail about influential cases).

4.0 Results

4.1 Descriptive Statistics

See Tables 1-3 for descriptive statistics and bivariate correlations. In short, the sample was largely white (79.4%), female (58.8%), middle aged (M = 52.6), and reported an average of approximately 4 chronic life difficulties of moderate to severe intensity with a range of 0-18.

4.2 Inferential statistics

The results of inferential statistical models are presented below, by hypothesis. Additionally, complete results are presented in table format for select models (See Tables 4 and 5).

H1: The number of moderate or severe life stressors will be positively associated with cardiometabolic risk.

The number of moderate or severe life difficulties as measured by the LEAP was ***negatively*** associated with cardiometabolic risk ($F(246) = 6.48$, $b = -0.03$, $p = .03$).

H_2: The number of moderate or severe life stressors will be positively associated with GCR.

The number of moderate or severe life difficulties as measured by the LEAP was ***negatively*** associated with GCR ($F(246) = 2.63$, $b = -1.26$, $p = .01$). That is to say, as individuals experienced a greater number of stressors, they also demonstrated increased immune cell sensitivity to dexamethasone. While not included in the original data analysis plan, we performed this analysis

again, while controlling for stimulated IL-6 values, as has been described elsewhere (e.g. Natale et al., 2022). In this model, there was not a significant association between stimulated IL-6 on GCR, and the relationship between life difficulties and GCR remained statistically significant ($F(238) = 2.55$, b = -1.23, p = .01).

H_3: GCR will be positively associated with cardiometabolic risk.

GCR was not significantly associated with cardiometabolic risk ($F(246) = 5.39$, b < .0001, p = .99).

H4: GCR will partially mediate the association between life stressor exposure and cardiometabolic risk.

Given that GCR was negatively associated with stress exposure, and was not associated with cardiometabolic risk, and that life stressors were negatively associated with cardiometabolic risk, we did not proceed with testing a mediating hypothesis as planned.

H_5: GCR will be positively associated with circulating concentrations of IL-6.

Circulating concentrations of IL-6 were not significantly associated with GCR ($F(246) = 7.67$, b < .001, p = .79).

H_6: Circulating concentrations of IL-6 will be associated with cardiometabolic risk.

Circulating concentrations of IL-6 were positively associated with cardiometabolic risk ($F(246) = 23.12$, b = .90, p < .001).

H7: Circulating concentrations of IL-6 will partially mediate the association between GCR and cardiometabolic risk.

Given that GCR was unrelated to cardiometabolic risk and circulating IL-6 concentrations, we did not proceed with testing the mediating hypothesis as planned.

4.3 Sensitivity Analyses

The results of models in which potentially influential cases were excluded showed slight increases in effect sizes and reductions in p-values. This appears to reflect that a small number of individuals were having a mild suppressive effect on the relationship between stress and stress and cardiometabolic risk as well as stress and IC50. However, as these relatively influential individuals displayed plausible values for all relevant variables, and showed no evidence of being systematically different from the rest of the sample, thus we do not feel that these individuals ought to be excluded from analyses. Furthermore, the direction of the effect did not change, and the effect sizes remained in the small to moderate range.

4.4 Exploratory Analyses

Several exploratory analyses were conducted after discovering the opposite pattern of findings than expected. These included examining whether different operationalizations of stress using the LEAP produced different patterns of results. After considering the possibility that the impact of stress exposure on biological functioning may vary for moderate as opposed to severe stressors, we created a measure which included only severe stressors. Next, noting that the previous studies were often of caregivers, we developed a measure that counted only caregiving stressors

and a measure that indicated the presence vs absence of caregiving. Also, noting that timing of difficulties might result in different patterns of biological functioning, we also created a dichotomous measure assessing presence vs. absence of one or more current difficulties as reported by the LEAP Additionally, we experimented with controlling for the temporal proximity of the most recent difficulty, to further address the role of timing. Also, seeing that Cohen et al., (2012) included both life events and difficulties in their stress measure, we employed a similar approach, and created a measure of the number of events or difficulties of moderate to severe intensity. Across all of these operationalizations using the LEAP, we found either null results or the same small, negative association as originally observed.

In light of the nascency of the LEAP measure, and the possibility that the unexpected pattern of results may be due to the use of this measure, we also examined the relationship between GCR, cardiometabolic risk and negative life events as measured by the PERI life events checklist. Negative PERI events were unrelated to both cardiometabolic risk and GCR. Given that previous studies were conducted in samples that showed marked psychological distress, we also examined whether depressive symptoms (measured by the CES-D) and perceived stress (measured by the PSS) were associated with GCR and cardiometabolic risk, and we found null results across these analyses. While all models using alternative conceptualizations of stress produced non-significant results, it is perhaps worth noting that in all of these cases, the beta coefficients were positive, i.e., the relationships were in the expected direction.

Other exploratory analyses included using area under the curve with respect to increase and with respect to ground, as opposed to IC-50 values, as alternative quantifications of GCR. These models produced similar results as the models using IC-50 scores. Additionally, noting that the LEAP asks respondents about stressful events that occur within their social network, we

considered whether the number of social contacts and perceived social support accounted for the negative associations we observed—but controlling for these variables had no effect on the associations between stress and cardiometabolic risk and GCR. We also considered the possibility that effects of stressor exposure might be confounded or moderated by individual differences in coping ability. To account for this, we examined whether trait reappraisal was associated with outcomes of interest, and found null results. Lastly, we examined the moderating roles of age and sex in the models using the LEAP stress-exposure operationalization. Results remained non-significant in all cases. In analyses stratified by sex, we likewise found null results.

5.0 Discussion

The present study sought to extend the literature on the relationship between stress and health by examining whether chronic stressful life events are associated with GCR (GCR) in a sample of disease-free midlife adults, and whether GCR, in turn, was associated with cardiometabolic risk. Secondarily, we sought to determine whether in-vitro measures of GC sensitivity correlated with concentrations of systemic cytokines in circulation. Contrary to predictions, exposure to stressful life events was negatively associated with cardiometabolic risk and GCR, there were no effects of GCR on cardiometabolic risk, and in-vitro measures of GCR were uncorrelated with systemic concentrations of IL-6. There are multiple possible explanations for the unexpected pattern of results, and our results suggest a need for further research to clarify which groups are likely to experience immune and cardiometabolic dysregulation in response to stress. Additionally, our results suggest a need to carefully consider extant theories that relate stress to negative health outcomes via dysregulated glucocorticoid signaling.

As previously mentioned, our results contrast previous findings in this field, which have generally found an association between chronic stress exposure and GCR in immune cells (see Walsh et al., 2021 for review) and a positive association between stressful life events and cardiometabolic risk. Indeed, our data show small, negative relationships between exposure to stress, GCR and cardiometabolic risk. We also failed to observe an association between in-vitro measures of glucocorticoid sensitivity in immune cells and circulating concentrations of the cytokine, IL-6—suggesting that in-vitro measures of GCR may add little to our understanding of the levels of inflammation in the body. We did not observe significant associations between stress and GCR and cardiometabolic risk when using alternative stress measurement techniques and

operationalizations (e.g. PSS, PERI, & depressive symptoms were unrelated to outcomes of interest). Additionally, slight variations in LEAP operationalization (e.g. including moderate and severe vs only severe stressors) often resulted in non-significant associations. Together with the body of existing findings, we are therefore inclined to conclude that the unexpected negative associations that we observed may be due to chance.

If results were to replicate, however, they would appear to indicate that for some individuals, chronic stress can sensitize immune cells to the effects of glucocorticoids. Additionally, our results appear to indicate that exposure to prolonged stressful life events can be associated with reduced cardiometabolic risk, and that individual differences in GCR are not associated with individual differences in cardiometabolic risk. The only hypothesis that was confirmed in our findings was a positive relationship between circulating IL-6 and cardiometabolic risk, which has been previously noted in numerous studies (e.g. Marsland et al., 2010).

Potential Explanations: Sample Differences

Differences in sample characteristics between the present study and previous research may explain the disparate findings. Indeed, our sample was significantly older, less distressed, and not comprised primarily of caregivers of those with cancer (c.f. Miller et al., 2002, Cohen et al., 2012; Walsh et al., 2018; Rohelder et al., 2009) relative to previous studies. With respect to age, our sample was nearly a decade and a half older, on average, than the majority of previous studies which found a positive association between stress and GCR (but see also, Rohleder et al., 2009). We attempted to address this point of difference, to the extent possible, by co-varying for age and testing for an interaction between age and stressor exposure outcomes of interest. There were no significant results in these exploratory analyses. However, given the restricted age range of the

current sample relative to previous studies, this approach would not be sufficient to rule out the influence of age on the relationship between stress and GCR. Thus, it is possible that midlife adults, such as those recruited in our sample, are less likely to develop GCR and cardiometabolic risk after exposure to stressful life events.

Research on other biological pathways relating stress to disease risk has found moderating effects of age and there is some evidence suggesting that change in glucocorticoid sensitivity after acute stress exposure is decreased among elderly men (Rohlder et al., 2002). However, it is not clear that this would extend to context of chronic stress. Interestingly, age related changes in inflammation have been found, with concentrations of circulating and stimulated cytokines increasing with age (for review see Franchesci et al., 2000; Pawelec et al., 2014), but it is not clear that this would provide any insight into invitro measures of glucocorticoid regulation of the immune response, as we found these biomarkers to be unrelated. Given this constellation of findings, it appears that alterations in the regulation inflammation via glucocorticoid sensitivity with age is possible, but there is little existing evidence with which to make specific hypotheses.

With respect to distress, previous studies recruited individuals who demonstrated high levels of psychological distress. In fact, the averages reported in these studies often exceeded commonly used clinical cutoffs that designate patients as meeting diagnostic criteria for distress related disorders such as depression. In contrast, our sample demonstrated very low levels of distress, well below commonly used clinical cutoffs. While this also appears to be a promising explanation for the failure to replicate previous results, it should be noted that Miller et al., (2002) directly addressed whether the effect of caregiving stress on glucocorticoid sensitivity was mediated by levels of psychological distress. They found that depressive symptoms were unrelated to GCR, and that controlling for depressive symptoms did not reduce the strength or significance

of the association between caregiving status and GCR. Additionally, in exploratory analyses, we found no evidence of a relationship between depressive symptoms or perceived stress and GCR(albeit distress was low in our sample). This would seem to suggest that levels of distress do not account for changes in glucocorticoid sensitivity. Interestingly, however, Walsh et al., (2018) also assessed whether changes in distress over time predicted changes in GCR, and found evidence of a positive association between these measures. Thus, there is inconsistent evidence regarding the role of psychological distress in GCR.

An additional difference between our study and previous reports is that most of the previous literature in this area examined caregiving stress. It is possible that there are unique aspects of caregiving stress that contribute to GCR. Duration and severity of stress are two dimensions that hypothetically may differentiate chronic caregiving stress and the type of stress exposure measured in the present study and explain discrepant findings. We sought to evaluate these possible explanations in exploratory analyses. First, we experimented with different operationalizations of life difficulties which varied by severity and found no differences in the pattern of results. For instance, we explored whether mild, moderate and severe difficulties were differentially related to outcomes of interest, rather than grouping moderate and severe difficulties together. The null results of these models appear to suggest that differences in the severity of caregiving stress as opposed to stress from generalized life difficulties, does not explain the observed discrepancies between studies.

Next, we attempted to explore the influence of stressor duration, as previous studies of caregivers often recruited individuals who were under extreme duress for months and even years, whereas in our sample, typical stressor duration was shorter. We explored the potential confounding role of duration by including a control variable that was the duration, in days, of the

longest difficulty. Additionally, we created categorical variables wherein individuals were classified as having at least one severe stressor that lasted either between 1 and 3 months, between 3 and 6 months, between 6 months and 1 year, and lasting a year or more. Given the fact that the majority of our sample reported multiple chronic stressors occurring at varying times over the previous year, it was not possible to produce simple measures of stressor duration. Nonetheless, these exploratory analyses yielded null results.

Potential Explanations: Measurement Error

Another potential explanation for the null and unexpected findings is that our measures of stress and biomarkers are not comparable to those used in previous reports. For instance, it is possible the LEAP interview does not capture the same experience that has been approximated by stress measurement techniques in previous studies. While the LEAP is a newer measure, it was built to mimic assessment using the LEDS, a gold standard measure of stress exposure, that has been linked to a variety of health outcomes (including GCR). Moreover, in our exploratory analyses, we employed the PERI life events checklist, a widely used measure of stressful life events, and found no correlations between these measures and outcomes of interest.

Nonetheless, we note that the previous study which employed the LEDS measure (Cohen et al., 2012), operationalized stress exposure as the presence or absence of a life event or difficulty of marked long term threat. This operationalization appears to indicate that there were relatively few individuals with more than one or two stressors of this severity in their study. By contrast, we found that, on average, our participants reported nearly 5 moderate to severe life difficulties (note that we excluded events) and fewer than 10% reported zero life difficulties of this severity. This appears to reflect that either: a.) despite efforts to match previous measurement techniques, we

measured stress differently than did Cohen et al. (2012); or b.) that our sample experienced more stress than did the participants in Cohen et al., (2012). Given the low levels of psychological distress observed in our sample, possibility b.) seems unlikely. Without normative data for the LEAP, or previous use of the measure in clinical populations, it is difficult to determine the precise meaning of the level of stress exposure in our sample—a necessary datum to determine whether the LEAP performed as expected. In sum, it is possible that measurement technique played a role in our unexpected findings. However, this explanation does not extend to the null findings of exploratory analyses which employed comparatively common measures of stress (i.e., PSS, CESD, and PERI scales) or exploratory analyses in which we dichotomized stress exposure in the same manner as Cohen et al., (2012).

It is also possible that our techniques for the measurement of GCR played a role in the unexpected findings. We attempted to address this possibility by comparing our GCR scores to those obtained in other studies. This was not as straightforward as it may seem, however, as previous studies have used slightly different laboratory procedures (e.g. using cortisol as opposed to dexamethasone or varying the concentrations of compounds slightly) when calculating in vitro GCR. One previous study from our group utilized sufficiently similar laboratory techniques for comparison (Lindsay et al., 2021) and we note comparable IC50 scores. While limited, this data would seem to indicate that our measurement techniques were at least as reliable as previous, published studies.

Another possible reason that stress may not have been found to be associated with cardiometabolic risk in this sample was because cardiometabolic risk was measured via a composite score technique, which assumes that the direction of the effect of stress on each of the individual components of the composite score would be in the anticipated direction. We addressed

this by examining whether LEAP-defined stress exposure was associated with each component of cardiometabolic risk in separate, exploratory models, and found no evidence that the absence of a relationship between cardiometabolic risk and stress was due to component level discrepancies.

Other Considerations and Future Directions

It is unclear why the present study found largely null, and occasionally inverse, associations between stress and cardiometabolic risk. Landmark reviews have concluded that stress is associated with cardiovascular disease risk and that metabolic dysfunction is thought to be a key driver of this relationship (Steptoe and Kivimaki, 2013). There are studies which employed very similar designs to our own, operationalizing stress as exposure to life events, and cardiometabolic risk using standardized techniques, and these have found positive associations between life events and cardiometabolic risk (e.g. Raikkonenn et al., 2007; Pyykkonen et al., 2010). Interestingly, one these studies found that work related stressors, specifically, were related to cardiometabolic risk (Pyykkonen et al., 2010). Accordingly, in exploratory analyses we examined the relationship between work related negative life events and cardiometabolic risk and found a significant and positive association. This association was on the border of statistical significance, however, and did not hold after correction for multiple comparisons.

After the failure to identify individual differences that may explain our unexpected results in exploratory analyses, we returned to the literature to reevaluate whether the relationship between stress and our outcomes of interest were as robust as we had previously thought. While previous literature shows an apparently consistent relationship between chronic stress exposure and GCR, a careful review of the adjacent literature on depression and GCR reveals a more complex pattern of results. Indeed, we note five studies which found null results when examining relationships

between depression or high-perceived stress and GCR, or *enhanced* sensitivity in depressed subjects (Carvahlo et al., 2008; Miller, Freedland, and Carney, 2005; Miller, Rohleder, Stetler, & Kirschbaum 2005; Nikkelslat et al., 2015; Lindsay et al., 2021 unpublished results).One would not intuit that the pattern of results in chronically stressed individuals would differ markedly from depressed individuals as many depressed subjects have experienced chronic stress exposure, and that the symptoms of chronic stress exposure and depression are similar. It seems that future studies should examine the interplay between stress exposure and the development of major depression on GCR.

The present study was in part motivated by the theoretical assumption that both hypercortisolism and inflammation are key biological processes that underly the relationship between stress and cardiovascular disease risk. However, it is important to note that the literature supporting the hypothesis that chronic stress exposure is related to increased cortisol concentrations is mixed (for review see Miller, Chen, and Zhou, 2007). Indeed, while excess glucocorticoids may be detrimental to cardiovascular health (Walker, 2007), chronic psychological stress may not reliably lead to increased cortisol concentrations. In this light, glucocorticoid resistance of immune cells might not be required to explain relationships between stress, HPA axis activity, inflammation and CVD risk. Nonetheless, glucocorticoid resistance could theoretically still play a role in the relationship between stress and CVD risk insofar as it indexes immune functioning thought to play a role in the pathogenesis or progression of CVD. The results of this study provide preliminary evidence on this latter possibility, in that they showed that in-vitro glucocorticoid resistance of immune cells was not associated with cardiometabolic risk factors or systemic cytokines—known risk factors for CVD.

With respect to the notion that in-vitro glucocorticoid resistance would predict systemic concentrations of IL-6, it is important to note that immune cells constitute just one of multiple sources of IL-6, including adipocytes (Fain, 2006) and that these cells are regulated by factors beyond glucocorticoid concentration, including ANS hormones (Walsh et al., 2021). It is possible that cells could be resistant to glucocorticoids and IL-6 levels be simultaneously elevated if IL-6 variation was driven by an alternative source or if an alternative regulatory influence altered the functioning of immune cells, or some combination of these factors. Additionally, it is important to note that IL-6 secretion was measured in response to immunological challenge—it is possible, and perhaps likely, that under basal conditions, differences in sensitivity have no bearing on systemic concentrations of IL-6, as circulating cells may not be secreting significant amounts of IL-6 in these contexts. Therefore, except in cases of widespread infection, we may see little correspondence between systemic IL-6 and in-vitro glucocorticoid resistance but may see positive correlations only at local sites of infection, where cells are in states of heightened activity. Indeed, the only study to find a correlation between IL-6 and glucocorticoid resistance, was the study of cold likelihood in Cohen et al., (2012), in which IL-6 concentrations were derived from infected nasal tissues. In this light, it may be fruitful to consider whether glucocorticoid resistance may be associated with atherosclerosis, and thereby CVD risk, rather than through systemic concentrations of IL-6.

Another important future direction in this research area involves validating the measurement techniques for assessing GCR and standardizing procedures across studies. GCR is an umbrella term that includes a wide range of operationalizations. These include functional measures such as those employed here, assessment of GR polymorphisms, systemic dexamethasone suppression tests, DNA methylation techniques and many others. It remains

unclear whether these different operationalizations are interchangeable, and more research is needed to determine how highly correlated these measures are. Even within a single operationalization, there is considerable heterogeneity across laboratory protocols. For instance, studies utilizing in-vitro, functional GCR assessment vary in the amount and type of glucocorticoid used to suppress cytokine production, as well as incubation time and the amount of immunological challenge. This precludes direct comparison of scores across studies. In short, more research is needed to establish standardized procedures and correlate different operationalizations of GCR.

Additionally, little is known about the measurement reliability, stability and functional correlates of immune cell GCR. With respect to reliability, it should be noted that the field does not have normative data on immune cell GCR, and it is not clear to what extent GCR can be expected to vary within an individual. While the one existing longitudinal study of stress and GCR noted significant changes in group means over time, these differences were small in magnitude relative to inter-individual variability at a given timepoint (Walsh et al., 2018). Additionally, as no other study has utilized the same laboratory protocol, it is not clear whether this is representative of normal amounts of variability. Additional, longitudinal studies of stress and GCR, with repeated assessment, appear necessary.

With respect to the functional correlates of GCR, one previous study found that GCR was associated with increased risk for developing colds (Cohen et al., 2012), but no other study, to our knowledge, has found an association between GCR and health outcomes of interest. To our knowledge, our study was the first to test whether GCR predicted a health outcome since Cohen et al., (2012). Thus, it appears that more research is required to determine whether GCR is a mechanism leading to inflammatory disease risk as has been hypothesized. It may be the case that

the “glucocorticoid resistance model of stress and disease” applies to infectious disease contexts, but does not explain the relationship between stress and chronic, non-infectious illness.

5.1 Conclusion

The present study did not find evidence that stress, as measured by exposure to stressful life events, was positively associated with cardiometabolic risk or GCR. On the contrary, it appears that stress, as presently defined and in healthy mid-life adults, may be negatively associated with GCR and cardiometabolic risk. The present study found no evidence of an association between GCR and cardiometabolic risk, or between GCR and circulating IL-6 concentrations. These unexpected findings warrant careful consideration of the limits of generalizability of findings pertaining to stress and GCR.

6.0 Figures and Tables

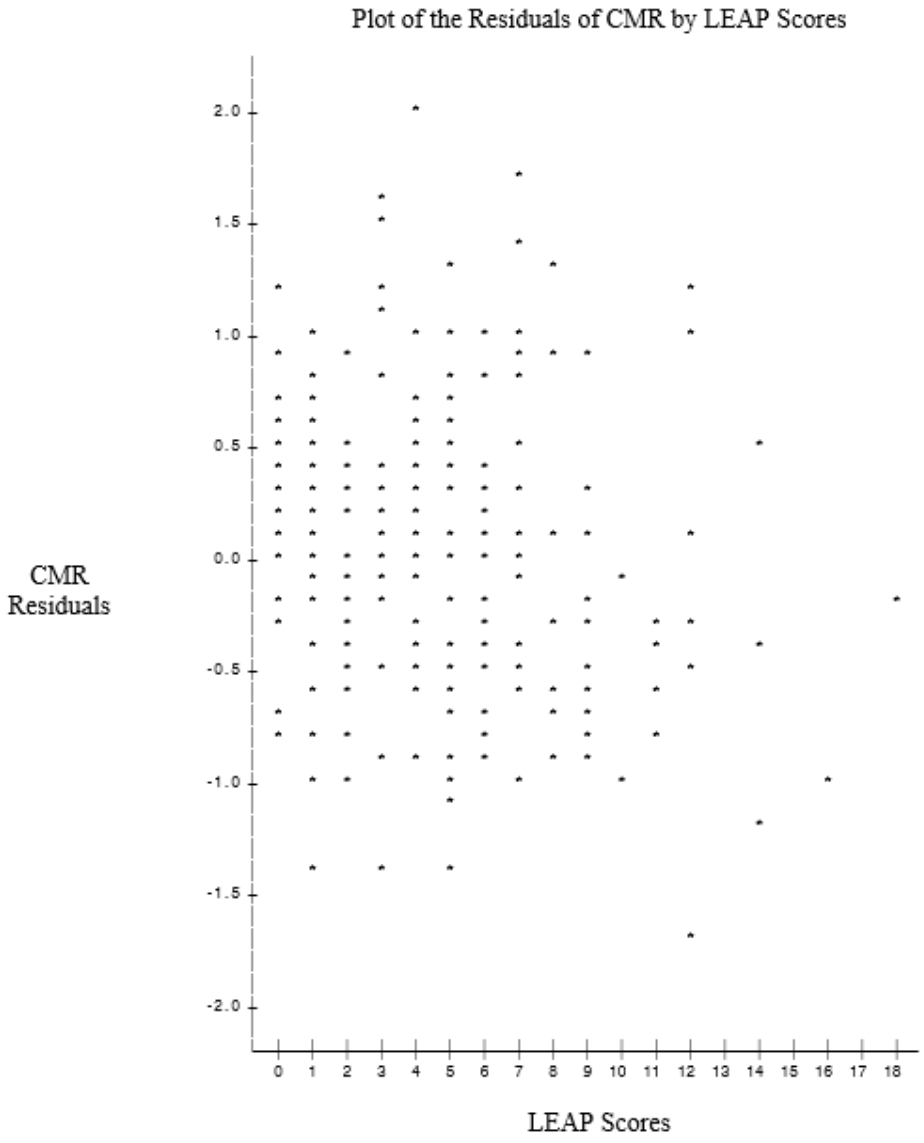

Figure 1. Plot depicts the relationship between the number of LEAP chronic stressors and the residuals for cardiometabolic risk, adjusted for demographic covariates.

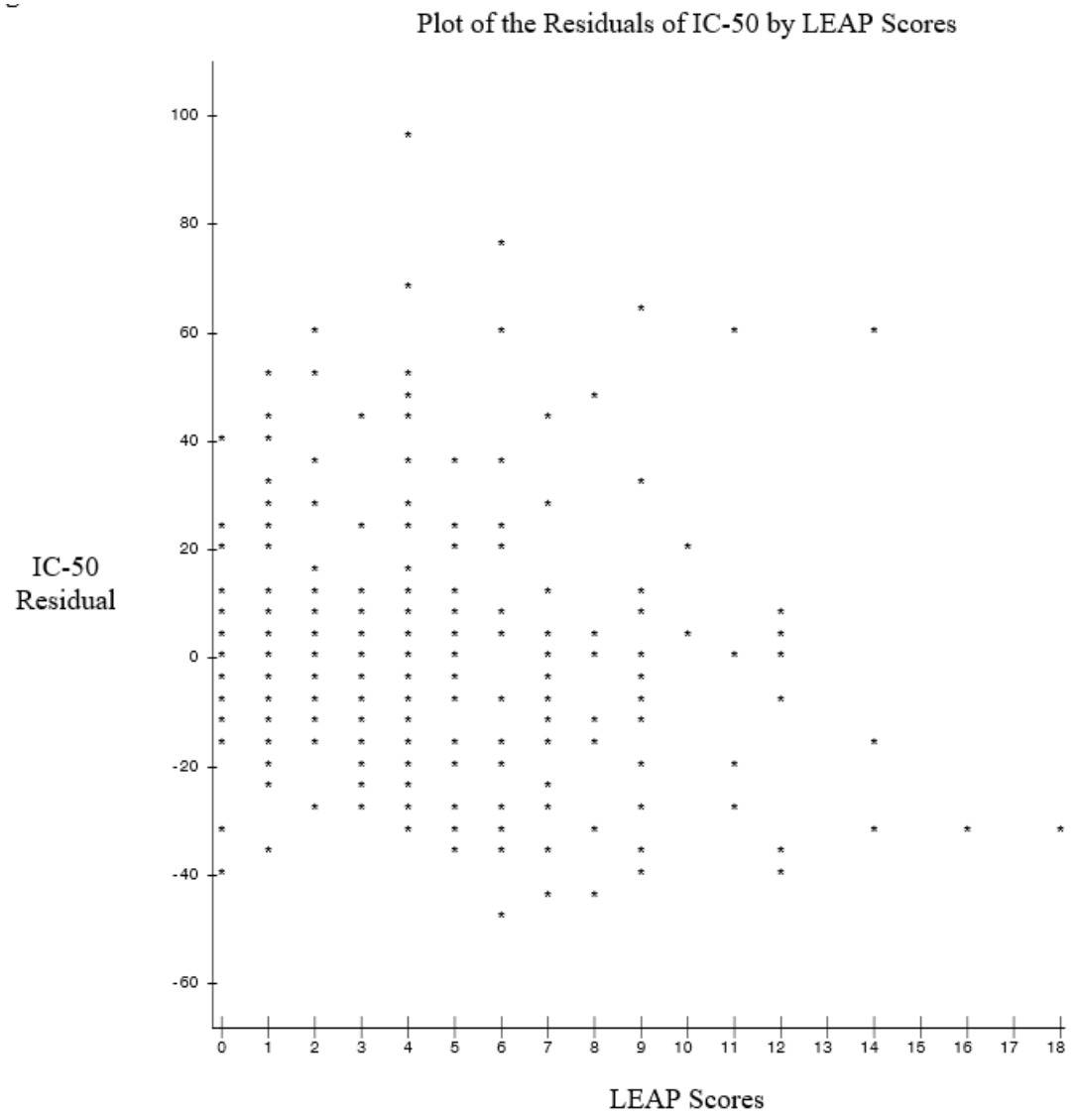

Figure 2. Plot depicts the relationship between the number of LEAP chronic stressors and the residuals for IC-50, adjusted for demographic covariates.

Table 1

Descriptive Statistics for Primary Study Variables

Variable	Mean or %	SD
Sex (% Female)	58.70%	*NA*
Race (% White vs Non-White)	79.35%	*NA*
Education (% Bachelors or higher)	66.80%	*NA*
Age	52.65	7.44
Glucose	91.18	12.42
Triglycerides	97.78	52.97
Mean Systolic Blood Pressure	118.75	12.71
Mean Diastolic Blood Pressure	76.34	6.98
HDL Cholesterol	59.00	15.81
Waist Circumference	88.81	14.51
IC-50 (IL-6)	49.09	25.03
Circulating IL-6	2.75	9.72
LEAP Chronic Stressful Events	4.58	3.40
PERI Negative Life Events	3.34	3.85

Note: N = 247. LEAP and PERI measures assessed exposure over the previous year. Race was presented as white/non-white due to low heterogeneity in racial composition of sample. *NA* = statistic not applicable.

Table 2

Bivariate Correlations Between Demographic Characteristics and Outcome Variables (N = 247)

Variable	1	2	3	4	5	6	7
1.Age							
2.Sex	< 0.01						
3.Race	-0.14*	0.08249					
4.Education	0.05	-0.09	-0.07				
5.LEAP Chronic Stressful Events	-0.13*	0.17**	-0.07	-0.06			
6.IL-6 IC-50	0.06	-0.06	-0.12*	-0.05	-0.17**		
7.Circulating IL-6	0.06	0.15*	0.24***	-0.25***	-0.08	< -0.01	
8.Cardiometabolic Risk	0.05	-0.17***	0.07	-0.24***	-0.16**	0.02	0.50***

Note: N = 247. * <.05, ** <.01, *** < .001.

Table 3

Bivariate Correlations Between Cardiometabolic Risk Components (N = 247)

Variable	1	2	3	4	5	6
Cardiometabolic Risk Composite Score						
Glucose	0.66 ***					
Triglycerides	0.71***	0.31***				
Systolic BP	0.47***	0.21***	0.16*			
Diastolic DBP	0.56***	0.23***	0.26***	0.73***		
HDL Cholesterol	-0.66***	-0.30***	-0.49***	-0.03	-0.20**	
Waist Circumference	0.76***	0.44***	0.44***	0.26***	0.35***	-0.47***

Note: N = 247. * <.05, ** <.01, *** < .001.

Table 4

Regression Analysis Cardiometabolic Risk as a Function of Chronic Stress and Demographic Characteristics (Hypothesis 1)

Variable	*b*	*SE*	*t*	*p*	Partial Eta-Square
Age	< 0.01	< 0.01	0.92	0.36	< 0.00
Sex	**-.22**	**0.08**	**-2.74**	**< 0.01**	**0.03**
Race (white/non-white)	0.10	0.10	0.97	0.33	< 0.01
Education	**-.19**	**0.04**	**-4.25**	**<.0001**	**0.07**
Number of Chronic Life Events from LEAP Interview	**-.026**	**0.01**	**-2.21**	**0.03**	**0.02**

Note: N = 247. R^2 = .12. Significant effects bolded. Cardiometabolic risk was measured using a composite score technique.

Table 5

Regression Analysis Predicting Glucocorticoid Resistance as a Function of Chronic Stress and Demographic Characteristics (Hypothesis 2)

Variable	*b*	*SE*	*t*	*p*	Partial Eta-Square
Age	0.08	0.22	0.37	0.71	< 0.01
Sex	-1.58	3.25	-0.49	0.63	< 0.01
Race (white/non-white)	**-8.25**	**3.95**	**-2.09**	**0.04**	**0.02**
Education	-1.99	1.77	-1.12	0.26	< 0.01
Number of Chronic Life Events from LEAP Interview	**-1.26**	**0.48**	**-2.65**	**<.01**	**0.03**

Note: N = 247. R^2 = .05. Significant effects bolded. Glucocorticoid resistance was measured as the inhibitory concentration of interleukin-6 by dexamethasone after cells were exposed to lipopolysaccharide in-vitro.

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