Concept Paper

Provisional Paper Title: The association of stressful life events and inflammation in midlife: Differential impacts on suPAR, C-reactive protein, and Interleukin-6

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Objective of the study:

The experience of stressful life events is associated with poor cardiovascular health. For example, people with high levels of perceived stress and those with PTSD have a 27% and 55% increased risk of developing coronary heart disease, respectively[1-2]. One mechanism that might explain how stressful experiences lead to poorer health is through higher levels of systemic inflammation. A large body of literature has linked higher levels of inflammation to increased risk of cardiovascular diseases and cardiovascular events[3-4]. Stressful life events—such as adversity in childhood, divorce, and PTSD—have also been associated with higher levels of systemic inflammation[5-9]. If stressful life events lead to increased inflammation, it could contribute to poorer downstream cardiovascular health.

There are several ways to assess systemic inflammation, each with advantages and disadvantages in their cost, utility, and predictive validity. One marker of systemic inflammation, suPAR, has been recently identified as a more effective measure of chronic stress than other, more traditional measures of systemic inflammation used in longitudinal cohort studies, such as hsCRP and IL-6[10-11]. Although there is evidence that suPAR better assesses the burden of chronic stressors cross-sectionally, there remains a need to link chronic stressors and suPAR prospectively to compare and contrast these associations with other measures of systemic inflammation. This need is particularly acute given the potential cardiovascular health implications of inflammation—suPAR has been linked to poorer cardiovascular endpoints in a similar way to other measures of systemic inflammation and substantially improves the prediction of cardiovascular health outcomes when combined with CRP[12]. The Dunedin Study is well-suited to address this open question, as the recent phase 38 and 45 assessment included measurements of suPAR, hsCRP, and IL-6.

Data analysis methods:

In pursuit of these aims, we propose the following analyses:

Aim 1: Investigate the association of stressful life events at from phase 32 to 38 (reported at phase 38) with suPAR, hsCRP, and IL-6 (pending availability) levels at phase 38 in independent multiple regression models.

Aim 2: Investigate the association of stressful life events at from phase 38 to 45 (reported at phase 45) with change in suPAR, hsCRP, and IL-6 (pending availability) from phase 38 to 45 in independent multiple regression models.

Additional considerations: It is additionally possible that the impact of stressful life events depends on childhood adversity, consistent with prior research[13]. We will investigate this possibility if main effect models do not demonstrate reliable results. Also, IL-6 will be included pending availability in the sample

General analysis methods: All models will include a variety of relevant demographic, childhood, and health behavior covariates. All models will be run in MPLUS[14] using full information maximum likelihood estimation to account for missing data[15]. A *p* value < 0.05 is a priori designated as statistically significant.

Variables needed:

- Systemic Inflammation
    - suPAR at 38 and 45
    - hsCRP at 38 and 45
    - IL-6 at 38 and 45 (pending availability)
- Stressful life events
    - Assessed at 38 and 45 using count of events reported in the life history calendar (LHC) interview:
        - Death of a close family member or friend (LCH18)
        - Job loss (LCH18)
        - Serious physical illness or injury to self (LCH18)
        - Serious physical illness or injury to friend or family (LCH18)
        - Legal trouble (LCH18)
        - Physical or sexual assault (LCH18)
        - Serious financial difficulty (LCH18)
        - High number of moves (LHC1)
        - Homelessness (LHC3 and 4)
        - Incarceration (LHC15)
        - Divorce or separation of married/cohabitating relationship (LHC6)
        - Other spontaneously reported events (LCH18)
        - Will exclude items in phase 38 that do not match the phase 45 LCH18 (if possible)
    - "Adult Stress" composite variable from phase 26[16]
    - Childhood prospective ACEs
- Childhood covariates
    - Childhood SES
    - Childhood IQ (WISC-R averaged across ages 7, 9, and 11 years)
    - Self-control
- Demographic and health behavior covariates
    - Sex
    - Smoking status (current smoking status and pack years) at 38 and 45
    - BMI at 38 and 45

Significance of the Study (for theory, research methods or clinical practice):

Determining the mechanisms through which stressful life events impact cardiovascular health is an important step towards effectively preventing poor health across the lifespan. The current study will help provide additional evidence for one biologically-plausible pathway through which stressful life events might be linked to poorer health: systemic inflammation. This study will also provide evidence as to the specificity of associations between suPAR and ongoing stressors, as compared to hsCRP and IL-6. One particular strength of the proposed study is the ability to compare the relative contributions of stressful life events in early childhood, early adulthood, and more proximal events to systemic inflammation. The use of suPAR in the sample will also extend prior work in the Dunedin study to include longitudinal change in suPAR over time. If implicated in the pathway from stressful life events to cardiovascular health, systemic inflammation would be a candidate mechanism through which to improve health in midlife, such as through behavioral treatments, psychopharmacological treatments, or the use of anti-inflammatory medication.

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Data Security Agreement

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KB	My project is covered by Duke or Otago ethics committee OR I have /will obtain ethical approval from my home institution.
KB	I will treat all data as "restricted" and store in a secure fashion. My computer or laptop is: a) encrypted (recommended programs are FileVault2 for Macs, and Bitlocker for Windows machines) b) password-protected c) configured to lock-out after 15 minutes of inactivity AND d) has an antivirus client installed as well as being patched regularly.
KB	I will not "sync" the data to a mobile device.
KB	In the event that my laptop with data on it is lost, stolen or hacked, I will immediately contact Professor Moffitt or Caspi. (919-684-6758, tem11@duke.edu, ac115@duke.edu)
KB	I will not share the data with anyone, including my students or other collaborators not specifically listed on this concept paper.
KB	I will not post data online or submit the data file to a journal for them to post. *Some journals are now requesting the data file as part of the manuscript submission process. The Dunedin Study Members have not given informed consent for unrestricted open access, so we have a managed-access process. Speak to Terrie or Avshalom for strategies for achieving compliance with data-sharing policies of journals.*
KB	I will delete all data files from my computer after the project is complete. Collaborators and trainees may not take a data file away from the office. The data remains the property of the Study and cannot be used for further analyses without an approved concept paper for new analyses.

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