ENVIRONMENTAL-RISK (E-RISK) LONGITUDINAL TWIN STUDY
CONCEPT PAPER FORM

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Social isolation, loneliness, and inflammation

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Objective of the study and its significance:

The importance of social relationships for health and longevity is supported by a large and growing body of evidence (Umberson & Montez, 2010). According to a recent meta-analysis, social isolation, loneliness and living alone are each associated with a 25-30% increase in risk for mortality (Holt-Lunstad et al, 2015). Numerous mechanisms for this association have been proposed, including maladaptive health-related behaviours, increased blood pressure and impaired sleep quality.

Another pathway through which social relationships could influence health is inflammation. According to a hypothesis based on evolutionary theory (Cacioppo et al, 2006), loneliness is an adaptive response to social disconnection that prepares individuals to face an unsafe environment without protection from kith and kin. Based on this premise, it is hypothesised that loneliness is accompanied by changes in immune functioning that would bolster an individual's ability to fight infection in event of wounding. Consistent with this, individuals high in feelings of loneliness exhibit a pattern of pro-inflammatory changes in gene expression (Cole et al, 2007).

A limitation of the existing literature on social connection and inflammation is that studies have often

conflated social isolation and loneliness, or only examined one of the two. These are frequently co-occurring but conceptually distinct phenomena, and it is not clear which is the 'active ingredient'. Those studies that have examined both in parallel (e.g. Shankar et al, 2011) suggest that objective social isolation, rather than loneliness, is the more relevant risk factor for this particular outcome. However, this research has been cross-sectional in nature and cannot address the possibility that individuals suffering from inflammatory disease may, as a result, be restricted from engaging in social activities. Longitudinal studies have found that childhood social isolation predicts increased inflammation in adults (Danese et al, 2009; Lacey et al, 2014), but there is limited longitudinal research on loneliness to compare this to. A further issue still is that loneliness is highly correlated with depression (Matthews et al, 2016), and studies that fail to take their co-occurrence into account may yield associations that are confounded.

There are several ways to assess systemic inflammation, each with advantages and disadvantages in their cost, utility, and predictive validity. We have recently identified one such marker of systemic inflammation, suPAR (*soluble urokinase plasminogen activator receptor*), as a more effective measure of chronic stress than other, more traditional measures of systemic inflammation used in clinical or research settings, i.e., high-sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6). Specifically, we found increased suPAR levels in individuals who had been exposed to early life risk factors, such as adverse childhood experiences, or specific victimization experiences (Rasmussen et al., 2019a; 2019b).
In contrast to CRP and IL-6, both of which are acute-phase reactants and markers of acute inflammation and infections (Rhodes et al., 2011; Hunter & Jones, 2015), suPAR appears to be less affected by acute conditions (Lyngbæk et al., 2012) and therefore potentially a better measure of systemic chronic inflammation. The plasma level of suPAR is thought to reflect a person's overall level of immune activity, and elevated suPAR is observed in a wide range of diseases and pathological conditions (Hayek et al., 2015; Persson et al., 2014; Schaefer et al., 2017; Theilade et al., 2015). Research indicates that suPAR is associated with clinical outcomes independent of CRP and IL-6 (Rasmussen et al., 2016; Botha et al., 2015).

Here, we wish to use data from the E-Risk and Dunedin studies to investigate the associations between social isolation and loneliness with markers of systemic inflammation, including the acute inflammation markers CRP and IL-6 and the chronic inflammation marker suPAR.

Statistical analyses:

Aim 1 (primary analysis): Investigate the association of childhood social isolation and adolescent/adulthood loneliness with suPAR, hsCRP, and IL-6 levels at age 18 in the E-Risk Study and at age 38 in the Dunedin Study, in separate models of social isolation or loneliness, and in models including both social isolation and loneliness.

Aim 2 (secondary analysis): Investigate the association of depression with suPAR, hsCRP, and IL-6 levels at age 18 in the E-Risk Study and at age 38 in the Dunedin Study. Test whether the associations found in Aim 1 are robust to controls for depression.

Associations will be tested using multivariable linear regression analyses. All models will include a variety of relevant demographic, childhood, and health behaviour covariates, such as sex, body mass index, and smoking. Further adjustments will be made for childhood SES. A p value < 0.05 is a priori designated as statistically significant.

Variables Needed at Which Ages (names and labels):

	E-Risk:	**Dunedin:**
General:	familyid Unique family identifier atwinid Twin A ID (ex chkdg) btwinid Twin B ID (ex chkdg) rorderp5 Random Twin Order risks Sample Groups cohort Cohort sampsex Sex of Twins: In sample	Sex

	zygosity Zygosity	
Childhood:	Age 5: sisoe5 Social isolation scale (mum & teacher average) - P5 – Elder sisoy5 Social isolation scale (mum & teacher average) - P5 – Younger Age 7: sisoe7 Social isolation scale (mum & teacher average) – P7 – Elder sisoy7 Social isolation scale (mum & teacher average) – P7 - Younger Age 10: sisoe10 Social isolation scale (mum & teacher average) – P10 - Elder sisoy10 Social isolation scale (mum & teacher average) – P10 - Younger Age 12: sisoe12 Social isolation scale (mum & teacher average) – P12 – Elder sisoy12 Social isolation scale (mum & teacher average) – P12 – Younger lonelye12 Loneliness – P12 – Elder lonelyy12 Loneliness – P12 – Younger cdie12 Depression scale – CDI – Elder cdiy12 Depression scale – CDI - Younger	Social isolation (Zisolkid)
Age 18:	socisoe18 Social Isolation scale - P18 – Elder socisoy18 Social Isolation scale - P18 – Younger lonelye18 Loneliness scale – P18 – Elder lonelyy18 Loneliness scale – P18 – Younger mdesxe18 MDE – symptom scale – P18 – Elder mdesxy18 MDE – symptom scale – P18 – Younger dxmdee18 Major depressive episode, dsm4 – P18 – Elder dxmdey18 Major depressive episode, dsm4 – P18 - Younger suPAR (suPARngml_E, suPARngml_Y) CRP (CRPmgLE18, CRPmgLY18) IL-6 (IL6pgmLE18, IL6pgmLY18) Smoking: - smkcure18, smkcury18 - smkcnume18, smkcnumy18 - smkpkyre18, smkpkyry18 Body mass index (bmie18, bmiy18)	
Age 38:		suPAR (suPAR38ngml) CRP (CRP38np, LNCRP38np) IL-6 () Smoking (Smoker38, PackYrLifTm38, CigsDay38) Body mass index (bmi38np) Current illness (SickToday38, sickat38)

		Loneliness (Lonely38, LonelyUCLA38)

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Data Security Agreement

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Proposing Author	Timothy Matthews / Line Jee Hartmann Rasmussen
Today's Date	17 September 2019

Please keep one copy for your records
(Please initial your agreement)

TM/LR I am familiar with the King's College London research ethics guidelines (https://www.kcl.ac.uk/innovation/research/support/ethics/about/index.aspx) and the MRC good research practice guidelines (https://www.mrc.ac.uk/research/policies-and-guidance-for-researchers/good-research-practice/).

TM/LR My project has ethical approval from my institution.

TM/LR I am familiar with the EU General Data Protection Regulation (https://mrc.ukri.org/documents/pdf/gdpr-guidance-note-3-consent-in-research-and-confidentiality/), and will use the data in a manner compliant with its requirements.

TM/LR My computer is (a) encrypted at the hard drive level, (b) password-protected, (c) configured to lock after 15 minutes of inactivity, AND (d) has an antivirus client which is updated regularly.

TM/LR I will treat all data as "restricted" and store in a secure fashion.

TM/LR I will not share the data with anyone, including students or other collaborators not specifically listed on this concept paper.

TM/LR I will not merge data from different files or sources, except where approval has been given by the PI.

TM/LR 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 E-Risk Study cannot be shared because the Study Members have not given informed consent for unrestricted open access. Speak to the study PI for strategies for dealing with data sharing requests from Journals.

TM/LR Before submitting my paper to a journal, I will submit my draft manuscript and scripts for data checking, and my draft manuscript for co-author mock review, allowing three weeks.

TM/LR I will submit analysis scripts and new variable documentation to project data manager after the manuscript gets accepted for publication.

TM/LR I will delete the data after the project is complete.

TM/LR **For projects using location data:** I will ensure geographical location information, including postcodes or geographical coordinates for the E-Risk study member's homes or schools, is never combined or stored with any other E-Risk data (family or twin-level data)

TM/LR **For projects using genomic data:** I will only use the SNP and/or 450K data in conjunction with the phenotypes that have been approved for use in this project at the concept paper stage.

Signature: .....