









STUDY PROTOCOL

Life course programming of stress responses in adolescents and young adults in India: Protocol of the Stress Responses in Adolescence and Vulnerability to Adult Non-communicable disease (SRAVANA) Study [version 1; referees: 2 approved]

GV Krishnaveni ¹, Kalyanaraman Kumaran¹, Murali Krishna¹, Sirazul Sahariah ², Giriraj Chandak³, Sarah Kehoe⁴, Alexander Jones⁵, Dattatray Bhat ⁶, Vijay Danivas⁷, Krishnamachari Srinivasan⁸, J Suguna Shanthi¹, SC Karat⁹, Mary Barker ⁴, Clive Osmond ⁴, Chittaranjan Yajnik ⁶, Caroline Fall⁴

¹Epidemiology Research Unit, CSI Holdsworth Memorial Hospital, Mysore, India

²Centre for the Study of Social Change, Mumbai, India

³Centre for Cellular and Molecular Biology, Hyderabad, India

⁴MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK

⁵Department of Paediatrics, University of Oxford, Oxford, UK

⁶Diabetes Unit, KEM Hospital Research Centre, Pune, India

⁷Mysore Medical College and Research Institute, Mysore, India

⁸St. John's Research Institute, St. John's National Academy of Health Sciences, Bangalore, India

⁹Department of Pediatrics, Jerudong Park Medical Centre, Jerudong Park, Brunei

v1 First published: 10 May 2018, 3:56 (doi: [10.12688/wellcomeopenres.14583.1](https://doi.org/10.12688/wellcomeopenres.14583.1))

Latest published: 10 May 2018, 3:56 (doi: [10.12688/wellcomeopenres.14583.1](https://doi.org/10.12688/wellcomeopenres.14583.1))




Abstract

Background: Early life nutrition may affect individuals' susceptibility to adult non-communicable diseases (NCD). Psychological stress is a well-recognised NCD risk factor. Recent evidence suggests that impaired foetal nutrition alters neuro-endocrine pathways, and hypothalamic-pituitary-adrenal axis feedback systems, resulting in abnormal stress responses, and NCD risk. This study aims to examine adolescent cortisol and cardiovascular stress responses in relation to maternal nutrition and contemporaneous NCD risk markers.

Methods: The study sample will be drawn from three well-established birth cohorts in India; the Parthenon cohort, Mysore (N=550, age~20y), the SARAS KIDS prenatal intervention cohort, Mumbai (N=300, age~10-12y) and the Pune Rural Intervention in Young Adults/ PRIYA cohort, Pune (N=100, age~22y). We will perform the 'Trier Social Stress Test (TSST)', a well-accepted stress-test module which involves participants performing 5-minutes each of public speaking and mental arithmetic tasks in front of unfamiliar 'judges' (stressor). Repeated measures of salivary cortisol and autonomic cardiovascular outcomes relative to the stressor will be assessed. Measures of psychological stress, cognitive function, blood pressure, glucose-insulin metabolism and depression will be carried out. Mechanistic studies including DNA methylation in gluco-corticoid receptor (*NR3C1*) and *11β-HSD2* gene loci and neuroimaging will be carried out in a subsample. Qualitative interviews and focus group discussions in a subsample of the Parthenon cohort will explore

Open Peer Review

Referee Status:  

	Invited Referees	
	1	2
version 1 published 10 May 2018	 report	 report
1 Candace M. Raio , New York University, USA		
2 Giridhara R. Babu  , Public Health Foundation of India, India		

Discuss this article

Comments (0)

the perception of stress and stressors among the youth. We will convert repeated measures into time-weighted averages before analysis. We will carry out multivariable regression analysis to test the associations. We will further refine the analyses using the mixed-model regression and conditional analyses for the association with repeated measures.

Ethics and dissemination: This study has been approved by the research ethics committee of CSI Holdsworth Memorial Hospital, Mysore. The findings will be disseminated locally and at international meetings, and reports will be submitted to open access peer reviewed journals.

Keywords

stress response, adolescents, TSST, India, non-communicable disease, maternal nutrition



This article is included in the [Wellcome Trust/DBT India Alliance](#) gateway.

Corresponding author: GV Krishnaveni (gv.krishnaveni@gmail.com)

Author roles: **Krishnaveni G:** Conceptualization, Data Curation, Funding Acquisition, Investigation, Methodology, Project Administration, Supervision, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Kumaran K:** Data Curation, Investigation, Methodology, Project Administration, Supervision, Writing – Review & Editing; **Krishna M:** Investigation, Methodology, Writing – Review & Editing; **Sahariah S:** Investigation, Methodology, Project Administration, Writing – Review & Editing; **Chandak G:** Data Curation, Methodology, Resources, Writing – Review & Editing; **Kehoe S:** Methodology, Writing – Review & Editing; **Jones A:** Methodology, Writing – Review & Editing; **Bhat D:** Methodology, Resources, Writing – Review & Editing; **Danivas V:** Methodology, Writing – Review & Editing; **Srinivasan K:** Methodology, Supervision, Writing – Review & Editing; **Suguna Shanthi J:** Methodology, Supervision, Writing – Review & Editing; **Karat S:** Methodology, Writing – Review & Editing; **Barker M:** Methodology, Supervision, Writing – Review & Editing; **Osmond C:** Formal Analysis, Methodology, Supervision, Writing – Review & Editing; **Yajnik C:** Investigation, Methodology, Project Administration, Resources, Supervision, Writing – Review & Editing; **Fall C:** Conceptualization, Formal Analysis, Methodology, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

How to cite this article: Krishnaveni G, Kumaran K, Krishna M *et al.* **Life course programming of stress responses in adolescents and young adults in India: Protocol of the Stress Responses in Adolescence and Vulnerability to Adult Non-communicable disease (SRAVANA) Study [version 1; referees: 2 approved]** Wellcome Open Research 2018, 3:56 (doi: [10.12688/wellcomeopenres.14583.1](https://doi.org/10.12688/wellcomeopenres.14583.1))

Copyright: © 2018 Krishnaveni G *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution Licence](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Grant information: The current study is supported by the Wellcome Trust through a Wellcome Trust-DBT India Alliance personal fellowship [IA/CPHS/16/1/502655 to GVK].

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

First published: 10 May 2018, 3:56 (doi: [10.12688/wellcomeopenres.14583.1](https://doi.org/10.12688/wellcomeopenres.14583.1))

Introduction

Psychological stress is an unpleasant subjective experience, caused by an awareness of situational demands exceeding an individual's adaptive capacity¹. It has been identified as an important lifestyle risk factor for adult non-communicable disease (NCD)². During stress, the body triggers physiological changes through the actions of the hypothalamic-pituitary-adrenal (HPA) axis (and its end product cortisol) and the autonomic nervous system, to maintain homeostasis³. It is suggested that repeated psychological stress induces dysregulated HPA axis activity and autonomic cardiovascular control, reflected in either exaggerated or blunted responses of these systems to acute stress. Over time, these abnormalities can provoke the cardiometabolic, neuroendocrine and immunological disorders responsible for NCDs. Studies have shown increased cardiovascular and mental health disorders in relation to abnormal stress responses^{3,4}.

The "Developmental Origins of Health and Disease (DOHaD) hypothesis" proposes that variations in nutrition during foetal development influence individual's susceptibility to adult NCDs⁵. Both intra-uterine growth restriction (resulting in low birthweight), and accelerated growth caused by maternal gestational diabetes (GDM), increase the risk of NCDs⁶⁻⁸. This phenomenon is thought to reflect permanent effects ('programming') of unbalanced foetal nutrition on physiological systems⁹. Recent evidence suggests that altered neuroendocrine responses to stress may be a pathway linking early life nutrition to the development of NCDs in later life¹⁰. Maternal nutrients, particularly those involved in the one-carbon metabolism are vital for many neuro-developmental processes, and their deficiencies may alter neuroendocrine structure and function^{11,12}, impair HPA axis feedback systems^{13,14}, and influence stress reactivity. A few studies in adults and children have found associations between lower birth weight (as a proxy for maternal undernutrition) and higher cortisol and cardio-sympathetic responses to stress¹⁵⁻¹⁸. However, relationships of maternal nutritional status and maternal hyperglycemia *per se* with offspring stress responses are not known.

There is an escalating prevalence of NCDs in India; ~80 million people in the country are expected to develop type 2 diabetes by 2030¹⁹, and the estimated prevalence of GDM is ~15% currently²⁰. With recent socio-economic transition, resulting in energy dense diets and physical inactivity, these conditions are increasingly affecting younger age groups²¹. Recent reports suggest that stress levels are also increasing steadily among youth, resulting in a growing prevalence of anxiety disorders, depression and suicide^{22,23}. In India, widespread maternal undernutrition, with specific micronutrient deficiencies, as well as high rates of maternal GDM, may confer long-term cardiometabolic and neuro-cognitive adverse effects on the offspring²⁴. However, there is limited evidence for the early life programming of stress responses as a risk factor for NCDs in India. Our own studies at Holdsworth Memorial Hospital (HMH), Mysore showed that increased HPA axis activity, measured using fasting plasma cortisol concentration, was associated with higher cardiometabolic risk markers in adults and children^{25,26}. Subsequently we observed that the adolescent offspring of GDM

mothers (OGDM) exhibited exaggerated cardiovascular responses to acute stress²⁷.

Adolescence, the developmental period from the onset of puberty until the attainment of social independence, is associated with intense negative emotions, stress perception and reactivity²⁸. These attributes are thought to result from extensive remodelling of cortical brain structure and changing efficiency of cognitive and emotional control occurring during this period²⁸. Stress-related behavioural and physiological changes during adolescence and early adulthood may initiate risk trajectories for NCDs, and may also have adverse implications for future offspring through programming effects, thus perpetuating the risk cycle. Various biological, environmental and social factors may determine variations in stress-reactivity²⁹. Understanding these factors, critical life course periods during which they influence changes in stress responses, and mechanisms underlying these changes may lead to interventions to mitigate their adverse effects on health. The current study proposes to build on the previous evidence to understand the life course factors that predict adolescent stress responses.

Aims and objectives

The overarching aim of the study is to understand modifiable factors that might reduce stress responses and guide the development of an integrated intervention to reduce stress reactivity, and therefore future risk of NCDs in adolescents and young adults in India.

The key objectives are to:

1. Test the persistence of the association of birth weight, and maternal GDM and micronutrient status with cortisol and autonomic cardiovascular responses to acute stress in adolescents
2. Examine the association of stress responses with their psychological and cardiovascular risk markers
3. Test the role of prenatal nutritional intervention in reducing physiological stress responses in the adolescent offspring
4. Test whether nutritional supplementation during adolescence/young adulthood is associated with improved stress responses in young adults exposed to maternal nutritional deficiency
5. Investigate specific epigenetic (methylation) changes, and neurocognitive characteristics that relate to stress responses, and
6. Develop a socio-cognitive intervention model to be used in a future study to reduce stress responses in adolescents and young adults

Protocol

Study sample

The "Stress Responses in Adolescence and Vulnerability to Adult Non-communicable disease (SRAVANA) study" will

adopt a multi-centric approach, leveraging on the unique characteristics of three Indian birth cohorts to achieve specific, but complementary objectives.

The Mysore Parthenon Cohort was established at HMH in 1997–1998 to investigate the long-term effects of maternal GDM and nutritional status on offspring NCD risk³⁰. The cohort comprised 663 normal offspring of 830 women whose GDM status had been assessed; ≈6% of them were OGDM³¹. Offspring follow-up continued every 6–12 months after birth for anthropometry, body composition, pubertal staging, cardiometabolic and cognitive assessments. The study showed high rates of low maternal B12 and vitamin D status, and GDM, suggesting that undernutrition and overnutrition co-exist in this transitioning population^{31–33}. Offspring exposed to maternal GDM had greater adiposity, insulin resistance and systolic BP, and higher cardiovascular stress responses during adolescence than those of mothers with normal glucose tolerance^{27,34}. Higher risk markers were also observed in relation to lower offspring birthweight and maternal nutritional imbalances (vitamin D, folate and homocysteine)^{32,33,35}.

The cohort participants are currently aged 20, and ≈85% of the original cohort are still being followed up (N=550). For the present study, we will measure NCD risk markers, chronic stress in the whole cohort, and acute stress responses in a subsample (N~250) who participated in earlier studies of stress reactivity at the age of 13.5 years²⁷. See below for a detailed description of these assessments.

The Mumbai Maternal Nutrition Project (MMNP or “Project SARAS”; ISRCTN 62811278) was a community-based randomised trial of nutritional supplementation for women living in urban slums, pre-conceptionally and throughout pregnancy³⁶. The primary outcome was birth weight, but the study was designed to have enough power to study long-term effects on body composition, cardiometabolic risk markers and cognitive function in the children. The intervention was a daily snack (such as samosas and fritters) made from micronutrient-rich foods (milk, green leafy vegetables and fruit). Women in the control group received a similar snack made from foods of lower micronutrient content such as potato and onion. A total of 6513 women were randomised, 2291 women became pregnant, and 1962 delivered live singleton newborns during 2006–2012. The study showed that supplementation increased offspring birthweight among women of normal or high body mass index³⁶. The incidence of GDM was halved in the intervention mothers compared to controls³⁷.

SARAS KIDS is the ongoing follow-up study of the offspring born to these women. In the SRAVANA study, we will randomly select 150 offspring each from the maternal intervention and control groups and all available OGDM during early adolescence (~10–12y). We will measure NCD risk markers and stress responses. We will test the hypothesis that ‘prenatal nutritional supplementation normalises cortisol and cardiovascular stress responses, especially in OGDM’.

The Pune Maternal Nutrition Study (PMNS) was a community based observational study set up in rural Pune in 1993³⁸. Non-pregnant, married women were identified, and followed up until they became pregnant (N=797). Pregnant women were assessed for diet, micronutrient status and foetal growth. The 753 offspring formed the PMNS cohort, and had detailed anthropometry at birth and at 6-monthly intervals. Maternal undernutrition (low BMI and vitamin B12 status) was widespread in this population. Low B12 status in the mothers was associated with higher insulin resistance in the children.

The PMNS offspring are now part of a community-based intervention study (ISRCTN 32921044), with the hypothesis that physiological doses of micronutrient supplementation during adolescence/young adulthood will improve the birthweight, B12 status, and newborn and childhood body composition in the next generation³⁹. The cohort members (N=557) were enrolled when they were ~16–18y old, and were randomised to receive either i) B12 2µg, ii) B12 2µg plus multiple micronutrients plus milk protein (MMN), or iii) placebo daily. The intervention is ongoing. We will recruit ~50 participants each from the intervention and placebo groups at 22–23y of age. We will measure NCD risk markers and stress responses. We will test the hypothesis that current micronutrient supplementation is associated with improved cortisol and cardiovascular responses to stress in young adults exposed to maternal nutritional deficiencies.

Assessments (Table 1)

The Trier Social Stress Test (TSST) is a valid method of ethically inducing acute psychological stress, particularly in adolescents⁴⁰. The test is based on ‘uncontrollability, unpredictability and social evaluative threat (perception of negative assessment of the self by others)’ for inducing stress. We have previously adapted the TSST modified for children (TSST-C) for use in the Parthenon adolescents, and have shown that it is valid for Indian conditions⁴¹. The TSST involves participants performing 5-minutes each of public speaking and mental arithmetic tasks in front of unfamiliar evaluators (stressor). Pre-test and post-test saliva samples will be collected for cortisol assessment. Systolic and diastolic blood pressure (BP), cardiac output, stroke volume, heart rate and total peripheral resistance, as measures of autonomic nervous system, will be measured continuously before and during the TSST using a portable hemodynamic monitor (Nexfin, BMEye). Acute stress response will be estimated as the change in post-stress cortisol and cardiovascular parameters from pre-test values (baseline). These measures form the primary outcome of the study.

Secondary outcomes

Psychological assessments: We will administer standard questionnaires, valid for Indian adults/adolescents for psychological measures of chronic stress and behavioural assessment.

1) **Perceived stress scale**¹, a commonly used 14-item scale to measure the degree to which situations in one’s life during the past month are appraised as stressful, and gives a measure of chronic stress.

Table 1. List of assessments and outcomes of the proposed study.

Parameters tested	Method	Outcome	Mysore	Mumbai	Pune
Stress response	Trier Social Stress Test	Cortisol concentration; continuous blood pressure, heart rate, cardiac output, stroke volume and total peripheral resistance Cortisol and cardiovascular stress response	✓	✓	✓
	Cardiometabolic Parameters	Anthropometry	Weight, height; mid-upper arm, head, waist and hip circumference; subscapular and triceps skinfold	✓	✓
	Body composition (bioimpedance)	Body fat and lean mass	✓	✓	✓
	Hand grip strength (dynamometer)	Muscle strength	✓	✓	✓
	Blood pressure	Resting systolic and diastolic blood pressure; pulse rate	✓	✓	✓
	Oral glucose tolerance test	Diabetes/glucose intolerance	✓	☑	☑
	Fasting blood samples	Fasting insulin for insulin resistance (HOMA-IR); total cholesterol, triglycerides, HDL-cholesterol	✓	☑	☑
Psychological Parameters	Wechsler's Adult Intelligence Scale-IV	Cognitive function	✓	☑*	☑
	PHQ-9	Depression	✓	-	✓
	MINI		✓	-	☑
Behavioural Parameters	Perceived Stress Scale	Chronic stress	✓	-	✓
	Stressful Life Events Scale		✓	-	✓
	Strengths and Difficulties Questionnaire	Internalising and externalising behaviour	✓	✓	✓
Lifestyle Indicators	Food Frequency Questionnaire	Dietary intake pattern	✓	✓	☑
	24-hour Food Recall	Nutrient intake quantity	✓	✓	✓
	International Physical Activity Questionnaire	Physical activity pattern, intensity and magnitude	✓	✓	✓
	Smoking and Alcohol intake	Addictive behaviour, substance Abuse	✓	-	✓
Mechanistic Assays	Epigenetic Assay	DNA methylation: <i>NR3C1</i> , <i>11β-HSD2</i>	✓	✓	-
	Magnetic Resonance Imaging for brain	Regional cerebral volumes	✓	✓	☑
Socio-demographic Indicators	Standard of Living Index	Socio-economic Status	✓	✓	✓

✓ : SRAVANA study assessments; ☑: Assessed as part of ongoing/recently completed follow-up.

HOMA-IR: Homeostasis Model Assessment for Insulin Resistance; MINI: Mini International Neuropsychiatric Interview; PHQ-9: Patient Health Questionnaire-9

*Kauffman's Assessment Battery and additional tests

2) Stressful Life Events Scale is a 40-item scale to assess chronic stress caused by life events among participants in the past one year⁴².

3) [Strengths and Difficulties Questionnaire](#), a behavioural screening questionnaire for children and adolescents.

4) Depression and other psychological health issues will be identified using the Patient Health Questionnaire-9 (PHQ-9) and MINI (Mini International Neuropsychiatric Interview)^{43,44}. The [PHQ-9](#) is a screening questionnaire for depression, and gives a score that measures severity of depression, while MINI is a diagnostic interview schedule for diagnosing depression.

5) Cognitive function will be assessed in Mysore, and Pune (as part of ongoing follow-up), using Wechsler Adult Intelligence Scale-Fourth Edition - India (WAIS-IV^{India}) a widely used standard IQ test designed to measure intelligence and cognitive ability in adults and older adolescents⁴⁵. In Mumbai, cognitive function has been recently assessed for the SARAS KIDS children using three core tests from the Kauffman's Assessment Battery and some additional tests⁴⁶⁻⁴⁸.

Cardiometabolic assessments: We will measure weight (digital scale), height (adult stadiometer), head, mid-upper arm, waist and hip circumferences (anthropometric tape), subscapular and triceps skinfold thickness (Harpenden callipers), hand-grip strength (Jamar dynamometer), and body composition (bioimpedance). Waist-to-hip ratio will be calculated for central adiposity. Resting blood pressure will be measured on the non-dominant arm using an automatic device after the participants have been seated relaxed for five minutes.

An oral glucose tolerance test ([WHO protocol](#)) will be administered in Mysore after an overnight fasting for plasma glucose, insulin and lipid concentrations. In Mumbai and Pune OGTTs using the WHO protocol have been carried out recently as part of ongoing follow-up.

Lifestyle indicators: Dietary intake will be assessed using a purpose-designed food frequency questionnaire (FFQ)⁴⁹ and a 24-hour recall questionnaire ([Supplementary File 1](#) and [Supplementary File 2](#)). Physical activity will be measured using the [International Physical Activity Questionnaire – Short version](#). Information on smoking and alcohol intake will be documented.

Laboratory assays: All assays will be carried out using standard protocols. Salivary samples for cortisol assay will be collected using synthetic swabs (Salivette[®]Cortisol, Sarstedt, Germany). Samples will be stored at -20° till analysis.

A part of the whole blood sample at all time points (EDTA vacutainer) will be transferred to 2 ml micro centrifuge tubes for a hemogram. The remaining blood will be centrifuged (2500 g × 15 min) within 30-minutes of collection, and the separated plasma stored at -80°C until further analysis. Packed cells will be stored for DNA.

Biochemistry analyses will be undertaken for various measurements:

- Hemogram: Cell Counter hematology analyser on the day of collection.
- Plasma glucose and lipids: Automated biochemistry analyser (Hitachi 902, Roche Diagnostics, Germany) using standard enzymatic kits.
- Insulin: ELISA kit (Mercodia AB, Uppsala, Sweden).
- Cortisol: ELISA kit (Alpco Diagnostics, Salem, NH)

Epigenetic assays: In the Parthenon and SARAS KIDS cohorts, we will use a candidate gene approach to assess epigenetic characteristics (DNA methylation) at the gluco-corticoid receptor (*NR3C1*) and *11β-HSD2* gene loci in relation to maternal factors and offspring stress responses, and explore the effect of maternal nutritional supplementation on these associations. Studies in humans and animals have shown that methylation levels in the promoter regions of these genes may be influenced by maternal GDM and/or undernutrition and have adverse implications for offspring stress responses⁵⁰⁻⁵².

Neuroimaging: We will collect the following Magnetic Resonance Imaging (MRI) biomarkers in subsamples of participants in Mysore and Mumbai (N=100 each): 1) whole brain, hippocampal and amygdale volumes and cortical thickness and surface area, 2) cortical structural complexity measured using fractal dimension from volumetric images, 3) white matter integrity quantified by fractional anisotropy, radial diffusivity and mean diffusivity from diffusion tensor imaging and 4) functional connectivity of networks quantified from resting state fMRI.

T1-weighted volumetric sequences will be acquired for the assessment of brain regional volumes, cortical thickness, cortical surface area, gyrification index, fractal dimension, voxel base morphometry analysis.

Neuroimaging data will be investigated with the aim of identifying significant correlations of cortisol and cardiovascular stress responses with structural and functional brain networks.

Other: Information will be collected on medical and drug history, recent stressful events, education, occupation and socio-economic status, and parental diabetes and cardiovascular disease.

Intervention development

We propose to develop a socio-cognitive intervention method for stress management. We intend to use a person-based approach to develop a culturally-appropriate intervention design within the overarching framework of the MRC guidance on complex intervention development^{53,54}. 'A person-based approach to intervention development' aims to understand, and accommodate, the perspectives and psychosocial context of the intervention users in a systematic manner, through qualitative research, thus strengthening evidence-based interventions.

We will carry out three distinct pieces of work that will run parallel with the other components as below.

i) Systematic review: will be carried out to understand what works in reducing perceived stress in adolescents and young adults. We will follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement⁵⁵.

Eligibility criteria, search strategy and identification of literature: We will consider any non-therapeutic psychological interventions as our exposure of interest. Outcome will be 'perceived stress' in adolescents and young adults. We will include all intervention studies published in English until the start of the review process. We will search Medline/PubMed and the Cochrane Library databases using the medical subject headings (MeSH) terms and text word terms and reference lists of retrieved literature.

Data extraction and quality assessment: Data extraction and quality assessment of each article will be carried out independently by GVK and another assessor. Discrepancies between assessors will be resolved by discussion with a third independent assessor.

Evidence synthesised from this will inform the content and delivery form of the intervention.

ii) Formative qualitative research: We will conduct qualitative interviews with ~25 adolescents and young adults in Mysore, soliciting their views on stresses and stressors that affect their daily life, and the ways to tackle them. Specific suggestions from the participants for activities and techniques to be used in the intervention design will be discussed (eg. digital sources of support). Analysis of the pooled data from this work will identify 'guiding principles' on the key issues that must be addressed by the intervention, and specific ideas about the most effective types of support that could be offered.

iii) Development of intervention format and content: This work will produce 1) a co-created set of intervention materials that are ready to be tested, and 2) a decision on the intervention delivery format. To understand intervention users' perspective of what might or might not be helpful in reducing stress, we will conduct 'focus group discussions' with 8–10 adolescents/young adults to explore intervention formats (eg. smartphone-based) and content (eg. applied techniques) based on existing resources as means of support for managing stressful situations. Participants will also be invited to discuss their experiences of using any other resources.

A set of intervention materials will be produced based on the results of the above three steps. '**Think aloud**' interviews will be conducted where participants will be asked to verbalise their thoughts as they go through the intervention materials, which will identify features and technologies that they particularly enjoy and think they will use, and those that would irritate them⁵⁶. Materials will be adapted iteratively between interviews. In a longitudinal study, 5–10 young adults will be asked to use the

intervention materials for several weeks before being interviewed about their experiences.

The final intervention design will incorporate the full range of techniques known to be effective, and identified by young adults as being acceptable, in reducing and helping to manage stress. The intervention module will be pilot tested in a future study.

Data analysis

We will summarise repeated measures of cortisol and cardiovascular response to stress in a time weighted average (TWA). Linear and logistic regression models will be used to examine associations of stress responses (TWA) with risk outcomes (eg. blood pressure, depression). These models will be adjusted for age, sex, socio-economic status, and relevant maternal and offspring co-variates.

We will use linear mixed-model regression analysis to examine associations of maternal exposures with repeated measures of cortisol and cardiovascular parameters to account for within-group correlations.

We will use the conditional analysis method⁵⁷ to examine the change in stress responses from early adolescence to adulthood in Mysore, to explore tracking.

Metanalysis: We will also do metanalyses of data from Mysore, Mumbai and Pune components of the study on the association of stress responses with 1) maternal nutritional status and 2) offspring NCD risk markers.

Qualitative interviews: Transcripts from qualitative interviews and conversations will be subject to thematic analysis, using a standard thematic analysis methodology involving constant comparative methods.

Sample size and power calculation

Mysore: The sample size of the Mysore study component is based on the previous stress-responses study carried out during 2011–2013 among the Parthenon cohort participants⁴¹. The previous sample size was calculated based on the only available study in children on the association between birth weight and stress response¹⁵. We estimated that our sample will have 80% power to detect an effect size of 2.9 nmol cortisol increase per kg increase in birth weight at 5% significance level. In the current study, we aim to follow-up all the children who had participated in the above study. This sample size will have 80% power at the 5% significance level to detect an association of 0.18 SD of a continuous outcome (eg. TWA salivary cortisol) per SD of an exposure (eg. birth weight).

Mumbai: The sample size calculation for the Mumbai component of the study is based on the Mysore sample size. The estimated sample size will be able to detect an association of 0.16 SD of a continuous outcome per SD of a continuous exposure, and will detect a difference in outcome of 0.23 SD between the intervention and the control arms.

Pune: As there are no earlier studies on the effect of current nutritional intervention on stress responses, we based the sample size for this study on the findings from comparison of effect size between the offspring of B12 deficient and normal B12 status mother. The estimated sample size will detect an association of 0.28 SD of a continuous outcome per SD of an exposure. The study will detect a difference of 0.56 SD in the outcome between the control and intervention group.

Ethics and dissemination

We follow Indian Council of Medical research (ICMR) guidelines for the ethical conduct of biomedical research. We have already received approval for the entire study from the ethics review committee of CSI Holdsworth Memorial Hospital, the host institution. Ethical permission will be sought additionally from the institutional ethical review committees in Mumbai and Pune prior to the start of these study components.

The participants will be given written information and the study is explained verbally, either telephonically or by home visit by research assistants/health workers. The participants will be encouraged to ask questions before obtaining consent. Written informed consent will be obtained for adult participants, and from parents in case of children <18 years (Mumbai) before commencing data collection. Verbal assent will be obtained from the children. Consent for genomic samples will be taken specifically.

The data generated from this study will be shared with the scientific community at local, national and international conferences, and published in open access peer review journals. The major findings will be shared with the study participants and their families in lay terms through public engagement initiatives.

Study status

The follow-up of the Mysore Parthenon cohort participants is currently underway. By the end of April 2018, 82 participants have completed the TSSTs and 309 participants have undergone OGTT and assessments for other secondary outcomes except cognitive function. Qualitative interviews have been carried out among 5 adolescents/young adults.

Conclusion

Altered neuro-endocrine reactivity to stressful situations is a risk factor for cardiovascular and mental health disorders in humans. Previous studies in India and other parts of the world have shown consistent associations between impaired early life nutrition and increased risk of these disorders. There is limited evidence for a role of early nutritional programming of stress responses, though this is considered one of the pathways for increased adult disease risk. Micronutrient deficiencies, especially of one-carbon nutrients, are associated with psychological

manifestations of stress in humans⁵⁸. However, there are no studies examining the effect of nutritional supplementation on physiological stress responses in young individuals. Our study attempts to fill gaps in this area of research and explore in detail behavioural, environmental, epigenetic and neurological aspects linking early nutritional exposure to variations in stress reactivity.

To our knowledge, this is the first study to explore the role of life course nutrition on adolescent stress responses in humans. The study will generate robust evidence for the role of intra-uterine nutritional imbalances in the development of NCDs through programming of abnormal stress responses. Capitalising on the existing intervention designs in two well-known Indian birth cohorts, it will determine whether 1) prenatal nutritional supplementation and 2) current nutritional supplementation mitigates abnormal stress responses in vulnerable adolescents. The secondary analysis of these data, along with the existing past data, will guide the development of multi-faceted interventions for reducing NCD risks through a life course approach.

Declarations

Ethics approval

The study has been approved by the Ethics Committee of the CSI Holdsworth Memorial Hospital, Mysore (June 2017; no. CSIHMH/ERU2017/1). The committee membership follows Indian Council of Medical Research guidelines and is composed of a total of nine members (2 clinicians, 3 scientists, 1 legal expert, 1 social scientist/spiritual leader, 1 academician and 1 lay person).

Data availability

All data underlying the results are available as part of the article and no additional source data are required

Competing interests

No competing interests were disclosed.

Grant information

The current study is supported by the Wellcome Trust through a Wellcome Trust-DBT India Alliance personal fellowship [IA/CPHS/16/1/502655 to GVK].

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

Our sincere thanks to Kiran KN, Saroja A, Somashekara R, Santosh N, Ramya MC, Praveen Kumar, Chaitra Urs, Divyashree K, Asha Dhube and Shama Joseph who are part of the research team at CSI Holdsworth Memorial Hospital, Mysore. We also thank SNEHA-India for its support.

Supplementary material

Supplementary File 1: Food frequency questionnaire.

[Click here to access the data.](#)

Supplementary File 2: Food recall questionnaire.

[Click here to access the data.](#)

References

- Cohen S, Janicki-Deverts D, Miller GE: **Psychological stress and disease.** *JAMA.* 2007; **298**(14): 1685–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
- McEwen BS: **Protective and damaging effects of stress mediators: central role of the brain.** *Dialogues Clin Neurosci.* 2006; **8**(4): 367–81.
[PubMed Abstract](#) | [Free Full Text](#)
- Chida Y, Steptoe A: **Greater cardiovascular responses to laboratory mental stress are associated with poor subsequent cardiovascular risk status: a meta-analysis of prospective evidence.** *Hypertension.* 2010; **55**(4): 1026–32.
[PubMed Abstract](#) | [Publisher Full Text](#)
- deRooij SR: **Blunted cardiovascular and cortisol reactivity to acute psychological stress: a summary of results from the Dutch Famine Birth Cohort Study.** *Int J Psychophysiol.* 2013; **90**(1): 21–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hanson M, Gluckman P: **Developmental origins of noncommunicable disease: population and public health implications.** *Am J Clin Nutr.* 2011; **94**(6 Suppl):1754S–1758S.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Barker DJP: **Mothers, babies and health in later life.** Ed. Churchill Livingstone. 2nd edition. 1998.
[Reference Source](#)
- Schlutz W, Phillips DI: **Fetal origins of mental health: evidence and mechanisms.** *Brain Behav Immun.* 2009; **23**(7): 905–16.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Dabelea D: **The predisposition to obesity and diabetes in offspring of diabetic mothers.** *Diabetes Care.* 2007; **30** Suppl 2: S169–74.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hanson MA, Gluckman PD: **Early developmental conditioning of later health and disease: physiology or pathophysiology?** *Physiol Rev.* 2014; **94**(4): 1027–76.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Phillips DI, Barker DJ, Fall CH, *et al.*: **Elevated plasma cortisol concentrations: a link between low birth weight and the insulin resistance syndrome?** *J Clin Endocrinol Metab.* 1998; **83**(3): 757–60.
[PubMed Abstract](#) | [Publisher Full Text](#)
- McGarel C, Pentieva K, Strain JJ, *et al.*: **Emerging roles for folate and related B-vitamins in brain health across the lifecycle.** *Proc Nutr Soc.* 2015; **74**(1): 46–55.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Roy S, Kale A, Dangat K, *et al.*: **Maternal micronutrients (folic acid and vitamin B₁₂) and omega 3 fatty acids: implications for neurodevelopmental risk in the rat offspring.** *Brain Dev.* 2012; **34**(1): 64–71.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kajantie E: **Fetal origins of stress-related adult disease.** *Ann N Y Acad Sci.* 2006; **1083**(1): 11–27.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Phillips DI, Jones A, Goulden PA: **Birth weight, stress, and the metabolic syndrome in adult life.** *Ann N Y Acad Sci.* 2006; **1083**: 28–36.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Jones A, Godfrey KM, Wood P, *et al.*: **Fetal growth and the adrenocortical response to psychological stress.** *J Clin Endocrinol Metab.* 2006; **91**(5): 1868–71.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Jones A, Beda A, Osmond C, *et al.*: **Sex-specific programming of cardiovascular physiology in children.** *Eur Heart J.* 2008; **29**(17): 2164–70.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Feldt K, Räikkönen K, Pyhälä R, *et al.*: **Body size at birth and cardiovascular response to and recovery from mental stress in children.** *J Hum Hypertens.* 2011; **25**(4): 231–40.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Wüst S, Entringer S, Federenko IS, *et al.*: **Birth weight is associated with salivary cortisol responses to psychosocial stress in adult life.** *Psychoneuroendocrinology.* 2005; **30**(6): 591–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Wild S, Roglic G, Green A, *et al.*: **Global prevalence of diabetes: estimates for the year 2000 and projections for 2030.** *Diabetes Care.* 2004; **27**(5): 1047–53.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Rajput R, Yadav Y, Nanda S, *et al.*: **Prevalence of gestational diabetes mellitus & associated risk factors at a tertiary care hospital in Haryana.** *Indian J Med Res.* 2013; **137**(4): 728–33.
[PubMed Abstract](#) | [Free Full Text](#)
- Ramachandran A, Snehalatha C, Yamuna A, *et al.*: **Insulin resistance and clustering of cardiometabolic risk factors in urban teenagers in southern India.** *Diabetes Care.* 2007; **30**(7): 1828–33.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Aggarwal S, Berk M: **Evolution of adolescent mental health in a rapidly changing socioeconomic environment: a review of mental health studies in adolescents in India over last 10 years.** *Asian J Psychiatr.* 2015; **13**: 3–12.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Patel V, Ramasundarahettige C, Vijayakumar L, *et al.*: **Suicide mortality in India: a nationally representative survey.** *Lancet.* 2012; **379**(9834): 2343–51.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Yajnik CS, Deshmukh US: **Maternal nutrition, intrauterine programming and consequential risks in the offspring.** *Rev Endocr Metab Disord.* 2008; **9**(3): 203–11.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ward AM, Fall CH, Stein CE, *et al.*: **Cortisol and the metabolic syndrome in South Asians.** *Clin Endocrinol (Oxf).* 2003; **58**(4): 500–5.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Krishnaveni GV, Veena SR, Dhube A, *et al.*: **Size at birth, morning cortisol and cardiometabolic risk markers in healthy Indian children.** *Clin Endocrinol (Oxf).* 2014; **80**(1): 73–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Krishnaveni GV, Veena SR, Jones A, *et al.*: **Exposure to maternal gestational diabetes is associated with higher cardiovascular responses to stress in adolescent Indians.** *J Clin Endocrinol Metab.* 2015; **100**(3): 986–93.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Casey BJ, Jones RM, Levita L, *et al.*: **The storm and stress of adolescence: insights from human imaging and mouse genetics.** *Dev Psychobiol.* 2010; **52**(3): 225–35.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kudielka BM, Hellhammer DH, Wüst S: **Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge.** *Psychoneuroendocrinology.* 2009; **34**(1): 2–18.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Krishnaveni GV, Veena SR, Hill JC, *et al.*: **Cohort profile: Mysore parthenon birth cohort.** *Int J Epidemiol.* 2015; **44**(1): 28–36.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hill JC, Krishnaveni GV, Annamma I, *et al.*: **Glucose tolerance in pregnancy in South India: relationships to neonatal anthropometry.** *Acta Obstet Gynecol Scand.* 2005; **84**(2): 159–165.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Krishnaveni GV, Veena SR, Winder NR, *et al.*: **Maternal vitamin D status during pregnancy and body composition and cardiovascular risk markers in Indian children: the Mysore Parthenon study.** *Am J Clin Nutr.* 2011; **93**(3): 628–35.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Krishnaveni GV, Veena SR, Karat SC, *et al.*: **Association between maternal folate concentrations during pregnancy and insulin resistance in Indian children.** *Diabetologia.* 2014; **57**(1): 110–21.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Krishnaveni GV, Veena SR, Hill JC, *et al.*: **Intrauterine exposure to maternal diabetes is associated with higher adiposity and insulin resistance and clustering of cardiovascular risk markers in Indian children.** *Diabetes Care.* 2010; **33**(2): 402–4.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Krishnaveni GV, Veena SR, Wills AK, *et al.*: **Adiposity, insulin resistance and**

- cardiovascular risk factors in 9-10-year-old Indian children: relationships with birth size and postnatal growth. *J Dev Orig Health Dis*. 2010; 1(6): 403–11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
36. Potdar RD, Sahariah SA, Gandhi M, *et al.*: Improving women's diet quality preconceptionally and during gestation: effects on birth weight and prevalence of low birth weight—a randomized controlled efficacy trial in India (Mumbai Maternal Nutrition Project). *Am J Clin Nutr*. 2014; 100(5): 1257–68.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
37. Sahariah SA, Potdar RD, Gandhi M, *et al.*: A Daily Snack Containing Leafy Green Vegetables, Fruit, and Milk before and during Pregnancy Prevents Gestational Diabetes in a Randomized, Controlled Trial in Mumbai, India. *J Nutr*. 2016; 146(7): 1453S–60S.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
38. Yajnik CS, Deshpande SS, Jackson AA, *et al.*: Vitamin B₁₂ and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. *Diabetologia*. 2008; 51(1): 29–38.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
39. Kumaran K, Yajnik P, Lubree H, *et al.*: The Pune Rural Intervention in Young Adolescents (PRIYA) study: design and methods of a randomised controlled trial. *BMC Nutrition*. 2017; 3: 41.
[Publisher Full Text](#)
40. Gunnar MR, Talge NM, Herrera A: Stressor paradigms in developmental studies: what does and does not work to produce mean increases in salivary cortisol. *Psychoneuroendocrinology*. 2009; 34(7): 953–67.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
41. Krishnaveni GV, Veena SR, Jones A, *et al.*: Trier social stress test in Indian adolescents. *Indian Pediatr*. 2014; 51(6): 463–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
42. Aggarwal S, Prabhu CH, Anand LC, *et al.*: Stressful life events among adolescents: The development of a new measure. *Indian J Psychiatry*. 2007; 49(2): 96–102.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
43. Kochhar PH, Rajyadhyaksha SS, Suvarna VR: Translation and validation of brief patient health questionnaire against DSM IV as a tool to diagnose major depressive disorder in Indian patients. *J Postgrad Med*. 2007; 53(2): 102–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
44. Sheehan DV, Lecrubier Y, Sheehan KH, *et al.*: The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry*. 1998; 59(Suppl 20): 22–33; quiz 34–57.
[PubMed Abstract](#)
45. Wechsler D: *Manual for the Wechsler Adult Intelligence Scale Revised*. New York: Psychological Corporation; 1981.
[Reference Source](#)
46. Kaufman AS, Kaufman LN: *Kaufman assessment battery for children*. 2nd ed: Manual. Circle Pines (MN): AGS Publishing; 2004.
[Reference Source](#)
47. Korkman M, Kemp SL, Kirk U: Effects of age on neurocognitive measures of children ages 5 to 12: a cross-sectional study on 800 children from the United States. *Dev Neuropsychol*. 2001; 20(1): 331–54.
[PubMed Abstract](#) | [Publisher Full Text](#)
48. Kohs SC: *Intelligence measurement: a psychological and statistical study based upon the Block-design tests*. New York: Macmillan; 1923.
[Publisher Full Text](#)
49. Kehoe SH, Krishnaveni GV, Veena SR, *et al.*: Diet patterns are associated with demographic factors and nutritional status in South Indian children. *Matern Child Nutr*. 2014; 10(1): 145–58.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
50. Meaney MJ, Szyf M: Environmental programming of stress responses through DNA methylation: life at the interface between a dynamic environment and a fixed genome. *Dialogues Clin Neurosci*. 2005; 7(2): 103–23.
[PubMed Abstract](#) | [Free Full Text](#)
51. El Hajj N, Pliushch G, Schneider E, *et al.*: Metabolic programming of MEST DNA methylation by intrauterine exposure to gestational diabetes mellitus. *Diabetes*. 2013; 62(4): 1320–28.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
52. van der Knaap LJ, Oldehinkel AJ, Verhulst FC, *et al.*: Glucocorticoid receptor gene methylation and HPA-axis regulation in adolescents. The TRAILS study. *Psychoneuroendocrinology*. 2015; 58: 46–50.
[PubMed Abstract](#) | [Publisher Full Text](#)
53. Yardley L, Morrison L, Bradbury K, *et al.*: The person-based approach to intervention development: application to digital health-related behavior change interventions. *J Med Internet Res*. 2015; 17(1): e30.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
54. Craig P, Dieppe P, Macintyre S, *et al.*: Developing and evaluating complex interventions: the new Medical Research Council guidance. *BMJ*. 2008; 337: a1655.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
55. Moher D, Liberati A, Tetzlaff J, *et al.*: Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med*. 2009; 151(4): 264–9, W64.
[PubMed Abstract](#) | [Publisher Full Text](#)
56. Collins D: *Cognitive Interviewing Practice*. London, SAGE publications. 2014.
[Publisher Full Text](#)
57. Tu YK, Tilling K, Sterne JA, *et al.*: A critical evaluation of statistical approaches to examining the role of growth trajectories in the developmental origins of health and disease. *Int J Epidemiol*. 2013; 42(5): 1327–39.
[PubMed Abstract](#) | [Publisher Full Text](#)
58. Folstein M, Liu T, Peter I, *et al.*: The homocysteine hypothesis of depression. *Am J Psychiatry*. 2007; 164(6): 861–7.
[PubMed Abstract](#) | [Publisher Full Text](#)

Open Peer Review

Current Referee Status:  

Version 1

Referee Report 06 July 2018

doi:[10.21956/wellcomeopenres.15878.r33412](https://doi.org/10.21956/wellcomeopenres.15878.r33412)



Giridhara R. Babu 

Indian Institute of Public Health (IIPH) Bangalore, Public Health Foundation of India, Bangalore, Karnataka, India

The protocol of the “Stress Responses in Adolescence and Vulnerability to Adult Non-communicable disease (SRAVANA) study” is written well.

The objectives are outlined well. The authors propose using the validated Trier Social Stressor scale apart from several psychological and biological measures.

My comments are noted below:-

HPA-axis activation and inhibition involves several psychosocial stressors and buffers¹⁻². While sufficient details are provided on how the investigators measure stressors including cortisol levels, it would be useful to know how buffer mechanisms are measured³⁻⁴. For example, evidence suggests that social support⁵⁻⁶, secure attachment⁵ and social identity⁷ buffers stress response including and cortisol levels⁸.

The investigators mention that they propose to develop a socio-cognitive intervention method for stress management. It is useful to know whether the data for such an intervention is generated from the proposed study. If so, the assessments for cognitive function are explained as part of the secondary outcomes. However, details on how social environment is measured are missing. The authors may clarify regarding this.

Given that this study is multi-centric and involves three Indian birth cohorts; it will be useful to know how the team is planning to integrate the efforts of the different cohorts. A table mentioning common components in all the three cohorts, and specific examinations within individual cohorts can be useful. Also, it will be useful to know how the variations in population composition, outcomes and initial assessments amongst the three cohorts are accounted for.

Minor:-

Understanding the role of prenatal nutritional intervention in reducing physiological stress responses in the adolescent offspring is a worthy objective. The readers will get benefited if the theory and evidence regarding the mechanisms involved between nutrition and physiological stress are explained. This will also justify the role of testing nutritional supplementation during adolescence/young adulthood is associated with improved stress responses in young adults exposed to maternal nutritional deficiency.

The authors mention that all assays will be carried out using standard protocols. It will be useful to provide brief details about the protocols they will follow. Alternatively, they can upload the links to manuals or recommendations they are following. For example, it is useful to know how many samples of salivary cortisol will be collected? At what time of the day are these collected?

References

1. Selye H: Homeostasis and heterostasis. *Perspect Biol Med*. 1973; **16** (3): 441-5 [PubMed Abstract](#)
2. Jackson JS, Knight KM, Rafferty JA: Race and unhealthy behaviors: chronic stress, the HPA axis, and physical and mental health disparities over the life course. *Am J Public Health*. 2010; **100** (5): 933-9 [PubMed Abstract](#) | [Publisher Full Text](#)
3. Smeets T, Dziobek I, Wolf OT: Social cognition under stress: differential effects of stress-induced cortisol elevations in healthy young men and women. *Horm Behav*. 2009; **55** (4): 507-13 [PubMed Abstract](#) | [Publisher Full Text](#)
4. von Dawans B, Fischbacher U, Kirschbaum C, Fehr E, Heinrichs M: The social dimension of stress reactivity: acute stress increases prosocial behavior in humans. *Psychol Sci*. 2012; **23** (6): 651-60 [PubMed Abstract](#) | [Publisher Full Text](#)
5. Ditzen B, Schmidt S, Strauss B, Nater UM, Ehler U, Heinrichs M: Adult attachment and social support interact to reduce psychological but not cortisol responses to stress. *J Psychosom Res*. 2008; **64** (5): 479-86 [PubMed Abstract](#) | [Publisher Full Text](#)
6. Hostinar CE, Johnson AE, Gunnar MR: Parent support is less effective in buffering cortisol stress reactivity for adolescents compared to children. *Dev Sci*. 2015; **18** (2): 281-97 [PubMed Abstract](#) | [Publisher Full Text](#)
7. Häusser J, Kattenstroth M, van Dick R, Mojzisch A: "We" are not stressed: Social identity in groups buffers neuroendocrine stress reactions. *Journal of Experimental Social Psychology*. 2012; **48** (4): 973-977 [Publisher Full Text](#)
8. Kikusui T, Winslow JT, Mori Y: Social buffering: relief from stress and anxiety. *Philos Trans R Soc Lond B Biol Sci*. 2006; **361** (1476): 2215-28 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Partly

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 19 June 2018

doi:[10.21956/wellcomeopenres.15878.r33254](https://doi.org/10.21956/wellcomeopenres.15878.r33254)

**Candace M. Raio**

Center for Neural Science, New York University, New York, NY, USA

This is a proposal for a study that aims to measure acute stress reactivity in a large sample of adolescents drawn from three birth cohorts in India (Mysore Parthenon cohort; SARAS KIDS prenatal intervention cohort; and PMNS cohort). The authors nicely motivate the study based on the extant literature pointing to maternal nutrition as an important factor that shapes risk for NCDs later in life. One pathway, they convincingly argue, through which this might occur is by programming of stress responses. Therefore, they propose to examine how early life factors confer risk for dysregulation of stress responses that may later give rise to NCDs.

Their key objectives are outlined clearly, and include identifying prenatal factors (e.g. maternal nutrition/metabolic health) that predict stress responses in adolescents, how these response relate to psychological and cardiovascular health in adolescents, how nutritional interventions (both to pregnant mothers and their offspring) relate to improvements in adolescent stress reactivity and, finally, how socio-cognitive intervention models can help reduce stress responses in these adolescents.

The authors propose using the Trier Social Stressor, which is well-validated and reliable, and propose collecting both neuroendocrine (cortisol) and autonomic arousal (cardiovascular) measures to index stress reactivity. A number of other psychological and biological measures will be collected, including subjective stress, cognitive function, blood pressure, etc. Imaging and genetics will be collected on a subset of participants.

The sample sizes are large, well-powered and sample from different age ranges. The authors propose that ~250 20-year old participants from the Parthenon cohort, 300 adolescents (half from mothers who underwent a nutritional intervention and half who were controls) from the MMNP, and a subset of young adults (~150) involved in an ongoing nutritional supplementation program, will undergo the acute stressor. The authors clearly describe their psychological, cardiovascular, metabolic and other behavioral measures.

The data will be useful to acquire a better understanding of how prenatal nutrition shapes trajectories of stress response in offspring and will facilitate our understanding how these responses may confer vulnerability to NCDs.

I would suggest the following:

- (1) That the authors should indicate the age and gender of the evaluators for the TSST, as this can influence stress reactivity in children and adolescents (i.e., are these evaluators 'peers', which we know are highly salient to adolescents, or are they adult observers?).
- (2) Given that uncontrollability and unpredictability are seminal features of the stress response, the authors may want to consider adding a measure of both. For example, the Intolerance of Uncertainty Scale or the updated Locus of Control Inventory (4th ed.) could provide important information regarding what feature of the Trier stressor is driving their individual stress reactivity. These are brief and easy to administer.
- (3) Will the database be made publicly available after the study is complete? Currently, the authors mention their data will be disseminated through publications but it would be good to know if this important

data set will be available to other researchers/collaborators.

(4) I assume both genders will be represented in the data set. The authors should consider documenting female menstrual cycle in the samples of young adults and any other hormonal medication that can affect cortisol responses.

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

Referee Expertise: acute/lifetime stress effects on affective control and decision-making

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.
