Study Protocol: Emotion-Diet Interactions in Pregnancy

NCT04430439

Date of Document: 10/20/2022

C.1 Overview. This research will represent the first investigation of objectively measured diet-stress interactions in human pregnancy, thus providing critical insight to the contribution of psychosocial stress in influencing important gestational metabolic pathways that have implications for maternal and child health and disease risk. The *working hypothesis* is that acute psychosocial stress will exacerbate the postprandial plasma glucose/insulin and free fatty acid (FFA) response to a standardized meal, and that this response will be exaggerated after consumption of a high GI versus a low GI meal. The *overall strategy* is to enroll a cohort of non-diabetic pregnant women with overweight and obesity to test the postprandial metabolic response to a standardized meal of varying GI, with and without psychosocial stress exposure.

C.2 Study Design. This is a two-armed, randomized, cross-over study in which N=80 women will be randomly assigned in a parallel design to either a low or high GI standardized breakfast. Within each arm, N=40 women will attend two test study visits in a randomized, cross-over design, to undergo either the TSST challenge task or a control non-stress task immediately following consumption of their assigned meal type.

C.3 Clinical Research Setting. The study will be performed at the PI's research facility on the UCI campus in Irvine and will utilize additional clinical facilities of the UCI Institute of Clinical and Translational Science (ICTS).

C.4 Study Site and Population. Subjects will be primarily recruited from UCI-affiliated clinics; the UCI Medical Center faculty private practice, and UCI-affiliated family health clinics in surrounding districts within Orange County, CA, which serve women from an urban metropolitan area encompassing a population base of over 2.75 million residents. Recent demographic profiles of this population indicate that 68% of the patients are multiparous; 65% are married, and 58% belong to ethnic minority groups (predominantly Hispanic). Over the past year, the PI has independently enrolled N=33 Hispanic women from this prenatal population.

C.5 Subjects and Recruitment

C.5.1 Selection Criteria: *Inclusion criteria*: *a*) ≤22 week's gestation at recruitment, *b*) >18 years age, *c*) singleton intrauterine pregnancy, *d*) non-smoker, *e*) pre- or early-pregnancy BMI 25.0-39.9 kg/m^2, *f*) non-diabetic. *Exclusion criteria*: *a*) presence of any prior and present obstetric risk conditions such as placental or cord abnormalities, uterine anomalies, congenital malformations, fetal chromosomal abnormalities, infection, diabetes, hypertension, *b*) psychiatric disorders such as clinical depression or PTSD, *c*) any conditions that may dysregulate neuroendocrine, metabolic or cardiovascular function, such as diabetes, hepatic, renal, or autoimmune disorders, *d*) use of lipid-lowering or anti-diabetic medications, *e*) use of corticosteroids in the last month or any prior use of psychotropic medications, *f*) current smoker or use of tobacco products within 1 year prior to pregnancy, *g*) current or history of alcohol abuse or illicit drug use, *h*) pre- or early-pregnancy BMI <25.0 or ≥35.0 kg/m^2.

C.5.2 Participant Recruitment: Pregnant women will be actively recruited from UCI-affiliated prenatal clinics. A partial waiver of HIPAA will be requested to review the appointment lists and screen the electronic medical record for potential eligibility. Potential participants will be explained the study, provided written study information and invited to participate. The recruitment strategy will be supplemented by passive methods involving IRB-approved mass media announcements through UCI resources (flyers, employee-wide emails and online media advertisements). All disseminated study advertisement material will include the PI's name and contact information, enabling interested and potentially eligible pregnant women to enquire for further details and eligibility screening. All recruited study participants will provide informed signed consent at the first study (screening) visit.

C.6 Visit Protocols and Study Measures.

C.6.1 Screening & consent visit: This visit will entail informed consent and confirmation of full study eligibility in order to proceed with subsequent test visits. An interview with a trained research coordinator will determine whether any medical conditions, psychiatric disorders or medication usage may render them ineligible to continue participation. Peripheral blood glucose will be tested by glucometer to detect potential undiagnosed diabetes (random glucose ≥200 mg/dl). Those considered eligible to continue participation will be provided instructions to complete an online survey at home before their first test study visit. This will assess maternal sociodemographic factors, psychosocial states (e.g. chronic stress, anxiety, resiliency) and behaviors (e.g. usual dietary intake, sleep, exercise habits). A standardized healthy evening meal of medium GI will be delivered to participant homes prior to each test study visit to control for recent dietary influences on morning glycemia.

C.6.2 Test visit 1: Participants will attend the research lab facility in the morning following an overnight fast (minimum 8 hours), between 20-22 week's gestation. A research nurse will place an indwelling catheter to the antecubital vein to allow frequent blood sampling throughout the test period with minimal discomfort. After catheter placement, women will rest comfortably for 30 mins. A fasting blood and saliva sample will be collected immediately prior to consumption of the assigned standardized breakfast meal, followed by performance of either the psychosocial stress or non-stress protocol. Postprandial blood and saliva samples will be collected at +30 (pre-task), 45 (post-task), 60, 75, 90, 120 & 150 mins. A heart rate variability monitor will be worn throughout the

duration of each test study visit. Immediately pre- and post-task protocols, participants will complete questionnaires to assess momentary affective state and self-appraisal of upcoming/completed tasks.

C.6.3 Test visit 2: All participants will be allowed a 1-week "wash-out" period before returning to the research lab for test visit 2. Participants will undergo the same protocol as test visit 1 with the same meal assignment, except with the alternative stress/non-stress paradigm as performed on visit 1.

C.6.4 Experimental stress/non-stress paradigm: The TSST is a standardized, validated experimental procedure to effectively induce psychosocial stress among healthy adults, including pregnant women.[59-61] The TSST takes 15 mins to complete and involves a 5 min speech preparation, 5 min speech performance under stern observation of an evaluative committee while being video-taped, and 5min complex verbal arithmetic task. For the control non-stress condition, women will have a relaxed 15 min conversation with a familiar researcher.

C.6.5 Test meal composition: Test meals will be prepared by research staff under direction of the study PI, a registered dietitian. Both types of test meals will have comparable caloric content but will vary in fiber, carbohydrate and fat content to alter the GI value (*Table 1*). The pre-visit standard evening meals will have a medium GI value. All participants will be asked about food allergies or intolerances and the meals will be adapted as appropriate, while maintaining an equivalent energy content and macronutrient distribution.

Table 1: approximate nutritional composition of standard evening and test visit meals

	Standard pre-visit evening meal	**Low GI test meal**	**High GI test meal**
Energy, kcal	600	300	300
% carbohydrate	55	47	76
% protein	15	19	13
% fat	30	33	12
Fiber, g	15	4.5	0.7

C.6.6 Bio-sample processing and analysis: Fasting and postprandial blood samples will be centrifuged at 1500rpm for 15min and the separated plasma aliquoted to microtubes. All plasma and saliva will be stored at -80°C until analysis. Plasma will be assayed for glucose and insulin and saliva for cortisol by ELISA.[62] Plasma FFA will be measured by LC-MS/MS.[63] HOMA-IR will be computed from fasting glucose and insulin values[64] and Adipo-IR from fasting insulin and FFA values.[65] The $AUC_{glu/ins}$ and AUC_{FFA} will be determined for each visit.

C.6.7 Randomization procedures: A biostatistician will generate the randomization sequence for a 1:1 assignment to the meal group arms (*i.e*. high GI or low GI), and for the sequence of stress or non-stress protocols at the test study visits (*i.e.* stress at v1 and non-stress at v2, or non-stress at v1 and stress at v2) within each arm. Randomization sequences will be contained in sealed, opaque envelopes labeled by sequential study IDs and only revealed by research coordinators after completion of a screening visit for a fully eligible participant.