Method and Protocol

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Diet and the gut microbiota-immune axis in the context of perinatal mental health: Protocol for a prospective cohort study

Caroline JK Wallace[1,2] and Marie-Claude Audet[1,2,3]

Abstract

Background: Physiological and psychosocial changes experienced by women during the perinatal period may put them at risk for postpartum mental health disturbances. Accumulating evidence suggests that dietary patterns may influence mental health through the modulation of the gut microbiota and its effects on host immune activity. Thus, targeting the gut microbiota via dietary intake could serve as both a preventative and therapeutic strategy in improving perinatal mental health.

Objectives: Here, we present a protocol for a prospective cohort study that primarily aims to determine if diet quality during pregnancy is protective against postpartum depression severity. Secondary objectives will examine if microbiota- and blood-based inflammatory markers may be associated with the relationship between prenatal diet quality and postpartum depression severity, as well as with associations between additional dietary and mental health outcomes.

Methods and Analysis: Dietary patterns and mental health symptoms will be documented in 100 pregnant women at 4 time points during pregnancy and postpartum. Participants will also provide stool and blood samples at the same time points to determine microbiota composition and predicted function and inflammatory factors, respectively. Stool microbiota will be analyzed using 16S ribosomal RNA gene sequencing and bioinformatics tools (QIIME 2/PICRUSt2). Inflammatory factors will be determined using high-sensitivity antibody-based immunoassays. Statistical analyses will include linear mixed models and hierarchical linear mixed effect models.

Ethics: The study was approved by the Research Ethics Boards of the Royal Ottawa Health Care Group (#2022002) and of the University of Ottawa (#H-06-22-8013). Informed consent will be obtained from all participants before their enrollment.

Discussion: Findings from this study will help develop evidence-based dietary recommendations and potential interventions for women susceptible to or suffering from postpartum mental health issues that are accessible, noninvasive, and have potential to play a role in prevention and treatment.

Keywords

anxiety, depression, diet, gut microbiota-immune axis, perinatal period, postpartum, pregnancy, study protocol, women's mental health

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Introduction

The perinatal period, which typically encompasses pregnancy, parturition, and 1-year postpartum,[1] is marked by physiological and psychosocial changes that may put women at risk for postpartum mental health disturbances, especially depression. In Canada, approximately 18% of women experience symptoms consistent with postpartum depression, with the prevalence rates of postpartum anxiety symptoms reaching 14%.[2] Available first-line pharmacotherapeutics for

[1]School of Nutrition Sciences, Faculty of Health Sciences, University of Ottawa, Ottawa, ON, Canada
[2]University of Ottawa Institute of Mental Health Research at The Royal, Ottawa, ON, Canadaa
[3]Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada

Corresponding author:
Caroline JK Wallace, School of Nutrition Sciences, University of Ottawa; University of Ottawa Institute of Mental Health Research at The Royal.
Email: caroline.wallace@uottawa.ca

depression lack efficacy in about two-thirds of individuals with the illness and are often coupled with intolerable side effects leading to poor treatment compliance.[3] Due to existing, albeit variable, evidence of maternal antidepressant use as a risk factor for adverse outcomes in the offspring,[4,5] physicians are hesitant to recommend antidepressant medication for women who are pregnant or breastfeeding and many women themselves are reluctant to use pharmacological interventions during the perinatal period.[6] Untreated postpartum depression has been associated with poor physical and psychological health, reduced quality of life, and difficulties with relationships in the mother, impaired cognitive and language development, poor quality of sleep, and overall health concerns in the infant, as well as altered mother–child bonding and difficulty breastfeeding.[7] The demonstration that postpartum depression may lead to an environment that is harmful to both the mother and the child highlights the need to develop effective strategies to maintain optimal mental health in the perinatal period and mitigate the burden of the illness.

During the course of a healthy pregnancy, key biological systems undergo substantial changes to support the growth of the fetus and to prepare the woman's body to the metabolic demands of breastfeeding. Along with fluctuations in sex and stress hormones and in metabolic and immune factors,[8] there are indications that the gut microbiota becomes less rich and is being remodeled over the course of pregnancy,[9–12] although stability of the fecal microbial community across gestation has also been reported.[13–15] While a consensus on the fecal bacteriome across pregnancy has yet to be established, the view that its community may be sensitive to pregnancy-specific changes in the host and/or environmental influences, which can greatly vary between individuals, is being increasingly recognized.[9,15] Pregnancy thus represents a unique period during which adaptive changes in stress, inflammatory, and metabolic systems in the mother, as well as fluctuations in the gut microbiota, would otherwise be reflective of pathological states in nonpregnant individuals.[16] It is thus not surprising that additional perturbations to these systems resulting from maternal stress, poor diet quality, and/or a fragile perinatal mental health could create the perfect conditions for postpartum mental outcomes to emerge,[17,18] especially as these perturbations are apparent in nonpregnant women with mental health disorders,[19,20] as well as in pregnant and postpartum individuals experiencing higher levels of stress and more severe depressive symptoms.[21,22]

The gut microbiota is receiving increasing attention as a potential target for preventative and therapeutic interventions in mental illnesses[23,24] and it has been suggested that its community could influence mental health through its actions on the inflammatory signaling routes between the intestinal environment and the brain of the host (the gut microbiota-immune-brain axis).[25,26] Beyond microbiota-targeted dietary supplementation (e.g., probiotics, prebiotics, or synbiotics)[27–29] and single-nutrient interventions (e.g., polyunsaturated fatty acids [PUFAs]),[30] whole-of-diet approaches that comprise plant-based and whole foods are associated with a reduced incidence of depression and anxiety[31–33] and are effective in reducing mental health symptoms in both clinical and healthy populations.[34,35] In particular, diets high in fruits, vegetables, nuts, plant-based proteins, fermented foods, fiber and prebiotics, monounsaturated fatty acids and PUFAs, and polyphenols, and very low in processed and ultra-processed foods generally have positive effects on mental health.[23,31,32,36] Notably, most of these foods or the nutrients they are composed of have been shown to influence inflammatory immune processes within and beyond the gut, likely through their actions on the gut microbiota.[23,36,37]

While dietary components and patterns that may improve mental health in individuals with depression and/or anxiety diagnoses or those with subclinical symptoms have been documented, very few reports have examined these outcomes during pregnancy and postpartum and thus diets promoting mental health during these periods are still poorly understood. Three systematic reviews demonstrated that better diet quality during pregnancy was associated with less prenatal depressive and anxiety symptoms, but inconsistent results were found for postpartum depressive symptoms,[38–40] leading to the conclusion that beyond the lack of consensus on what is an optimal diet for mental health during pregnancy, robust studies examining this line of research are still needed. In addition, to the best of our knowledge, no studies to date have examined the molecular underpinnings that may be driving the relationship between perinatal diet and mental health. In a context where dietary habits may change during pregnancy, potentially owing to variations in hormones, stress, and/or nutritional and energy needs,[41] and where diets with higher inflammatory potential during pregnancy have been associated with lower abundances of taxa with known anti-inflammatory actions,[18] it is possible to think that diet-related changes to the normal trajectory of microbiota fluctuations over the course of pregnancy, with impacts on inflammatory processes, could affect mental health during this period and beyond.

Study aims

By assessing dietary intake and mental health symptoms throughout a critical portion of the perinatal period, the primary objective of this study is to determine whether better diet quality in the prenatal phase is protective against depressive symptoms in the postpartum phase. By determining biomarkers relevant to the gut microbiota-immune axis during the same period, secondary objectives are to examine whether inflammatory-based microbiota (taxa with documented pro- and anti-inflammatory properties) and circulating (pro-inflammatory cytokines and acute-phase proteins) factors could be associated with the relationship between prenatal diet quality and postpartum

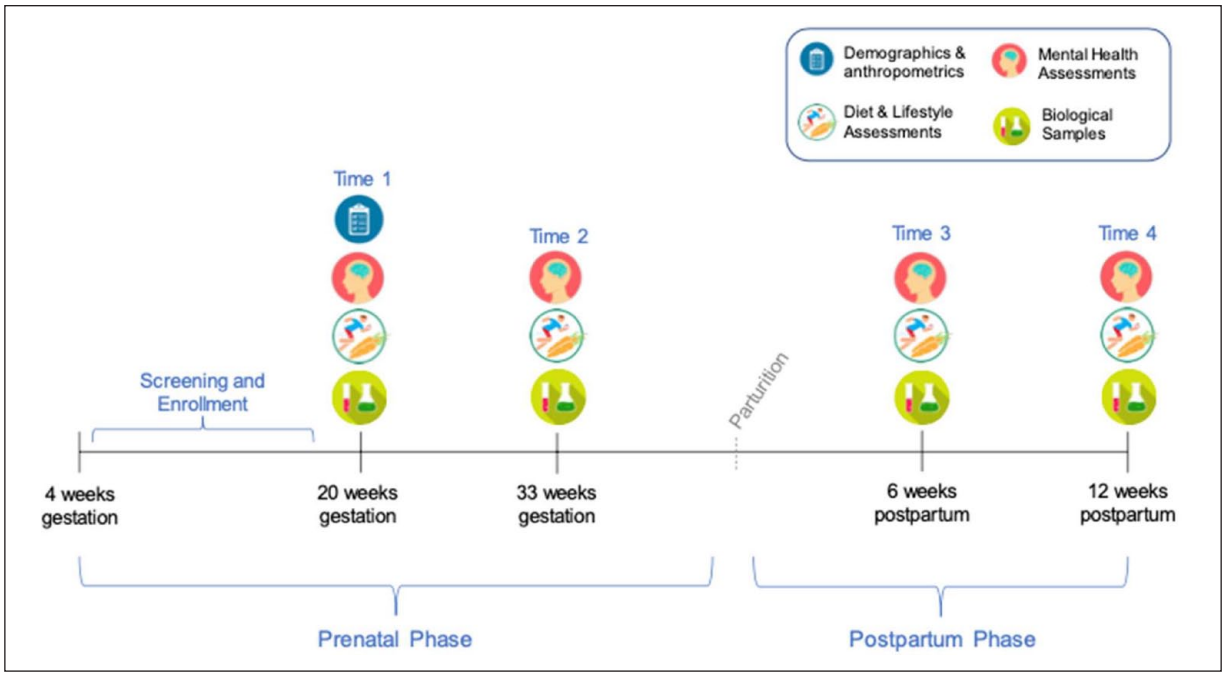

Figure 1. Study design including timeline of enrollment and study visits during the prenatal and postpartum phases and overview of the data collected at each time point. Complete information is described in the section "Study outcome measures" and in Table 1 of Appendix 1.

depression severity, as well as with associations between additional dietary and mental health outcomes.

Methods

Study design

This is a prospective cohort study in which dietary patterns, mental health symptoms, as well as stool microbiota and circulating inflammatory markers will be determined at four key time points across two phases of the perinatal period. Other measures related to lifestyle and social environments will also be documented. Data collection is expected to take place over 3 years.

The study design is outlined in Figure 1. Complete details on data collection at each study visit can be found in Table 1 of Appendix 1.

Prenatal phase. Participants will be enrolled at 20 weeks of gestation (Time 1: halfway through the second trimester of pregnancy) where they will undertake a first study visit. A second visit to the study site will take place at 33 weeks of gestation (Time 2: halfway through the third trimester of pregnancy). These time points were selected as fecal microbiota fluctuations during gestation have been mostly documented toward the end of pregnancy, with the first trimester being comparable to nonpregnant individuals,[9–12] and as postpartum mental disturbances have been associated with abnormalities in hormonal or inflammatory gestational trajectories mostly during the second and third trimesters.[42–44] Each study visit will involve completing a clinician-rated scale, self-report questionnaires, and a web-based food frequency questionnaire (FFQ), having a blood sample drawn, and receiving instructions to collect a stool sample at home. At Time 1, information including demographics, anthropometrics, and medical history will also be collected. At each study visit, participants will be asked about changes in their weight, substance/medication/supplement usage, treatment from a mental health professional, social support group participation, practice of complementary and/or alternative medicine, metabolic changes, consistency of their feces (constipation), as well as recent infections, including COVID-19.

Postpartum phase. Participants will make two visits to the study site at 6-week (Time 3) and 12-week (Time 4) postpartum to complete identical study procedures as at Time 2 of the prenatal phase. These time points were chosen based on previous reports showing that fecal microbiota shifts from pregnancy were apparent at 6-week postpartum[14] and that mental disturbances associated with hormonal and inflammatory gestational fluctuations were mostly documented at 6- and 12-week postpartum.[43,45]

Study setting

Study visits for data collection will take place at a research institute within a tertiary-care mental health hospital in

Ottawa, Ontario, Canada. Stool samples will be collected by participants at home. Stool and blood samples will be analyzed at the University of Ottawa.

Study participants

A total of 120 pregnant women will be recruited from the community in the Ottawa-Gatineau area of Ontario and Québec, Canada, via paper- and web-based advertisements as well as through local midwifery and family medicine practices. To be included in the study, participants will have to be aged 18–40 years, able to understand, read, and speak English to provide written informed consent and complete self-report questionnaires, and plan to be available for the duration of the study. Potential participants will be excluded if they are at less than 4 or more than 20 weeks of pregnancy at the time of enrollment, have a previous diagnosis of major depressive disorder, generalized anxiety disorder, bipolar disorder, or obsessive-compulsive disorder, currently use antidepressant, anxiolytic, and/or antipsychotic medication, have a history of inflammatory bowel disease, irritable bowel syndrome, or other gastrointestinal disorders, cardiovascular diseases, diabetes, liver cirrhosis, fatty liver diseases, or other inflammatory disorders, or have a current alcohol or substance abuse and/or dependence.

Although we acknowledge that antibiotics and microbiota-targeted supplements (e.g., probiotics, prebiotics, synbiotics) strongly impact the gut microbiota, we will not exclude or withdraw participants from the study should they require a course of medications or supplements during pregnancy and/or postpartum for retention purposes. The intake of any medications or substances that could modify the gut microbiota will be documented at each study visit as part of a Medical History Form, should we need to consider their impact on study outcomes during data analysis and exclude specific time points if required.

Study outcome measures

Primary outcome measures. The primary outcome is the association between diet quality in the prenatal phase (Times 1 and 2) and severity of depressive symptoms specific to the perinatal period in the postpartum phase (Times 3 and 4). Dietary patterns will be determined using the Diet History Questionnaire-III (DHQ-III; a food frequency log assessing intake of foods, beverages, and supplements for the past month),[46] from which the Healthy Eating Index (HEI)[47] will be computed to provide an index of overall diet quality based on compliance with dietary guidelines. Severity of perinatal-specific depressive symptoms will be assessed using the self-report questionnaire Edinburgh Postnatal Depression Scale (EPDS).[48]

Secondary outcome measures. Secondary outcomes include associations between additional dietary and mental health measures, moderations of the dietary-mental health associations by stool microbiota (taxa with documented pro- and anti-inflammatory actions) and circulating (pro-inflammatory cytokines, acute-phase proteins) inflammatory factors, as well as changes in dietary, mental health, and biological factors over the prenatal and postpartum phases.

Additional dietary measures. The Mediterranean Diet Adherence Screener (MEDAS)[49] will assess the consumption of foods and food habits related to the Mediterranean Diet. The Dietary Inflammatory Index (DII; computed from the DHQ-III scores)[50] will provide an overall picture of the inflammatory potential of diet. Dimensions of dietary patterns, reflecting how intakes of foods, beverages, and supplements (as per the DHQ-III outputs) load together, will be determined using principal component analysis.

Additional mental health measures. The clinician-rated Montgomery–Åsberg Depression Rating Scale (MADRS)[51] will measure changes in the severity of depressive symptoms, whereas the self-report questionnaires Perinatal Anxiety Screening Scale (PASS)[52] and Perceived Stress Scale (PSS)[53] will determine the severity of perinatal-specific anxiety symptoms and perceptions of stressors' unpredictability, uncontrollability, or overload, respectively. The presence of disordered eating behaviors, which could influence dietary intake and related measures, will be documented using the SCOFF Questionnaire.[54]

Stool microbiota characteristics. Stool samples will be collected within 2 days of each study visit using a stool collection kit (Norgen Biotek Corp., Thorold, ON, Canada) by the participant at home. Participants will be advised to store the collection tubes containing their stool samples in a safe and dry area, at room temperature, as instructed by the collection kit manufacturer and to return their samples to the study staff at the next visit where they will be stored at −80°C until analysis. Participants will also be asked to complete the Bristol Stool Scale[55] each time they collect a stool sample to determine the consistency of their feces. Isolation and purification of total genomic DNA from stool samples will be conducted using a stool DNA isolation kit (Norgen Biotek Corp., Thorold, ON, Canada). The 16S ribosomal RNA (16S rRNA) gene amplicons will be prepared by amplifying the V3 and V4 hypervariable regions of the 16S rRNA gene and sequenced using a 600-cycle MiSeq Reagent Kit v3 and a MiSeq system according to the manufacturer's instructions (Illumina, San Diego, CA, USA).

After data processing using QIIME 2[56] and DADA2,[57] reads will be aligned to taxa using the Silva database[58] to identify the abundance of bacteria at various taxonomic levels.

MicrobiomeAnalyst[59] will be used to determine the Chao1 and Shannon alpha-diversity indices and the Bray-Curtis dissimilarity beta-diversity index. Beta-diversity will be calculated using Bray-Curtis distance and visualized

using principal coordinate analysis and permutational multivariate analysis of variance will be used as the statistical method to confirm between-group differences. The Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUSt2) software[60] will be used to infer metagenomes and predict Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway abundances from the 16S rRNA data.

Circulating levels of pro-inflammatory factors. Whole blood samples will be collected by a phlebotomist at the study site. Immediately after collection in pre-coated ethylenediaminetetraacetic acid tubes, blood samples will be put on wet ice, centrifuged at 4°C, and plasma will be aliquoted and stored at −80°C until analysis. The pro-inflammatory cytokines interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α as well as the acute-phase protein C-reactive protein (CRP) will be assessed through high-sensitivity antibody-based immunoassays. These pro-inflammatory factors have been selected based on their documented associations with perinatal depression.[61,62]

Other outcome measures

Demographics, anthropometrics, and medical history. Age, sex at birth, gender identity, ethnicity, level of education, marital status, household income level, as well as food insecurity and proximity to food swamps will be collected as part of demographic information at Time 1. Anthropometric information such as height, weight, and body mass index (BMI), as well as a brief medical history that includes questions about previous and current pregnancies (e.g., whether pregnancy was intended) will also be documented at that same study visit.

Lifestyle. Physical activity levels, social support, and loneliness will be assessed using the International Physical Activity Questionnaire,[63] the Social Provisions Scale,[64] and the 8-item UCLA Loneliness Scale,[65] respectively. These measures, along with demographics, anthropometrics, and medical history information, will be used to characterize the sample.

Sample size

The sample size required for our study was calculated to detect an association between diet quality (HEI scores) during the prenatal phase and severity of perinatal-specific depressive symptoms (EPDS scores) during the postnatal phase, with 80% of statistical power, 0.05 alpha level of probability level of significance, and an anticipated effect size ranging from small (0.02) to medium (0.15) using 0.08 as the mid value. Because the study design is longitudinal, we may face retention challenges related to attrition. By recruiting 120 participants during the prenatal phase, we expect that we will lose about 15–20 participants due to attrition,[66] leading us to at least 100 participants at the postpartum phase. This would provide a sufficient sample size to establish a significant predictive relationship between prenatal diet quality and postpartum depression severity, our primary outcome.

Statistical analyses

The primary outcome will be analyzed using linear mixed models, with diet quality in the prenatal phase as the independent variable and severity of perinatal-specific depressive symptoms in the postpartum phase as the dependent variable. Additional linear mixed models will be used to analyze predictive associations between secondary dietary (e.g., MEDAS or DII scores, dimensions of dietary patterns) and mental health (e.g., MADRS, PASS, or PSS scores) outcomes. Hierarchical linear mixed effect models will be used to examine whether the relationships between dietary measures during the prenatal phase and mental health symptoms during the postpartum phase are moderated by selected inflammatory-based microbiota and blood markers. These will primarily include taxa with known pro-inflammatory (e.g., Proteobacteria, *Enterobacteriaceae*) and anti-inflammatory (e.g., *Faecalibacterium*) properties as well as KEGG pathways related to inflammatory activity (e.g., *lipopolysaccharide* biosynthesis pathway, peptidoglycan biosynthesis pathway) and pro-inflammatory factors listed previously (IL-6, IL-8, TNF-α, CRP). Models will be adjusted using one or more of the following covariates: pre-pregnancy BMI, age, ethnicity, level of education, marital status, household income level, food insecurity, and constipation. Changes in primary and secondary outcome measures over time (across the four study visits) will be analyzed using a series of analyses of variance with repeated measures on the factor Time.

Anticipated results

We expect that better overall diet quality during the prenatal phase, indicated by higher HEI scores, will predict less severe perinatal-specific depressive symptoms during the postpartum phase, indicated by lower EPDS scores. We anticipate that these associations will be moderated by specific microbiota (e.g., higher abundance of butyrate-producers like *Faecalibacterium*) and circulating (e.g., reduced concentrations of pro-inflammatory cytokines like IL-6) inflammatory markers. Similar associations between additional dietary and mental health measures, as well as their moderation by microbiota and inflammatory factors, are also expected.

Discussion

The gut microbiota-immune axis has been identified as a promising target for the prevention and treatment of mental health disturbances using dietary interventions.[23,26,36] Approaches based on dietary modifications may be

enticing for pregnant and breastfeeding women given their unique and vulnerable biopsychosocial status. However, rigorous longitudinal cohort studies examining this within the perinatal period are lacking. The consideration of two time points for dietary evaluation during the prenatal phase will also allow the examination of changes in eating behaviors that often occur during pregnancy, a concern already highlighted by Sun et al.[67] Our multidisciplinary approach will contribute to the body of literature documenting the relationships between diet quality during pregnancy and severity of depressive symptoms at postpartum and will be the first to comprehensively examine whether inflammatory-related microbiota and blood factors are linked to the relationships between dietary patterns and mental health in the context of the perinatal period.

Potential limitations

Numerous external variables may influence our primary study outcome, namely whether diet quality during the prenatal phase predicts severity of depressive symptoms specific to the perinatal period in the postpartum phase. How women perceive the social support they get can predict if they experience maternal stress and anxiety.[68] Low socioeconomic status has also been associated with more perinatal depressive symptoms,[69] and when tied into the well-established association with diet quality,[70] these relationships can be difficult to disentangle. The effects of these confounding factors and more will be mitigated in our study by collecting data related to household income, education level, food insecurity, gravidity and parity, and experiences of social support and loneliness to better characterize our sample.

Further, the validity of FFQs in epidemiological research remains a topic of ongoing discussion, with accurately measuring dietary and nutritional intake being notoriously difficult.[71] There are several methods of capturing this information in clinical studies, such as 24-hour diet recalls, food records, dietary journals, food and nutrition apps, and FFQs, all of which presenting both strengths and weaknesses.[72] In the present study, the DHQ-III was selected as an FFQ to measure dietary intake due to its strengths in capturing usual intake estimates over longer periods of time and its cost-effectiveness. However, limitations with FFQs exist such as the use of closed-ended questions and potential for low accuracy due to recall bias.[73] Although the present study will use past-month recall as opposed to the also commonly used past-year recall to reduce these inherent biases, this will remain a limitation to consider.

Impact

The field of nutritional psychiatry, which focuses on the role of diet and nutrition in mental health, is a rapidly expanding area of research. Yet, very few studies have examined diet and mental health in women during dynamic life periods such as pregnancy and postpartum.

These findings will be highly influential in the field of women's mental health research, especially as this population has been, unfortunately, largely neglected in clinical studies. Specifically, this will contribute to a growing body of evidence required to develop dietary recommendations for women experiencing postpartum mental health issues. By understanding postpartum depression and anxiety within the context of the gut microbiota-immune axis, the results of this study will help to overcome the shortcomings of available treatment options and uncover novel treatment targets that are accessible, noninvasive, and have potential to play a role in prevention.

Declarations

Ethics approval and consent to participate

Ethics approval was obtained by The Royal Ottawa Health Care Group (#2022002) and the University of Ottawa Office of Research Ethics and Integrity (#H-06-22-8013) Research Ethics Boards. Prior to starting any study activities, written informed consent will be obtained and any questions participants may have about the study will be answered.

Consent for publication

Not applicable.

Author contribution(s)

Caroline JK Wallace: Conceptualization; Methodology; Writing – original draft; Writing – review & editing; Project administration; Funding acquisition.
Marie-Claude Audet: Conceptualization; Methodology; Funding acquisition; Writing – review & editing; Supervision.

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Competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials

Not applicable.

ORCID iDs

Caroline JK Wallace https://orcid.org/0009-0000-1462-6213
Marie-Claude Audet https://orcid.org/0000-0002-3694-1510

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Appendix 1

Study visit schedule

Table 1. Schedule of assessments completed at each study time point.

Procedure	Time point			
	Time 1 (20-week gestation)	Time 2 (33-week gestation)	Time 3 (6-week postpartum)	Time 4 (12-week postpartum)
Informed consent	•			
Demographics	•			
Anthropometrics	•			
Medical history	•	•	•	•
Mental health assessments				
EPDS	•	•	•	•
MADRS	•	•	•	•
PASS	•	•	•	•
PSS	•	•	•	•
SPS	•	•	•	•
ULS-8	•	•	•	•
SCOFF	•	•	•	•
Diet and lifestyle assessments				
DHQ-III	•	•	•	•
MEDAS	•	•	•	•
IPAQ	•	•	•	•
Biological samples				
Stool samples	•	•	•	•
Blood samples	•	•	•	•

EPDS: Edinburgh Postnatal Depression Scale; MADRS: Montgomery-Åsberg Depression Rating Scale; PASS: Perinatal Anxiety Screening Scale; PSS: Perceived Stress Scale; SPS: Social Provisions Scale; ULS-8: 8-item UCLA Loneliness Scale; DHQ-III: Diet History Questionnaire-III; MEDAS: Mediterranean Diet Adherence Screener; IPAQ: International Physical Activity Questionnaire.