PROTOCOL FOR THE COVID-19 WELLBEING AND STRESS STUDY: A LONGITUDINAL STUDY OF PARENT DISTRESS, BIOLOGICAL STRESS AND CHILD BIOPSYCHOSOCIAL DEVELOPMENT DURING THE PANDEMIC AND BEYOND

Jennifer E Khoury,1 Leslie Atkinson,2 Susan Jack,3 Teresa Bennett,4,5 Sandeep Raha,6 Eric Duku,5 Andrea Gonzalez2,4,5

ABSTRACT

INTRODUCTION

Since the beginning of the global COVID-19 pandemic, declared on 11 March 2020,1 the lives of millions of individuals have been impacted. Given the imminent risk to public health, governments globally implemented public health measures to mitigate the transmission of SARS-CoV-2, including travel restrictions, physical and social distancing, school and daycare closures, and restricted access to in-person healthcare. In addition to the high rates of morbidity and mortality, the economic and social ramifications of the pandemic led to dramatic changes to daily life. Since 2020, it has become clear that the pandemic has had a heavy burden on the mental health of the global population. Pregnant individuals and parents of young children have uniquely experienced this mental health burden.2,5 The potential adverse effects that prenatal stress can have on fetal development are well documented,6–10 however, the long-standing COVID-19 pandemic represents an unfortunate but rare opportunity to investigate the enduring effects of a chronic stressor on pregnant people and their developing children.

PRENATAL STRESS AND CHILD DEVELOPMENT

The perinatal period is a time of vulnerability for both the mother and fetus. The Developmental Origins of Health and Disease (DOHaD) hypothesis suggests that the prenatal and early postnatal period signifies a time of rapid growth and development,6–10 where stress can impact fetal biomarkers,


Preparation of this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/ bmjopen-2023-071926).

Received 16 January 2023
Accepted 22 May 2023

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Prospective longitudinal design from pregnancy to year 3 postpartum (six time points) during the COVID-19 pandemic.
⇒ Multimethod approach beginning in the prenatal period, including biomarkers (cortisol, telomeres), questionnaires, behavioural observation and developmental assessments.
⇒ Demographically low-risk sample.
⇒ Exclusive reliance on self-report of distress and mental health symptoms.

The COVID-19 pandemic has had a unique impact on the mental health and well-being of pregnant individuals and parents of young children. However, the impact of COVID-19-related stress during pregnancy on early child biopsychosocial development, remains unclear. The COVID-19 Wellbeing and Stress Study will: (1) investigate the impact of different forms of prenatal stress experienced during the pandemic (including objective hardship, perceived psychological distress and biological stress) on child stress biology, (2) examine the association between child stress biology and child developmental outcomes, (3) determine whether child stress biology acts as a mechanism linking prenatal stress to adverse child developmental outcomes and (4) assess whether gestational age at the onset of the COVID-19 pandemic or child sex, moderate these associations.

Methods and analyses The COVID-19 Wellbeing and Stress Study is a prospective longitudinal study, consisting of six time points, spanning from pregnancy to 3 years postpartum. The study began in June 2020, consisting of 304 pregnant people from Ontario, Canada. This multimethod study is composed of questionnaires, biological samples, behavioural observations and developmental assessments.

Ethics and dissemination This study was approved by the Hamilton Integrated Research Ethics Board (#11034) and the Mount Saint Vincent University Research Ethics Board (#2020-187, #2021-075, #2022-008). Findings will be disseminated through peer-reviewed presentations and publications, community presentations, and electronic forums (social media, newsletters and website postings).
including the developing brain, which form the building blocks critical to lifelong health and development.\textsuperscript{7,8,11,13} Prior research indicates that maternal self-reported distress during pregnancy is linked to diverse developmental outcomes (for reviews see, references 14–17) including adverse birth outcomes,\textsuperscript{18,19} infant temperament,\textsuperscript{20} as well as later child developmental outcomes, including delays in language and motor development,\textsuperscript{21,22} academic challenges,\textsuperscript{23} and mental health problems.\textsuperscript{24}

The prenatal stress literature is often limited by the exclusive reliance on (mainly retrospective) self-reports of prenatal stress. Stressful events that impact many individuals, such as natural disasters, epidemics and pandemics, are important for disentangling the components of prenatal stress, that is, determining whether the effect of prenatal stress on child development can be attributed to the mother’s (A) objective exposure to a stressor, (B) subjective experience of distress or (C) biological stress response. Indeed, the natural disaster literature demonstrates that these three components of prenatal stress differentially impact child development.\textsuperscript{25–27} Given the heavy burden that the pandemic has had on the mental health and well-being of pregnant individuals and parents, it is essential to understand the role that COVID-19-related prenatal stress may have in impacting child development, in both the short term and long term, and elucidating the mechanisms responsible for these effects.

The impact of COVID-19 on pregnant people and parents

Pregnant individuals and parents of young children have been strongly affected by the pandemic.\textsuperscript{2–5} Pregnant people have endured the additional burden related to perinatal COVID-19 infection, with possible health implications for themselves and their fetus,\textsuperscript{28} as well as uncertainty regarding birthing processes due to hospital restrictions, and limited access to social support, during a time when such support is critical.\textsuperscript{29,30} In addition, reductions in access and increased restrictions to healthcare heavily impacted pregnancy. Results from the current study underscore the nature of healthcare disruptions, such that 23% of the sample had prenatal appointments cancelled, 73% switched to telehealth care and 48% had difficulty accessing prenatal classes or other services during pregnancy,\textsuperscript{31} which is in accordance with a nationwide Canadian sample where 40% experienced cancelled prenatal care appointments.\textsuperscript{32} A scoping review indicated that reductions in prenatal care visits and limits to support people during labour adversely affected maternal mental health and well-being.\textsuperscript{33} In addition, parents, particularly those caring for infants and young children, were more likely to experience heightened stress due to increased caregiving demands, isolation from extended family and community supports, elevations in relationship conflict and financial hardship.\textsuperscript{34,35}

The stressful burden of the COVID-19 pandemic on perinatal women is evinced by elevations in mental health problems worldwide, with prevalence rates of psychological distress reaching 70%, depression between 25%–31% and anxiety 34%–42%, all rates exorbitantly higher than those measured prepandemic.\textsuperscript{2–5} During the initial months of the pandemic, a Canada-wide study showed that 37% experienced clinically elevated symptoms of depression and 57% endorsed clinically significant anxiety.\textsuperscript{36} It is essential to uncover how elevated perinatal distress is associated with child outcomes.

COVID-19 and infant and early child outcomes

Research has begun to explore the effects of COVID-19-related prenatal stress on infant and early childhood outcomes, however, to date, longitudinal data are limited. In a longitudinal study of American women, prenatal distress was associated with higher risk for preterm birth.\textsuperscript{37} Similarly, in a Canadian sample, fear of COVID-19 during pregnancy was associated with greater risk for lower gestational age at birth and lower infant birth weight.\textsuperscript{38} Additional research is beginning to demonstrate the impact of COVID-19 prenatal stress on later infant development. For example, higher levels of postnatal stress during the pandemic were indirectly associated with infant regulatory capacity at age 3 months through higher parenting stress and mother–infant bonding, however, prenatal stress was retrospectively reported at birth in this study.\textsuperscript{39} In addition, higher pregnancy distress was associated with greater infant socioemotional problems through continued experience of stress in the postpartum period.\textsuperscript{40} Similarly, in a longitudinal sample, complex interactions between trait level anxiety and COVID-19 related fear during pregnancy were related to infant language and motor development at 12 months.\textsuperscript{41} In contrast, another longitudinal study found that prenatal COVID-19-related stress and hair cortisol during the COVID-19 lockdown were not significantly associated with infant temperament at 6 months.\textsuperscript{42} Additional longitudinal research is needed to understand how prenatal stress related to COVID-19 is associated with diverse developmental outcomes across the first years of life.

In addition to COVID-19-related stress during pregnancy, caregivers are also experiencing elevated distress and mental health problems.\textsuperscript{2,43–45} Like the prenatal environment, the postnatal caregiving environment is critical to child development, such that caregiver postpartum distress,\textsuperscript{15–17} mental health,\textsuperscript{17} 46,47 as well as caregiving behaviour,\textsuperscript{48–50} contribute to child psychosocial outcomes. It is possible that the effects of prenatal stress may be mitigated or exacerbated by the postnatal environment. Although prior research well documents the adverse parent and child outcomes associated with natural disasters and epidemics, the longer-term effects of a chronic stressor with the widespread scope as the COVID-19 pandemic, remains unclear.

How prenatal stress alters development: fetal programming of biological stress markers

In addition, it is critical to understand the mechanisms responsible for how prenatal stress impacts child development. The fetal programming hypothesis suggests that
excessive exposure to stress during pregnancy alters the structure and function of fetal stress biology which can, in turn, impact different facets of later development. There are several biological stress systems that begin developing during the prenatal period. First, the primary stress system in humans, the hypothalamic–pituitary–adrenal (HPA) axis, is highly vulnerable to the effects of prenatal stress. Exposure to stress during pregnancy is strongly associated with altered child acute and chronic HPA activity, indexed by salivary and hair cortisol levels, respectively. A handful of studies have examined the impact of the COVID-19 pandemic on cortisol levels, indicating increases in child and adolescent hair cortisol levels over the course of the pandemic and increases in youth cortisol levels following lockdowns specifically. In a study of mothers and their children aged 5–14 years, several pandemic-related stress factors, including working from home, family job loss and social isolation were correlated with both mother and child hair cortisol levels, and maternal hair cortisol was positively associated with child internalising problems. Thus far, the one study to examine maternal hair cortisol levels during pregnancy did not find significant associations with infant affect or regulation at 6 months. However, cortisol samples in this study may be subject to wash-out effects due to sampling and assay procedures. Thus, given that the developing HPA axis is impacted by prenatal stress, it is important to understand how differences in maternal HPA activity during pregnancy can influence offspring HPA activity and subsequent health and development.

Second, telomere biology is also an important mechanism for fetal programming. Telomeres are DNA proteins that sustain chromosome length and prolong cell death. Telomere biology is, in part, shaped by the prenatal and early postnatal environment. There is a natural developmental trend for telomere erosion from birth to age 3, thus there is great value in assessing the rate of telomere shortening during this period, as an important marker for later health. Prior research demonstrates that elevated prenatal stress is associated with shorter telomeres at birth and later in development, however, research has yet to explore the effect of prenatal COVID-stress on infant telomere length. A novel aspect of this prospective longitudinal study is the measurement of maternal HPA activation during pregnancy and child biological stress markers, including HPA activity and telomere attrition, during early childhood.

In summary, prenatal stress can influence the developing fetus' stress biology, with evidence that stress in pregnancy can impact the development of the child’s HPA axis (ie, cortisol levels) and cellular ageing (ie, telomere length). Long-term HPA activity can be assessed retrospectively using cortisol extracted from hair, and telomere shortening (attrition) can be measured longitudinally. This study will examine how different measures of prenatal stress (objective, subjective distress, HPA activity) are associated with both offspring HPA activity (hair cortisol levels) and telomere shortening, both of which can have subsequent impact on child health and development.

Child sex and gestational age
It is also important to assess timing of stress exposure during gestation and fetal sex as they may contribute to differing child outcomes. Prenatal stress impacts offspring biological stress and psychosocial outcomes differentially depending on the timing of prenatal stress exposure, likely related to sensitive periods of development for specific child outcomes. For example, prenatal stress experienced in early pregnancy, related to natural disasters (storms, floods), has more pronounced effects on child cognitive and language outcomes. In contrast, other research has found that stress exposure during the third trimester was more strongly linked to neurodevelopmental disorders. However, prenatal stress exposure early and late in pregnancy has a greater impact on child HPA activity and telomere shortening, compared with mid-pregnancy stress. In addition, there are sex differences related to the effects of prenatal stress, however, these findings are heterogeneous. For example, prenatal stress has been associated with lower infant birth weight, smaller head circumference and shortened gestational age in male but not female infants. In contrast, other studies have found stronger associations between prenatal stress and adverse birth outcomes and HPA axis functioning in females compared with males. Taken together, it is important to include timing of exposure to prenatal stress as well as sex when understanding the effects of the pandemic on child outcomes.

Study purpose
The overarching goal of the COVID-19 Wellbeing and Stress Study is to prospectively examine the impact of prenatal stress on early child biological stress markers and developmental outcomes. Study findings will continue to uncover the biopsychosocial consequences of the pandemic for both maternal and child health and will further inform prevention and intervention strategies.

Primary aims and hypotheses
This study has four primary aims (figure 1). First, we aim to examine the impact of prenatal stress, including objective hardship, perceived psychological distress and biological stress on child stress biology. Second, we will assess the association between child stress biology and child developmental outcomes. We hypothesise that prenatal stress is associated with indices of elevated child biological stress (accelerated telomere shortening and altered cortisol levels) and that these child stress profiles are associated with more adverse developmental outcomes. Third, we aim to examine whether child stress biology acts as a mechanism linking prenatal stress to adverse child developmental outcomes. We hypothesise that, over time, child stress biology plays a mechanistic role,
accounting for the indirect association between prenatal stress and child developmental outcomes, consistent with the biological programming and the DOHaD hypotheses. Fourth, we will assess whether gestational age (4a) and child sex (4b), moderate the aforementioned associations. Given inconsistencies in the literature, we do not hypothesise the direction of moderating effects for gestational age or child sex.

Secondary aims
This study also aims to understand how psychosocial factors are associated with perinatal stress, child stress biology and developmental outcomes. We will explore whether psychosocial factors moderate the impact of prenatal stress on child outcomes. We will examine psychosocial factors related to coping (coping, emotion regulation) as well as historical adversity in interpersonal relationships (ie, adverse childhood experiences) and current quality of interpersonal relationships (eg, social support, marital conflict, family functioning).

Lastly, this study aims to assess the influence of the postnatal environment on child outcomes. We are interested in examining the role of parenting practices, parenting behaviour and parent distress in the postpartum period, in relation to child outcomes. We will also examine the potential role of these postnatal environmental factors as moderators of the link between prenatal stress and child outcomes.

METHODS
Study design
This is a prospective longitudinal study (figure 2). A total of 304 pregnant people were recruited in Ontario, Canada between June and July 2020 (T1). This study consists of six time points: T1 (pregnancy, n=304), T2 (6 weeks postpartum, n=265), T3 (6 months postpartum, n=180), T4 (15 months postpartum, n=190), T5 (25 months postpartum, n=to be determined (TBD)) and T6 (35 months postpartum, n=TBD). This multimethod study is composed of (1) questionnaires, (2) biological samples, (3) behavioural observations and (4) developmental assessments. Across all time points, participants will complete online questionnaires to assess objective and subjective distress, interpersonal relationships, and child development. Mothers will provide hair samples to assess HPA activity at all time points, child hair samples will be collected from T4 to T6, and mother and child saliva will be collected from T4 to T6 to assess telomere attrition. Self-reported birth outcomes will be assessed at 6 weeks postpartum (T2). Lastly, parent–child interactions will be assessed via virtual (or home, T5–T6) videotaped interactions from 6 to 35 months postpartum (T3–T6).

Participant recruitment and eligibility
Participants include individuals living in Ontario, Canada, who were pregnant during the COVID-19 pandemic. Participants were recruited through social media (Facebook and Instagram) advertisements, pamphlets distributed to midwifery groups and word of mouth. As such, this work is based on an opportunistic sample and may not be representative of the diverse population in Ontario.
Canada. Inclusion criteria were that participants must (1) live in Ontario, Canada, (2) read and write in English, (3) be 18 years of age or older and (4) be ≤36 weeks gestation at the time of recruitment. Recruitment of pregnant individuals began in June 2020 and completed at the end of July 2020. Participants receive an Amazon gift card for completion of each study assessment.

Sample description
In total, 304 individuals completed the T1 surveys during pregnancy. At T1, online surveys were completed between June and August 2020. Thus far, participants have completed four waves of data collection (T1: pregnancy, n=304; T2: 6 weeks postpartum, n=265; T3: 6 months postpartum, n=180; T4: 15 months postpartum, n=190). In addition, T5 (25 months postpartum) data collection is currently ongoing and T6 (35 months postpartum) data collection will commence in summer 2023. A total of six participants withdrew after T1 (four due to miscarriage, two for undisclosed reasons), the remaining eligible participants who did not complete subsequent assessments did not respond to follow-up survey requests. T1 surveys were completed between June and August 2020, T2 surveys were completed between July 2020 and May 2021, T3 surveys were completed between February 2021 and October 2021, and T4 surveys were completed between October 2021 and June 2022. For context, a state of emergency was declared by the provincial government of Ontario three times between 17 March 2020 and 7 April 2021, which included over 300 days of lockdown.

At the onset of the study, participants were between 4 and 26 weeks gestation (M=21.44, SD=8.93 weeks), with 45.1% (n=137) of participants in the first trimester, 24.3% (n=74) in the second trimester, and 30.6% (n=93) in the third trimester of pregnancy. In total, 52.6% of the sample were primiparous at T1. Participants ranged from 19 to 44 years old (M=32.09, SD=4.27 years). A large portion of the sample identified as white (84.9%), married or in a common-law relationship (94.4%) and completed high school education (96.1%). Participants reported a median annual household income range of $C110 000–$C149 999. Compared with national statistics (Statistics Canada, 2016, 2021), the current sample reports higher income and education, and is racially more homogeneous, likely due to sample recruitment methods. Of note, this sample is demographically similar to that of a Canada-wide COVID pregnancy sample.36

Patient and public involvement
Research questions and measures were informed by past research and consideration of participant needs. Participants were not involved in study development or recruitment. Study findings will be disseminated to participants through newsletters, social media and website postings.

Study measures
Table 1 provides a full list of measures and the corresponding administration time point.

Questionnaires
Questionnaires are administered at each time point. Sociodemographic, pregnancy-related information and delivery/labour experiences were captured during pregnancy and post-delivery. Objective stress related to COVID-19 focused on the impact of COVID-19 on health (including infection), finances, social connections and relationships, and child adjustment. Subjective distress included anxiety, depression, stress and substance use. Coping and the quality of interpersonal relationships, including parenting, were also assessed. We also assessed sleep and physical activity. Measures are listed in Table 1 and a complete list of questionnaires, along with brief descriptions, is included in online supplemental table S1.

Biological samples
Hair cortisol
Prenatal exposure to biological stress was assessed using hair cortisol samples collected during pregnancy and 6 weeks postpartum. Maternal and child hair cortisol was also sampled at 6 months, 15 months, 25 months and 35 months postpartum to capture parent and child biological stress in the postpartum period. Hair cortisol is a valid biomarker for chronic HPA activity,85–88 is a reliable index of stress during pregnancy,86,87 infancy and early childhood,88,92–94 and is linked to early risk and child psychosocial outcomes.10 Given the chronic nature of the COVID-19 pandemic, it is essential to use a biomarker that effectively measures long-term levels of cortisol. Mothers and children will provide hair samples (3 mm diameter, ~50 strands) that will be cut into 3 cm segments to reflect cortisol production in the prior 3 months.98 Cortisol will be assayed in duplicate using Cortisol ELISA Kits from Salimetrics

Salivary telomeres
The rate of telomere shortening during early childhood will be assessed as a biomarker of stress, as well as an important marker for later health. Given the link between parent and child telomere length,99 both mother and child saliva will be sampled and assayed for telomere length (and attrition) at 15 months, 25 months and 35 months postpartum (T4–T6). Telomeres will be measured using swabs to absorb saliva, collected from participant’s cheeks. Given normative telomere attrition early in life,67 68 we will examine the rate of telomere shortening. DNA will be collected using Oragene kits and buccal cell telomere length will be determined by using a qPCR relative telomere length assay.106–108 We will use DNA extracted from Human TR146 cells, a model of human buccal cells (Millipore-Sigma, Oakville ON), as an internal quality control.

Child development outcomes
Child social-emotional, cognitive and language development will be assessed at 6 months, 15 months, 25 months and 35 months postpartum (T3–T6) via questionnaires.
### Table 1: Study measures

<table>
<thead>
<tr>
<th>Measures</th>
<th>Time points</th>
<th>T1</th>
<th>T2 6 weeks postpartum</th>
<th>T3 6 months postpartum</th>
<th>T4 15 months postpartum</th>
<th>T5 25 months postpartum</th>
<th>T6 35 months postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section A: background and demographic information</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Consent</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Demographic and socioeconomic information</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Section B: pregnancy and postdelivery</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Pregnancy details (trimester, prenatal care)</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Post-delivery questionnaire (birth outcomes)*</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Breastfeeding questionnaire*</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Section C: objective stress (COVID)</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>COVID-19 Experiences Questionnaire*</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>The COVID Stress Scales</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Section D: subjective distress</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Centre for Epidemiological Studies Depression short form</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Edinburgh Postnatal Depression Scale</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Cambridge Worry Scale</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Generalised Anxiety Disorder Questionnaire</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Perceived Stress Scale</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Alcohol Use Disorders Identification Test-Concise (AUDIT-C) Questionnaire</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Section E: coping</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Brief COPE</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Difficulties in Emotion Regulation Questionnaires (DERS)</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>DERS Short Form</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Section F: social relationships and parenting</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Multidimensional Scale of Perceived Social Support</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Martial Conflict Questionnaire</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Parenting Behaviour</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Circle Questionnaire</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Adverse Childhood Experiences Scale</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Section G: sleep and activity</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Insomnia Severity Index</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Pittsburgh Sleep Quality Index</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Frequency &amp; Extent of Physical Activity*</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Section H: child outcomes</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Ages and Stages Questionnaire</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Brief Infant Toddler Social Emotional Assessment Scale</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>MacArthur Bates Communicative Development Inventories</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Preschool Language Scale-5**</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Section I: biological stress</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Maternal hair cortisol</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Child hair cortisol</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Mother and infant salivary telomeres</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Section J: behavioural observation</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Parent-child free play</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Child imitation sorting task**</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Child toy frustration task**</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Child delay of gratification task**</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

* Measure created for this study. ** Measure only administered for subset of home visits.
and behavioural observation (for those who complete home visits, T5–T6).

Child development questionnaires
Child development will be measured at 6 months, 15 months, 25 months and 35 months postpartum using the gold-standard, norm-referenced and parent-reported Ages and Stages Questionnaire-3 (ASQ-3).103 The ASQ-3 developmental screener captures child development in areas of language/communication, gross and fine motor, problem solving, and personal social. The ASQ-3 is a valid tool for capturing dimensional variations in development and is highly sensitive for detecting infants with developmental delays. Language development will also be measured using the MacArthur-Bates Communicative Developmental Inventory Third Edition104 at 15 months, 25 months and 35 months postpartum and the Preschool Language Scale5 at 25 months and 35 months postpartum for those completing in-person home assessments. In addition, child social-emotional functioning will be assessed using the Brief Infant Toddler Social Emotional Assessment Scale,106 which is a standardised assessment of socioemotional problems and competencies in children ages 12–35 months.

Behavioural observation of child development
Home visit assessments at 25 months and 35 months postpartum will include additional behaviour tasks designed to observe child development (see table 1). These tasks include the Imitation Sorting Task,107 designed to assess early cognitive and executive functioning, the Toy Frustation Task,108 designed to elicit frustration and emotion regulation strategies, and the Delay of Gratification task,109 designed to assess ability to forego a smaller, immediate reward in favour of a future, larger reward. Child affect and behaviour will be coded from these observation tasks.

Behavioural observation of parent–child interaction
At 6 months, 15 months, 25 months and 35 months postpartum, behavioural observation will occur at participants’ homes either via virtual (Zoom) or in-person observation. Parents and children will be observed during a 12 min free-play interaction, consisting of 5 min of playing with toys, 5 min playing without toys and 2 min of a divided attention task. Parental sensitivity to the child will be coded using the 25-item short Maternal Behaviour Q-Set (MBQS-25).110 The MBQS-25 is highly reliable and is related to markers of perinatal stress, early child cognitive development and social-emotional functioning.110-112

Data management
Questionnaire data will be collected and managed using Qualtrics, a secure web-based application to enable data collection for research studies. Qualtrics software stores data on a secure server housed in Canada. Personal identifiable data (eg, name, email address, DOB) will be stored separate from deidentified (questionnaire response) data and once downloaded will be stored in password encrypted electronic databases separate from other research data.

Data analysis plan
First, data visualisation and normality tests will be conducted to determine whether variables need to be transformed prior to analyses. A combination of regression-based analyses and longitudinal structural equation modelling (SEM) will be used to examine study hypotheses. Prior to the main analyses, potential covariates will be explored using bivariate correlations. For example, sociodemographic variables, pregnancy-related variables (eg, pregnancy health, access to prenatal care), infant birth-related variables and COVID-19-related variables (eg, time since initial lockdown at T1), will be explored as potential covariates. Regression analyses will be used to assess the associations between prenatal stress and child stress biology (Aim 1) and child stress biology and developmental outcomes (Aim 2). SEM will be used to assess longitudinal mediation models (Aim 3) and moderation effects of sex and gestational age (Aim 4a and 4b). Gestational age will be assessed as a continuous moderator and sex will be assessed using subgroup analyses. Separate models will be conducted to test each potential moderator. Prior to SEM, factor analysis will be used to derive latent factors for pregnancy stress (perceived distress, objective stress and biological stress), child stress biology (hair cortisol, telomere length), and child development (social-emotional, cognitive, language). Should these indicators not load onto the expected latent factors, the number and composition of latent factors will be adjusted, or variables will be treated as manifest variables.

Participant attrition
Attrition can be difficult to avoid when conducting longitudinal studies, and attrition has been particularly prevalent for studies conducted during the COVID-19 pandemic.39 113 To address potential biases that might influence participant attrition, prior to conducting main analyses, we will compare participants who completed follow-up surveys with participants who did not, based on sociodemographic information and primary variables of interest. Any variables that are associated with attrition will be included in regression and SEM models as covariates or auxiliary variables.114 115 In addition, models will be conducted using full information maximum likelihood estimation with robust or bootstrapped standard errors to account for missing data.116-117

Power analysis
Power analyses for the most complex SEM model were assessed using the RMSEA method, a widely accepted method to determine SEMs power requirements.118 119 Using conservative RMSEA H0 =0.05, RMSEA H1 =0.01119, with 160° of freedom (based on the number of model parameters minus the number of free parameters), and power=0.80, we require a minimum sample size of 136.
Ethics and dissemination
This study was initially approved by the Hamilton Integrated Research Ethics Board under Project #11034, on 3 June 2020. It has also been approved by the Mount Saint Vincent University Research Ethics Board under Project #2020-187, #2021-075 and #2022-008. At each new study assessment, caregivers provide consent for their continued participation and are informed of their right to stop participation, or not participate in any aspect of the study, at any time. Participants are required to sign the electronic informed consent form prior to providing data.

Deidentification and data storage plans comply with the Tri-Council Policy Statement-2. All questionnaire data collected are deidentified, labelled only with the data collection date and unique participant ID. Identifiable data are stored separately and is password protected. Videos are stored on secure/encrypted drives on password-protected computers within locked laboratories. Biological samples are labelled with unique participant ID and stored in locked cabinets and freezers within locked laboratories.

Study findings will be disseminated in several ways, including peer-reviewed journal articles; presentations at researcher conferences; presentations to members of the community, policy partners and practitioners; and updates to study participants through electronic newsletters and website postings.

As of January 2023, data collection is complete up to 15 months postpartum (T4) and 25 months postpartum data collection is underway. Initial findings related exclusively to questionnaire data have been published.28 31

DISCUSSION
Extensive evidence indicates that pregnancy and the early postpartum are critical periods which set the stage for later health and development. Initial pandemic research shows that pregnant people and parents are experiencing heightened levels of distress, and that this distress is, in turn, associated with adverse birth and early developmental outcomes.2–5 37–40 Related literature demonstrates that the COVID-19 pandemic has had a significant impact on child and adolescent mental health and learning outcomes. However, very little is known about the long-term impact of COVID-19 on infants and toddlers and what mechanisms are responsible for these effects. This longitudinal study will elucidate how prenatal stress during the pandemic is associated with biological stress and developmental outcomes in the first 3 years of life.

Furthermore, we will identify risk and resilience factors that amplify or ameliorate these associations. This research will address public health priorities by understanding how the pandemic is impacting early childhood biopsychosocial development and how to foster child health and well-being.

REFERENCES
5 Yan H, Ding Y, Guo W. Mental health of pregnant and postpartum women during the coronavirus disease 2019

Author affiliations
1 Department of Psychology, Mount Saint Vincent University, Halifax, Nova Scotia, Canada
2 Department of Psychology, University of Toronto, Toronto, Ontario, Canada
3 School of Nursing, McMaster University, Hamilton, Ontario, Canada
4 Psychiatry and Behavioural Neuroscience, McMaster University, Hamilton, Ontario, Canada
5 Offord Centre for Child Studies, McMaster University, Hamilton, Ontario, Canada
6 Department of Biochemistry & Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada

Twitter Jennifer E Khoury @JenKhoury

Acknowledgements We’d like to thank all of the pregnant people who participated in this research as well as the research assistants who worked on this project.

Contributors All authors (JEK, LA, AG, SJ, TB, ED and SR) contributed to the study conception, design and funding applications. Material preparation was performed by JEK, LA and AG. JEK oversaw data collection and management. JEK and ED contributed to statistical analyses. SR contributed to telomere methodology. JEK wrote the first draft of the manuscript. All authors (JEK, LA, AG, SJ, TB, ED and SR) contributed to editing the manuscript and have approved the final manuscript.

Funding This work was supported by the Canadian Institute of Health Research (CIHR) Project Grant-PA: Pandemic/FAQ Pandemic Emergencies Research, grant number 465280. This work was also supported by a Tier II Canadian Research Chair (CRC) in Interdisciplinary Studies in Neurosciences awarded to JEK and a Tier II CRC in Family Health and Preventive Interventions awarded to AG.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s).

Provenance and peer review Not commissioned; externally peer reviewed.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iDs
Jennifer E Khoury http://orcid.org/0000-0002-6703-4198
Andrea Gonzalez http://orcid.org/0000-0003-0087-830X

See: http://creativecommons.org/licenses/by-nc/4.0/.


54 Catalani A, Alemà GS, Cinque C, et al. Maternal corticosterone effects on hypothalamic-pituitary-adrenal axis regulation and...


95 Ferro MA, Gonzalez A. Hair cortisol concentration mediates the association between parent and child psychopathology. *Psychoneuroendocrinology* 2020;114.


