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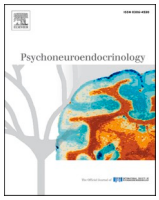

# Relationship between psychological stress and ghrelin concentrations in pregnant women with overweight or obesity

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ABSTRACT

Exposure to, perception of, and response to stress have all been shown to influence appetite and dietary behaviors in non-pregnancy human and animal studies, mediated in part by the appetite stimulating hormone ghrelin. Yet, the impact of prenatal stress on biological pathways associated with appetite in the context of pregnancy is not well understood. The objective of this study was to assess the relationship between these layered dimensions of stress with fasting and postprandial plasma ghrelin concentrations among Hispanic pregnant women with overweight or obesity, a population known to experience heightened levels of stress. Thirty-three non-diabetic Hispanic women with pre-pregnancy body mass index of 25.0–34.9 kg/m$^2$ participated in a crossover study at 28–32 weeks' gestation. At each visit, participants provided fasting blood and saliva samples, consumed a standardized mixed-meal, and completed a 15-minute task: friendly conversation (control) or the Trier Social Stress Test (experimental stress exposure). Six timed blood and saliva samples were collected up to 2 h from baseline and assayed for ghrelin and cortisol, respectively, and area-under-the-curve (AUC) values were computed. Day-to-day stress levels were assessed by the Perceived Stress Scale. Physiological and psychological stress reactivity was determined by cortisol AUC and change in self-reported affect state, respectively, during the experimental stress visit. Maternal perceived stress was positively associated with ghrelin concentrations in the fasted ($\beta = 0.06$, $p = 0.02$) and postprandial state ($\beta = 0.05$, $p = 0.02$). Mean ghrelin AUC was not significantly different following acute stress versus control. Measures of acute stress reactivity were not associated with ghrelin AUC. Contrary to our hypothesis, among Hispanic pregnant women with overweight and obesity, exposure to an acute stress induction task did not alter postprandial ghrelin concentrations, and changes in individual psychological and physiological stress reactivity did not associate with postprandial ghrelin. However, our findings suggest that maternal report of general perceived stress over the last month is associated with higher fasting and postprandial ghrelin concentrations. Differences in the effects of short-term stress exposure versus day-to-day perception of stress on appetite and food intake in pregnancy deserves further investigation.

## 1. Introduction

Numerous studies have examined the adverse effects of prenatal stress on pregnancy outcomes, such as preterm delivery and low birth weight (Dunkel Schetter, 2011). Similarly, the importance of healthy nutrition in pregnancy to support adequate fetal growth, reduce the risk of perinatal complications, and influence long-term health outcomes for the offspring, is well established (Barger, 2010; Wu et al., 2004). Yet, the potential for stress to influence maternal nutrition is only beginning to be recognized in the context of pregnancy (Baskin et al., 2015; Lindsay et al., 2017). Further, the impact of prenatal stress on biological pathways that may influence appetite and food choices during pregnancy, which subsequently influences overall diet quality, has been considerably under-studied.

Throughout pregnancy, exposure to low-grade, day-to-day stress is pervasive and perceptions of that stress may be heightened due to a multitude of factors such as concerns for fetal development, pregnancy complications, worries about birth, and anxiety over future childrearing responsibilities. Prior research found that 78% of pregnant women reported low-moderate stress levels and 6% endorsed high stress in

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pregnancy (Woods et al., 2010). Recent global events such as the ongoing COVID-19 pandemic has further aggravated concerns for well-being in pregnancy (Ahmad and Vismara, 2021; Preis et al., 2020). Certain maternal characteristics may trigger additional stress during pregnancy. For example, over 28% of pregnant women with obesity reported experiencing weight stigma during prenatal healthcare visits, which is associated with higher levels of depressive symptoms and perceived stress (Incollingo Rodriguez et al., 2019, 2020). Individuals from minority groups may also experience higher stress levels associated with acculturation (Chasan-Taber et al., 2020), racial and ethnic discrimination (Giurgescu et al., 2017), poverty (Silveira et al., 2013a, 2013b), and inadequate social support (Robinson et al., 2016) as they navigate their journey through pregnancy. In a large cohort study of Hispanic pregnant women in the United States, 27% were classified as having high levels of perceived stress in early pregnancy, which correlated with incidence of poor health behaviors such as alcohol intake and smoking (Silveira et al., 2013a, 2013b). In more recent studies, it has been observed that Hispanic women have poor diet quality and high rates of sedentariness before and during pregnancy (Kominiarek et al., 2021; Thomas Berube et al., 2019). Although the determinants of these health behaviors were not determined, we speculate that elevated levels of perceived stress may play a role. Therefore, Hispanic pregnant women are an important subpopulation to consider with respect to the effects of stress on the underlying mechanisms that may drive health behavior decision making.

Emerging evidence suggests that the quality and quantity of food consumed in the maternal diet and the physiological response to food intake may be influenced by psychological factors such as stress (Lindsay et al., 2017). The concept of stress has multiple dimensions, which may each play a role in influencing diet and nutritional metabolism (Crosswell and Lockwood, 2020). Firstly, stressors or stressful exposures are conditions or environmental events that are taxing or demanding on an individual's psychological or physiological state. Secondly, perceived stress is the characterization of an individual's feelings or thoughts about how much stress they are under at a given point in time or over a recent period, such as the past month. Thirdly, stress reactivity is a measure of the degree of psychological (e.g., affect/mood state) or physiological (e.g., hormonal (cortisol), heart rate variability, skin moisture levels) changes in response to a stressful condition/event or to a perceived stress. It is important to note that measures of psychological and physiological reactivity to a stressor do not necessarily correlate, such that an individual may exhibit increase in negative affect without discernible change in cortisol levels. Further, the timing or duration of exposure to a stressor may also determine the perception of and responsivity to that stress. In the context of human pregnancy, several observational studies report associations between self-reported perceived stress or other mental health indicators and maternal diet quality (Baskin et al., 2017; Hurley et al., 2005; Lindsay et al., 2020). However, the cross-sectional nature of these studies and collection of only self-report data precludes our ability to determine directionality and biological mechanisms underlying these associations. Advancing our understanding of these mechanisms, while considering the three layers of stress as described above, could better inform the development of future prenatal interventions that aim to support healthy maternal diet and lifestyle for improved pregnancy outcomes.

Ghrelin is an orexigenic (i.e., appetite stimulating) peptide primarily secreted from the stomach that regulates energy metabolism, among other roles (Ibrahim Abdalla, 2015). Plasma ghrelin concentrations are normally reduced following food intake, supporting satiety. However, dysregulated ghrelin signaling, exhibited by reduced suppression (i.e., elevated concentration) of ghrelin after a meal, may contribute to overeating (le Roux et al., 2005). Psychological stress may be an important external factor contributing to elevated ghrelin concentrations, supported by evidence from human experimental studies in non-pregnant adults (Chuang and Zigman, 2010; Li et al., 2015). A recent systematic review reported that ghrelin concentrations increase following acute mental or physical stress exposures (Bouillon-Minois et al., 2021). Moreover, changes in the key stress hormone, cortisol, are shown to significantly and positively correlate with ghrelin concentrations from the pre- to postprandial states among non-pregnant women with obesity (Sarker et al., 2013). Cortisol is secreted in response to activation of the hypothalamic-pituitary-adrenal (HPA) axis under conditions of chronic stress or during recovery from acute stress. In turn, cortisol promotes desire for and intake of hyperpalatable foods, typically those high in fat and sugar, which activates a hedonic state and sense of reward when these foods are consumed, thereby reducing unpleasant emotions related to stress (Dallman et al., 2006). Growing evidence also implicates ghrelin as a key hormone involved in the regulation of energy metabolism in response to different physical and social stressors via alterations in appetite cues and preferences for high carbohydrate diets. In addition, ghrelin targets mesolimbic brain regions to modulate dopaminergic reward processes that, in turn, help to protect against neurological damage, anxiety and depressive symptoms associated with chronic stress (Abizaid, 2019). Under conditions of persistent or elevated perceived stress, these activities of cortisol and ghrelin may contribute to stress-induced eating behaviors, such as excess intake of hyperpalatable foods and eating in the absence of hunger.

While we understand that psychological report of stress and physiological response to stress (e.g., cortisol levels) influence ghrelin levels and appetite signaling in the non-pregnant state, ghrelin concentrations and its determinants of variation are not well understood in the context of pregnancy. Furthermore, research on the determinants and effects of ghrelin concentrations during pregnancy may be complicated by natural gestational-induced fluctuations in circulating concentrations of this hormone. Prior research reports that maternal ghrelin concentrations peak mid-pregnancy and are at their lowest in the third trimester (Fuglsang et al., 2005), which may reflect a physiological adaptation to changes in maternal weight and energy requirements (Makino et al., 2002). Understanding the effect of stress on ghrelin concentrations before and after meal intake at defined gestational timepoints in pregnant women with elevated pre-pregnancy weight status could help determine if stress management approaches may be a tool to support healthy eating behaviors that provide adequate nourishment for healthy pregnancy outcomes. It is also important to consider whether maternal ghrelin levels are altered under situations of day-to-day perceived stress and/or acute stress exposures during pregnancy, so that interventions may be targeted appropriately to support healthier maternal eating behaviors that may not be achieved through dietary prescription alone.

In controlled human studies evaluating the effects of stress, the Trier Social Stress Test (TSST) is a safe, validated, and standardized method of inducing temporary psychosocial stress so that psychological, physiological, or behavioral processes in response to this stress exposure can be measured (Allen et al., 2017). The present study utilizes the TSST as a stress induction task among Hispanic pregnant women with overweight and obesity. The objective of this study is to determine the association between maternal measures of perceived stress, as well as psychological and physiological stress reactivity to a stress induction task, with ghrelin concentrations in the fasting and postprandial states in the third trimester of pregnancy. We chose to study Hispanic pregnant women as they have higher levels of baseline perceived stress compared to non-Hispanic women and are at higher risk of adverse maternal and child health outcomes, potentially as a consequence of heightened perceived stress and associated poor health behaviors (Silveira et al., 2013a, 2013b). We also studied women with pre-pregnancy overweight or obesity as elevated body weight status has been linked to dysregulated ghrelin signaling (le Roux, 2005). We hypothesized that:

1. higher levels of maternal perceived stress would be associated with higher fasting ghrelin concentrations and higher postprandial ghrelin concentrations over 2-hours after consuming a standardized meal
2. postprandial ghrelin concentrations would be elevated following an acute stress induction task versus a control non-stress condition

3. higher physiological and psychological stress reactivity (measured by total salivary cortisol output or change in affect state following an acute stress exposure) would positively correlate with post-prandial ghrelin concentrations measured over 2 h.

## 2. Methods

This study is a secondary analysis of data collected as part of a crossover study designed to test the postprandial glucose-insulin response to a standardized meal under acute psychological stress versus non-stress control conditions. Each participant provided written informed consent and the study was approved by the Institutional Review Board at the University of California, Irvine.

### 2.1. Participant eligibility and recruitment

Pregnant women obtaining prenatal care from UCI Health-affiliated obstetric clinics in Orange County, California, were screened through the electronic medical record for potential eligibility between February 2018 and March 2020. Identified women were approached in person at their scheduled prenatal visits, or by phone, to provide a detailed explanation of the study and undergo a screening questionnaire to determine eligibility. Women were eligible if they were of self-reported Hispanic ethnicity, aged 18–40 years, had a pre-pregnancy BMI 25.0–34.9 kg/m$^2$, carrying a singleton pregnancy, less than 30 week's gestation, non-diabetic with a normal result on the standard glucose challenge test at 24–28 weeks, non-smoker, and fluent in either English or Spanish. The parent study restricted eligibility to women of Hispanic ethnicity to limit inter-individual variability which could influence the metabolic biomarkers being studied. Also, within the geographic location of this study, people of Hispanic ethnicity are known to experience higher rates of psychological stress attributed to various social and demographic factors compared to non-Hispanic individuals.

### 2.2. Study design

For this crossover study, participants attended two laboratory visits that were 1–2 weeks apart between 28- and 32-weeks' gestation in non-randomized order. At the first study visit, each participant underwent a control non-stress task which involved a 15-minute friendly conversation with a research coordinator. At the second study visit, participants completed the TSST, a validated, standardized protocol of 15 min duration used to elicit an acute psychological and physiological stress response (Kirschbaum et al., 1993). The TSST has been previously used in studies with pregnant women (Deligiannidis et al., 2016) and involves 5 min of speech preparation, 5 min to deliver a speech in front of stern evaluators while being videotaped, and 5 min to perform a complex, verbal arithmetic task. The order of tasks at each visit was not randomized in order to prevent a woman from experiencing a stressful event on her first visit, which may deter them from returning for the second visit or produce anticipatory stress on arrival at the second visit. The participants, however, were blinded to the task allocation of each visit.

### 2.3. Data collection

On the first study visit, participants completed questionnaires concerning their sociodemographic characteristics and usual stress levels using the 14-item Perceived Stress Scale (PSS), a widely used subjective measure of the perception of stress over the preceding month (Cohen et al., 1983). The PSS score has a possible range of 0–40 with values with values from 14 to 26 indicating moderate stress and values from 27 to 40 indicating high stress levels. On both visits, participants completed the Positive and Negative Affect Schedule (PANAS) immediately before and after the task period to assess changes in mood state as influenced by the control task or TSST. The PANAS generates separate scores for positive affect and negative affect, each ranging from 10 to 50 with higher scores representing higher levels of either affect state. Change in positive affect and negative affect scores were used as a proxy measure of psychological stress reactivity to the task procedures.

At each study visit, blood samples were collected by intravenous catheter and saliva samples by cotton swab at baseline (fasting- pre-meal sample) and at 15-, 30-, 45-, 60-, 90- and 120-minutes post baseline sample. Participants consumed the same standardized breakfast drink (Boost Plus, 237 ml bottle; 360 kcal, 15 g protein, 45 g carbohydrate, 22 g sugar, 3 g fiber, 14 g fat) at each visit immediately following the fasting sample collection and asked to drink all of it within 10 min. Participants were instructed to rinse their mouth with water after consuming the drink 5-minutes before the next saliva sample collection to avoid contamination of the swabs. The non-stress task or the TSST was performed between the 15 and 30-minute sample collection timepoints at the first and second study visits, respectively.

### 2.4. Biosample processing and analysis

Blood samples were collected in 3 ml EDTA tubes and saliva samples were collected using Salimetrics oral swabs, which were placed under the tongue for 3 min and then inserted into a Salivette tube (Sarstedt). Both blood and saliva samples were immediately centrifuged upon collection at 1500 g for 15 min, then aliquoted and stored at − 80 °C until analyzed. Total ghrelin concentrations were measured using a multiplex from Meso Scale Discovery (Rockville, MD). Saliva samples were assayed for cortisol using a commercially available enzyme immunoassay (Salimetrics, LLC). All assays were performed in duplicate and the average of the duplicate was used in statistical analyses. The average intra- and inter-assay coefficients of variability were for 2.7% and 6.3% for ghrelin and 3.5% and 3.3% for cortisol, respectively.

### 2.5. Statistical analysis

All statistical tests were performed using SPSS version 28. Maternal characteristics and perceived stress and affect scores are presented using descriptive statistics. Intraclass correlation coefficients and their 95% confidence intervals were calculated to test the reproducibility of fasting ghrelin and cortisol measurements across study visits using a single measurement, absolute-agreement, 2-way mixed-effects model. The following summary measures of ghrelin were computed for each visit: total area-under-the-curve (AUC), mean ghrelin concentrations across seven sample timepoints, and change in ghrelin from baseline (fasting) to the final sample at 120 mins postprandial. The AUC for cortisol at each visit was also computed. The values for all summary measures of ghrelin and cortisol were natural log-transformed for normality. Associations between either PSS score, fasting ghrelin, mean ghrelin, change in ghrelin, ghrelin AUC or cortisol AUC at each visit with maternal characteristics (age, pre-pregnancy BMI, education level, born in the U.S., Government healthcare, parity, gestational age at each visit) were assessed by Pearson or Spearman correlation tests for continuous or categorical variables, respectively, to identify potential covariates to include in the analyses. None of these variables were significantly correlated with either PSS score, ghrelin or cortisol values. However, we conducted subsequent analyses with and without adjustment for gestational age at the time of assessment and pre-pregnancy BMI given that both ghrelin and cortisol concentrations are known to be affected by stage of pregnancy, and ghrelin is usually associated with BMI of which there was reasonable variability in this population.

Differences in within-person summary ghrelin measures, cortisol AUC, and pre-post task change in positive and negative affect scores between study visits were assessed using paired sample t-tests and by repeated-measures ANOVA adjusting for gestational age at the time of visit 2. Pre-pregnancy BMI was also added as a covariate in the models with ghrelin measures. To determine the association between maternal perceived stress and ghrelin concentrations, we used a linear regression

model with either fasting ghrelin or summary measures of postprandial ghrelin (AUC, mean of all values, change from baseline to endpoint) from the non-stress visit as the dependent variables, and PSS score as the independent variable, with and without adjustment for gestational age at visit 1 and pre-pregnancy BMI. Baseline (fasting) ghrelin concentration was also added to the models in which change in ghrelin from baseline to endpoint was the dependent variable. To determine the association of physiological and psychological stress reactivity with ghrelin concentrations, the linear regression model was constructed with summary measures of ghrelin from visit 2 as the dependent variable and cortisol AUC or change in negative affect score from pre-post task at the TSST visit as the independent variable, with and without adjustment for gestational age and pre-pregnancy BMI. Given that not all individuals respond equally to the TSST in terms of stress reactivity, participants were classified as high or less cortisol reactors based on a median split of cortisol AUC on visit 2. This dichotomous variable was then used to test for mean differences in summary measures of ghrelin between groups using the independent sample t-test and the general linear model adjusting for gestational age and pre-pregnancy BMI. Due to the timing of the TSST administration after the meal was consumed, it was not possible to determine the association between measures of stress reactivity to the stress induction task and fasting ghrelin concentrations. Significance was indicated at $p < 0.05$ for all analyses.

## 3. Results

### 3.1. Population characteristics and ghrelin concentrations

A total of 38 pregnant Hispanic women were enrolled in the parent study. Of those, 5 participants were withdrawn after study visit 1 due to reasons of preterm delivery (n = 1), unwillingness to drink the breakfast drink (n = 1), restricted availability and unable to complete visit 2 within the required gestational timeframe (n = 3). Thus, complete data were available for the present analysis in n = 33. Sociodemographic variables, pre-pregnancy BMI and PSS scores are described in Table 1. All participants self-identified as being of Hispanic ethnicity. The mean pre-pregnancy BMI was 28.8 ± 2.7 kg/m$^2$ and 33% were classified as having obesity. The mean PSS score was 13.4 ± 3.5 suggesting that, on average, this population of pregnant women had borderline moderate levels of perceived stress in their daily lives. The mean baseline negative affect score was low and showed little variation across participants, while the mean baseline positive affect score fell in the mid-range but had wider variability.

The average ghrelin concentrations across time during each visit are displayed in Fig. 1. The intraclass correlation coefficient values for fasting ghrelin (0.86, 95% CI: 0.76 – 0.93) and cortisol (0.69, 95% CI: 0.45 – 0.83) across visits indicate good and moderate levels of reproducibility, respectively. However, we note that the reproducibility ratings do not consider variations explained by advancing gestational age between measurements.

**Table 1**
Maternal characteristics and baseline psychological states of study population (n = 33).

| | Mean ± SD or N (%) |
|---|---|
| Gestational Age (days) at visit 1 | 207.5 ± 8.7 |
| Gestational Age (days) at visit 2 | 214.8 ± 8.6 |
| Maternal Age (years) | 30.2 ± 5.1 |
| Pre-pregnancy BMI (kg/m$^2$) | 28.8 ± 2.7 |
| Overweight | 22 (66.7%) |
| Obese | 11 (33.3%) |
| Multiparous | 29 (76.3%) |
| Born in the U.S. | 15 (46.9%) |
| Governmental Health insurance | 28 (73.7%) |
| Perceived Stress Score | 13.4 ± 3.5 |
| Positive affect score (pre-task) at visit 1 | 33.2 ± 8.9 |
| Negative affect score (pre-task) at visit 1 | 12.0 ± 2.3 |

BMI, body mass index.

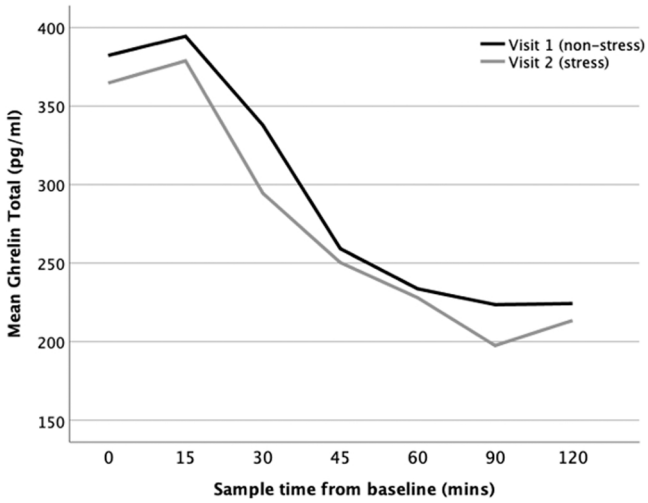


**Fig. 1.** Ghrelin concentration across each study visit, from baseline (fasting) to 2 h after consumption of the standardized meal.

### 3.2. Associations between perceived stress and ghrelin concentrations without stress induction

Maternal report of perceived stress in the last month was significantly and positively associated with fasting ghrelin (β = 0.059, p = 0.017, 95% CI=0.011, 0.106), ghrelin AUC (β = 0.045, p = 0.020, 95% CI=0.008, 0.082), and mean ghrelin concentrations (β = 0.046, p = 0.022, 95% CI=0.007, 0.085) at visit 1, under control non-stress conditions (Fig. 2 A-1 C). After adjusting for gestational age and pre-pregnancy BMI, these relationships remained significant (p = 0.013, 0.021, 0.020, respectively). Perceived stress was not associated with absolute change in ghrelin concentrations from fasting to 2-hours postprandial.

### 3.3. Effects of the stress induction task on measures of stress reactivity and ghrelin concentrations

On average, salivary cortisol AUC was significantly higher after acute stress compared to the non-stress control condition (3.91 ± 0.40 vs 4.11 ± 0.38, t = −3.911, p = 0.001). However, after adjusting for gestational age, the difference between visits in cortisol AUC was no longer significant (p = 0.432), suggesting that gestational age rather than exposure to the TSST may explain the higher cortisol concentrations at the second study visit. Positive affect significantly decreased and negative affect significantly increased after the TSST on visit 2 compared to the control task on visit 1 (Fig. 3). After adjusting for gestational age, the pre-post task difference in negative affect score between visits remained significant (p = 0.032), indicating that the TSST successfully elicited a psychological stress response. On both study visits, our measure of physiological stress (cortisol AUC) was not found to correlate with indices of baseline psychological states (perceived stress, positive affect, negative affect at visit 1), or with psychological stress reactivity to the TSST (change in negative affect score on visit 2).

Mean ghrelin concentrations across the visit and the ghrelin AUC following the standardized meal were significantly lower at the second visit after the acute stress induction task, compared with the non-stress visit (p = 0.030 and 0.014, respectively) (Table 2). However, after adjusting for gestational age at the time of the stress visit and pre-pregnancy BMI, there were no significant differences in any of the summary ghrelin measures between visits (Table 2).

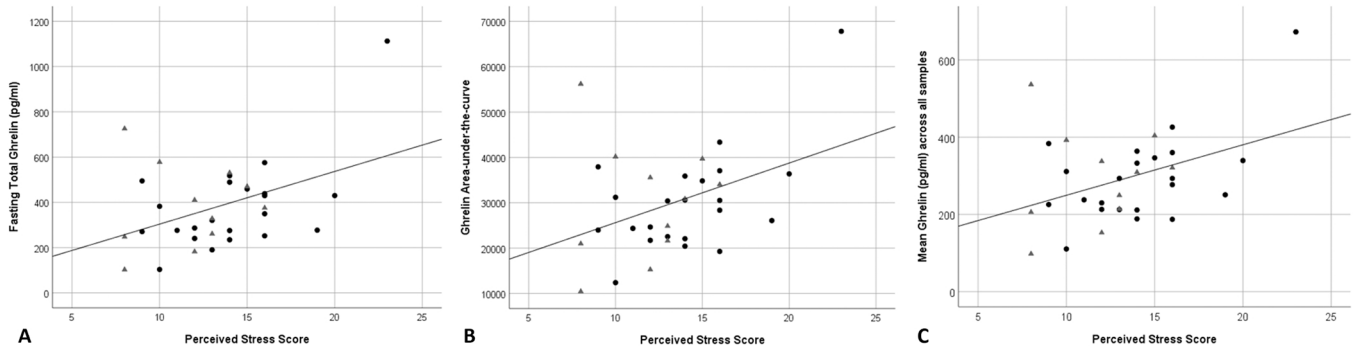


**Fig. 2.** Association between baseline perceived stress levels with fasting and postprandial ghrelin concentrations under a non-stress condition. The association between perceived stress score and the dependent variable, fasting ghrelin (A), ghrelin area-under-the-curve (B), or mean ghrelin concentrations across seven samples from fasting to 2 h postprandial (C). In all panels, black circles denote participants with pre-pregnancy overweight and grey triangles denote participants with pre-pregnancy obesity.

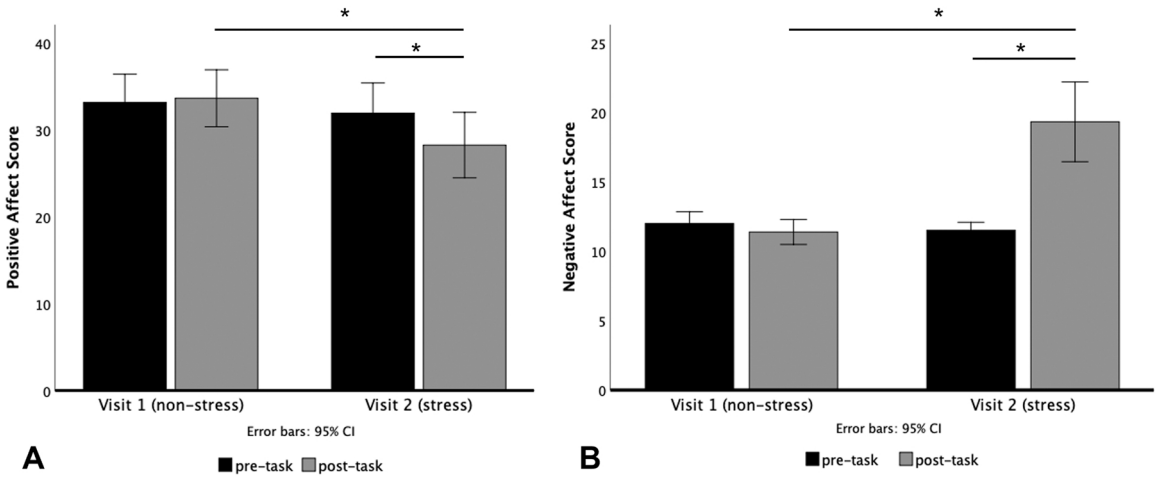


**Fig. 3.** Positive and negative affect scores at each study visit. A: Positive affect score at pre- and post-task in the non-stress visit and stress visit. B: Negative affect score at pre- and post-task in the non-stress visit and stress visit. Asterisks and horizontal lines denote significant difference between bars.

**Table 2**
Difference between summary ghrelin measures under non-stress conditions and following an acute stress task after consumption of a standardized breakfast shake.

| | Visit 1 (non-stress) Mean ± SD | Visit 2 (stress) Mean ± SD | t | P (unadjusted) | P (adjusted) |
|---|---|---|---|---|---|
| Ghrelin AUC | 10.24 ± 0.40 | 10.19 ± 0.37 | 2.28 | 0.030 | 0.589 |
| Mean ghrelin | 5.61 ± 0.41 | 5.55 ± 0.38 | 2.57 | 0.015 | 0.632 |
| Change in ghrelin from baseline to 2-hr postprandial | 4.69 ± 1.06 | 4.75 ± 0.79 | -0.43 | 0.708 | 0.823 |

AUC, area under the curve. Adjusted models included gestational age at visit 2 and pre-pregnancy body mass index as covariates.

### *3.4. Associations of stress reactivity with ghrelin concentrations*

Neither physiological stress reactivity (salivary cortisol AUC) nor psychological stress reactivity (change in negative affect) to the TSST were significantly associated with postprandial ghrelin summary measures following consumption of a standardized meal, before or after adjusting for covariates (Table 3). Furthermore, postprandial ghrelin summary measures on the TSST visit did not differ between those classified as high versus low cortisol reactors (all $p > 0.05$).

## 4. Discussion

This study explores the relationship between layered dimensions of stress (day-to-day perception of stress, exposure to an acute stressor, and measured physiological and psychological stress reactivity) and ghrelin concentrations among Hispanic pregnant women with overweight and obesity. Our findings indicate that the relationship between stress and ghrelin may differ according to the dimension of stress in question, providing the first evidence in human pregnancy about the roles of persistent low-grade perceived stress versus acute psychological stress exposure or stress reactivity in influencing maternal appetite cues.

Firstly, we identified a significant positive relationship between maternal report of perceived stress in the last month and fasted and postprandial ghrelin after consuming a standardized meal. This supports our hypothesis that day-to-day stress during pregnancy may contribute to greater appetite stimulation (via higher ghrelin concentrations). This result also corroborates previous findings from non-pregnancy research in animals and humans, showing that chronic stress exposure is associated with increased circulating concentrations of ghrelin (Yousufzai et al., 2018). In contrast, exposure to an acute psychosocial stress induction task did not induce any change in postprandial ghrelin concentrations, nor did measured physiological or psychological stress reactivity associate with postprandial ghrelin. This indicates that

**Table 3**
Association between physiological and psychological stress reactivity measures following the TSST and postprandial ghrelin concentrations after consumption of a standardized breakfast.

| | Beta | Standard Error | 95% Confidence Interval | | Adjusted R square | P (unadjusted) | P (adjusted) |
|---|---|---|---|---|---|---|---|
| Dependent variable: Ghrelin AUC | | | | | | | |
| Salivary cortisol AUC | -0.018 | 0.173 | -0.374 | 0.337 | -0.035 | 0.916 | 0.923 |
| Change in negative affect score | -0.012 | 0.009 | -0.030 | 0.006 | 0.029 | 0.174 | 0.782 |
| Dependent variable: Mean ghrelin | | | | | | | |
| Salivary cortisol AUC | -0.002 | 0.177 | -0.364 | 0.360 | -0.036 | 0.990 | 0.972 |
| Change in negative affect score | -0.012 | 0.009 | -0.030 | 0.006 | 0.030 | 0.173 | 0.213 |
| Dependent variable: Change in ghrelin from baseline to 2-hrs postprandial | | | | | | | |
| Salivary cortisol AUC | 0.065 | 0.203 | -0.352 | 0.481 | 0.694 | 0.753 | 0.568 |
| Change in negative affect score | -0.001 | 0.010 | -0.021 | 0.020 | 0.718 | 0.950 | 0.771 |

AUC, area under the curve; TSST, Trier Social Stress Test. Adjusted models included gestational age at visit 2 and pre-pregnancy body mass index as covariates. Models with change in ghrelin as the dependent variable are also adjusted for baseline ghrelin concentrations.

short-term, acute stress exposure of mild intensity during pregnancy does not induce changes in ghrelin signaling in this population of Hispanic pregnant women, whereas the psychological burden of day-to-day perceived stress over a longer duration may influence ghrelin concentrations and possibly, appetite cues.

Based on evidence from non-pregnancy studies, we speculate that the relationship between maternal perceived stress and ghrelin concentrations could translate to higher appetite for and intake of hyperpalatable foods during pregnancy, which generally provide a rapid energy source with little nutritional value. In a cohort of non-pregnant women, Jaremka et al. (2014) found that greater frequency of self-reported interpersonal stressors was associated with higher ghrelin and lower leptin concentrations and a higher caloric intake compared to women who reported less interpersonal stressors. Given that ghrelin is involved in appetite stimulation and leptin acts to suppress appetite, this combination of high ghrelin and low leptin in individuals experiencing high levels of stress may represent an underlying biological pathway for stress-related overeating behaviors (Sominsky and Spencer, 2014). In another study of non-pregnant women with overweight, cravings for highly palatable foods and overall caloric intake was positively associated with ghrelin concentrations, and ghrelin was also associated with flatter diurnal cortisol slopes over the course of three days (Buss et al., 2014). A flatter diurnal cortisol slope is indicative of chronic psychosocial stress via dysregulated hypothalamic-adrenal-axis functioning and is a precursor to many inflammatory related health conditions (Adam et al., 2017). Although our study did not evaluate the relationship of stress or ghrelin concentrations with maternal eating behaviors, evidence from non-pregnancy literature suggests that ghrelin may moderate stress-induced comfort eating (Schellekens et al., 2012). This is an important area for future research to consider as it is as yet unknown if maternal stress-induced alterations in ghrelin concentrations translate to unhealthy eating patterns and reduced diet quality in pregnant women, which in turn could influence pregnancy outcomes. For example, emerging evidence suggests that emotional or disinhibited eating behaviors may be associated with excess gestational weight gain (Blau et al., 2018) and rapid fetal growth (Savage et al., 2019), although this is an area of research requiring further investigation.

Contrary to our hypothesis, plasma ghrelin AUC, representing total ghrelin output after a meal, was lower following the stress induction task compared to the control condition. However, this difference was no longer significant after adjusting for gestational age, suggesting that advancing gestation is associated with lower ghrelin concentrations rather than exposure to an acute psychological stress task. All participants in this study were in their third trimester and at a slightly later gestation on their second visit compared to their first visit. Given that maternal ghrelin concentrations have been reported to be at their lowest in the third trimester of pregnancy (Fuglsang et al., 2005), it is possible that gestational-induced suppressed ghrelin concentrations at the time of the TSST precluded the ability to identify any effect of the acute stress induction task on ghrelin.

Our study results also did not identify an association between physiological stress reactivity (salivary cortisol AUC) to the acute stress task and post-prandial plasma ghrelin concentrations (after a standardized meal). It is possible that meal consumption influenced the relationship between physiological stress and ghrelin concentrations in this study. Food intake acts to inhibit ghrelin secretion but also may activate the parasympathetic nervous system (D'Alessio et al., 2001) thereby moderating the physiological stress response. However, we note that cortisol is secreted as the end result of HPA-axis activation, not via autonomic nervous system activation, and there is a lack of evidence to suggest that meal intake can interfere with cortisol response to stressors. Previous research with non-pregnant participants demonstrated a short-term elevation in ghrelin concentrations following the TSST but without food intake (Monteleone et al., 2012; Raspopow et al., 2010). However, similar to our results, Monteleone et al. did not find a significant correlation between subjects' cortisol and ghrelin AUC in response to the TSST (Monteleone et al., 2012). There may be an alternative biological pathway by which stress could influence ghrelin concentrations, or perhaps the TSST in our study was simply unsuccessful in eliciting a substantial physiological stress response in order to test its relationship with ghrelin. While we observed a heightened cortisol AUC after the TSST compared to the control condition, this difference was not significant after adjusting for gestational age. Although we did find that, on average, participants exhibited significant psychological stress reactivity to the TSST as measured by an increase in negative affect, this measure also was not associated with postprandial ghrelin concentrations. However, we were unable to determine the effects of either measure of stress reactivity with fasting ghrelin concentrations.

The exact mechanisms linking acute stress exposure and ghrelin regulation are not yet known. However, ghrelin receptors have been found in sympathetic-regulated areas to stimulate appetite and energy consumption. Additionally, they have been located in ventral segmental and hippocampal regions of the brain, which are responsible for regulation of rewarding processes that help dampen the unpleasant emotional sensations under conditions of stress (Abizaid, 2019). Differences in the creation and regulation of these neurological systems may help to explain inter-individual variation in ghrelin concentrations under stressful circumstances. Studies of ghrelin receptor activity, specifically the growth hormone secretagogue receptor (GHS-R1a), reveal that dimerization of GHS-R1a with other G-coupled proteins that target reward pathways may affect ghrelin signaling activity with respect to appetite regulation (Schellekens et al., 2013). Further research into the neurobiological mechanisms linking ghrelin receptor activity and reward processes under stress could help further understanding of susceptibility to stress-induced appetite and food cravings. However, as previously noted, gestational physiology independently alters concentrations of both ghrelin and cortisol such that their concentrations differ at various stages in pregnancy compared to non-pregnant physiology, regardless of underlying psychological stress. Thus, future research specific to the context of pregnancy is also required to elucidate if the

same stress-reward pathways involved in emotional eating behaviors in the non-pregnant state act differently under gestational physiology.

Strengths of this study include ghrelin measurements in both the fasting and postprandial state, however, the main limitation of this study is the measurement of total ghrelin rather than the active form, acylated ghrelin, which is responsible for the appetite-stimulating effects. Therefore, we cannot conclude with certainty that the observed associations (or lack thereof) between our dimensions of stress and ghrelin concentrations translate to effects on appetite cues in this population of pregnant women with overweight and obesity. Another strength is that we characterized psychological states that reflect day-to-day perceptions of stress as well as acute psychological stress reactivity in pregnant women so that differences in stress duration in relation to ghrelin could be explored. However, given the absence of other available tools to measure momentary psychological stress, a limitation is that we relied on changes in maternal negative affect as a proxy measure of psychological stress reactivity to the TSST. While we also included measurement of physiological stress reactivity using salivary cortisol under lab-based conditions, we did not measure physiological stress levels in everyday settings and therefore cannot conclude that cortisol and ghrelin are unrelated across different situations in which stress may occur. Reliance on the TSST as an acute stress exposure may also be considered a study limitation. The TSST is one of the most common methods used to induce acute psychological stress and has been previously used in pregnancy cohorts (Deligiannidis et al., 2016). Although the TSST mimics a real-world acute stress situation, not all participants may find the TSST stressful. While we did observe a significant difference in maternal affect state pre- and post-TSST, our physiological measure of stress reactivity (i.e., cortisol AUC) did not differ after accounting for gestational age. We attempted to address this by characterizing high and low cortisol responders, yet the limited sample size may be insufficient to adequately detect a relationship between acute stress reactivity and ghrelin concentrations, especially considering that the parent study was not powered to address this outcome. Another limitation was the lack of randomization for visit order, such that the second visit included the stress task for all participants. As a result, being later in the third trimester of pregnancy at the time of stress exposure may have impacted the results since ghrelin has been shown to decrease (Fuglsang et al., 2005) and cortisol to increase (Mastorakos and Ilias, 2003) with advancing gestation. Randomizing the visit order in future experimental studies of this nature could help account for the effects of gestational age on these biomarkers. Lastly, our focus on Hispanic pregnant women is a strength of the study as this population have been reported to experience high rates of psychological stress associated with acculturation and low-income levels (Chasan-Taber et al., 2020; Silveira et al., 2013a, 2013b). However, we did not assess the underlying socio-cultural and economic conditions or experiences that may be driving perceived stress in our population, which would be a worthwhile consideration for future work with respect to the influence on ghrelin concentrations in pregnancy. Perceived stress has also been positively associated with risk of impaired glucose tolerance in Hispanic pregnant women (Silveira et al., 2014), and the potential for stress-induced reward-related eating behaviors or poor diet quality to moderate this association deserves further research. Although our crossover study design strengthens the validity of our data for Hispanic pregnant women with overweight or class 1 obesity, our results cannot be generalized to non-Hispanic women or those with pre-pregnancy BMI in the normal weight or in the obesity class 2 and above categories.

## 5. Conclusions

Based on our findings, greater perceived stress among Hispanic pregnant women with overweight and obesity may contribute to higher ghrelin concentrations both in the fasted and postprandial state. However, counter to our hypothesis, exposure to and reactivity to a standardized acute stress induction task did not impact postprandial ghrelin concentrations in this population of women with overweight and obesity in the third trimester of pregnancy. The differential findings in this regard may reflect the differences in duration and type of stress captured by these constructs, such that the psychological burden of day-to-day perceived stress over a longer duration may be more influential on ghrelin concentrations compared to a temporary stress exposure in a controlled setting. Future studies with pregnant women may include larger sample sizes, randomization, measurements earlier in gestation, and evaluation of eating behaviors related to ghrelin concentrations. Furthermore, extensive characterization of the different types of psychological stressors that impact Hispanic pregnant women and those from other underserved populations is required to fully investigate the potential links between prenatal stress, ghrelin and eating behaviors and how these associations may be altered under different socio-cultural contexts. These approaches may help advance our understanding of the role of stress-induced ghrelin alterations and the potential impact on maternal health and pregnancy outcomes in diverse populations.

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## CRediT authorship contribution statement

KL and LG conceived and carried out the experiments. JM and KL analyzed data. All authors were involved in writing the paper and had final approval of the submitted and published versions.

## Declaration of interest

None.

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