



Brief report

## Impact of stress reduction instructions on stress and cortisol levels during pregnancy

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### Abstract

This pilot study examined whether giving stress reduction (SR) instructions to pregnant women would be effective in regulating stress, mood, and cortisol levels during pregnancy. Forty-one predominantly low-income Latina women, receiving prenatal services at a public county hospital, completed measures of stress and mood (depressive symptoms, positive and negative affect) and provided morning and evening saliva samples to measure cortisol prior to and after receiving SR instructions. We hypothesized that adherence to these SR instructions would result in lower levels of stress, negative mood states, and cortisol levels when compared to baseline values. Repeated measures ANOVA analyses demonstrated significantly lower levels of stress ( $P < 0.001$ ), lower symptoms of depression and negative affect ( $P < 0.01$ ), and lower levels of morning cortisol ( $P = 0.01$ ) under the SR condition, compared to baseline. Health behaviors that were engaged in during the SR condition and implications for prenatal health interventions are discussed.

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### 1. Introduction

Elevated levels of stress during pregnancy have been associated with a number of prenatal complications and negative birth outcomes, such as pre-term labor, low infant birthweight

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and APGAR scores, and increased use of neonatal intensive care unit services, even after controlling for parity and obstetric risk factors (Dole et al., 2003; Paarlberg et al., 1995; Williamson et al., 1989). Elevated levels of stress hormones, such as cortisol, have been suggested as a potential biological mechanism leading to health complications in pregnant women (Austin and Leader, 2000; Sandman et al., 1997; Wadhwa et al., 2001). Support for this relationship comes from studies showing that pregnant women with elevated cortisol levels are more likely to have pre- and post-mature births, have infants with low APGAR scores, or have infants who need resuscitation assistance at birth (McCool et al., 1994; Ponirakis et al., 1997); however, additional research is needed to identify the critical threshold by which elevated prepartum cortisol levels become dangerous for the mother and infant. Nevertheless, given the number of health complications associated with elevated stress and cortisol levels during pregnancy, studies examining the efficacy of prenatal interventions that target these risk factors are needed.

The purpose of this pilot study was to assess whether giving stress reduction (SR) instructions to pregnant women would be effective in regulating stress, mood, and cortisol levels during pregnancy. We hypothesized that adherence to these instructions would result in improvements in stress and mood, and lower cortisol levels, when compared to baseline values.

## 2. Method

### 2.1. Participants

Pregnant women participating in a longitudinal study of risk identification for maternal depression were recruited for the current study. Women were approached following their medical appointment in an outpatient prenatal clinic of a public county hospital in California. Study eligibility criteria, based on a review of medical records, included women that were: (1) 18 years of age or older; (2) 6–32 weeks pregnant; (3) fluent in either Spanish or English; and (4) without any current major medical or substance abuse problems. Following their appointment with their health care provider, eligible women participated in a brief session in which the study protocol and issues of confidentiality were described in detail. Those interested provided written informed consent and were included in the study. Of the 60 eligible participants approached for the study, 12 (20%) were lost to contact and seven (12%) did not return their saliva samples, resulting in a sample size of 41. Participants did not differ from non-participants by age, parity status, gestational age, education, or income level.

Of the 41 participants, a large proportion were Spanish-speaking Latina women (81%), who were born in Mexico (57%), and were on average 19 years of age when they immigrated to the US (S.D. = 7.4). Participants were approximately 26 years of age (S.D. = 4.6), in their 27th week of pregnancy (S.D. = 8.0; 70% in second trimester), and had less than 12 years of education ( $M = 11$  years, S.D. = 3.5). Most participants were married or living with a partner (71%), had one or more children (62%), and were unemployed (68%). Eighty-three percent of participants' partners were employed, with 68% earning an annual income under \$20,000.

## 2.2. Procedure

After participants completed the informed consent form, they took part in a 45-min session in which a research assistant explained and demonstrated how to collect saliva samples and complete the stress and mood measures at home over a 10-day period, under two separate conditions: non-stress reduction (NSR) and stress reduction (SR) condition.

The NSR condition was a baseline collection, occurring under daily living conditions, where participants were instructed to collect saliva samples (45 min after waking up; 8:00 p.m.) and complete stress and mood measures (depressive symptoms, positive and negative affect) over the span of 2 days (see Table 1 for average collection time). Following the first 2 days of collection, participants returned the saliva samples and stress and mood measures to research staff. They were then administered a questionnaire assessing their behaviors on the days of collection, as well as any medications they were taking, to screen for cortisol-altering substances, such as certain foods or anti-inflammatory medications.

Participants were then asked to repeat the cortisol collection procedures and complete the stress and mood measures at home under a second, SR condition consisting of adhering to the following instructions: “Eliminate things that are stressful and/or participate in things that increase your level of relaxation.” Participants were instructed to begin planning for the SR condition the night before the ninth day of collection, and to continue engaging in stress reduction behaviors the following day until collection was completed. On the 10th day, participants returned the saliva samples and stress and mood measures to research staff and

Table 1  
Means and standard deviations of stress and mood measures and salivary cortisol for pregnant women, by intervention condition

	Intervention condition	
	Non-stress reduction day <i>M</i> (S.D.)	Stress reduction day <i>M</i> (S.D.)
Stress and mood measures		
Stress rating		
Morning	25.4 (21.1)	13.4 (17.2)**
Evening	25.3 (20.8)	13.9 (16.3)**
CES-D	17.9 (10.5)	12.4 (8.2)*
PANAS		
Positive affect	2.8 (0.8)	2.9 (0.8)
Negative affect	1.6 (0.6)	1.4 (0.4)*
Salivary cortisol		
Cortisol (log scores)		
Morning	1.07 (0.19)	0.96 (0.29)*
Evening	0.58 (0.40)	0.58 (0.39)
Collection time (hour:minutes (minutes))		
Morning	8:46 (59)	8:48 (60)
Evening	8:09 (26)	8:02 (20)

\*  $P \leq 0.01$ .

\*\*  $P \leq 0.001$ .

were then administered a questionnaire assessing their behaviors on the day of collection, as well as the extent they felt they were able to have a day of stress reduction.

For both study conditions, participants were instructed not to smoke, eat, brush their teeth, use mouthwash, exercise, or take medication for at least 60 min before collecting their saliva samples in order to avoid contamination by factors known to interfere with cortisol analysis (Gröschl et al., 2001). After each morning and evening collection, participants were instructed to immediately store their samples in the freezer and call the research office to leave a voicemail message indicating the saliva sample had been collected.

### 2.3. Measures

#### 2.3.1. Stress measure

A 100-point analog scale (0: not at all stressed, 100: extremely stressed) used to represent how stressed participants felt at the time of each cortisol collection.

#### 2.3.2. Mood measures

Center for Epidemiologic Studies—Depression Scale (CES-D; Radloff, 1977), a 20-item, self-report measure assessing affective and somatic symptoms of depression, with higher scores reflecting greater symptoms of depression ( $\alpha = 0.91$ ); Positive and Negative Affect Schedule (PANAS; Watson et al., 1988), a 20-item measure of both positive and negative dimensions of mood, with higher scores reflecting greater frequency of positive and negative mood states (positive affect:  $\alpha = 0.91$ ; negative affect:  $\alpha = 0.86$ ). All materials were provided in either Spanish or English, based on participant preference.

#### 2.3.3. Cortisol collection questionnaire

Following the NSR and SR conditions, questions related to the frequency and duration of physical activity, and use of any cortisol-altering substances during collection were asked. Following the SR condition, participants were asked to rate whether or not they were able to have a SR day, to what extent, and to list what activities helped or did not help in having a SR day.

#### 2.3.4. Cortisol assays

Participants collected saliva using a Salivette sampling device from Salimetric to obtain levels of cortisol (Sarstedt, Newton, NC). This device consisted of a small, absorbent cotton swab, which participants chewed for at least 2 min or until the cotton swab became soaked with saliva. This cotton swab was then placed into a small plastic tube and stored at  $-20^{\circ}\text{C}$  in the participants' freezers until analysis. Once these samples were returned to the hospital, they were analyzed in the hospital's laboratory using the same radioimmunoassay kit to keep all procedures constant across samples. Samples were thawed and centrifuged at  $1500 \times g$  for 30 min, resulting in a clear supernatant, which was frozen and stored at  $-20^{\circ}\text{C}$  until assayed. Each sample was assayed in duplicate using the same salivary cortisol kit, with the mean of duplicates reported in micrograms per deciliter. A standard curve and a low and a high control were run with each assay. Cortisol levels were determined by employing a time-resolved enzymatic immunoassay. The intra- and interassay coefficients of variation were both under 8%.

## 2.4. Analysis

Morning and evening salivary cortisol levels, collected over the first 2 days, were tested for reliability, resulting in an intraclass correlation coefficient of 0.66 and 0.78 ( $P < 0.001$ ), respectively; therefore, the three morning samples and two evening samples were averaged. Similarly, due to high reliability between morning ( $r = 0.93$ ) and evening ( $r = 0.93$ ) stress ratings ( $P < 0.001$ ) on the first 2 days, both the morning and evening stress ratings were averaged.

The distribution of morning and evening cortisol levels for both the NSR and SR conditions was positively skewed, and so a logarithmic transformation (base 10, cortisol values converted from  $\mu\text{g/dl}$  to  $\text{nmol/l}$ ) was conducted to ensure that scores approximated a normal distribution. Stress, mood (depression, positive and negative affect), and cortisol (morning and evening) levels were compared across the NSR and SR conditions using repeated samples ANOVA. Given that cortisol levels naturally rise during pregnancy (Harris, 1996), and that participants entered our study at different points in their pregnancy, gestational age was controlled for when analyzing changes in cortisol levels over time. Finally, the association among stress, mood, and cortisol levels, for both conditions, was estimated and tested using Pearson's correlation analyses.

## 3. Results and discussion

### 3.1. Adherence to cortisol protocol and stress reduction instructions

We observed that 39 of the 41 participants (95%) adhered to the protocol procedures by recording the dates and times they collected cortisol samples at home and leaving a voicemail to research staff when the protocol was completed (see Table 1 for average collection times; range of missing data: 0–7%). None of the 41 participants reported consuming any foods or taking any medications known to interfere with cortisol levels. Of 38 responses, 95% of participants reported being able to have a SR day. Of two respondents that indicated they were not able to have a SR day, one person indicated it was due to having gastrointestinal problems that day, while the second attributed it to having to work. The latter respondent was only one of 13 employed women (8%) that were not able to have a SR day. When asked how stressful the SR day was compared to a usual day (5-point Likert scale, “not at all” to “extremely”), 58% of participants reported “not at all stressful or very slightly,” 34% reported “a little stressful,” and 8% reported “moderately stressful.”

### 3.2. Health behaviors

At baseline, 55% of participants reported engaging in some form of exercise, with 95% of those participants identifying walking as being the most common exercise activity. These participants reported walking an average of 3–4 days a week for 30-min bouts. Only two participants (5%) reported exercising on the NSR day. For the SR condition, participants were asked what activities were most helpful in reducing stress that day. Content analysis of participant responses demonstrated that 58% engaged in household activities (e.g., cooking, watching TV, reading, listening to music), 51% engaged in outdoor activities (e.g.,

getting out of the house, walking alone or with others, shopping), 35% sought out positive interactions with others (e.g., playing with children, visiting or speaking with friends and family), and 8% reported modifying their thoughts (e.g., decreasing frequency of worrisome thoughts, thinking more optimistically). Approximately 43% of participants reported engaging in more than one of these activities and 21% reported that it was helpful to be conscious of knowing they were supposed to have a day of stress reduction.

### 3.3. *Impact of stress reduction instructions on stress, mood, and cortisol levels*

Repeated measures ANOVA analyses demonstrated significant decreases in morning ( $F(1, 40) = 27.9, P < 0.001$ ) and evening stress ratings ( $F(1, 37) = 13.5, P = 0.001$ ), symptoms of depression (CES-D;  $F(1, 39) = 11.6, P = 0.002$ ), and negative affect (PANAS;  $F(1, 40) = 9.2, P < 0.01$ ) under the SR condition, compared to the NSR condition (Table 1). In addition, a  $2 \times 3$  (condition  $\times$  trimester of pregnancy) repeated measures ANOVA revealed significant decreases in morning cortisol levels ( $F(1, 38) = 7.1, P = 0.01$ ) after controlling for gestational age. The sphericity assumption associated with multiple repeated measures analyses was tested using Mauchly's test of sphericity and results indicated it was not violated.

Positive affect and evening cortisol levels were not significantly different across conditions. The non-significant difference for evening cortisol levels appeared to be due to differences in gestational age ( $F(2, 38) = 4.8, P < 0.05$ ). Post-hoc analyses demonstrated that participants in their first trimester of pregnancy had significant decreases in evening cortisol levels ( $P < 0.01$ ), compared to participants in their second or third trimester who demonstrated slight increases in cortisol from the NSR to the SR condition.

Independent samples *t*-tests comparing exercisers versus non-exercisers on stress, mood, and cortisol levels, were not significant. These results are not consistent with previous findings demonstrating that pregnant women who report engaging in leisure-time physical activity have better overall psychological well-being than pregnant women who do not engage in physical activity (Da Costa et al., 2003); this inconsistency may be due to differences in sample size and/or the quantity of time spent on physical activity. Future studies are needed to examine the impact of exercising during pregnancy on cortisol levels.

Independent samples *t*-tests were also conducted to compare participants' ratings of how stressful the SR day was compared to a usual day on stress, mood, and cortisol levels. Results showed that participants who rated the SR day as "not at all stressful or very slightly stressful" reported lower symptoms of depression than participants who rated the SR day as "a little or moderately stressful" ( $P < 0.01$ ).

Approximately 62–77% of employed and unemployed women in our sample completed the NSR and SR conditions on a weekday; however, there were no significant differences on stress, mood, or cortisol levels for participants who completed the NSR or SR condition on a weekday versus weekend.

### 3.4. *Relationship among stress, mood, and cortisol levels*

In general, none of the correlations testing the association among stress, mood, and cortisol levels across both conditions were statistically significant. However, evening cortisol

levels for the SR condition were positively and significantly associated with positive affect for both the NSR ( $r = 0.35$ ,  $P < 0.05$ ) and SR ( $r = 0.34$ ,  $P < 0.05$ ) conditions (opposite of the hypothesized direction).

In summary, our results are the first to examine the impact of a prenatal intervention aimed at regulating stress *and* cortisol levels. Results of this pilot study suggest that when pregnant women are given instructions to reduce stress on a given day, they are successful in decreasing self-perceived levels of stress and mood, as well as morning cortisol levels. In particular, this simple intervention was effective for a predominantly low-income, Latina sample, and suggests that future studies try to replicate our findings in other populations. This pilot study is limited by: (1) small sample sizes for employed pregnant women and women in their first and third trimesters of pregnancy, and (2) the fact that order of the NSR and SR conditions was not counterbalanced. Nevertheless, future studies might tailor similar health promotion efforts on coping strategies that target psychosocial and biological risk factors during pregnancy.

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