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Physiologic responses to cognitive challenge during pregnancy: effects of task and repeat testing

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Abstract

Physiological responses to stress during pregnancy are believed to influence birth outcomes. Researchers have studied pregnant women in laboratory stressor paradigms to investigate these associations, yet normative data on cardiovascular and respiratory responses to laboratory challenge during pregnancy are not yet established. To begin to establish such normative data, this study examined the effects of task and repeat stressor exposure on reactivity in third-trimester pregnant women. Thirty-one healthy pregnant women (mean age = 27 years; range 18–36) between the 33rd and 39th week of pregnancy, were instrumented for continuous electrocardiography, blood pressure (BP), and respiration data. Subjects rested quietly for a 5-min baseline and then performed both a mental arithmetic stressor and a Stroop color–word-matching task, each 5 min in length and each followed by a 5–min recovery period. The order of the tasks was counterbalanced. After each 5-min period, subjects rated the period on a 10-point stress scale. Averaged across task type and challenge period, systolic and diastolic BP and respiration rate increased significantly in response to cognitive challenge, but heart rate (HR) did not. When data were examined for task and period effects, the following results emerged: the Stroop task elicited significantly greater systolic BP and HR reactivity than the arithmetic task, yet subjects rated the arithmetic task as more stressful. Averaged across task type, subjects showed greater systolic BP reactivity during the second challenge period compared to the first. Finally, women's BP tended to drift upward and did not return to baseline during the first recovery period. These findings indicate that averaging data across tasks and periods can obscure the time course of response patterns that may be important in the study of associations between maternal stress and perinatal development, as well as in other research on reactivity to repeat stress exposure.

Keywords: Cardiovascular reactivity; Pregnancy; Blood pressure; Stress; Task type; Repeat stress exposure

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1. Introduction

The study of physiological responses to stress during pregnancy is the focus of three different lines of research: (1) the role of life stress in adverse birth outcomes (Wadhwa et al., 1993, 1998; Lobel, 1994; Sandman et al., 1994, 1999; Copper et al., 1996; McCubbin et al., 1996; Pressman et al., 1998); (2) the role of life stress in neurobehavioral development (Groome et al., 1995; DiPietro et al., 1996; Pressman et al., 1998; Monk et al., 2000); and (3) the role of reproductive hormones in regulating autonomic nervous system reactivity (Matthews, 1989; Matthews et al., 1992). Researchers have begun to use standard laboratory mental stress tests to explore these questions (Matthews et al., 1992; McCubbin et al., 1996; Monk et al., 2000). Many studies have investigated normative cardiovascular response patterns to laboratory stressors and the parameters that modulate reactivity in men and non-pregnant women (Linden, 1985; Linden et al., 1985; Linden, 1991; Kamarck et al., 1992). However, despite recent government mandates to include women of childbearing age in research protocols, there are no studies characterizing the effects of specific test conditions on cardiovascular reactivity to laboratory stressors in pregnant women.

This lack of studies is especially unfortunate in light of the enormous physiological and psychological changes that occur during pregnancy. Dramatic alterations in hemodynamic, hormonal, and endocrine functioning are induced, including a 45% rise in blood volume, 30–50% increase in cardiac output [due both to increases in stroke volume and heart rate (HR)], a decrease in systemic vascular resistance (Monga and Creasy, 1994), a five-fold increase in estradiol and a two-and-a-half-fold increase in the level of circulating cortisol (Monga and Creasy, 1994). These and other changes occur at different times and at varying rates over the course of pregnancy. For example, blood pressure (BP) begins to decrease as early as the seventh week of pregnancy, reaching its lowest point between 24 and 32 weeks, and then rises to pre-pregnancy levels near term. Levels of corticotrophin-releasing hormone (CRH) binding protein are relatively constant throughout pregnancy until the 36–38th week, when they fall by approximately 60%, resulting in a dramatic increase in bioactive CRH during the latter part of pregnancy (Perkins et al., 1995). Psychologically, pregnant women report greater pregnancy-specific stress in the first and third trimesters compared to the second trimester (Da Costa et al., 1999), and elevated levels of non-specific anxiety and depression in the latter part of pregnancy (O'Hara et al., 1990; Da Costa et al., 1999). Up to 70% of women report depressive symptoms throughout the course of pregnancy, although this figure is confounded by some of the characteristics of pregnancy that overlap with features of depression (e.g. fatigue and sleep changes) (Klein and Essex, 1995). Pregnant women also describe difficulties with concentration and memory (Parsons and Redman, 1991; Keenan et al., 1998; Janes et al., 1999).

These biological and psychological alterations of pregnancy suggest that a unique profile of reactivity to laboratory challenge might be expected in this group. Furthermore, methods that are most effective and reliable for eliciting a stress response from non-pregnant subjects may not be optimal for pregnant women.

Although the three published reports on mental stress reactivity during pregnancy (Matthews et al., 1992; McCubbin et al., 1996; Monk et al., 2000) all used similar cognitive tasks (i.e. mental arithmetic and Stroop), there were several methodological differences among the studies. Key differences include: (1) stage of pregnancy; (2) duration and number of challenges; and (3) statistical treatment of the reactivity scores. In our work, we used a 5-min mental challenge period with only third trimester pregnant women. In contrast to the other two studies based on either a similar but shorter (2 min) mental stressor with second trimester pregnant women, or a similar but longer (10 min) mental stressor with women in all stages of pregnancy (Matthews et al., 1992; McCubbin et al., 1996), we found no change in maternal HR and we found smaller changes in diastolic blood pressure (DBP). In our study and the one by Matthews et al., the increase in systolic blood pressure (SBP) was comparable (approx. 7 mmHg increase). However, as with most psychophysiology studies of laboratory stressor reactivity (Kamarck et al., 1992), the Matthews et al. results were based on aggregated scores (averaging reactivity across two stressor periods), while ours was in response to a single challenge period.

These differences in findings raise important methodological questions concerning tests of pregnant women. Does the task matter? How long should the stressor period last? Are there changes in reactivity with repeated stress periods? Answers to these questions have implications for the establishment of optimal designs for studies of cardiovascular reactivity during pregnancy.

The overall aim of this study was to begin to establish normative cardiovascular and respiratory responses to laboratory challenge during pregnancy and to characterize potentially relevant parameters that could modify the results. The specific aims were to examine: (1) differences between Stroop versus arithmetic; and (2) multiple testing effects (first versus second challenge period in third trimester pregnant women exposed to laboratory stressors).

2. Methods

2.1. Subjects

Through posted announcements and signs in obstetricians' offices, 36 pregnant, non-smoking women with singleton fetuses ranging in gestational age from 33–39 weeks were recruited at the Columbia–Presbyterian Medical Center (CPMC). Women were excluded from the study if there were any maternal or fetal complications, including hypertension, diabetes mellitus, suspected fetal growth restriction, or a fetal structural anomaly on ultrasound. None of the subjects reported smoking while pregnant, nor drinking more than two glasses of wine throughout the entire pregnancy. The racial mix of the group was 53% Latina, 22% Caucasian, 17% African American, 3% Asian and 5% were of different races. For all subjects, English was the primary language. The mean maternal age was 27 years (range 18–36). Nearly 40% of the sample had had some college education and 28% had earned post-graduate degrees; 61% were married; 65% were nulliparous; and 55% were working full-time outside the home. Because this sample was drawn from an urban hospital and included doctors and support staff, as well as patients, the average annual family income was above the national average, yet included women receiving public assistance (M = $59 666; range $12 000–200 000). This study was approved by the Institutional Review Board, CPMC. Informed consent was obtained from each subject.

At the time of testing the average fetal gestational age was 36 weeks (S.D. = 1.5, range 33–39 weeks) as determined by a combination of last menstrual period and sonogram. All fetuses were born after 35 weeks (mean = 40, range 35–42 weeks) and none was small for dates. The average weight at birth was 3427 g (range 2298–3944 g).

2.2. Procedure

Women made a single visit to the laboratory that began at approximately 11:00 h and ended at 13:00 h. After a review of the experimental procedures, they were interviewed briefly about their pregnancy and living situation. Electrodes for electrocardiographic (ECG) and respiration monitoring then were attached near their right shoulder, on the left anterior axillary line at the 10th intercostal space and in the right lower quadrant. An additional set of three electrodes was placed on the women's abdomen to record fetal ECG (see below). The subject then was placed in a semi-recumbent position. A Finapres BP cuff (Ohmeda) was placed on the middle finger of the non-dominant hand and a numeric keypad for responding to the tasks was secured in a comfortable position relative to the dominant hand. Subjects could not see the keypad during either task, but could identify the keys by feel. Although the decision to use a keypad was based on our decision to follow standard procedures used in our laboratory and others (e.g. Sloan et al., 1997) and was not designed to increase subjects' stress responses, it is possible that it had that effect. An ultrasound transducer (Advanced Medical Systems) was placed on the subject's abdomen as an alternative method to record fetal HR. BP readings from the Finapres were checked against a manual sphygmomanometer; adjustments in the Finapres cuff fitting and non-dominant hand position were made until the difference between the sphygmomanometer and Finapres readings was less than ± 10 mmHg SBP. Subjects were given instructions regarding the cognitive tasks and were allowed to practice each task for 1 min. At the start of data collection, subjects were instructed to remain silent throughout the procedures. Subjects rested quietly for a 5-min baseline and then performed both a mental arithmetic stressor and a Stroop color–word task, each 5 min in length and followed by 5-min recovery periods. The order of the tasks was counterbalanced.

2.3. Cognitive stressors

2.3.1. Mental arithmetic

In this task, the subjects were presented with a four-digit number on a computer monitor and were instructed to subtract serially by seven starting with this number, which disappeared after the first answer was entered. Subjects entered their answers on the numeric keypad. Their answers were not visually displayed, nor did they receive feedback on the correctness of their responses. At 1-min intervals, the experimenter gave the subject verbal prompts, e.g. to work faster. This task was not paced by the computer, but the subjects were instructed to subtract as quickly and as accurately as possible.

2.3.2. Stoop color–word task

In this version of the Stroop task, the subjects were presented with color names (blue, green, yellow and red) in colored letters that were either congruent or incongruent with the names. The subject's task was to press the key on the keypad that corresponded to the color of the letters as indicated by a legend on the computer screen. The task was paced by the computer, and an incorrect response or failure to respond rapidly enough resulted in a message indicating 'incorrect' on the screen. At 1-min intervals, the experimenter gave the subject verbal prompts, e.g. to work faster.

2.3.3. Self-report of stress

The subjects were asked to rate the stress they experienced on a 1–10 (none at all to extreme stress, respectively) scale after each of the four periods of the experiment (a rating of subjective stress was not elicited after the second recovery period).

2.4. Acquisition and processing of maternal ECG and respiration signals

Electrodes were attached to a heart/respiration monitor (Hewlett-Packard 78292A) and the analog ECG and respiration impedance waveforms were digitized and collected by a microcomputer. Analog ECG signals were digitized at 500 Hz by a 16-bit A/D card (National Instruments 16XE50). Specially written software was used to mark R-waves and create files of RR-intervals. Artifacts in the RR-interval series were defined as values below 0.4 s (HR $>$ 150 beats/min) or above 1.5 s (HR $<$ 40 beats/min). When artifacts were detected, the RR-interval file was examined. Artifacts were rejected or corrected following established procedures (Berntson et al., 1990). Respiration was sampled at 50 Hz. Post-acquisition software was used to mark the peaks and troughs of the impedance waveform. These marks were verified by visual inspection and were then used to calculate respiratory rate.

2.5. Acquisition and processing of maternal BP signals

BP was measured on a beat-to-beat basis by an Ohmeda Finapres 2300 monitor. The analog pressure waveform was digitized at 250 Hz. Systolic BP (SBP) and diastolic BP (DBP) values were marked by peak/trough detection software and errors in marking were corrected interactively.

2.6. Data reduction and analyses

Due to technical failure, one of the 36 subjects did not undergo a complete laboratory session.

Two subjects were omitted from data analyses as outliers, because their baseline HRs were 65 and 119 beats/min respectively, which were at least $2 \times$ S.D. from the mean (M = 94.51 beats/min; S.D. = 11.35) and two others were omitted from analyses because their baseline SBPs were 95 and 171 mmHg, respectively, which were at least $2 \times$ S.D. from the mean (M = 127.12 mmHg; S.D. = 16.64). This report is based on data from the remaining 31 subjects.

In addition, the following data were lost due to equipment and experimenter errors: an entire set of BP data was lost for one subject; BP data during recovery were lost for another subject; and stress ratings were unrecorded for two subjects.

2.6.1. Comparison groups

Sixteen subjects underwent the Stroop challenge as their first test and the arithmetic task as their second challenge (ST → AR); the order was reversed for the other 15 subjects, i.e. arithmetic first, Stroop second (AR → ST).

2.7. Statistical analyses

Subjects' responses for both first and second mental challenge periods were changes from baseline values, which were computed as the average of min-3 and -4 of the 5-min baseline period. The first and second minute were excluded, as subjects were often still adapting to instrumentation and within-subject variability was high. The fifth minute was excluded, as towards the end of this period subjects were told to prepare for the upcoming mental challenge. Reactivity to the tasks were computed as within-subject change scores, the difference between the mean for cardiovascular values during each of the 5-min stressors and the average of min-3 and -4 of the initial baseline period. The correlations between baseline and delta scores were non-significant, except for respiration ($r = -0.50$, $P < 0.01$ for first stressor; $r = -0.43$, $P < 0.05$ for second). Thus, statistical control for baseline–reactivity relationships, such as the use of residualized scores, was unnecessary, except for respiration data (Llabre et al., 1991).

For the first set of analyses, data for the two stressors were averaged across period and task type, and t-tests were used to analyze differences from the baseline. To answer questions about the differential effects of the individual tasks and repeat testing, repeated-measure ANOVAs were used to analyze the data generated from the crossover design.

3. Results

3.1. Self-reports of stress

At baseline, the subjects reported a level of 2 (± 1.8) on the 10-point self-report stress scale. The mean self-reported level of stress during the challenge tasks (averaged across task type and challenge period) was 6.4 (± 1.8), a significant increase ($P < 0.001$).

3.2. Cardiovascular and respiratory responses to cognitive challenge

In Table 1, values for HR, SBP, DBP, and respiratory rate averaged across task type and challenge period are presented for baseline, task, and change scores. BP and respiration rate increased significantly in response to cognitive challenge, but HR did not change significantly.

3.3. Self-reports of stress: effects of task and repeat testing

There was no significant difference between groups (ST → AR versus AR → ST) on baseline ratings of self-reported stress. A repeated-mea-

Table 1
Mean ($\pm$S.D.) baseline, tasks (averaged), and delta values of HR, BP, and respiration rate

Parameter	Baseline	Tasks (averaged)	Delta Score
HR (beats/min)	95.1 ± 9.9	96.6 ± 10.0	1.5 ± 6.4
SBP (mmHg)	126.8 ± 14.6	135.3 ± 15.2	$9.8 \pm 10.1^{***}$
DBP (mmHg)	76.7 ± 10.3	82.2 ± 10.4	$5.4 \pm 6.1^{***}$
Respiratory rate (counts/min)	18.6 ± 5.2	22.5 ± 6.1	$3.9 \pm 7.4^{**}$

$^{**}P < 0.01$.
$^{***}P < 0.001$.

Table 2
Mean (±S.E.) of HR, BP, and respiration rate changes by task type

Parameter	Task		P
	Stroop	Arithmetic	
HR (beats/min)	2.5 ± 4.6	0.56 ± 4.5	0.01
SBP (mmHg)	12.5 ± 7.7	7.2 ± 9.2	0.05
DBP (mmHg)	6.5 ± 5.0	4.2 ± 5.8	NS
Respiratory rate (counts/min)	4.4 ± 6.2	3.3 ± 4.7	NS

sure ANOVA for self-report stress ratings indicated that there was a main effect for task on stress ratings, $F(1,27) = 10.84$, $P < 0.01$. Third trimester pregnant women rated the arithmetic task as more stressful than the Stroop task. There was no significant period by task interaction, indicating that subjects found the arithmetic task more stressful, regardless of whether it was the first or second stressor.

3.4. Cardiovascular and respiratory reactivity: effects of task

Table 2 presents the results from repeated measures ANOVAs based on the crossover design. There were no significant differences between groups (ST → AR vs. AR → ST) on any of the baseline measures. In addition, subjects' HR and respiration rate values did not differ significantly between the baseline and first recovery period (the rest period prior to the second stressor period).[1] However, this was not true for the BP values, as will be discussed below.

Repeated-measures ANOVAs showed significant main effects of task for SBP [$F(1,28) = 4.36$, $P < 0.05$] and HR [$F(1,29) = 10.75$, $P < 0.01$]. When averaged across the two challenge periods, the Stroop task elicited significantly greater SBP and HR reactivity than the arithmetic task. There were no differences in DBP and respiration rate reactivity between the Stroop and arithmetic tasks. There were no significant interactions between task and challenge period, indicating that the effects of Stroop versus arithmetic on SBP and HR change were not influenced by the sequence in which the tasks were given.

3.5. Cardiovascular and respiratory reactivity: effects of repeat testing

When subjects' responses were collapsed across tasks, subjects showed greater SBP and DBP reactivity during the second challenge period compared to the first [$F(1,28) = 9.06$, $P < 0.01$ and $F(1,28) = 11.02$, $P < 0.01$, respectively] (see Figs. 1 and 2). Although there was a trend for HR reactivity to be larger during the first compared to the second stressor period (2.1 ± 6.3 vs. 0.93 ± 6.9 beats/min; $P = 0.09$), there were no differences in respiration rate reactivity between these two periods. Stressor period did not interact significantly with task in these analyses, indicating that the effects of period on BP reactivity were not influenced by the sequence in which the tasks were given. However, these results from the analyses of BP reactivity are confounded by the findings that, independent of task and task × period interactions, DBP was higher during the first re-

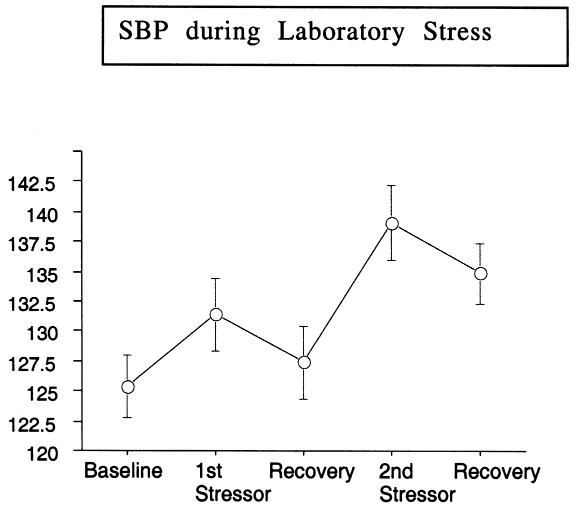

Fig. 1. Mean (±S.E.) SBP during each period, collapsed across tasks.

[1]Recovery was calculated as the average of min-3 and -4 of the recovery period. The first and second minute were excluded because subjects were still returning towards baseline, and min-5 was excluded because during this period subjects were warned to prepare for the upcoming mental challenge.

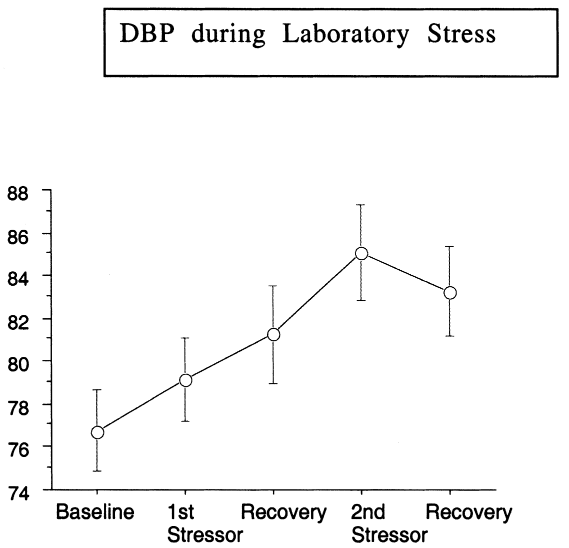

Fig. 2. Mean (±S.E.) DBP during each period, collapsed across tasks.

covery period compared to the initial baseline, and that there was a tendency for SBP to be elevated in the first recovery compared to the initial baseline [$F(1,27) = 9.31$, $P < 0.01$ and $F(1,27) = 3.08$, $P = 0.09$, respectively]. Since BP values were elevated immediately before the second stressor period, change scores to the second stressor taken from the initial baseline may be inflated. However, for SBP, but not DBP, post hoc analyses revealed that when subjects' responses were collapsed across tasks and compared to the most proximal baseline period (i.e. second stressor values compared to values from the first recovery period), subjects still showed greater SBP reactivity during the second challenge period compared to the first [$F(1,27) = 4.55$, $P < 0.05$].

4. Discussion

In this study, we assessed third trimester pregnant women in a standardized psychophysiology laboratory stress paradigm to examine effects of task type and multiple testing on cardiovascular and respiratory reactivity.

The average baseline systolic and diastolic blood pressures of these 31 pregnant women were above established norms for pregnant women (116–120 systolic; 70 diastolic) (Margulies et al., 1987; Reiss et al., 1987), but were comparable to data from non-pregnant subjects anticipating a mental challenge task (Sloan et al., 1997; Monk et al., 1999). The average baseline heart rate was only marginally higher than that recorded from supine pregnant women in previous studies (Clark et al., 1991; Monk et al., 2000), and consistent with the finding of elevated pulse rate during pregnancy (Ashmead and Reed, 1997).

When data from this sample were aggregated across task and testing period, subjects showed significant increases to laboratory stressors in SBP, DBP, and respiration rate, but not in HR. Except for HR, these findings are consistent with results from other laboratory studies in which data is averaged across two tasks. Two studies using similar challenge tasks (arithmetic and Stroop) have reported an increase in HR to laboratory stressors during pregnancy (Matthews et al., 1992; McCubbin et al., 1996) and one did not (Monk et al., 2000). However, in Matthews et al., there was a trend for the HR change to laboratory stress to be smaller in pregnant compared to non-pregnant women. Because these studies did not sample women at the same times during pregnancy, and because stroke volume declines over pregnancy while resting HR increases until its maximum is reached at approximately 32 weeks, these discrepancies could be due to differences in baseline physiologic states associated with the varying stages of pregnancy (Monga and Creasy, 1994).

In this sample, subjects increased their SBP and HR more in response to the Stroop than the arithmetic task. However, they reported that the arithmetic task was more stressful. In addition, pregnant women's BP did not return to baseline during the first recovery period and, for SBP, continued to rise during the second stressor period. Taken together, these data indicate that pregnant women in the third trimester show greater SBP and HR reactivity to the Stroop vs. arithmetic task and have elevated BP values during recovery, and, for SBP, during a second challenge period, compared to the initial baseline values. Finally, self-reported stress was not consistent with physiological reactivity.

Several hypotheses have been considered to account for the task, stress ratings, and period effects. It is possible that the average responses of this sample of women had more cardiovascular reactivity to Stroop compared to arithmetic because: (1) they were uncomfortable with arithmetic as a result of school and socialization experiences and disengaged from the demands of the task (Hall et al., 1999; Casey et al., 1997; Frome and Eccles, 1998); or, (2) subjects were less affected by the arithmetic task and therefore, less physiologically reactive to it, due to the lack of response feedback on our version of the arithmetic task (compared to our version of the Stroop task). However, whether women were disengaged or less affected by arithmetic, they rated it as more stressful. This finding is consistent with previous research showing that women tend to perform less well on serial subtraction compared to men, and to rate this task as more difficult and frustrating compared to other challenge tasks (Matthews, 1988). These results suggest the possibility that physiological reactivity may be affected more by feedback on performance than task difficulty. Consistent with this interpretation, in a study by Linden et al., 1985), greater physiological reactivity was also associated with increased performance pressure.

Prior studies indicate that BP tends to drift upwards (from a fractional amount to 3 mmHg) over successive baseline periods during laboratory stress paradigms (Jennings et al., 1992; Anderson et al., 1989; de Geus et al., 1990). However, in our sample of third trimester pregnant women, in addition to BP increasing during the first recovery (a second baseline period), post hoc analysis indicates that during a second stressor period, SBP was further elevated compared to the recovery *and* first stressor values.

Interactions between psychosocial and physiological processes may account for this BP data. Perhaps in the context of increased subsyndromal anxiety and stress during late pregnancy (Da Costa et al., 1999), exposure to a second challenge period is experienced as even 'more stressful' and pregnant subjects react accordingly. However, as with the inconsistent ratings of stress and task-type reactivity, this hypothesis is not supported by the stress ratings, as subjects did not report the second period as more stressful than the first. Alternatively, the drift upward in BP values from original baseline to recovery, and the increase in SBP reactivity from the first to the second stressor period, occurred over the course of 20 min, after approximately 45 min of instrumentation time. It is possible that a humoral stress response was initiated at the start of the testing session, which only became physiologically evident over the course of the testing session, and, in particular, during the second challenge period. Another possible hypothesis is based on the interaction of stress and reproductive hormones. While research on hormone replacement therapy suggests that estrogen increases the density and sensitivity of beta adrenergic receptors, leading to increased cardiac output as well as reduced vasoconstriction and diminished BP reactivity in laboratory challenge tests (Matthews et al., 1989; Grewen et al., 2000), the time course of this effect in response to repeated laboratory challenge has not been established. Estrogens are also known to prolong the biological actions of catecholamines seen in reduced and prolonged recovery from sympathetic activation in women compared to men (Iversen, 1973; Claustre et al., 1980; Drake et al., 1998). Perhaps under the conditions of repeated exposure to stress, the prolonged recovery from the effects of increasing levels of circulating catecholamines is observable in the drift upward of BP during a second rest period (recovery), and contributes to the further increase in SBP during the second challenge period. Furthermore, body position, in part, could account for pregnant women's BP data. For this paradigm, pregnant women responded to the second stressor after 15 min of semi-supine reclining. It is possible that in this position they experienced mild impedance to venous return that initiated a baroreflex-mediated pressor response. Thus, the greater BP reactivity during the second challenge period occurred in the context of increasing vasoconstriction that might account for some of the BP increase.

Our findings of greater BP values during recovery compared to an initial baseline and, for SBP, an increase over first stressor values, suggest that the data from Matthews et al. (1992) showing diminished DBP responses during second trimester pregnancy compared to pre-pregnancy values might be an *underestimate* of the magnitude of buffered reactivity during pregnancy to a *first* mental challenge, given that the data were based on reactivity values collapsed across a first and second stressor period.

Laboratory studies of stress reactivity generally aggregate subjects' responses across challenge tasks to improve reliability (Kamarck et al., 1992), or, alternatively, expose subjects to only one stressor (Sloan et al., 1996). Both approaches tend to produce data showing significant increases in subjects' HR and BP in response to a laboratory challenge. However, these approaches do not allow for the analysis of differential reactivity to various mental challenges, nor of the time course of reactivity to repeated stressors, despite evidence that certain mental challenges elicit greater cardiovascular reactivity (Linden et al., 1985; Kamarck et al., 1992) and that response magnitude varies as a function of period and/or sequence effects (Linden et al., 1985). When the reactivity data of the pregnant women from our study were aggregated, these differences in the magnitude of responses between the two tasks and over the course of the laboratory session were obscured.

The differences in response magnitude and in the time course of reactivity may have significant methodological implications for future studies using laboratory challenge paradigms to examine the effects of maternal stress on fetal outcome, as well as for those investigating the influence of reproductive hormones on stress reactivity. Use of only one 5-min stressor, the Stroop, may be sufficient to answer questions about the associations between maternal cardiovascular and respiratory stress responses and fetal behavior. This is an important finding, given that a semi-supine position is uncomfortable for pregnant women to maintain for a long duration. On the other hand, the use of repeated mental challenge tasks may be important for research examining the relationship between the influence of reproductive hormones on sympathetic activation, and, in particular, the effects of reproductive hormones on sympathetic reactivity in the face of multiple challenges over a longer period of time.

Specific characteristics of our laboratory challenge paradigm may make our results less generalizable to other laboratories and/or samples. For example, instead of verbalizing responses, our subjects used a keypad, which they were not permitted to view, to respond to them. Searching for keys may increase their stress responses. Our timed verbal prompts encouraging subjects to work faster may also have induced greater reactivity. Finally, as previously suggested, the difference between the Stroop and arithmetic tasks in feedback vs. no-feedback on performance may account for some of our results.

To begin to establish a methodological approach designed specifically to test the cardiovascular effects of stress throughout pregnancy, the examination of task and period effects should be applied to studies uniformly made up of women in the first and second trimesters of pregnancy. Identification of the most reliably potent stress-elicitor may obviate the need for two challenge periods. On the other hand, related to the renewed interest in the health-related significance of cardiovascular indices during the recovery period following laboratory stress (Linden et al., 1997; Rutledge and Linden, 2000), attention to the pattern of stress responses over two challenge periods may provide additional information on mechanistic and psychosocial processes that mediate overall stress reactivity. In particular, investigating the pattern of subjects' responses to repeated challenge might be a more ecologically valid paradigm for characterizing reactivity to repeated stress exposure. Finally, though in this study we looked at the generalizability of stress reactivity between tasks and the pattern of reactivity over the two stressor periods in a physiologically and psychologically distinct sample, third trimester pregnant women, our approach may have implications for laboratory stress studies with other populations.

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