Regulation of Sympathetic Nerve Activity During the Cold Pressor Test in Normotensive Pregnant and Nonpregnant Women

Charlotte W. Usselman, Paige K. Wakefield, Rachel J. Skow, Michael K. Stickland, Radha S. Chari, Colleen G. Julian, Craig D. Steinback,* Margie H. Davenport*

Abstract—Baseline neurovascular transduction is reduced in normotensive pregnancy; however, little is known about changes to neurovascular transduction during periods of heightened sympathetic activation. We tested the hypothesis that, despite an exacerbated muscle sympathetic nerve activity (microneurography) response to cold pressor stimulation, the blunting of neurovascular transduction in normotensive pregnant women would result in similar changes in vascular resistance and mean arterial pressure (Finometer) relative to nonpregnant controls. Baseline neurovascular transduction was reduced in pregnant women relative to controls when expressed as the quotient of both total resistance and mean arterial pressure and sympathetic burst frequency (0.32±0.07 versus 0.58±0.16 mm Hg/L/min/bursts/min, P<0.001 and 2.4±0.7 versus 3.6±0.8 mm Hg/bursts/min, P=0.001). Sympathetic activation was greater across all 3 minutes of cold pressor stimulation in the pregnant women relative to the nonpregnant controls. Peak sympathoexcitation was also greater in pregnant than in nonpregnant women, whether expressed as sympathetic burst frequency (+17±13 versus +7±8 bursts/min, P=0.049), burst incidence (+17±9 versus +6±11 bursts/100 h, P=0.03), or total activity (+950±660 versus +363±414 arbitrary units, P=0.04). However, neurovascular transduction during peak cold pressor–induced sympathoexcitation remained blunted in pregnant women (0.25±0.11 versus 0.45±0.08 mm Hg/L/min/bursts/min, P<0.001 and 1.9±1.0 versus 3.2±0.9 mm Hg/bursts/min, P=0.006). Therefore, mean arterial pressure (93±21 versus 99±6 mm Hg, P=0.4) and total peripheral resistance (12±3 versus 14±3 mm Hg/L/min) were not different between pregnant and nonpregnant women during peak sympathoexcitation. These data indicate that the third trimester of normotensive pregnancy is associated with reductions in neurovascular transduction, which result in the dissociation of sympathetic outflow from hemodynamic outcomes, even during cold pressor-induced sympathoexcitation. (Hypertension. 2015;66:00-00. DOI: 10.1161/HYPERTENSIONAHA.115.05964.)

Key-Words: blood pressure □ cold pressor test □ neurovascular transduction □ pregnancy □ sympathetic nerve activity

The risk of developing cardiovascular disease is lower in young women when compared with similarly aged men. However, pregnancy represents a period of increased susceptibility to disordered cardiovascular regulation for women. Pregnant women have an elevated risk for orthostatic intolerance. Moreover, ≤8% of pregnancies are associated with the development of de novo hypertension. The cause of such hypertensive pregnancy disorders remains unclear, and as a result, there exist few approaches by which clinicians can predict, screen, or treat the development of the hypertensive disorders of pregnancy (gestational hypertension and preeclampsia).

Previous data have shown that the vascular response to the cold pressor test (CPT) is blunted in normotensive women compared with women in the nonpregnant, postpartum state. However, an elevated pressor response to the CPT has been observed in women with or who subsequently develop preeclampsia. As such, the CPT has been suggested as a simple screening tool for pregnancy-related hypertensive disorders. The mechanisms driving these divergent responses remain unknown; however, sympathetic nervous system activity is a primary determinant of the pressor response to the CPT.

Differing vascular responses to the CPT may be mediated by altered sympathetic responses or altered translation of sympathetic activity into a vascular outcome (neurovascular transduction). Studies conducted over the past 2 decades have indicated, albeit not universally, that normotensive
pregnancy is associated with elevations in muscle sympathetic nerve activity, which occurs progressively across the trimesters of pregnancy. This increase in baseline sympathetic activity occurs concomitantly with elevations in circulating nitric oxide and reductions in vascular resistance. As a result, neurovascular transduction is reduced in normotensive pregnancy. Moreover, the vascular changes more than offset the increase in sympathetic activity because mean arterial pressure (MAP) is reduced in the first and second trimesters until a return toward prepregnancy blood pressures in the third trimester. However, it remains unclear whether sympathetic responsiveness or neurovascular transduction is altered during the CPT in normal pregnancy.

Therefore, the purpose of this study was to compare sympathetic responsiveness during the CPT and quantify the resultant hemodynamic outcomes between normotensive pregnant women and nonpregnant controls. We hypothesized that sympathetic responses to CPT would be exacerbated in pregnant women, indicating increased sympathetic reactivity. We further hypothesized that neurovascular transduction would be blunted in pregnant women, resulting in no differences in hemodynamic outcomes between pregnant and nonpregnant women.

Methods

Participants

Eighteen pregnant and 16 nonpregnant women (Table 1) participated in the research study after providing written, informed consent. All participants were normotensive nonsmokers and free of respiratory, cardiovascular, and neurological diseases. All pregnant women had singleton pregnancies and were tested in the third trimester (range, 29–40 weeks’ gestation; mean, 33±4 weeks). None of the participants reported a history of gestational diabetes mellitus, gestational hypertension, or preeclampsia. Postpartum follow-up with all pregnant women (maternal recall) confirmed that none of the participants developed these conditions during their pregnancy. All nonpregnant women were white, and all pregnant women except 2 Asian women were white. Nonpregnant women were tested during the early follicular phase of the menstrual cycle, with the exception of those taking oral contraceptives (Micronor, n=1; Yaz, n=1) and those using intrauterine devices inhibiting menses altogether (Minera, n=3); 45° from supine.

Experimental Protocol

Participants arrived at the laboratory at 8:00 AM after a 12-hour fast and having abstained from caffeine, alcohol, and strenuous exercise for 12 hours. Participants were fed a light, standardized meal and were instructed to void their bladder. Participants were seated in a semirecumbent position (sitting with upper body 45° from supine) in a dentist-style chair in a laboratory maintained at 20°C. After all instrumentation was complete, participants rested for a minimum of 10 minutes before baseline measures were begun. After 3 minutes of established baseline, the left hand was placed in ice water up to the wrist for 3 minutes.

In a clinical laboratory, the participant was asked to sit on a comfortable chair and place their hand in ice water. After a period of 3 minutes, the participant was asked to raise their hand and hold it in a horizontal position for 10 minutes. Throughout this period, the participant's heart rate and blood pressure were monitored. After this period, the participant was returned to their initial seated position and the process was repeated for a second 3-minute period.

Data Analysis

All data were stored for offline data analysis (PowerLab/16SP with LabChart 7; ADInstruments, Colorado Springs, Colorado). Baseline muscle sympathetic nerve activity signals were obtained in 12 pregnant and 12 nonpregnant participants. Responses to the 3-minute CPT were analyzed in 1-minute bins. In participants in whom adequate sympathetic recordings were maintained during the CPT (n=9 nonpregnant and n=9 pregnant), the 1-minute period corresponding to peak sympathoexcitatory period was selected for all subsequent analyses. The mode of peak sympathoexcitatory period was the first minute of CPT in nonpregnant women and the second minute of CPT in pregnant women. However, the mean of the peak sympathoexcitatory period was not different between nonpregnant (1.6±0.7) and pregnant women (2.1±0.8; P=0.1). Therefore, these bins (1st min in nonpregnant and 2nd min in pregnant women) were selected for analysis of hemodynamics in those participants in whom sympathetic recordings were not obtained.

Bursts of sympathetic activity were identified using semiautomated peak detection and confirmed by a trained observer. Sympathetic activation was quantified as burst frequency (bursts/min) and burst incidence (bursts/100 hb), as well as burst amplitude and total muscle sympathetic nerve activity (amplitudes×frequency). Burst amplitude was normalized within each participant by expressing all burst amplitudes relative to the largest burst observed at baseline, which was assigned a value of 100. To compare burst amplitudes at baseline, probability distribution curves were generated, and the median of these distributions were compared between groups.

Basal neurovascular transduction was calculated as TPR/sympathetic burst frequency and MAP/sympathetic burst frequency. In all hemodynamic and sympathetic outcomes, relative responses to CPT were calculated by subtracting baseline values from CPT values. Neurovascular reactivity during CPT was subsequently calculated by dividing the relative changes in TPR and MAP by the relative changes in sympathetic activity.

Table 1. Participant Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonpregnant</th>
<th>Pregnant</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation, weeks</td>
<td>n/a</td>
<td>33±4</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>29±6</td>
<td>31±3</td>
<td>0.2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>166±6</td>
<td>165±5</td>
<td>0.6</td>
</tr>
<tr>
<td>Pregnant BMI, kg/m²</td>
<td>n/a</td>
<td>27±3</td>
<td></td>
</tr>
<tr>
<td>Non/Prepregnant BMI, kg/m²</td>
<td>25±5</td>
<td>23±3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Data are mean±standard deviation. BMI indicates body mass index.
Statistical Analyses
Baseline values and peak CPT responses were compared between pregnant and nonpregnant women using Student’s unpaired t tests (all 2-tailed). The effects of CPT (ie, across all 1-minute time bins) and group and the interactions between them were examined using 2-way split-plot ANOVA. Alpha was set at 0.05 for all comparisons. Relationships between sympathetic nerve activity and vascular outcomes were assessed using linear regression analyses.

Results
Baseline
Baseline blood pressures were similar between pregnant women and controls (Table 2). Heart rate and Q were elevated in the pregnant women, whereas TPR was reduced relative to the nonpregnant controls. Sympathetic burst frequency, incidence, and total activity were higher in the pregnant women (Table 3). However, burst amplitude distribution curves were not different between pregnant and nonpregnant women (Table 3). Baseline sympathetic neurovascular transduction (TPR/sympathetic burst frequency) was significantly reduced in the pregnant women relative to nonpregnant (Figure 1; P<0.001). Similarly, transduction expressed as MAP/sympathetic burst frequency was reduced in pregnant women relative to nonpregnant (2.4±0.7) relative to nonpregnant women (3.6±0.8 mm Hg/ bursts/min; P=0.001).

Responses to the Cold Pressor Test
Hemodynamic and sympathetic responses across 3 minutes of CPT are shown in Figure 2. All variables were increased significantly across stages of the CPT. Heart rate was higher and TPR was lower across all time points in the pregnant women relative to the nonpregnant participants. A group×CPT interaction was observed in MAP, with pregnant women having lower pressure during the third minute of CPT (P=0.04). However, the relative changes in hemodynamics during the minute of peak sympathetic activation during CPT were similar between groups (Figure 3).

Sympathetic burst amplitude did not change during CPT and remained similar between groups (Figure 2). Sympathetic burst frequency, incidence, and total activity were higher during CPT in the pregnant women compared with the nonpregnant women. Further, during peak sympathoexcitation, the relative increases in sympathetic burst frequency, incidence, and total activity were also larger in the pregnant women (Figure 3).

Neurovascular transduction was reduced in both pregnant and nonpregnant women during the CPT (Figure 1) and remained blunted in the pregnant women relative to the nonpregnant women. In contrast to the difference between groups in neurovascular transduction at rest, neurovascular responsiveness during the CPT, expressed as the change in TPR for a given change in sympathetic activity, was similar between groups (P=0.6; Figure 1).

Sympathetic–Hemodynamic Associations
Under baseline conditions, no relationships were observed between sympathetic nerve activity and hemodynamic outcomes in either the pregnant or the nonpregnant women (Figure 4). In nonpregnant women, sympathoexcitation in response to CPT was associated with the development of significant, positive relationships between sympathetic burst incidence and both TPR and MAP. In contrast, prevailing sympathetic nerve activity remained unassociated with MAP, Q, or TPR in the pregnant women during CPT.

Discussion
The present study provides direct evidence for heightened sympathoexcitatory responses during pregnancy. Specifically, generalized increases in sympathetic nerve activity evoked through the application of the CPT were greater in pregnant women relative to similarly aged nonpregnant women. However, as at baseline, exaggerated sympathetic activity in the pregnant women was not associated with greater resultant vascular resistance or arterial pressure. Together, these data indicate that although the third trimester of normotensive pregnancy is associated with a pronounced increase in sympathetic reactivity, substantial reductions in neurovascular

Table 2. Baseline Hemodynamics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonpregnant</th>
<th>Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>72±6</td>
<td>82±10*</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>89±5</td>
<td>87±12</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>115±9</td>
<td>112±15</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>72±5</td>
<td>70±10</td>
</tr>
<tr>
<td>Cardiac output, L/min</td>
<td>6.2±1.0</td>
<td>7.5±1.5*</td>
</tr>
<tr>
<td>Total peripheral resistance, mm Hg/L/min</td>
<td>15±2</td>
<td>12±2*</td>
</tr>
</tbody>
</table>

Data are mean±standard deviation. *P<0.05 vs nonpregnant.
transduction occur concomitantly, resulting in the dissociation between sympathetic outflow and hemodynamic outcomes in pregnant women.

In the present study, we observed a marked sympathoexcitation in terms of burst frequency, incidence, and total muscle sympathetic nerve activity in normotensive pregnant women under baseline conditions. We also examined the burst amplitude component of the sympathetic signal, but observed no significant pregnancy-induced change in the regulation of baseline burst amplitude. During CPT, the burst frequency component remained elevated from baseline levels in the pregnant women relative to the nonpregnant women. Moreover, despite starting at a relatively higher level of sympathetic nerve activity, the pregnant women exhibited a larger peak increase in sympathetic activity from baseline when compared with the nonpregnant women. Conversely, the increases in burst amplitude which occurred in response to CPT were nearly identical between the pregnant and nonpregnant participants (effect of group, P = 0.8). That we observed that pregnancy-related increases in sympathetic activity exist only in the frequency component of the sympathetic signal, and not in the amplitude component, is indicative that sympathetic hyperactivity during pregnancy is because of mechanisms regulating the gating of sympathetic activity and not generalized central sensitization. This hypothesis is in keeping with our recent data demonstrating baroreflex resetting during pregnancy, favoring elevated burst incidence for a given arterial pressure.23

The present data are in line with most, although not all,12 previous studies which have indicated that pregnancy is associated with an increase in baseline sympathetic activity.4,13–15 To date, only one previous study has compared muscle sympathetic responses to the CPT between normotensive pregnant women and nonpregnant controls.12 Although similar responses to CPT were observed between groups, the group of pregnant women studied did not exhibit the marked elevations in baseline sympathetic activity, which have come to be associated with pregnancy4,13–15; therefore, these women may not be representative of the general population.

An important observation of this study was that, despite markedly elevated sympathetic activity with pregnancy, we observed a reduction in TPR in the pregnant women relative to the nonpregnant women, reflecting a blunting of neurovascular transduction. In fact, although strong, positive relationships between sympathetic activity and both peripheral resistance and MAP were observed during the CPT in the nonpregnant women, we observed no such relationships in the

Figure 2. Hemodynamic and sympathetic responses across 3 min of cold pressor test (CPT). All variables were increased significantly across stages of the CPT. Muscle sympathetic nerve activity (MSNA) and heart rate (HR) were consistently greater, and total peripheral resistance (TPR) was consistently reduced in the pregnant women relative to the nonpregnant controls. Data are mean±standard deviation. *P<0.05 vs nonpregnant. BF indicates burst frequency; BI, burst incidence; and MAP, mean arterial pressure.

Figure 3. Relative changes from baseline during peak cold pressor test sympathoexcitation. Changes in hemodynamics were not different between pregnant and nonpregnant women, whereas relative increases in muscle sympathetic burst frequency (BF), burst incidence (BI), and total activity were greater in pregnant women. Data are mean±standard deviation. *P<0.05 vs nonpregnant. HR indicates heart rate; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; and TPR, total peripheral resistance.

Figure 2: Relative changes from baseline during peak cold pressor test sympathoexcitation. Changes in hemodynamics were not different between pregnant and nonpregnant women, whereas relative increases in muscle sympathetic burst frequency (BF), burst incidence (BI), and total activity were greater in pregnant women. Data are mean±standard deviation. *P<0.05 vs nonpregnant. HR indicates heart rate; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; and TPR, total peripheral resistance.
pregnant women. These changes are likely because of the integration of several factors.

1. Changes in circulating hormone levels may exert a direct, excitatory effect on the sympathetic nervous system. Large increases in estradiol and progesterone and modest increases in testosterone occur during pregnancy. Estradiol-mediated excitatory central neural mechanisms may contribute to the pregnancy-induced sympathetic excitation. Likewise, both progesterone and testosterone have been positively associated with sympathetic nerve activity in nonpregnant women. The magnitude of change in circulating aldosterone has also been positively associated with the increase in baseline sympathetic activity during pregnancy. Therefore, it is likely that circulating hormone levels play a role in the pregnancy-induced sympathetic activation.

2. Current evidence is equivocal about changes in circulating norepinephrine (a primary sympathetic neurotransmitter) in healthy pregnant versus nonpregnant women. However, healthy pregnant women seem to exhibit a blunted increase in systemic vascular resistance in response to infusion of norepinephrine. Estradiol-mediated excitatory central neural mechanisms may contribute to the pregnancy-induced sympathetic excitation. Likewise, both progesterone and testosterone have been positively associated with sympathetic nerve activity in nonpregnant women. The magnitude of change in circulating aldosterone has also been positively associated with the increase in baseline sympathetic activity during pregnancy. Therefore, it is likely that circulating hormone levels play a role in the pregnancy-induced sympathetic activation.

3. The positive effects of estradiol on endothelial nitric oxide synthesis are well described. Moreover, in vitro studies suggest that estradiol also acts on vascular smooth muscle to evoke vasodilation. Therefore, direct actions of estradiol on the vasculature would encourage vasodilation in pregnant women relative to nonpregnant women, thereby opposing the vasoconstrictory influence of elevated sympathetic nerve activity and reducing neurovascular transduction.

4. Changes in β-adrenergic receptor density with pregnancy may also contribute to the blunting of neurovascular transduction. Contrary to α-adrenergic receptors, β-adrenergic receptors mediate vasodilation. Young healthy women seem to exhibit greater β-adrenergic responses relative to individuals with lower circulating estradiol levels (ie, men and postmenopausal women), indicating a favorable role for estradiol on β-adrenergic receptor densities. Moreover, some of the vasodilation elicited through β-adrenergic receptor activation is nitric oxide–dependent, and in that way, the effects of estradiol may be additive. It is possible that higher β-adrenergic binding in pregnant women would enhance vasodilation and thereby support a decreased basal neurovascular transduction. However, little evidence exists to suggest that this occurs, and in fact, some data suggest that β-adrenergic receptor density may be reduced in pregnancy.

The pregnancy-induced change in neurovascular transduction represents a possible mechanism which could potentially contribute to the development of the hypertensive disorders of pregnancy. Although vascular responses to the CPT have been shown previously to be blunted in normotensive pregnancy, an augmented blood pressure response to cold pressor stimulation has been observed in women with preeclampsia. Alongside data which indicate that CPT-mediated increases in sympathetic activation are not different between normotensive and hypertensive pregnancies, this suggests that an elevation in neurovascular transduction occurs during preeclamptic pregnancies. In fact, the magnitude of the pressor response to the CPT, as assessed during the second trimester of pregnancy, has been shown to be predictive of the subsequent development of preeclampsia during the third trimester.
Perspectives
In this study, we have shown that sympathoexcitatory responses to generalized sympathetic stress are exaggerated in normotensive pregnancy. However, neurovascular transduction is reduced in pregnant women, thereby dissociating sympathetic nerve activity from vascular resistance and arterial pressure during CPT. This uncoupling effect represents a possible mechanism which could become dysregulated during pregnancy and, thereby, contribute to the development of the hypertensive disorders of pregnancy, which constitute ≤8% of all pregnancies. Further research is required to investigate whether neurovascular transduction is, indeed, augmented in the hypertensive disorders of pregnancy and the mechanism(s) by which this occurs. An improved understanding of such sympathetic and vascular regulatory mechanisms may aid in the development of treatments and prevention of the hypertensive disorders of pregnancy.

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Disclosures
None.

Reference
34. Hart EC, Charkoudian N, Wallin BG, Curry TB, Eisenach J, Joyner MJ. Sex and ageing differences in resting arterial pressure regulation: the role


**What Is New?**

- These data provide the first human evidence for heightened responses to a generalized sympathoexcitatory stimulus during normotensive pregnancy.

- However, this increase in sympathetic reactivity is coupled with a reduction in the transduction of the sympathetic signal, thus preventing a hypertensive response during the sympathoexcitation.

**What Is Relevant?**

- The mechanisms which regulate blood pressure during pregnancy are not completely understood, nor are the factors which result in the development of de novo hypertension in ≤8% of pregnancies.

- The opposition of elevated sympathetic reactivity by reduced neurovascular transduction in normotensive pregnant women represents a possible mechanism which may be dysregulated in hypertensive pregnancies.

**Summary**

Sympathetic reactivity in response to the cold pressor test is increased in normotensive pregnancy relative to nonpregnant controls. However, as at baseline, neurovascular transduction remains blunted during sympathoexcitation in pregnant women, and as a result, blood pressure responses to the cold pressor test are similar or less than those observed in nonpregnant controls. Subsequent studies should determine whether this blunting of neurovascular transduction is observed in women with gestational hypertension and preeclampsia because this represents a possible mechanism by which hypertension develops in this clinical population.
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