Does hypercapnia-induced impairment of cerebral autoregulation affect neurovascular coupling? A functional TCD study

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Maggio P, Salinet ASM, Panerai RB, Robinson TG. Does hypercapnia-induced impairment of cerebral autoregulation affect neurovascular coupling? A functional TCD study. J Appl Physiol 115: 491–497, 2013. First published June 6, 2013; doi:10.1152/japplphysiol.00327.2013.—Neurovascular coupling (NVC) and dynamic cerebral autoregulation (dCA) are both impaired in the acute phase of ischemic stroke, but their reciprocal interactions are difficult to predict. To clarify these aspects, the present study explored NVC in a healthy volunteer population during a surrogate state of impaired dCA induced by hypercapnia. This study aimed to test whether hypercapnia leads to a depression of NVC through an impairment of dCA. Continuous recordings of middle cerebral arteries cerebral blood flow velocity (CBFv), blood pressure (BP), heart rate, and end-tidal CO2 were performed in 19 right-handed subjects (aged >45 yr) before, during, and after 60 s of a passive paradigm during normocapnia and hypercapnia. The CBFv response was broken down into subcomponents describing the relative contributions of BP (VBP), critical closing pressure (VCPCR), and resistance area product (VRAP). VRAP reflects myogenic activity in response to BP changes, whereas VCPCR is more indicative of metabolic control. The results revealed that hypercapnia significantly affected NVC, with significant reductions in the relative contribution of VCPCR to the paradigm-induced increase in CBFv. The present study suggests that hypercapnia impairs both dCA and NVC, probably acting through an impairment of the metabolic component of CBF control.

Subjects and measurements. Healthy volunteers were recruited from departmental staff and their relatives. Inclusion criteria were age of >45 yr and right-handedness according to the Edinburgh inventory.
paradigms) was repeated during CO₂ inhalation. Arterial BP was measured using a three-lead electrocardiogram (ECG), and end-tidal CO₂ was measured via nasal prongs (Salter Labs) by a capnograph (Capnocheck Plus). Bilateral insonation of the middle cerebral arteries (MCAs) was performed using TCD (Viasys Companion III; Viasys Healthcare) with a 2-MHz probe, which was secured in place using a head-frame. The MCAs were identified according to their signal depth and velocity characteristics (2). Hypercapnia was induced by the inhalation of a mixture of 5% CO₂ in air through a mask. During CO₂ breathing, the capnograph was connected to the mask via a sample line. During the entire procedure, subjects were in a supine position, and detailed instructions were given before taking measurements.

Heart rate interval was recorded using a three-lead electrocardiogram (ECG), and end-tidal CO₂ was measured via nasal prongs (Salter Labs) by a capnograph (Capnocheck Plus). Bilateral insonation of the middle cerebral arteries (MCAs) was performed using TCD (Viasys Companion III; Viasys Healthcare) with a 2-MHz probe, which was secured in place using a head-frame. The MCAs were identified according to their signal depth and velocity characteristics (2). Hypercapnia was induced by the inhalation of a mixture of 5% CO₂ in air through a mask. During CO₂ breathing, the capnograph was connected to the mask via a sample line. During the entire procedure, subjects were in a supine position, and detailed instructions were given before taking measurements.

After a period of 15 min of stabilization, participants performed a 5-min baseline recording and two passive motor paradigms during air breathing. Passive motor stimulation was shown to have better reproducibility than active or imagery motor paradigms (36). The same sequence of measurements (5-min baseline and two passive motor paradigms) was repeated during CO₂ inhalation. Arterial BP was measured with a sphygmomanometer before each measurement.

The passive paradigm consisted of an examiner performing repetitive flexion and extension of the subject’s elbow within a range of movement of ~90° at a rate of 1 Hz; subjects were instructed to relax and not move the arm. The frequency was given by the sound of a metronome. All paradigm recordings started with a 90-s baseline phase. Thereafter, the paradigm was performed over 60 s, with a 90-s recovery phase. During the rest and recovery periods, the examiner kept hold of the participant’s arm. The paradigm was performed only with the dominant arm.

Data analysis. Data were simultaneously recorded onto a data acquisition system (PHYSIDAS, Department of Medical Physics, University Hospitals of Leicester) for subsequent offline analysis. ECG, end-tidal CO₂, BP, and stimulus marker signals were sampled at 500 samples/s, and BP was calibrated at the start of each recording. All signals were visually inspected to identify artifacts and noise, and narrow spikes (<100 ms) were removed by linear interpolation. The channels were subjected to a median filter, and all signals were low-pass filtered with a cut-off frequency of 20 Hz. The R–R interval (i.e., the interval between two consecutive heartbeats recorded by the ECG) was then automatically marked from the ECG, and continuous heart rate was plotted against time. Occasional ectopic beats caused spikes in the heart rate signal; these were manually removed by remarking the R–R intervals for the time points at which they occurred. Mean BP and CBFv values were calculated for each cardiac cycle. The end of each respiratory phase was detected in the end-tidal CO₂ signal, linearly interpolated, and resampled with each cardiac cycle. The instantaneous relationship between BP and CBFv was estimated using the Welch method. The transfer function of BP-CBFv was then calculated with BP selected as the input and right then left CBFv as the output variables. An inverse fast Fourier transform was then applied, converting data back into the time domain, to calculate the CBFv step response (16). ARI was assigned to each recording by using the best least-squares fit between the CBFv step response and one of the 10 model ARI curves proposed by Tiecks et al. (38). ARI was computed for each subject separately for left and right hemisphere for normocapnia and hypercapnia.

Mean values of each variable were extracted from the 30 s before the start of the paradigm for baseline. Using the electrical output from the metronome, coherent averages were calculated for each variable synchronized by the beginning of the paradigm. Temporal paradigm-synchronized population averages of the first and second motor paradigms were compared, and the maneuver that achieved the highest amplitude of contralateral CBFv response was chosen to represent the participant’s response (36).

Decomposition of the CBFv changes into its main subcomponents was performed as described previously (26). In summary, the percent change in CBFv was decomposed into standardized subcomponents describing the relative contributions of BP (ΔBP), resistance area product (ΔRAP), and critical closing pressure (ΔCrCP). Therefore, the total change in CBFv during activation was represented as the sum of the three subcomponents, reflecting the separate contribution of parallel changes in BP, CrCP, and RAP. It has been suggested that ΔCrCP might reflect myogenic activity in response to BP changes, whereas ΔCrCP is more indicative of metabolic control (25, 26).

Statistical analysis. Student’s t-test for dependent variables was used to compare baseline values of CBFv, BP, heart rate, end-tidal CO₂, CrCP, RAP, and ARI between normocapnia and hypercapnia. To compare changes in CBFv and its subcomponents between normocapnia and hypercapnia, the area-under-the-curve (AUC) was calculated for their differences from the beginning of the maneuver up to 20 s after the end of passive arm movement. Differences between ipsi- and contralateral values of AUC were tested by two-way, repeated-measures ANOVA, also including the effects of hypercapnia. In the absence of significant differences between sides, values for the right and left MCA were averaged, and the effects of hypercapnia were tested by paired Student’s t-tests. Student’s t-test for dependent variables was also used to compare end-tidal CO₂ AUC values between the two groups. Multivariate ANOVA was performed to assess the overall effects of hypercapnia, taking into account the interactions between CBFv, BP, CrCP, and RAP. All statistical analyses were performed with Statistica software for Windows. A value of P < 0.05 (α) was adopted to indicate statistical significance. Bonferroni correction for multiple testing was performed by recalculation of the critical value of α, taking into account the correlation coefficient between variables.

RESULTS

Nineteen participants (8 men) of mean age 58.1 yr (SD 7.1, range 48–77) were recruited. Participant’s mean Edinburgh Inventory for right-handedness was 96.5% (SD 8.5). All participants completed measurements under normocapnia. During hypercapnia, all subjects completed the baseline recording and the first motor paradigm. Only 16 participants completed the second paradigm during hypercapnia since three participants did not tolerate continuous use of the face mask. In these three cases, we analyzed the only available passive motor paradigm recording to represent the participant’s response.

Baseline changes. The influence of hypercapnia on cerebral and peripheral hemodynamic variables during baseline recordings at rest is given in Table 1. Contralateral and ipsilateral baseline CBFv, BP, and end-tidal CO₂ were significantly applied to the data, and the cross- and auto-spectra were estimated using the Welch method. The transfer function of BP-CBFv was then calculated with BP selected as the input and right then left CBFv as the output variables. An inverse fast Fourier transform was then applied, converting data back into the time domain, to calculate the CBFv step response (16). ARI was assigned to each recording by using the best least-squares fit between the CBFv step response and one of the 10 model ARI curves proposed by Tiecks et al. (38). ARI was computed for each subject separately for left and right hemisphere for normocapnia and hypercapnia.
higher during CO2 inhalation than during air breathing. Contralateral CrCP was significantly lower during hypercapnia than during normocapnia. Ipsilateral CrCP was also lower, although this did not reach statistical significance. Heart rate and RAP were not different between air and 5% CO2. Both contralateral and ipsilateral autoregulation indexes were significantly impaired by hypercapnia (Table 1).

Neurovascular coupling. One subject did not show a CBFv response to the paradigm during normocapnia and was excluded from further analysis. Population averaged changes of CBFv, BP, CrCP, and RAP for normocapnia and hypercapnia are displayed in Figs. 1–4. Figure 5 depicts changes in AUC due to hypercapnia for both ipsi- and contralateral sides. No significant differences were observed between values for the right and left MCA, and these were averaged for further analyses. In addition to the shifts in baseline values described above, hypercapnia induced very significant effects on the response to passive motor stimulation of the arm (multivariate ANOVA F = 3.2; P = 0.00462). The increase of CBFv induced by the paradigm during normocapnia (AUC = 5.2 ± 4.1%) was significantly lower during hypercapnia (2.7 ± 0.68%; P = 0.013). The paradigm-induced increase in BP was reduced by hypercapnia (Figs. 2 and 5), although the AUC did not reach statistical significance (1.36 ± 0.8 vs. 0.18 ± 0.67%; P = 0.21).

DISCUSSION

Neurovascular coupling is a complex mechanism involved in the control of CBF and is regulated by myogenic (17), metabolic (12), and neural components (13). Its impairment observed during the acute phase of stroke (20) could be explained by direct ischemic neuronal injury, reduced oxidative and glucose metabolism (9), and/or compromised CA (3). To clarify these aspects, the present study explored NVC in a healthy volunteer population during a surrogate state of impaired dCA, as induced by CO2 inhalation. The subcomponent analysis of the CBF response to a passive motor stimulus was also applied to give further insights into different factors that might affect CBFv changes during hypercapnia.

Main findings. The significant changes in the NVC response to a passive motor paradigm, induced by hypercapnia, con-

**Table 1. Mean (SD) values of cerebral and systemic hemodynamic variables for baseline air and CO2 measurements**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Air</th>
<th>CO2</th>
<th>*P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBFv contr, cm/s</td>
<td>56.6 (11.8)</td>
<td>72.0 (14.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CBFv ipsi, cm/s</td>
<td>49.4 (9.1)</td>
<td>62.2 (11.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>99.4 (11.1)</td>
<td>109.1 (19.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>67.1 (8.1)</td>
<td>70.8 (10.6)</td>
<td>0.08</td>
</tr>
<tr>
<td>EtCO2, Torr</td>
<td>40.7 (3.3)</td>
<td>47.9 (3.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CrCP contr, Torr</td>
<td>21.5 (10.5)</td>
<td>9.0 (18.7)</td>
<td>0.02</td>
</tr>
<tr>
<td>CrCP ipsi, Torr</td>
<td>19.2 (13.3)</td>
<td>13.0 (17.6)</td>
<td>0.2</td>
</tr>
<tr>
<td>RAP contr, Torr-s/cm</td>
<td>1.45 (0.41)</td>
<td>1.42 (0.39)</td>
<td>0.7</td>
</tr>
<tr>
<td>RAP ipsi, Torr-s/cm</td>
<td>1.70 (0.47)</td>
<td>1.60 (0.48)</td>
<td>0.2</td>
</tr>
<tr>
<td>ARI contr</td>
<td>5.2 (1.4)</td>
<td>4.4 (1.1)</td>
<td>0.04</td>
</tr>
<tr>
<td>ARI ipsi</td>
<td>5.4 (1.4)</td>
<td>4.5 (1.3)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means (SD). CBFv, cerebral blood flow velocity; BP, mean arterial blood pressure; EtCO2, end-tidal CO2; CrCP, critical closing pressure; RAP, resistance-area product; ARI, autoregulation index; contr, contralateral to the paradigm; ipsi, ipsilateral to the paradigm. *P value of Student’s t-test dependent samples.
firmed our first hypothesis as shown by the overall multivariate ANOVA and more specifically by the differences in the CBFv response. Despite the absence of significant changes in the V_{RAP} response, our second hypothesis, that NVC was affected due to impairment in dynamic CA, was also confirmed by the much reduced response in V_{CrCP}. The demonstration that hypercapnia leads to the simultaneous impairment of dCA and NVC thus suggests a possible association between what is usually regarded as two different mechanisms. CA is a complex homeostatic mechanism influenced by myogenic (4, 39), metabolic (30), and neural activity (13). Our results suggest that CO_{2} impaired the metabolic component of dCA, as indicated by the changes in V_{CrCP} (25, 26, 27, 28). Conversely, the lack of changes in V_{RAP} suggests that the myogenic pathway was not affected by hypercapnia.

**Neurovascular coupling during normocapnia.** The CBFv pattern response to the passive motor paradigm during normocapnia displayed an initial peak followed by a plateau profile and a delay in return to baseline after the cessation of stimulation, in very good agreement with data previously reported by others (18, 21, 35). Interestingly, the breakdown of the CBFv response into its subcomponents revealed that, during normocapnia, the CBFv increase is mainly determined by positive contributions of BP and CrCP. Moody et al (21) described significant dynamic changes in heart rate induced by word or puzzle paradigm. However, these mental activation tasks are more demanding and possibly require a greater overall sympathetic activation than passive motor activity.

**Cerebrovascular effects of hypercapnia: baseline condition.** Hypercapnia induced a bilateral increase of CBFv, a rise in BP and end-tidal CO_{2}, and a decrease of ARI and contralateral CrCP, but did not affect RAP. These effects are well known and in agreement with those reported previously (1, 24). The increase in inspiratory CO_{2} is followed by an extracellular rise in H^{+} ions, a pH reduction, and vessel smooth muscle cell relaxation, which are thought to be the major mechanisms of a CO_{2}-mediated increase of CBFv. Other mechanisms such as nitric oxide (NO), prostaglandins, or neurogenic components might also be involved in hypercapnic vasodilation (14). Mean BP increase is consistent with previous studies (1, 10) and could be explained by an effect of CO_{2} on the sympathetic nervous system (22) or as a CO_{2}-positive inotropic effect on the heart (10). Furthermore, changes in CO_{2} concentrations affect vasomotor tone, which may in turn affect CBFv through a decrease of CrCP (10, 28). The lack of ipsilateral CrCP changes induced by hypercapnia could be attributed to its wide standard deviation and study power. RAP did not change during 5% CO_{2}, as previously observed (24). These results confirm a preliminary hypothesis raised by Panerai et al. (24), which postulated that CO_{2} may induce a parallel shift of the flow-pressure curve with a reduction of CrCP rather than changes in the slope of the curve, thus with a lesser influence on RAP.

**During motor paradigm.** Previous studies demonstrated that hypercapnia reduces the early peak response and induces a slower increase of CBFv in rat models during somato-sensorial stimulation (18) or in humans during visual stimulation (33). Our findings confirm these observations in humans using passive motor activation, a substantially reproducible paradigm (36), also feasible in stroke patients (20), where motor deficits could make a voluntary activity not possible. Unfortunately, further comparisons between our results and those of Rosen-
garten et al. (33) are limited by methodological and physiological differences. In fact, regional differences in NVC between the motor and visual areas have been demonstrated (6), as well as regional heterogeneity of the cerebral vascular response to hypercapnia (15). Moreover, visual and motor paradigm-induced changes in peripheral variables, including respiratory rate, BP, and heart rate, may be dissimilar, thus triggering different CBFv changes.

fMRI or PET studies exploring the effect of hypercapnia on hemodynamic response to neural activation are so far conflicting. Two main hypotheses have emerged, the first stating that local CBF changes induced by stimulation are independent of and additive to basal CBF (19), and the second affirming that the magnitude of the hemodynamic response to stimulation is attenuated during increased basal CBF, as induced by CO2 inhalation (7). This discrepancy may be explained by the type and the duration of the stimulus used and/or by methodological differences in end-tidal CO2 increase (steady state vs. transient modulation) and in respiratory pattern control. In agreement with our results, Stefanovic et al. (37) reported that hypercapnia reduces stimulus-evoked BOLD signal changes in the human motor cortex, and at high end-tidal CO2 values an almost complete loss of the functional flow response to stimulation has been demonstrated (31). However, it is important to note that all these neuroimaging studies have largely ignored the role of task-induced BP and arterial pCO2 changes as well as the interplay between NVC and dCA during hypercapnia; therefore, a comparison between our results and previous literature concerning peripheral changes in response to motor paradigms is not possible.

Metabolic effects. The subcomponent analysis of the CBFv response to neural activation revealed that hypercapnia significantly affects V_crCP, which mainly represents the metabolic pathway of CBF control (25, 26, 28).

The molecular mechanisms governing the interaction between neurons and cerebral blood vessels during hypercapnia and during functional hyperemia have been the subject of intense research.

A widely accepted hypothesis for NVC is that activation of glutamate receptors during synaptic transmission leads to post-synaptic increases in Ca2+, which in turn activates enzymes that produce vasoactive agents (12). Some of the mediators (including H+, K+, and NO) implicated in the CBF response to neural activation are similarly demonstrated to be involved in CO2-induced CBF increase (32, 41). Accordingly, it is reasonable to postulate that, since the CBFv increase induced by hypercapnia and by neural activation share common molecular pathways, a manipulation of CO2 blood concentration could interfere with neural or glial regulation of cerebral microvasculature, thus affecting NVC. Moreover, molecular (41) and electrophysiological evidence (42) support the hypothesis that CO2 acts by modulating neural cells, in other words that its action is neurogenic in origin.

A recent study by Kennerley et al. (18) analyzed the effect of hypercapnia on neural and hemodynamic responses during whisker stimuli in the rat. They observed that increased CO2...
concentrations inhibited both the hemodynamic and the neural response to a somato-sensory stimulus, but subsequent modelling suggested that the difference in neural response might thoroughly explain hemodynamic changes. This result supports our findings, since it suggests that the impairment of CBF response to activation may be attributed to a compromised neural (metabolic) ability to drive the hemodynamic shift.

**Myogenic effects.** In our sample, neither the $V_{\text{RAP}}$ nor the $V_{\text{BP}}$ component of CBFv response to motor paradigm were affected by hypercapnia. The exact mechanisms underlying the contribution of $V_{\text{RAP}}$ on neurovascular coupling are not yet completely understood. $V_{\text{RAP}}$ is supposed to reflect myogenic activity (25, 26), even if some evidence suggests an additional metabolic influence (25, 26, 27). One possible explanation for this finding is that the metabolic pathway of CBF control is so compromised during hypercapnia that the myogenic influence is subordinate and not detectable by means of TCD measurements.

Returning to the main hypotheses of this work, it is possible to accept that hypercapnia leads to a depression of NVC, but more caution needs to be applied to the second hypothesis, that depression of NVC results from dCA impairment, due to the lack of significant differences in $V_{\text{RAP}}$, which have been associated with the myogenic component of dCA. More work in this aspect of NVC impairment in acute stroke is needed, as discussed below.

**Clinical implications.** Given the previous considerations and evidence from the literature (24), how can we then explain that acute ischemic stroke patients have impairments of both CA and NVC? Since both mechanisms are the result of myogenic, metabolic, and neural control, their impairment during acute ischemic stroke could be attributed to metabolic, myogenic, or neural involvement or, more likely, to their reciprocal interaction. Stroke subtype and volume, ischemic penumbra, small vessel disease, cerebrovascular risk profile, and extra- or intracranial atherosclerosis may have a different impact on metabolic or myogenic impairment of CA, as well as on dCA influence on NVC. For instance, an impairment of CA myogenic control could be postulated in patients suffering from severe hypertension and small vessel disease and experiencing small lacunar stroke. On the other hand, patients with large MCA infarction may present both metabolic and myogenic CA impairment. The corresponding effect of dCA on NVC is a composite, but intriguing, subject. It not only provides benefits for a detailed understanding of the damaging effects of stroke but is also crucial for the development of more specific and targeted rehabilitation therapies.

**Limitations**

In our study, we took into account only impairment of CA due to blunting of the metabolic pathway, which could be regarded as a limitation to reproduce clinical conditions that also impair myogenic control, as could be the case with some instances of acute ischemic stroke. Further work needs to be done in this direction and attempt to apply a more refined model of impaired CA. The observation that Ca$^{2+}$-channel blockade makes the cerebral microcirculation more vulnerable to systemic BP fluctuations (39) and that purinergic receptors modulate the myogenic tone in cerebral parenchymal arterioles (4) may suggest novel strategies to induce a surrogate state of myogenic-impaired CA.

Therefore, it is worth mentioning that hypercapnia impairs CA but also leads to vasodilation. At a high CO$_2$ level, the pial arteries could be near to a limit of vasodilatation, which cannot be further influenced by neuronal activation induced by the paradigm. Using other surrogates of impaired dCA, different from hypercapnia, would help to clarify this issue.

Measurements of CBFv reflect changes in CBF as long as the diameter of the insonated vessel remains constant. Moreover, any variations in MCA diameter taking place during activation tasks would also change RAP, since it represents cerebrovascular resistance multiplied by MCA cross-sectional area (28). However, several studies have demonstrated that the cross-sectional area of the MCA changes minimally during large modifications in BP or end-tidal CO$_2$ (11), even if there is still limited evidence during motor paradigms.

Although the interpretation of changes in $V_{\text{GCP}}$ and $V_{\text{RAP}}$ as manifestations of metabolic and myogenic CBFv regulation is currently supported (25–28), it is still speculative, and further experimental work is needed. In effect, it is still not possible to confirm that each parameter (CrCP or RAP) is uniquely associated with the metabolic or myogenic control of CBFv response to the paradigm, respectively (25, 27). Moreover, CrCP can also be influenced by cerebral venous pressure (8), a variable affected by intrathoracic pressure.

We cannot rule out that part of the reduced CBFv response to motor paradigm may be attributed to increased sympathetic activation triggered by CO$_2$. Pial arteries and large surface arterioles are innervated by nerve fibers that originate in autonomic and trigeminal sensory ganglia (13), and CO$_2$ has been suggested to modulate sympathetic nervous system activity (22). Future work taking into account the neural component of CBFv response to motor paradigm would help to clarify this issue.

Finally, the lack of biceps and triceps electromyography recordings cannot exclude the absence of voluntary muscular activity during the passive motor paradigm. However, similar to other brain activation studies (35, 36), subjects were closely monitored during the experiment for any active elbow movement.

In conclusion, hypercapnia leads to impairment of dCA and depression of NVC. Analysis of different subcomponents of the CBFv response to neural activation demonstrated that depression of the NVC response was mainly caused by changes in CrCP, thus suggesting greater involvement of metabolic pathways rather than myogenic control of CA, which would be expected to be mainly expressed through RAP. These findings are pertinent to a better understanding of NVC alterations in pathological conditions such as stroke, where CA is often impaired. Further work in this area should consider alternative models of CA impairment, such as that induced by calcium channel blockers, to test the generalizability of our findings, as well as further refinements in analytical techniques to improve estimates of the different covariates influencing both CA and NVC.

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AUTHOR CONTRIBUTIONS
Author contributions: P.M. and A.S.M.S. performed experiments; P.M., A.S.M.S., and R.B.P. analyzed data; P.M., A.S.M.S., R.B.P., and T.G.R. interpreted results of experiments; P.M. prepared figures; P.M. drafted manuscript; P.M., A.S.M.S., R.B.P., and T.G.R. approved final version of manuscript; A.S.M.S., R.B.P., and T.G.R. conception and design of research; A.S.M.S., R.B.P., and T.G.R. edited and revised manuscript.

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