Interindividual relationships between blood pressure and cerebral blood flow variability with intact and blunted cerebrovascular control

Yu-Chieh Tzeng and Braid A. MacRae

Cardiovascular Systems Laboratory, Centre for Translational Physiology, University of Otago, Wellington, New Zealand

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Although the identification and treatment of excessively high average blood pressure dominates clinical management of chronic blood pressure disease, there is growing recognition that elevated blood pressure variability (BPV) across a wide range of time scales also predicts poorer health outcomes (18, 26, 30). Elevated short-term (minutes) and long-term (visit-to-visit) BPV have been linked to accelerated end-organ damage (34, 35), stroke incidence (33), and poststroke complications (e.g., hemorrhagic transformation, mortality) (12). These findings suggest that other aspects of blood pressure, such as BPV, are also valuable for population risk stratification (28). However, narrowly focusing on BPV belies the dependence of vital organs, such as the brain on perfusion (i.e., blood flow) rather than blood pressure per se. Because the brain has a high metabolic demand for oxygen, cerebral blood flow variability (CFV) and the integrity of flow-stabilizing mechanisms, such as autoregulation, may underlie the relationship between elevated BPV and end-organ disease.

The classic depiction of the relationship between blood pressure and cerebral perfusion is that blood flow is maintained at near-constant levels across a wide range of cerebral perfusion pressures (60–150 mmHg) (16). This “textbook” model has been highly influential, but is a limited concept as it evolved from classical techniques that enabled only static (i.e., steady state) cerebral blood flow quantification. The advent of transcranial ultrasonic pulsed Doppler enabled cerebral blood flow dynamics to be studied on a pulsatile basis (1, 2, 36). Combined with signal processing methods, such as transfer function analysis, the application of transcranial Doppler ultrasound led to the recognition that cerebrovascular response characteristics resemble a high-pass filter, wherein the pressure buffering capacity is greater against lower frequency fluctuations in cerebral perfusion pressure (6, 9, 44). Although these are widely acknowledged concepts, it is important to recognize that they refer exclusively to within-individual physiology (6, 9, 38, 40, 44). In contrast, the extent to which CFV is determined by BPV and cerebral autoregulation (CA) across individuals has received comparatively little attention, despite population relationships being equally important from an epidemiological risk-stratification perspective.

From a treatment perspective, Matsui et al. (19) recently showed that day-by-day BPV was lower in patients treated with an angiotensin II receptor blocker/calcium channel blocker combination compared with those treated with an angiotensin II receptor blocker/diuretic combination. This raised the possibility that elevated BPV can be treated using conventional anti-hypertensive agents (25), although using calcium channel blockers to reduce BPV may not necessarily decrease CFV, given the potential for concomitant CA impairment (39). The implication for conditions that are fundamentally due to disordered perfusion (e.g., cerebral ischemia) is that possible variability increasing hemodynamic effects may negate the benefits of reducing BPV.

In view of these considerations, the objectives of this study were twofold. First, we sought to determine the extent to which differences in CFV across individuals were determined by BPV. Second, we examined the cerebral hemodynamic effects of calcium channel blockade (CCB) on these variables and their relationships. Impairment of CA with CCB was useful in two ways in that it provided insight into the interplay between BPV and CA in determining CFV across individuals, and the potential for CCB to be used to stabilize hemodynamic profiles. Assuming CCB would influence both BPV and CA (via direct action of CCB on peripheral and cerebral vasomotor activity), the use of a healthy cohort, over a patient cohort with established CA impairment, allowed the influence of intact CA (before blockade) and blunted CA (following blockade) to be...
investigated. It is hypothesized that, across individuals, spontaneous BPV will be positively related to CFV, and that CCB will reduce spontaneous CFV concomitantly to reduced BPV, despite CA impairment.

METHODS

Twenty-three healthy subjects (12 women), without any history of cardiovascular, respiratory, or endocrine disease, signed written informed consent before participating. All subjects had abstained from exercise and caffeinated food and beverages for at least 12 h and were advised to have a light breakfast at least 2 h before the study commencement at 0900. The study was approved by the New Zealand Central Regional Ethics Committee and conformed to the standards set by the Declaration of Helsinki.

Experimental protocol. All subjects were studied in the supine position in a temperature-controlled laboratory (22–23°C). Six-minute baseline recordings were made before and ~50 min following ingestion of either 60 mg nimodipine (n = 12, 6 women; 26 ± 5 yr) or a placebo pill (n = 11, 6 women; 29 ± 9 yr). The duration between recordings was based on the peak pharmaco-dynamic activity of nimodipine on the cerebrovasculature (5); the placebo trials served as time controls. Subject allocation to active or placebo treatment was randomized.

Measurements. We recorded the electrocardiogram, noninvasive beat-to-beat blood pressure via finger photoplethysmography (Fimeter MIDI, MLE1054-V, Finapres Medical Systems), right middle CA blood flow velocity (MCAv; 2-MHz pulsed Doppler ultrasound, ST3 Digital Transcranial Doppler System, Spencer Technologies, Seattle, WA), and partial pressure of end-tidal CO₂ sampled from a nasal line (gas analyzer model ML206, ADInstruments, Colorado Springs, CO). Recalibration of the finger blood pressure device was performed periodically before each recording to ensure accuracy of measurement, which was also verified against manual brachial artery blood pressure measurements, as previously described by an experienced clinician (Y.-C. Tzeng) (31). Data were acquired continuously at 1 kHz per channel via an analog-to-digital converter (PowerLab/16SP, ADInstruments, Colorado Springs, CO). Each recording was first subdivided into five successive windows that were detrended, passed through a Hanning window, and subjected to fast Fourier transform analysis. From the recorded electrocardiogram, blood pressure, and MCAv waveforms, we determined the time of each R wave and beat-to-beat values for mean MCAv (MCAvmean) and mean arterial blood pressure (MAP). Data were processed and analyzed with custom software written in LabVIEW 11 (National Instruments).

Spectral and transfer function analysis. Beat-to-beat MAP and MCAvmean signals were spline interpolated and resampled at 4 Hz for spectral and transfer function analyses based on the Welch algorithm. Each recording was first subdivided into five successive windows that overlapped by 50%. The data within each window were linearly detrended, passed through a Hanning window, and subjected to fast Fourier transform analysis. The cross spectrum between MAP and MCAvmean was determined and divided by the MAP autospectrum to derive the transfer function phase, gain, and coherence indexes. In this study, we used phase as our primary indicator of CA function, based on previous work showing a clear linear response between phase and CA modulated across a wide range of arterial PCO₂ levels (37). Spontaneous MAP and MCAvmean spectral powers and the mean value of transfer function phase, gain, and coherence were each calculated in the very-low- (VLF, 0.02–0.07 Hz), low- (LF, 0.07–0.20 Hz), and high-frequency (HF, 0.20–0.40 Hz) ranges, as commonly defined (44). To account for interindividual differences in MCA diameter and potential treatment influences on MCA diameter (within individual), transfer function gain and MCAvmean spectral power were assessed using normalized units defined as the beat-to-beat values divided by the mean value (24). To account for potential changes in absolute blood pressure following CCB, MAP spectral power was assessed in both absolute and normalized units. For consistency of reporting with MCAvmean spectral power, the normalized values were presented, unless specifically stated otherwise.

Statistical analysis. Normality was assessed for all parameters using the Shapiro-Wilk test; log transformations were applied to all spectral variables, and values presented are as described in Tables 1 and 2 or Figures 1–5 legends. A priori defined comparisons investigating the differences in treatment effects between CCB vs. placebo on hemodynamic and power spectral variables were assessed using linear mixed-effects models (21, 43). For each dependent variable, the linear mixed model specified a main effect for treatment (before vs. after treatment), a main effect for group (CCB vs. placebo), and a treatment × group interaction. Statistically significant interactions indicated that the treatment response differs between the CCB and placebo groups. The effect of treatment (before vs. after treatment) on spectral parameters within each group (CCB or placebo) was defined a priori as comparisons of interest. These pairwise comparisons were performed as planned using paired t-tests adjusted for multiple comparisons (Holm-Bonferroni method) to control for inflated type 1 error (29). Potential sex differences were assessed with the inclusion of sex as a fixed factor and testing for a sex × treatment × group interaction. Significant relationships defined a priori between spectral powers were assessed using scatter plots and linear regression. Given that multiple relationships were examined, P values were also adjusted for multiple testing. All data were analyzed using SPSS 17 (SPSS, Chicago, IL). Unless otherwise stated in figure and table legends, all values are expressed as means ± SE and rounded to two significant figures. Statistical significance was set at P ≤ 0.05.

RESULTS

Effects of CCB on baseline parameters. Baseline cardiovascular, respiratory, and cerebrovascular variables under the conditions of this study are presented in Table 1. Significant treatment × group interactions were found for heart rate, MCAvmean, and MAP, indicating that CCB had treatment effects that were distinct from the placebo group. An increase in heart rate and reduction in MCAvmean were observed following CCB, but not the placebo. In contrast, MAP increased following the placebo, but not CCB. A clinically negligible, but statistically significant, reduction in partial pressure of carbon dioxide was observed following CCB, but not placebo. These results were confirmed with a Bonferroni corrected pairwise comparison of placebo vs. CCB, following the normality of data achieved with log transformation. No treatment × group interaction was observed for MAP, suggesting that the effect of treatment on MAP was consistent across groups.

Table 1. Summary of baseline variables before and after treatment with calcium channel blockade or placebo

<table>
<thead>
<tr>
<th>Variable</th>
<th>Calcium Channel Blockade</th>
<th>Placebo</th>
<th>Group × Treatment Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>Before: 64 ± 2.4, After: 71 ± 4.6*</td>
<td>Before: 60 ± 1.9, After: 57 ± 1.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MCAvmean, cm/s</td>
<td>Before: 64 ± 3.4, After: 56 ± 2.4†</td>
<td>Before: 65 ± 3.4, After: 65 ± 3.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>Before: 76 ± 3.5, After: 72 ± 2.4</td>
<td>Before: 78 ± 2.8, After: 84 ± 2.8*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PternCO₂, Torr</td>
<td>Before: 40 ± 0.72, After: 39 ± 0.87*</td>
<td>Before: 39 ± 0.82, After: 38 ± 0.71*</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Values are mean ± SE. HR, heart rate; MCAvmean, mean middle cerebral artery flow velocity; MAP, mean arterial blood pressure; PternCO₂, end-tidal partial pressure of carbon dioxide. P values presented in the table are for interactions in the linear mixed model. *P < 0.05 vs. before treatment. †P < 0.01 vs. before treatment. Paired comparisons (before vs. after) were Holm-Bonferroni corrected.

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end-tidal CO₂ was observed following treatment in both groups (~1 Torr; Table 1).

Effects of CCB. The influence of CCB on MCAvmean and MAP spectral powers for one example subject is shown in Fig. 1. The treatment effects of both CCB and placebo on MCAvmean and MAP spectral powers are summarized in Fig. 2. A reduction in MCAvmean spectral power was observed in the VLF range following CCB treatment that differed from the placebo effect (interaction effect $P < 0.01$). Paired comparisons showed that CCB treatment reduced LF MCAvmean spectral power (vs. before treatment), but the interaction effect was not significant ($P = 0.12$). Treatment response was also statistically similar between groups (CCB and placebo) in the HF band (interaction effect $P = 0.14$).

A reduction in VLF MAP power was observed following CCB, but not following the placebo (interaction effect $P < 0.05$). In contrast, treatment with CCB produced responses that were similar to placebo for both LF and HF MAP power (interaction effects were not significant; both $P > 0.10$). The outcomes of the statistical analyses for absolute MAP power were consistent with those presented here for normalized MAP power. In support of the summary statistics for defined bands (Fig. 2), the group responses plotted on a continuous spectrum illustrate that reductions in both MCAvmean and MAP spectral...
power following CCB are most evident within the VLF range (Fig. 3).

The effects of CCB and placebo treatment on cross-spectral coherence, phase, and gain are presented in Table 2. VLF and LF phase were each reduced following CCB, although a significant interaction effect was seen only in the LF range. Treatment responses for HF phase were similar between CCB and placebo (Table 2). Responses were invariably similar for coherence and gain between CCB and placebo for all band definitions (VLF, LF, HF; Table 2). There were no statistically significant sex interactions for MAP and MCAvmean spectral power or transfer function parameters in any frequency band (all \(P > 0.1\)).

**Interindividual relationships between hemodynamic parameters.** Simple linear regressions relating MCAvmean and MAP spectral powers before treatment (pooled) and following CCB are shown in Fig. 4 for the VLF, LF, and HF bands. MAP power was a positive predictor of MCAvmean power in each frequency band, both before treatment (\(R^2 = 0.34–0.67\), all \(P < 0.01\)) and following CCB (\(R^2 = 0.53–0.61\), all \(P < 0.02\)). Baseline MAP was unrelated to MCAvmean, both before (\(R^2 = 0.002\), \(P = 0.84\)) and following CCB (\(R^2 = 0.19\), \(P = 0.20\)).

To determine whether CA might also be a predictor of MCAvmean power, we performed multiple linear regression analyses with either phase or gain included as a covariate. Analyses showed that, before treatment, the combination of MAP power and gain explained \(73\%\) of the variance in the VLF range (\(R^2 = 0.73\)), and \(56\%\) in the LF range (\(R^2 = 0.56\)). It was found that VLF MAP power significantly predicted VLF MCAvmean power (\(\beta = 0.87 \pm 0.14\), \(P < 0.01\)), as did VLF gain (\(\beta = 1.2 \pm 0.25\), \(P < 0.01\)). Likewise, both LF MAP power (\(\beta = 0.83 \pm 0.19\), \(P < 0.01\)) and LF gain (\(\beta = 1.0 \pm 0.30\), \(P < 0.01\)) were significant predictors of LF MCAvmean power.
0.29, \( P < 0.01 \) significantly predicted LF MCA_{\text{mean}} power. A similar pattern of relationships was observed following CCB treatment. MAP power and gain collectively explained 82\% of the variance in the VLF range (\( R^2 = 0.82 \)) and 74\% in the LF range (\( R^2 = 0.74 \)). VLF MAP power significantly predicted VLF MCA_{\text{mean}} power (\( \beta = 0.90 \pm 0.17, P < 0.01 \)), as did VLF gain (\( \beta = 1.7 \pm 0.39, P < 0.01 \)). Similarly, LF MAP power (\( \beta = 0.91 \pm 0.16, P < 0.01 \)) and LF gain (\( \beta = 1.7 \pm 0.50, P < 0.01 \)) both significantly predicted LF MCA_{\text{mean}} power. Ranking of the standardized \( \beta \)-coefficient showed that VLF and LF MAP power was always ranked higher than gain, both before and after CCB. In contrast to gain, phase was not a significant predictor of VLF or LF MCA_{\text{mean}} power under both conditions (all \( P > 0.1 \)).

Interindividual relationships between treatment effects. Where treatment with CCB resulted in significant changes in MCA_{\text{mean}} and MAP spectral powers (VLF band; Fig. 2), regression analysis was conducted to explore the relationship between these treatment effects. Following CCB, the magnitude of the reduction in MAP power was a positive predictor of MCA_{\text{mean}} power, accounting for 48\% of the variance (Fig. 5). That is, the within-individual effects of CCB on MCA_{\text{mean}} and MAP spectral powers were positively related across individuals. To determine whether changes in CA might also be a predictor, we included the change in phase with CCB treatment as an additional covariate. Results indicated that change (following CCB) in MAP power (\( \beta = 0.90 \pm 0.30, P < 0.01 \)), but not change in phase (\( \beta = 0.90 \pm 0.30, P = 0.33 \)) was a significant predictor of change in MCA_{\text{mean}} power (\( R^2 = 0.54 \)). In contrast, neither the change in MAP power (\( \beta = 0.60 \pm 0.29, P = 0.07 \)) or phase (\( \beta = -0.059 \pm 0.23, P = 0.81 \)) was related to change in MCA_{\text{mean}} power in the LF range.

**DISCUSSION**

This study is the first to examine the interindividual relationships between BPV and CFV in a cohort of subjects with an initially intact and subsequently blunted CA. Consistent with our hypotheses, we have demonstrated that, in the resting state: 1) spontaneous BPV is positively related to CFV across individuals; 2) treatment with CCB reduces both BPV and CFV, despite some impairment of CA; 3) these variability-dampening effects (on BPV and CFV) are predominately observed in the VLF band; and 4) in this band, the magnitude of change in BPV is positively related to the magnitude of the change in CFV.

Cerebral pressure-flow relationships. Although some proportionality between BPV and CFV might be expected between individuals, to date, the exact nature and strength of these associations have not been adequately explored. Our analysis showed that CFV and BPV were positively related across all of the frequency ranges studied (0.02–0.40 Hz) and that the coefficient of determination relating CFV and BPV ranged between 0.34 and 0.67 before and 0.53 and 0.61 after CCB. These findings show that BPV is a determinant of CFV across all frequency bands studied, irrespective of whether CA is intact or blunted, but also suggest that factors other than BPV are influential. Multiple-regression analysis incorporating MAP power and gain showed that both factors were positive predictors of MCA_{\text{mean}} power, indicating that, on average, individuals with higher BPV and poorer dynamic CA have greater CFV. However, we found that phase did not signifi-
tanty predict \( MCAV_{\text{mean}} \) power under any condition. This lack of convergence between two different CA metrics points to the need to confirm the relevance of CA in explaining interindividual variations in CFV in future studies using alternative measures of dynamic CA.

When accounting for the range of 0.02–0.40 Hz, significant treatment × group interactions for BPV and CFV were found only in the VLF range. The reduction in VLF BPV following CCB is consistent with work showing that VLF BPV is generated by L-type calcium channel-dependent mechanisms (15). Our results indicate that, within this band, the reduction in CFV is partly explained by the magnitude of the change in BPV. In contrast, no significant interaction effects for BPV or CFV were found in the LF or HF range, indicating that treatment responses following CCB were similar to placebo. In animals, CCB reduces LF BPV only at very high doses compared with that required to blunt VLF variability, suggesting that LF oscillations may be less dependent on L-type \( \text{Ca}^{2+} \) channels (15). This is also in keeping with the general consensus that LF BPV reflect sympathetic modulation of vascular tone, which can continue to occur in the presence of incomplete \( \text{Ca}^{2+} \) blockade, or if intracellular \( \text{Ca}^{2+} \) stores are not fully depleted.

Clinical significance. BPV has established recognition as a prognostic indicator of certain adverse health outcomes (28). However, given that many cerebrovascular disease processes involve disordered brain perfusion, it seems that perfusion itself (i.e., CFV) could explain why individuals with high BPV are at greater risk of stroke and subsequent complications. Such a causal pathway would require BPV to be related to CFV at a population (interindividual) level, and the present study supports this latter contention.

It has previously been demonstrated that the day-by-day BPV of hypertensive individuals can be reduced with CCB (19). There is concern, however, that CCB may also impair CA, reduce cerebrovascular resistance, and enhance blood pressure transmission to vital organs, such as the brain (13, 38). Similar concerns have been raised in studies of kidney function that show that many popular CA metrics exhibit poor convergent validity and, therefore, data interpretation needs to be done with an awareness of their individual strengths and limitations (e.g., chronic vs. acute phase of stroke) remain uncertain, but clearly warrant further study.

Methodological considerations. Inferences from this study should be made in cognizance of several important methodological considerations. Transcranial Doppler is based on measurements of cerebral blood flow velocity, not volumetric flow. Because flow is a product of the vessel cross-sectional area (\( \pi r^2 \)), multiplied by blood velocity, the latter can only be used as a valid surrogate of volumetric flow for comparisons across individuals, if the caliber of the insonated vessel is known. For this reason, we have focused our results and interpretations on relative rather than absolute changes in flow velocity variability (24). Furthermore, blood pressure was noninvasively monitored in this study using the Finometer device, which employs the volume-clamp method originally developed by Penaz (27). The standard output from the Finometer incorporates correction filters that attempt to reconstruct the brachial blood pressure waveform from the finger pressure waveform (31). However, the reconstruction filter is characterized by a gain >1 over the frequency range <0.5 Hz, which means that the Finometer output can potentially overestimate spectral powers within this range (17). In contrast, the unfiltered waveform (i.e., the classical Finapres signal) generates MAP spectral powers that are quantitatively similar to those obtained via direct intra-arterial blood pressure recordings (22). Thus, to enhance the accuracy of our results, all BPV spectral analyses were performed on the unfiltered blood pressure signal (17).

It is important to recognize that, while transfer function analysis is widely applied for CA assessment, there is currently no accepted gold-standard CA metric (37). Recent studies show that many popular CA metrics exhibit poor convergent validity and, therefore, data interpretation needs to be done with an awareness of their individual strengths and limitations (37). For example, VLF phase, VLF gain, and LF gain are known to be less sensitive to changes in arterial \( \text{PCO}_2 \) (potent modulator of CA), whereas LF phase tracks changes in arterial \( \text{PCO}_2 \) in a relatively consistent and linear fashion (37). Nevertheless, our assertion that CCB impairs CA is based on the knowledge that nimodipine attenuates dynamic cerebral vasomotion under direct visual examination (32) and not simply on the current observation that CCB reduced LF phase. Although CA impairment should result in a more pressure-passive (i.e., linear) circulation, we were not confident drawing inferences with coherence, because CCB reduced MAP spectral power (input) by ~32% in the VLF and ~20% in the LF range. These substantial reductions in input power can decrease coherence.
independently of CA, therefore, potentially masking increases in coherence resulting from CA impairment. To eliminate this source of confounding, it would have been necessary to augment blood pressure power to match the preblockade condition. This was not appropriate, as the study was designed explicitly to evaluate the effects of CCB on spontaneous BPV and CFV.

It is also worth recognizing that this study was conducted under resting conditions. Although we found CCB results in a more stable resting hemodynamic profile, this treatment could nevertheless compromise the ability of the vasculature to respond to active blood pressure perturbations, such as during changes in body posture or physical activity. Assessment under “dynamic” conditions was outside the scope of the present study because one key objective here was to explore the influence of CA impairment on the relationship between spontaneous BPV and CFV. To address this question, it was necessary to use a CCB that is known to affect cerebral vasomotor function. In this regard, nimodipine has been shown to impair arteriolar responses to blood pressure changes under direct visual examination (14). We found that low-dose oral nimodipine effectively reduced both BPV and CFV, despite causing impairment in one indicator of CA. This finding implies that variability-enhancing effects associated with somewhat blunted CA were not sufficient to negate the overall flow-stabilizing effects of reduced BPV. Based on this premise, we would expect CCB using agents that are less specific for the cerebrovasculature to be equally (if not more) effective in reducing spontaneous BPV and CFV in both resting and dynamic states. However, given that the present study was performed in healthy young subjects and after acute administration of a single dose of CCB, the results should not be directly extrapolated to patients under chronic CCB treatment or to patients with cerebrovascular disease without further clinical validation. Moreover, although we observed significant reductions in phase consistent with diminished CA, the average phase post-CCB was still greater than zero. Thus our findings reflect the changes associated with the blunting, not the complete abolishment, of CA.

Finally, the summary statistics reported in this study are based on previously defined frequency bands (44). These bands are somewhat subjective, and the extent to which they accurately delineate between distinct aspects of cerebrovascular physiology remains unclear (37). Such arbitrary truncation of the MAP and MCA\textsubscript{mean} power spectrum can result in the enhancement or loss of statistical power, depending on the nature of the underlying spectral response. Nevertheless, our findings shown in Fig. 3 indicate that most changes occurred in the VLF range. We acknowledge that, with a recording duration of 6 min, it is possible our VLF spectral power estimates were less reliable than in higher frequency ranges, and that it was not possible for us to quantify fluctuations below the VLF range, which require longer recordings.

Conclusion. Our findings show that BPV is a determinant of CFV across individuals within the 0.02- to 0.40-Hz frequency range, whether CA is intact or blunted. These findings raise the possibility that CFV may be a potential explanatory factor in the association between elevated BPV and adverse cerebrovascular outcomes and support the possibility of using CCB to improve hemodynamic stability under resting conditions.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: Y.-C.T. and B.A.M. conception and design of research; Y.-C.T. and B.A.M. performed experiments; Y.-C.T. and B.A.M. analyzed data; Y.-C.T. and B.A.M. interpreted results of experiments; Y.-C.T. and B.A.M. prepared figures; Y.-C.T. and B.A.M. drafted manuscript; Y.-C.T. and B.A.M. edited and revised manuscript; Y.-C.T. and B.A.M. approved final version of manuscript.

REFERENCES