The influence of age and sex on cerebrovascular reactivity and ventilatory response to hypercapnia in children and adults

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New Findings

What is the central question of this study?

This study investigated intracranial cerebrovascular and ventilatory reactivity to 6% CO_2 in children and adults and explored dynamic ventilatory and cerebrovascular onset responses.

What is the main finding and its importance?

We show cerebrovascular reactivity is similar in children and adults, but the intracranial blood velocity onset response was markedly attenuated in children. Sex differences were apparent, with greater increases in intracranial blood velocity in females, and lower ventilatory reactivity in adult females. Our study confirms the importance of investigating dynamic onset responses when assessing the influence of development on cerebrovascular regulation.

Abstract

The purpose of this study was to compare the integrated intracranial cerebrovascular reactivity (CVR) and hypercapnic ventilatory response (HCVR) between children and adults, as well as explore the dynamic response of the middle cerebral artery mean velocity (MCA_V). Children (n = 20; 9.9±0.7 years) and adults (n = 21; 24.4±2.0 years) completed assessment of CVR over 240s using a fixed concentration of inspired CO_2 (F_ICO_2, 0.06). Baseline MCA_V was higher in the adult females compared to the males ($p \leq .05$). MCA_V was greater in female children compared to male children ($p \leq .05$), and in female adults compared to male adults ($p \leq .05$) with hypercapnia. Relative CVR was similar in children and adults (3.71±1.06 vs. 4.12±1.32 %/mmHg; $p = .098$), with absolute CVR higher in adult females than males (3.27±.86 vs. 2.53±.70 cm/s/mmHg; $p \leq .001$). Likewise, HCVR did not differ between the children and adults (1.89±1.00 vs. 1.77±1.34 L/min/mmHg; $p = .597$), but was lower in adult females than males (1.815±.37 vs. 2.33±1.66 L/min/mmHg; $p \leq .05$). The heart rate response to hypercapnia was greater in children than adults ($p = 001$). A mono-exponential regression model was used to characterize the dynamic onset, consisting of a delay term, amplitude and time constant (τ). The results revealed that MCA_V τ was faster in adults than in children (34±18 vs .74±28 s; $p = .001$). Our study provides new insight into the impact of age and sex on CVR and the dynamic response of the MCA_V to hypercapnia.

Key words: Adults; cerebrovascular reactivity; children; hypercapnia; middle cerebral artery

Introduction

The partial pressure of arterial carbon dioxide ($PaCO_2$) plays the primary role in the regulation of cerebral blood flow (CBF) during wakefulness (Hoiland *et al.*, 2019). It is well known that elevations or reductions in $PaCO_2$ result in vasodilation or constriction, respectively, of the entire cerebrovascular tree from the extra- and intracranial arteries, to the pial vessels [reviewed extensively here (Willie *et al.*, 2014)]. This vasomotor mechanism is termed cerebrovascular reactivity (CVR) and provides a marker of the dynamic regulation of CBF and cerebrovascular reserve capacity (Fierstra *et al.*, 2013; Willie *et al.*, 2014).

The extensive neuronal development that occurs during childhood is reflected in a cerebral metabolic demand that is about twice that of adults (Vandekar *et al.*, 2017). Meeting this metabolic demand requires tight regulation of CBF. Although CVR has been extensively documented in adults [reviewed in: (Hoiland *et al.*, 2019)], far less is known about this response in healthy children and adolescents (Brouwers *et al.*, 1990; Leung *et al.*, 2016). There is, however, emerging evidence that age, sex and maturity may impact CBF during childhood and adolescence. Developmental trajectories reported from a large-scale (n=922) study of 8 to 22 year olds reveal that resting cerebral perfusion (assessed from magnetic resonance imaging (MRI) arterial spin-labelling) peaks between the ages of 8 to 10 years, with values approximately 30% higher than adults; these CBF elevations are commensurate with the higher cerebral metabolic needs of this age group (Satterthwaite *et al.*, 2014). Resting CBF declines from early to late puberty in boys; however, in girls, declines are only evident in early puberty, with increases in CBF mid to late puberty (Vandekar *et al.*, 2017). When intracranial blood velocity of the anterior and posterior cerebral circulation was compared between girls and boys (4-8 years), middle and basilar cerebral artery velocities were higher in the girls compared to the boys (Tontisirin *et al.*, 2007). In contrast, cerebral autoregulation, indexed from changes in cerebral blood velocity in response to changes in blood pressure, did not differ by sex (Tontisirin *et al.*, 2007). Sex differences have also been noted in women compared to

men, with higher cerebral blood velocities reported in women (Vriens *et al.*, 1989; Marinoni *et al.*, 1998).

Leung *et al.* (2016) have reported the development of grey and white matter CVR in a group (n=34) between 9 to 30 years of age. Here, CVR was lowest in the youngest children, with increases of ~33% and 50% in grey and white matter CVR respectively between the ages of 9 to 15 years. These differences declined with age and, in comparison to young adults, children had grey and white matter CVR values ~20% and 30% lower, respectively. Higher levels of resting cerebral perfusion noted in the younger child may limit the capacity for further vasodilation, as such a lower cerebrovascular reserve, and may account for the blunted CVR observed by Leung *et al.* (2016). Higher levels of resting cerebral blood velocity have been observed in girls compared to boys (Tontisirin *et al.*, 2007). However, whether there are sex differences in CVR in the healthy child is currently unknown. It is also possible that the blunted CVR of children compared to adults may relate to a heightened hypercapnic ventilatory response (HCVR; the change in VE in response to hypercapnia) that has been reported in children (Gratas-Delamarche *et al.*, 1993; Marcus *et al.*, 1994). It is thought that the elevated ventilatory sensitivity is due to the activation of the central chemoreflex increasing the drive to breathe in order to 'blow off' the CO_2 [reviewed in (Ainslie & Duffin, 2009)]. In adults, there is some evidence that a blunted CVR is reflected in elevations in the ventilatory sensitivity to CO_2 (Xie *et al.*, 2006); however, this is not a universal finding (Hoiland *et al.*, 2015). Onset, or dynamic responses, to hypercapnia have also been used to interrogate the integration of cerebrovascular and ventilatory responses to hypercapnia. At least in adults, close coupling of middle cerebral artery blood velocity (MCA_V) and $PaCO_2$ onset responses, in comparison to a slower onset in ventilation (V_E), may infer cerebrovascular reactivity to CO_2 modulates the central ventilatory chemoreflex (Ogoh *et al.*, 2008; Ainslie & Duffin, 2009).

The integrated CBF and ventilatory response to changes in end-tidal CO_2 ($P_{ET}CO_2$) has yet to be defined in children and has implications for understanding the control of V_E and CBF during

development. If an interaction exists between the chemoreceptor regulation of breathing and brain blood flow, changes in response to hypercapnia would be anticipated to be developmentally-dependent. Therefore, the purpose of this study was to compare intracranial CVR (indexed from the MCA_V) between children and adults and explore the dynamic response of MCA_V, V_E and $P_{ET}CO_2$ by characterizing the kinetic onset responses to hypercapnia. Our primary hypotheses were (i) in response to hypercapnia, a blunted CVR in children would be reflective of an increased HCVR CO_2 when compared to adults; (ii) the dynamic response of V_E to an increase in hypercapnia would be faster in children compared to adults, whereas the dynamic response of the MCA_V response to hypercapnia would be slower in children compared to adults; and (iii) females will have greater baseline MCA_V than males in both children and adults.

Methods

Ethical Approval

All experimental procedures and the protocols were approved by the University of British Columbia Clinical Research Ethics Board (H16-01281) and the study conformed to the standards set by the *Declaration of Helsinki*, except for registration in a database. Written informed consent was obtained from the adults and the parents of the children. Additionally, written and oral informed assent was obtained from each child.

Study design

Twenty–four children (13 females, 11 males; aged 8.3 to 10.8 years) and 26 young adults (14 females, 12 males; aged 20.8 to 27.8 years) participated in this experimental study. Adult participants were recruited from the University of British Columbia Okanagan and children were recruited from a local elementary school.

Following baseline screening, participants visited the laboratory on one occasion. Per recommendations (Ainslie & Duffin, 2009), participants were asked to refrain from eating high fat foods, consuming caffeine or alcohol, or exercising strenuously 24 hours prior to testing. Additionally, room temperature and time of day were held constant for all participants, and no visual stimulation was allowed during the protocol. Upon arrival, anthropometric measures were completed and the participants were instrumented. After instrumentation, the protocol consisted of a 10-minute supine rest period breathing room air, and four-minutes of hypercapnia in the same supine position. Prior work in adults has demonstrated a steady-state response to hypercapnia using a fixed CO_2 concentration is achieved between 2 to 3 minutes (Fisher, 2016), therefore we chose a fixed concentration of 6% inspired CO_2 (F_ICO_2) over 4 minutes to quantify the responses of MCA_V and V_E to hypercapnia in children and adults. During hypercapnia, 6% CO_2 was administered in 21% oxygen (balance nitrogen). A 3-way Hans Rudolph valve allowed inspiratory gases to be switched from room air to the 6% CO_2 mixture (using a 20L Douglas bag). Throughout the protocol MCA_V, $P_{ET}CO_2$, end-tidal O_2 ($P_{ET}O_2$), heart rate (HR), blood pressure (BP), V_E and its components tidal volume (V_T) and respiratory rate (R_R) were assessed continuously.

Measures

Anthropometrics and maturation. Body mass and stature were measured barefoot in light clothing to the nearest 0.1 kg and 0.1 cm respectively. Physical maturation was assessed from predicted age at peak height velocity (aPHV), using the Mirwald equation (Mirwald *et al.*, 2002) and from parental report of Tanner stage for pubic hair and genitalia (Rasmussen *et al.*, 2015). All children were >-1 year from aPHV (mean aPHV: -2.3 ± 0.9 years). Fourteen of the 20 children were Tanner stage 1 and 6 were Tanner stage 2 (3 boys, 3 girls). Tanner stage 1 is defined by the lack of secondary sexual characteristics i.e., no pubic hair, breast or genitalia development and considered pre-pubertal. Tanner stage 2 marks the onset of secondary sex characteristics i.e., sparse pubic hair, breast buds

or onset of male genitalia growth and is considered early-pubertal (Marshall & Tanner, 1969; Marshall & Tanner, 1970).

Cardiorespiratory measures: Beat-by-beat BP was assessed using a Finometer Pro (Finapres Medical Systems). HR was assessed using a 3-lead electrocardiogram (ECG; ADInstruments BioAmp ML132). V_E, R_R, V_T, $P_{ET}CO_2$ and $P_{ET}O_2$ were assessed breath by breath, using a metabolic cart (Oxycon Pro, Carefusion, USA). The metabolic cart was calibrated prior to each test using gases of a known concentration, in addition to calibration of volume. Respiratory variables were interpolated in second-by-second intervals and time-aligned with MCA_V, HR, and BP. We excluded data if there was relative hypocapnia at baseline – this was defined as $P_{ET}CO_2$ >2 standard deviations (SD) below the child or adult mean.

Cerebrovascular measures: MCA_V (right side) was assessed using a 2-MHz transcranial Doppler (TCD) ultrasound (Spencer Technologies). The TCD probe was secured via attachment to a headpiece (child-adapted and adult M600 bilateral head frame; Spencer Technologies). MCA insonation was performed through the trans-temporal window following previously described guidelines (Willie *et al.*, 2011). Our between-day coefficient of variation for resting MCA_V insonation is 4.7%.

Data Processing

Baseline and steady-state responses to hypercapnia.

Baseline values were calculated from 120 seconds of the supine rest. Response values were taken in the final 30s of the four-minute test and differences from baseline to hypercapnia (Δ) was calculated for HR, MAP, MCA_V, V_E, V_T, R_R, $P_{ET}CO_2$ and $P_{ET}O_2$.

Cerebrovascular resistance index (CVRi) was calculated as MAP divided by MCA_V (mmHg/cm/s) at baseline and during the last 30 seconds of hypercapnia.

There is no standardized analytical approach for the calculation of CVR (Willie *et al.*, 2012; Skow *et al.*, 2013); therefore, CVR was calculated both in absolute (cm/s/mmHg) and relative (%/mmHg) terms:

$$Absolute\ CVR = \frac{(response\ MCA_V\ - baseline\ MCA_V)}{(response\ P_{ET}CO_2 - baseline\ P_{ET}CO_2)}$$

$$Relative\ CVR = \frac{\left(\frac{(response\ MCA_V - baseline\ MCA_V)}{baseline\ MCA_V} * 100\right)}{(response\ P_{ET}CO_2 - baseline\ P_{ET}CO_2)}$$

HCVR (L/min/mmHg) was calculated as:

$$HCVR = \frac{(response\ V_E\ - baseline\ V_E)}{(response\ P_{ET}CO_2 - baseline\ P_{ET}CO_2)}$$

We also examined MAP (mmHg/mmHg) and HR (beats/min/mmHg) reactivity to CO_2 to account for the impact changes in F_ICO_2 may have on cardiovascular function:

$$MAP\ reactivity = \frac{(response\ MAP - baseline\ MAP)}{(response\ P_{ET}CO_2 - baseline\ P_{ET}CO_2)}$$

$$HR\ reactivity = \frac{(response\ HR - baseline\ HR)}{(response\ P_{ET}CO_2 - baseline\ P_{ET}CO_2)}$$

Dynamic onset responses to hypercapnia.

Previous studies investigating dynamic cerebrovascular responses to various stimuli including hypercapnia and exercise, have utilized monoexponential modelling, providing a robust fit for MCA_V, V_E and $P_{ET}CO_2$ responses (Ogoh *et al.*, 2009; Billinger *et al.*, 2017). Likewise, we conducted exploratory analyses of the kinetic response of MCA_V, V_E and $P_{ET}CO_2$ to hypercapnia using an iterative least-squares non-linear regression procedure, with corresponding 95% confidence intervals (95% CIs) to establish the precision of the estimate (GraphPad Prism Version 8.0.2, GraphPad Software,

San Diego, CA). The MCA_V, V_E and $P_{ET}CO_2$ onset responses for each participant were modelled using a monoexponential function including a delay term:

$$y(t) = y0+\Delta_A(1-e-(t-TD)/\tau)$$

where $y(t)$, $y0$, Δ_A, TD and τ are the response at a given time, the baseline value, the baseline corrected change in amplitude from baseline to asymptote, the time delay and the time-constant of the response, respectively. Each participant's response was modelled from the onset of the 6% CO_2 stimulus (t = 0 s). Goodness of fit (r^2>0.50) and normality of residuals were used to determine model acceptability.

Statistical Analysis

Statistical analyses were performed using SPSS (version 25, SPSS; Chicago IL). Normality was checked and verified using the Shapiro-Wilk test normality test for all variables. Two-way analyses of variance (ANOVA) were used to compare baseline and responses to hypercapnia by age and sex. T-tests were used to deconstruct the ANOVA main and interaction effects. One-way ANOVA was used to compare onset kinetic responses by age. Pearson product moment correlation coefficients were computed to assess the relationship between CVR, HCVR, HR and MAP reactivity. Statistical significance for main effects and interactions was set *a priori* at $p \leq 0.05$. Data are presented as mean ± SD unless stated otherwise.

Results

Of the 24 children and 26 adults recruited, four children and five adults were excluded from analyses. Three children were excluded who had a $P_{ET}CO_2$ <31.2 mmHg at baseline and the MCA could not be insonated in one child. Of the five adults who were excluded from the analysis, two were excluded due to low $P_{ET}CO_2$ (<34.7 mmHg) at baseline. The MCA could not be insonated in two adults and the signal acquisition for V_E was too poor for data to be processed in one adult. The data presented are from 20 children (11 females, 9 males) and 21 adults (10 females, 11 males). Age of

the children was 9.9 ± 0.7 years (stature: 1.42 ± .07 m; body mass: 33.4 ± 6.2 kg), while the age of the adults was 24.4 ± 2.0 years (height: 1.72 ± .06 m; body mass: 72.9 ± 10.4 kg).

Baseline and steady-state responses to hypercapnia.

Absolute values for all variables (HR, MAP, MCA_V, CVRi, V_E, V_T, R_R, $P_{ET}CO_2$, and $P_{ET}O_2$) at baseline and during the last 30s of 4 minutes of CO_2 administration are presented in Table 1. There were significant child-adult differences at baseline in HR, MAP, MCA_V, CVRi, V_T, R_R, $P_{ET}CO_2$, and $P_{ET}O_2$. An age difference remained for the Δ response to hypercapnia only for HR, CVRi, V_T and R_R (see Table 1). The ΔHR response to hypercapnia was significantly greater in children compared to adults, while the CVRi decline was much greater in the adults compared to children. There was no difference in V_E at baseline or in response to hypercapnia, but children achieved a similar V_E at all time points with a smaller V_T and faster R_R.

A main effect for sex was apparent for MCA_V, MAP, CVRi, V_E, $P_{ET}CO_2$, and $P_{ET}O_2$ (see Table 1). The MCA_V values were higher in adult females compared to males at baseline ($p \leq .05$) and in the final 30s of hypercapnia ($p \leq .05$). Baseline MCA_V values were similar in girls and boys, but greater in the final 30s of hypercapnia in the girls ($p \leq .05$).

< Insert Table 1 here >

There was a main effect for sex for the MAP response to hypercapnia, with follow up analyses revealing a greater MAP response to hypercapnia in adult females compared to males ($p \leq 0.05$), but not children. The CVRi response to hypercapnia also differed by sex in the adults only, decreasing more in the females than the males ($p \leq 0.05$).

Ventilatory responses to hypercapnia (absolute and ΔV_E) were greater in adult males compared to females ($p \leq 0.05$), but not in children. All males had a higher baseline $P_{ET}CO_2$, and a smaller $\Delta P_{ET}CO_2$

in response to hypercapnia ($p \leq .05$), whereas all females had a higher baseline $P_{ET}O_2$, but a smaller hypercapnic $\Delta P_{ET}O_2$ ($p \leq .05$).

Absolute and relative CVR are presented in Figure 1. There was no main effect for age in absolute CVR (see Figure 1, panel A and B), with values of 3.38 ± 0.89 cm/s/mmHg in children and 2.89 ± 0.85 cm/s/mmHg in adults. A main effect of sex was apparent ($p = .04$), with follow-up analyses indicated absolute CVR was greater ($p \leq .05$) in females (3.27 ± .86) than males (2.53 ± .70). When expressed in relative terms, no main effect for age or sex were found (see Figure 1, panel C and D), with values of 3.71 ± 1.17 %/mmHg in children and 4.12 ± 1.32 %/mmHg in adults.

< Insert Figure 1 here >

The HCVR, MAP and HR reactivity are presented in Figure 2. There was no main effect for age for absolute HCVR (see Figure 2, panel A). A main effect for sex was present (see Figure 2, panel B), with follow-up analyses indicating a higher ventilatory reactivity in adult males (2.33 ± 1.66 L/min/mmHg) compared to females (1.15 ± 0.37 L/min/mmHg; $p \leq .05$), but not in children (males: 2.32 ± 1.21 L/min/mmHg; females 1.53 ± 0.67 v; $p = .07$).

There was no main effect for age or sex for absolute MAP reactivity (see Figure 2, panel C and D). There was however, a main effect for age for absolute HR reactivity (see Figure 2, panel E), but not sex. HR reactivity was 1.05 beats/min/mmHg higher in children than in adults.

< Insert Figure 2 here >

There were no significant relationships between CVR, HCVR, MAP or HR reactivity in children or adults, when expressed in either absolute or relative terms.

Dynamic onset responses to hypercapnia.

The estimated kinetic responses at the onset of hypercapnia are shown in Table 2. Of the 20 children and 21 adults, acceptable models for MCA_V and $P_{ET}CO_2$ were determined in 14 children and 16 adults. Acceptable model fit for V_E was only achieved in 8 children and 10 adults. Given the smaller sample size of the dynamic responses, only a main effect for age was explored using one-way ANOVAs.

< Insert Table 2 here >

A typical MCA_V response profile for a child and an adult at the onset of hypercapnia is shown in Figure 3. One-way ANOVA analyses revealed significant main effects for age for MCA_V TD and τ (see Table 2), but not Δ_A. Children responded with a τ that was on average 42 s slower than adults. The MCA_V TD was longer in children, as was the $P_{ET}CO_2$ TD. There were no age differences in any of the V_E onset parameters.

< Insert Figure 3 here >

MCA_V and V_E τ values did not differ significantly in children (p=.383), but MCA_V τ was considerably slower than $P_{ET}CO_2$ τ (mean difference 43 s; $p \leq .001$). In contrast, MCA_V τ was on average 59 s faster than V_E τ in adults ($p \leq .001$), with no significant differences between MCA_V and $P_{ET}CO_2$ τ values (p=.299).

Discussion

This study is the first to report the impact of age and sex on CVR, HCVR and the dynamic onset response of MCA_V, V_E and $P_{ET}CO_2$ to hypercapnia in children and adults. In contrast to our primary hypothesis, we did not find a blunted CVR in children, or child-adult differences in HCVR. We do, however, observe a markedly attenuated MCA_V τ in children compared to adults. In partial keeping with our final hypothesis, we did find sex differences in MCA_V at baseline, but only in adults, with

higher MCA_V values in females. Sex differences in the MCA_V response to hypercapnia were however apparent in both children and adults, with a greater MCA_V response to 6%CO_2 in females compared to males.

Child to adult differences in reactivity to CO_2

The CVR of 3.8 %/mmHg we report in 9-10-year-old children was similar to the adult CVR of 4.1 %/mmHg. These values are similar to the average adult TCD derived MCA_V CVR of between 3 to 4 %/mmHg (Hoiland *et al.*, 2019) and would suggest that cerebrovascular reserve is similar in healthy children and adults. Leung *et al.* (2016) estimated white and grey matter CVR in children to be about 20 to 30% lower compared to adults of similar ages to those in our study, concluding this may indicate a reduced cerebrovascular reserve in the child. However, previous work by Leung *et al.* (2016) did not account for physical maturation and only 3 boys and 4 girls were under the age of 12 years, limiting conclusions regarding child-adult differences in CVR. Given the influence age and maturation have on CBF, our study adds to a scant literature on the development of CVR. Comparison of these two studies is limited because of methodological differences in the approaches used; however, the methodologies used and the impact they may have on the interpretation of the findings are important and discussed in more detail under '*Methodological Considerations'* section.

We hypothesized that HCVR would be amplified in children because of the striking developmental differences in the chemoreceptive responsivity to CO_2 previously demonstrated. We failed to find developmental differences in HCVR or a relationship between CVR and HCVR. This study was designed to explore the potential effect of CVR on the central ventilatory CO_2 chemoreflex, but the rise in V_E with hypercapnia resulted in elevations in $P_{ET}O_2$ in both children and adults. The failure to maintain an iso-oxic hypercapnic stimulus is a limitation given increases in $P_{ET}CO_2$ and $P_{ET}O_2$ induce peripheral and central respiratory chemoreflex loops. An alternative approach is the use of hyperoxic gas administration to blunt the peripheral chemoreceptor response and thus isolate the central V_E chemoreflex. Although the evidence is limited, the impact of hyperoxic hypercapnia is not

uniform in children and adults. Using a step protocol comprising a single breath of 15% CO_2 in 85% O_2, Gozal *et al.* (1994) demonstrate the slope of the ventilatory response to step hypercapnic stimuli (V_E vs $P_{ET}CO_2$) was 0.507 in children compared to 0.182 in adults. Further work is needed that explores the impact of iso-oxic and hyperoxic administration of CO_2, as well as modified re-breathing techniques (Keir *et al.*, 2019) to further probe the possible interplay between CVR and HCVR in children. Inclusion of the posterior cerebral circulation will be also important, given the central chemoreceptors are located near the ventral surface of the medulla and disparate blood flow regulation has been shown in the brainstem and cortex of adults (Willie *et al.*, 2012; Skow *et al.*, 2013).

An intriguing finding was the markedly slower MCA_V kinetic response in children compared to adults. The model fit was acceptable with normality residuals and mean r^2 of .78, .71 and .91 for MCA_V, V_E and $P_{ET}CO_2$ respectively. In adults, similar to prior work (Ogoh *et al.*, 2009), MCA_V τ was tightly coupled to $P_{ET}CO_2$ τ, with V_E in lag, supporting the premise that CVR influences the central ventilatory CO_2 chemoreflex. In contrast, in children this does not appear to be the case. The MCA_V τ was 42 seconds slower than $P_{ET}CO_2$ τ, and similar to V_E τ. These disparate findings suggest developmentally distinct regulatory mechanisms, which are discussed below.

Potential Mechanism(s)

Increasing F_ICO_2 has been shown to increase MAP and thereby cause increases in cerebral perfusion pressure (Battisti-Charbonney *et al.*, 2011). We found an 8-9% increase in MAP with the increased F_ICO_2 in both children and adults, a similar MAP reactivity to hypercapnia, no relationship between CVR and MAP reactivity, but a smaller decline in CVRi with hypercapnia in children. It is possible that child-adult differences in autonomic regulation and/or cerebral autoregulation during hypercapnia exist and influence the onset response of MCA_V, but further investigations are needed to substantiate this proposition. Alternatively, sex differences in the MAP response may have impacted

the MAP reactivity–CVR relationship since cerebral autoregulation is attenuated with hypercapnia and in females (Edgell *et al.*, 2012; Perry *et al.*, 2014; Labrecque *et al.*, 2019).

A notable difference between the children and adults in the current study was the elevated HR in children with the increased F_ICO_2. It is possible that this relates to altered sympathetic or parasympathic nervous activity with hypercapnia, but this is yet to be determined in children. Of note, the fraction of cardiac output (CO) delivered to the brain is highest during childhood, declining into adulthood (Wu *et al.*, 2016). Total cerebral blood flow to aortic outflow ratio is about 10% higher in 8-10-year-old children compared to adults (Wu *et al.*, 2016). As result of the increased cerebral oxygen consumption at this age (Vandekar *et al.*, 2017), it is possible that the increased HR reflects the role CO plays in meeting increased cerebrovascular demands in the child. This possibility, however, needs to be confirmed in future studies.

In adults, there is evidence that transitory increases in F_ICO_2 causes shear mediated vasodilation in the conduit extracranial vasculature, independent of changes in MAP, cardiac output or HR that often accompany sustained hypercapnia (Hoiland *et al.*, 2017). At least in adults, dilation of the MCA has been shown to occur with elevations in $P_{ET}CO_2$ >10 mmHg (Coverdale *et al.*, 2014). Half of our participants had an increase in $P_{ET}CO_2$ between 10-12 mmHg and it is possible this caused vasodilation of the MCA. If in fact hypercapnic hyperemia caused shear-mediated vasodilation in our participants, we are likely underestimating the change in cerebral perfusion. However, previous research has shown only increases of $P_{ET}CO_2$ approximately 15 mmHg led to significant dilation of the MCA (Verbree *et al.*, 2014), in which case it is likely no significant dilation occurred in the present study (see discussion within '*Methodological Considerations*' section). Future work will need to properly document the impact of hypercapnia on the cerebral vasculature vasomotion in children using volumetric techniques to help discern whether dilation occurs and if so whether hypercapnic hyperemia is shear mediated in this population.

Sex Differences in MCA_V

Women were found to have a significantly higher baseline MCA_V than men, however, baseline MCA_V was similar in girls and boys (see Table 1). The sex difference found in adults confirms our hypothesis and aligns with previous literature where significant sex differences were found in MCA_V of adults at rest (Vriens *et al.*, 1989; Marinoni *et al.*, 1998). However, the similarity between the girls (94.1 ± 9.5 cm/s) and boys (90.5 ± 9.8 cm/s) included in this data set is contrary to the tertiary hypothesis of the present study. The age of the sample population may provide a reasonable explanation for this finding. Using transcranial Doppler, Tontisirin *et al.* (2007) report higher levels of resting MCA_V in girls (99 ± 11 cm/s) compared to boys (92 ± 13 cm/s) in 48 children between the age of 4 and 8 years old. However, prior research reports no sex difference in baseline MCA_V in 76 children between any of the four age categories: 2-4 years; 5-9 years; 10-14 years; 15-19 years (Brouwers *et al.*, 1990). As the present study includes a range of children 8 to 11 years of age, an older population than those included by Tontisirin *et al.* (2007), yet within range of those included by Brouwers *et al.* (1990), it is reasonable that our results mirror the latter. However, future longitudinal work is needed to fully understand the influence of age on sex differences in resting intracranial blood velocities.

A number of reasons have been proposed for the elevated cerebral blood velocity response to hypercapnia in all females, including sex hormones, hemoglobin, cerebral metabolism, and shear stress (Vavilala *et al.*, 2002). Within the present study there was a lack of control for menstrual phase in the adult females. However, our findings are similar to others who detected sex differences in CVR without controlling for menstrual phase (Minhas *et al.*, 2018), but estrogen is known to impact vasodilation (Krause *et al.*, 2006) and we do not account for this. Additionally, estrogen has been linked to greater carotid artery compliance and distensibility in women compared to men (Marlatt *et al.*, 2013). Albeit within the peripheral vasculature, there is evidence of sex differences in vascular smooth muscle in children who were classified as Tanner stage 2 (McCue *et al.*, 2012). Despite only six of our participants being Tanner stage 2, the remaining 14 were Tanner stage 1, suggests other factors alongside sex hormones may account for the sex differences noted.

It is also important to consider whether sex differences in cerebral metabolic rate (CMR) account for the an elevated hypercapnic MCA_V response in the girls. In an early study of CMR using a modified Kety Schmidt procedure, Kennedy and Sokoloff (1957) report higher CMRs in children up to 11 years of age compared to adults. Tontisirin *et al.* (2007) also report an elevated MCA_V in girls compared to boys. In contrast, a recent investigation of sex differences in CMR in a large cohort of children and adolescents, showed a lower global CMR (expressed as a % of whole body resting metabolic rate) and lower cerebral glucose consumption in girls in comparison to boys (Vandekar *et al.*, 2017). This latter study highlighted the increased CMR in children compared to adults, alongside increased cerebral perfusion in the younger participants. The higher baseline perfusion and lower CVRi we note in the children compared to the adults is most likely an outcome of the higher CMR in the child.

Methodological considerations

How the CO_2 challenge is delivered is important given the impact this has on the time required to reach a steady state cerebrovascular response and the (in)ability to target end-tidal gases and standardize the end-tidal to arterial CO_2 gradient [reviewed in (Hoiland *et al.*, 2019)]. The present study implemented elevations in F_ICO_2 to experimentally manipulate $PaCO_2$. The benefit of this approach being it allows interactions between CVR and V_E to be explored since the $P_{ET}CO_2$ to $PaCO_2$ gradients are able to vary (Hoiland *et al.*, 2019). Leung *et al.* (2016) used prospective targeting of $P_{ET}CO_2$, alternating 60s of normoxic isocapnia [$P_{ET}CO_2$ of 40 mmHg] with 45s of normoxic hypercapnia [$P_{ET}CO_2$ of 45 mmHg]). Previous work in adults shows the time to steady state differs substantially between these two methods, with steady state attained during the prospective targeting technique within 1-15s, compared to the 1 to 2 minutes required when using a fixed concentration F_ICO_2 (Mark *et al.*, 2010; Fisher, 2016). Our dynamic onset data reveal a distinctly slower MCA_V onset in children compared to adults, and it will be valuable to understand differences in steady state between the F_ICO_2 and prospective targeting of $P_{ET}CO_2$ in children. Of note, we were only able to fit the monoexponential model to the ventilatory data in 8 children and 10 adults. The

ventilatory response to CO_2 usually requires more than four minutes to attain steady-state and it is likely our protocol was of insufficient length for the kinetic modelling of V_E (Ogoh *et al.*, 2009).

While using a fixed F_ICO_2 does allow the interactions between CVR and V_E to unfold, it does not permit precise control of the change in $P_{ET}CO_2$ (or $P_{ET}O_2$) and shifts in the $PaCO_2$ to $P_{ET}CO_2$ gradient can result (Hoiland *et al.*, 2019). It is important to note that resting $P_{ET}CO_2$ closely reflects $PaCO_2$ in both children and adults and is an acceptable, non-invasive surrogate (Ohuchi *et al.*, 1999). The baseline $P_{ET}CO_2$ and $P_{ET}O_2$ values we report for children (Table 1: 36.6±2.7 / 98.5±4.3 mmHg) are similar to those found in previous published work from our group (Ellis *et al.*, 2017) and others (MacLean *et al.*, 2016) reporting $P_{ET}CO_2$ 36.1±3.8 and $P_{ET}O_2$ 102.2±10.1 mmHg in healthy 9-12-year-olds. Hypercapnia can increase the physiological deadspace to tidal volume ratio that can inflate $P_{ET}CO_2$ to a greater extent than $PaCO_2$ and modify the MCA_V response. Interestingly, the response of $P_{ET}CO_2$ and $P_{ET}O_2$ to four minutes of increased F_ICO_2 in our study were similar between the children and adults. Although inter-individual variability in the $PaCO_2$ to $P_{ET}CO_2$ gradient is unavoidable using the fixed F_ICO_2 approach, this does not appear to have resulted in differences in the $P_{ET}CO_2$ response to hypercapnia between the children and adults in this study. It is worth noting also, similar to our previous findings (Ellis *et al.*, 2017), the pattern of response in MCA_V in children did not mirror the response pattern in $P_{ET}CO_2$; however, the changes in $P_{ET}CO_2$ and MCA_V seems to be aligned in adults. In order to properly discern the trajectory of change in CVR throughout childhood from methodology it will be important for future work to carefully consider the impact the experimental approach to delivery of CO_2, as well as the technique used to assess CBF or velocity has on the CVR response.

The implementation of TCD to assess of cerebral blood velocity has both limitations and benefits. TCD does not account for vessel diameter, only providing an assessment of blood velocity not blood flow (Hoiland *et al.*, 2019). As CO_2 is a known vasodilator, without a measure of vessel diameter the assessment of the change in CBF cannot be conducted and therefore the change in cerebral perfusion could be underestimated (Ainslie & Hoiland, 2014). Literature on the vasomotion of the

MCA with hypercapnia is convoluted, although it has been suggested that there is subtle vasodilation of the MCA with hypercapnia (Hoiland & Ainslie, 2016). However, the degree of the increase in $P_{ET}CO_2$ matters. For example, in a MRI study Verbree *et al.* (2014), reported significant vasodilation of the MCA after an increase in $P_{ET}CO_2$ from 36.9 ± 3.8 mmHg at baseline to 51.0 ± 4.5 mmHg in adults. However, no significant change in MCA diameter occurred with an increase from baseline values to 45.0 ± 3.8 mmHg (Verbree *et al.*, 2014), suggesting the magnitude of the change above baseline values influences the extent of vasomotion of the MCA. It is also unknown how the vasculature within the child reacts to hypercapnia and whether the vasomotion of the MCA changes in the same pattern as the adults. In the current study, the increase in $P_{ET}CO_2$ averaged 10.2 ± 1.9 mmHg within all participant values; however, $P_{ET}CO_2$ values of 49.7 ± 2.0 mmHg were reached in the adults (compared to 46.3 ± 1.8 mmHg in the children) due to their slightly higher resting $P_{ET}CO_2$ at baseline. It would seem reasonable that it is the delta change – rather than absolute value – in $PaCO_2$ that might influence the MCA diameter; therefore, both adults and children would have had the same vasomotion stimulus. Despite the noted limitations of both the fixed F_ICO_2 experimental approach and TCD for measurement of cerebral blood velocity, they do provide robust and commonly used methods for assessing cerebrovascular reactivity that are applicable to a paediatric population (Hoiland *et al.*, 2019).

We explored the link between cerebral blood velocity and V_E by insonating the MCA. The MCA is one of the two most commonly insonated vessels, the other being posterior cerebral artery, these vessels supply blood to the anterior and posterior regions of the brain, respectively. If a change (either increase or decrease) in V_E were expected to influence cerebral blood velocity through an interaction with the respiratory centers (e.g. via an influence on local metabolism), this would be expected to manifest within the *posterior* not *anterior* circulation. Therefore, insonation solely of the MCA as per this study, may not fully capture potential relationships between ventilatory and cerebrovascular control. At least in adults, studies have reported no differences in CVR to

hypercapnia between posterior and anterior circulations (Willie *et al.*, 2012; Hoiland *et al.*, 2015); however, if any regional differences are present in children should be considered in future studies.

Conclusion

These are the first findings to provide insight into the dynamic onset response of the MCA_V to hypercapnia in children compared to adults and to explore the impact sex may have on cerebrovascular reactivity in the child. We show a similar CVR, but markedly attenuated MCA_V onset response in the child, that may relate to differences in cerebral perfusion pressure, autonomic control or cerebral metabolism. Identifying which factors account for age, sex, and maturation differences in the MCA_V response to hypercapnia is challenging, but has relevant application to clinical settings when interpreting cerebral hemodynamic abnormalities in children. Future work will require careful consideration of the impact of the methodological approach employed, examination of regional differences in CVR in children and a longitudinal design to fully understand the development of cerebrovascular regulation.

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Additional Information

Disclosure/ Conflict of Interest

The authors declared no potential conflicts of interest.

Author Contribution

CT, DNF, PA & AM conceived the study design. CT, DNF & AM were involved with data collection. CM, AB, PA & AM were involved in data analysis and interpretation. CM, PA & AM drafted the manuscript. All authors critically reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Tables

Table 1. Ventilatory, cardiovascular, and cerebrovascular data at baseline and in the final 30s of hypercapnia in children and adults and by sex

		CHILDREN			ADULTS			ANOVA MAIN EFFECTS		
		Female	**Male**	**All Children**	**Female**	**Male**	**All Adults**	**Age**	**Sex**	**Age*Sex**
		(n = 11)	(n = 9)	(n = 20)	(n = 10)	(n = 11)	(n = 21)	*p value*	*p value*	*p value*
HR (beats/min)	BL	78 ± 8	78 ± 7	78 ± 8	69 ± 15	62 ± 12	65 ± 14	**.001**	.377	.292
	Hypercapnia	93 ± 10	92 ± 6	92 ± 8	73 ± 14	66 ± 8	69 ± 12	**.001**	.189	.427
	Δ	16 ± 6	13 ± 6	14 ± 6	4 ± 4	5 ± 6	4 ± 5	**.001**	.526	.492
MAP (mmHg)	BL	78 ± 10	78 ± 10	78 ± 9.5	88 ± 12	82 ± 9	85 ± 11	**.040**	.270	.384
	Hypercapnia	87 ± 10	83 ± 12	85 ± 11	97 ± 13	87 ± 10	92 ± 12	**.034**	**.048**	.403
	Δ	8 ± 6	5 ± 6	7 ± 6	9 ± 6	6 ± 7	7 ± 6	.687	.079	.957
MCA_V (cm/s)	BL	94.1 ± 9.5	90.5 ± 9.8	92.7 ± 9.5	79.6 ± 15.4	64.0 ± 7.4	71.4 ± 14.1	**.001**	**.008**	.086

	Hypercapnia	129.9 ± 11.9	118.1 ± 13.3	125.6 ± 12.7	115.8 ± 14.8	88.2 ± 11.6	101.7 ± 19.4	**.001**	**.001**	.053
	Δ	35.8 ± 8.0	27.6 ± 7.7	32.9 ± 9.6	36.2 ± 9.7	24.2 ± 6.2	29.9 ± 10.0	.548	**.001**	.456
CVRi (mmHg/cm/s)	BL	0.84 ± 0.16	0.86 ± 0.12	0.85 ± 0.14	1.17 ± 0.22	1.29 ± 0.19	1.24 ± 0.21	**.001**	.143	.252
	Hypercapnia	0.67 ± 0.08	0.70 ± 0.08	0.68 ± 0.08	0.70 ± 0.22	.88 ± 0.24	0.80 ± 0.25	**.001**	**.033**	.138
	Δ	-0.24 ± 0.17	-0.16 ± 0.07	-0.17 ± 0.09	-0.48 ± 0.28	-0.41 ± 0.15	-0.44 ± 0.22	.394	.478	.698
V_E (L/min)	BL	8.0 ± 1.5	7.7 ± 1.3	7.9 ± 1.3	8.2 ± 1.6	8.8 ± 1.9	8.5 ± 1.8	.196	.637	.366
	Hypercapnia	22.9 ± 5.4	27.0 ± 7.7	24.8 ± 6.7	20.7 ± 4.1	29.3 ± 11.7	25.3 ± 9.8	.978	**.014**	.369
	Δ	15.0 ± 5.2	19.3 ± 8.3	16.9 ± 7.0	12.6 ± 3.4	20.5 ± 11.4	16.7 ± 9.3	.812	**.016**	.468
V_T (L)	BL	0.42 ± 0.10	0.48 ± 0.15	0.45 ± 0.10	0.72 ± 0.07	0.91 ± 0.31	0.82 ± 0.25	**.001**	**.037**	.253
	Hypercapnia	0.88 ± 0.14	0.94 ± 0.21	0.91 ± 0.17	1.41 ± 0.18	1.87 ± 0.41	1.66 ± 0.40	**.001**	**.003**	**.021**
	Δ	0.46 ± 0.16	0.46 ± 0.23	0.46 ± 0.19	0.69 ± 0.21	.96 ± 0.32	0.84 ± 0.31	**.001**	.084	.087
R_R (bpm)	BL	20 ± 4	18 ± 4	19 ± 4	12 ± 3	11 ± 3	11 ± 3	**.001**	.142	.461
	Hypercapnia	28 ± 9	32 ± 11	30 ± 9	16 ± 3	16 ± 6	16 ± 5	**.001**	.373	.532
									.110	

	Δ	8 ± 7	14 ± 11	11 ± 10	4 ± 3	6 ± 6	5 ± 5	**.010**		.325
$P_{ET}CO_2$ (mmHg)	BL	36.0 ± 2.7	37.4 ± 1.4	36.6 ± 2.7	38.2 ± 2.0	40.2 ± 2.2	39.3 ± 2.3	**.001**	**.017**	.676
	Hypercapnia	46.4 ± 1.8	46.2 ± 1.9	46.3 ± 1.8	49.24± 1.8	50.1 ± 2.2	49.7 ± 2.0	**.001**	.641	.485
	Δ	10.4 ± 2.3	8.8 ± 1.5	9.7 ± 2.1	11.1 ± 1.1	9.9 ± 2.0	10.5 ± 1.7	.121	**.016**	.801
$P_{ET}O_2$ (mmHg)	BL	100.9 ± 4.1	96.6 ± 2.3	98.5 ± 4.3	96.1 ± 5.3	92.8 ± 4.9	94.4 ± 5.4	**.009**	**.004**	.482
	Hypercapnia	132.9 ± 4.3	133.0 ± 5.6	132.9 ± 4.3	125.7 ± 4.6	127.2 ± 5.4	126.5 ± 4.6	**.001**	.567	.610
	Δ	32.0 ± 3.0	37.4 ± 5.7	34.4 ± 5.1	29.6 ± 4.0	34.5 ± 6.4	32.1 ± 5.8	.094	**.002**	.870

Values are means ± SD. HR, heart rate; MAP, mean arterial blood pressure; CVRi, cerebrovascular resistance index; MCA_V, middle cerebral artery mean velocity; V_E, ventilation; V_T, tidal volume; R_R, respiratory rate; $P_{ET}CO_2$, end-tidal carbon dioxide; $P_{ET}O_2$, end-tidal oxygen; BL, baseline; Δ, absolute change between the pre-stimulus baseline to the last 30s of hypercapnia.

Table 2. MCA_V, $P_{ET}CO_2$ and V_E kinetic responses at the onset of hypercapnia in children and adults.

		Children (*n=14*)	**Adults** (*n=16*)	**ANOVA Main effect for age** (*p value)*
MCA_V	Δ_A (cm/s)	35.2± 8.1	29.2 ± 8.5	.081
	TD (s)	11 ± 5	5 ± 4	**.023**
	τ (s)	74 ± 28	34 ± 18	**.001**
$P_{ET}CO_2$	Δ_A (mmHg/s)	10.7 ± 1.6	9.7 ± 2.3	.321
	TD (s)	8 ± 2	4 ± 2	**.006**
	τ (s)	31 ± 11	27 ± 16	.622
		n=8	*n=10*	
V_E	Δ_A (L/min)	16.6 ± 6.0	26.9 ± 14.3	.127
n=8	TD (s)	11 ± 8	13 ± 7	.652
	τ (s)	102 ± 40	98 ± 24	.891

Values are means ± SD. MCA_V, middle cerebral artery mean velocity; $P_{ET}CO_2$, end-tidal carbon dioxide; V_E, ventilation. Δ_A delta amplitude, TD time delay, τ time constant.

Figure Legends

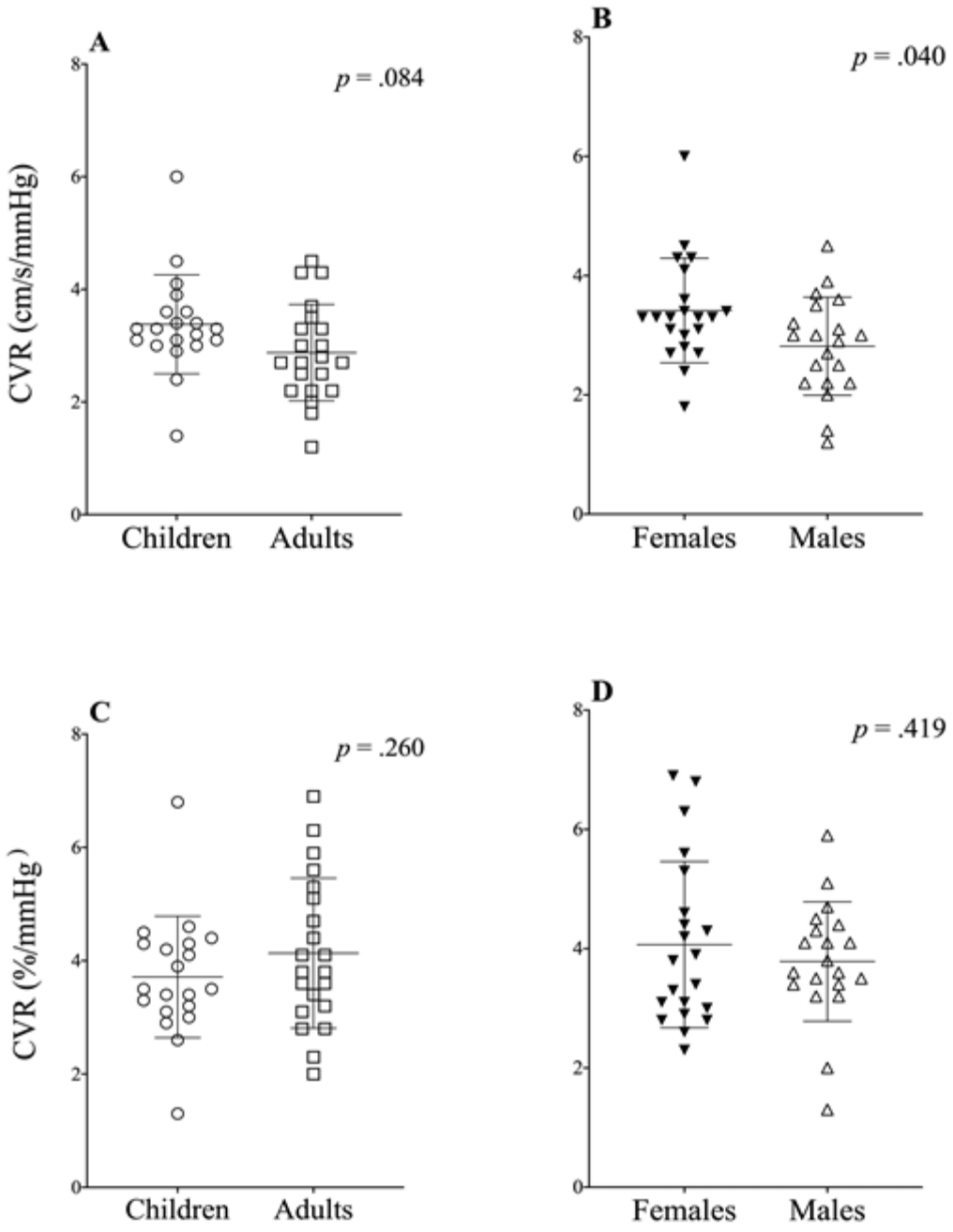

Figure 1. Relative and absolute cerebrovascular reactivity (CVR) to hypercapnia by age and by sex. *A,* absolute CVR in children and adults. *B,* absolute CVR in males and females. *C,* relative CVR in children and adults. *D*, relative CVR in males and females. *P* values indicate the main effects for age and sex from ANOVA analyses.

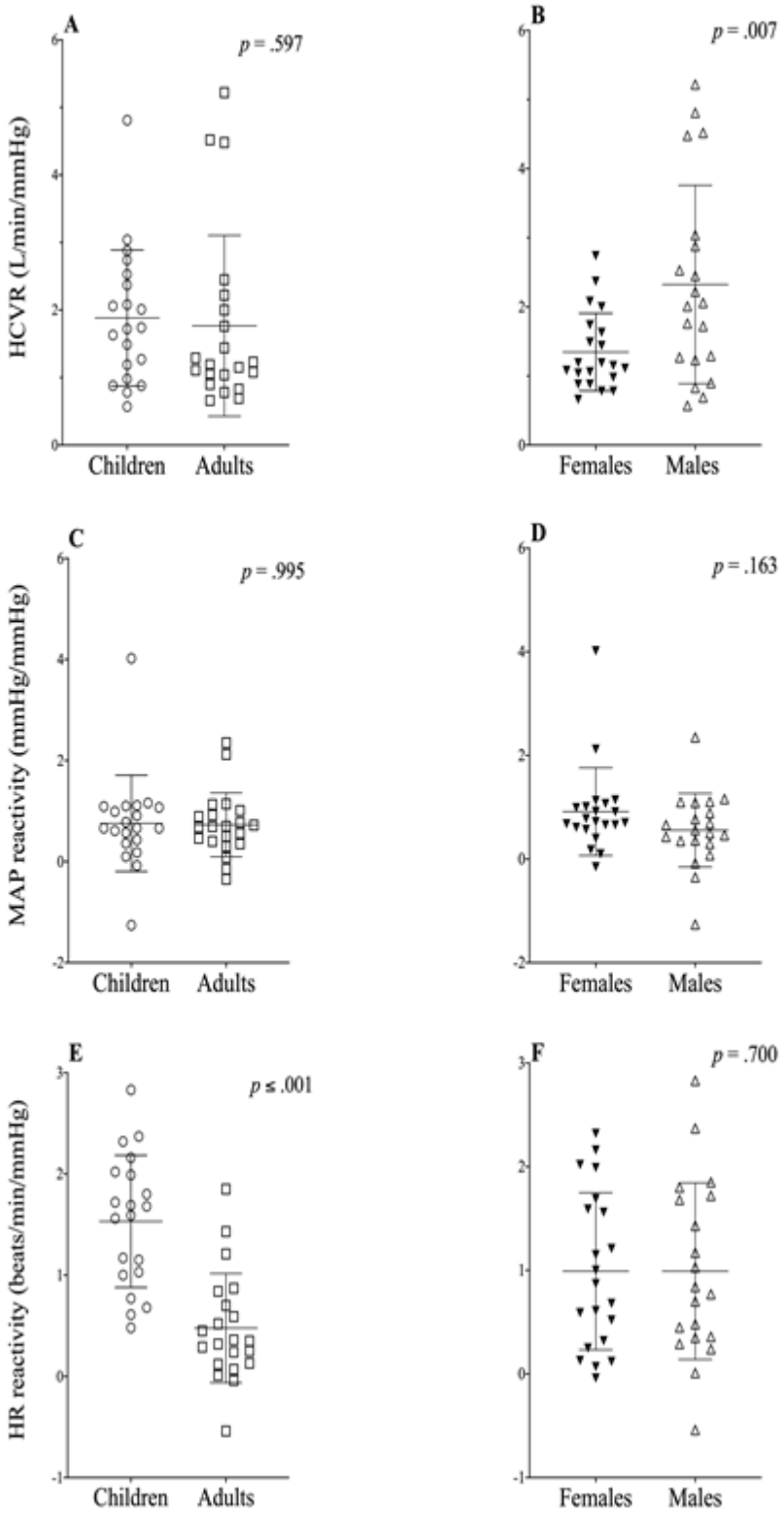

Figure 2. Hypercapnic ventilatory and cardiovascular reactivity by age and by sex. *A* hypercapnic ventilatory reactivity (HCVR) in children and adults. *B*, HCVR in males and females. *C,* mean arterial pressure (MAP) reactivity in children and adults. *D*, MAP reactivity in males and females. *E*, heart rate (HR) reactivity in children and adults. *F*, HR reactivity in males and females. *P* values indicate the main effects for age and sex from ANOVA analyses.

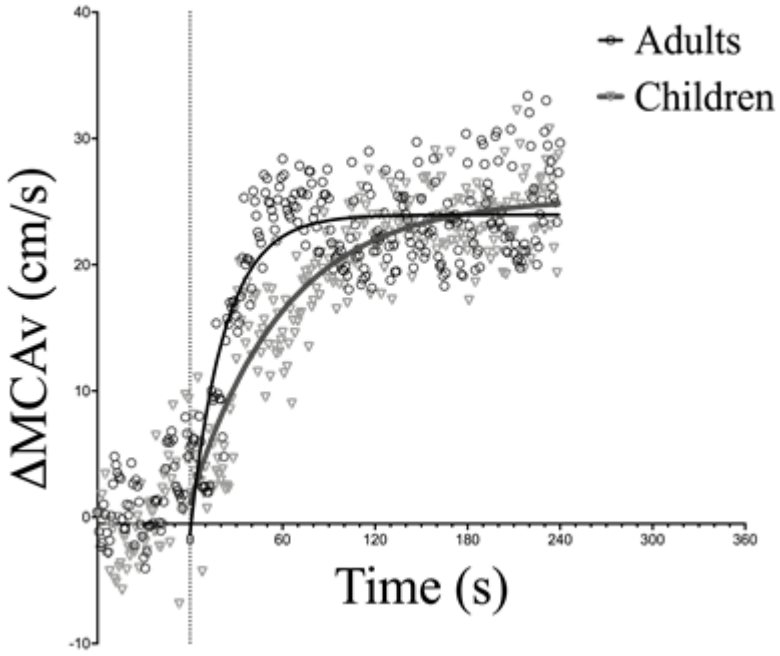

Figure 3. Dynamic onset response of MCA_V in representative child and adult subjects. The *continuous black and grey lines* represent the fitted monoexponential function in the adults and children respectively. The *vertical dotted line* signifies the onset of the CO_2 stimulus.