Cyclooxygenase-derived vasoconstriction restrains hypoxia-mediated cerebral vasodilation in young adults with metabolic syndrome

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Submitted 13 September 2013; accepted in final form 3 November 2013

Harrell JW, Schrage WG. Cyclooxygenase-derived vasoconstriction restrains hypoxia-mediated cerebral vasodilation in young adults with metabolic syndrome. Am J Physiol Heart Circ Physiol 306: H261–H269, 2014. First published November 8, 2013; doi:10.1152/ajpheart.00709.2013.—Poor cerebrovascular function in metabolic syndrome (MetSyn) likely contributes to elevated risk of cerebrovascular disease in this growing clinical population. Younger MetSyn adults without clinical evidence of cerebrovascular disease exhibit preserved hypercapnic vasodilation yet markedly impaired hypoxic vasodilation, but the mechanisms behind reduced hypoxic vasodilation are unknown. Based on data from rats, we tested the hypothesis that younger adults with MetSyn exhibit reduced cerebral hypoxic vasodilation due to loss of vasodilating prostaglandins. Middle cerebral artery velocity (MCAv) was measured with transcranial Doppler ultrasound in adults with MetSyn (n = 13, 33 ± 3 yr) and healthy controls (n = 15, 31 ± 2 yr). Isocapnic hypoxia was induced by titrating inspired oxygen to lower arterial saturation to 90% and 80% for 5 min each. Separately, hypercapnia was induced by increasing end-tidal CO2 by 10 mmHg above baseline levels. Cyclooxygenase inhibition (100 mg indomethacin) was conducted in a randomized double-blind, placebo controlled design. MCAv was normalized for group differences in blood pressure (healthy: 89 ± 2 mmHg vs. MetSyn: 102 ± 2 mmHg) as cerebral vascular conductance index (CVCi), and used to assess cerebral vasodilation. Hypoxia increased CVCi in both groups; however, vasodilation was ~55% lower in MetSyn at SpO2 = 80% (P < 0.05). Indomethacin tended to decrease hypoxic vasodilation in healthy controls, and unexpectedly increased dilation in MetSyn (P < 0.05). In contrast to hypoxia, hypercapnia-mediated vasodilation was similar between groups, as was the decrease in vasodilation with indomethacin. These data indicate increased production of vasoconstrictor prostaglandins restrains hypoxic cerebral vasodilation in MetSyn, preventing them from responding appropriately to this important physiological stressor.

MATERIALS AND METHODS

Subjects

Thirteen adults with metabolic syndrome (MetSyn, 33 ± 3 yr) and fifteen lean, healthy controls (31 ± 2 yr) participated in this study. In addition to providing written informed consent, each subject completed a physical activity and healthy history questionnaire. All subjects were sedentary (<60 moderate-intensity aerobic exercise/wk) and free of overt cardiovascular, neurological, cerebrovascular, metabolic, pulmonary, gastrointestinal, renal, and all other chronic diseases (self-report). Subjects were not taking regular medication of metabolic syndrome; cerebral blood flow; cyclooxygenase.

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any kind. Females were studied in the early follicular phase of their menstrual cycle (oral contraception allowed) and were required to take a urine pregnancy test to exclude pregnancy.

MetSyn participants met the NCEP-ATP III definition of metabolic syndrome as modified by the American Diabetes Association and International Diabetes Federation (13). Adults with MetSyn were required to meet three of the following five criteria: blood glucose $\geq 100$ mg/dl, resting blood pressure $\geq 135/\geq 85$ mmHg, waist circumference $> 102$ cm men, $> 88$ cm women, triglycerides $\geq 150$ mg/dl, and/or HDL <40 mg/dl women, <50 mg/dl men. Healthy controls were excluded if they met any of the criteria for MetSyn.

Subjects reported to the lab after a 12 h fast, including abstaining from exercise, alcohol, NSAIDs, and caffeine for 18 h. All procedures adhered to the Declaration of Helsinki and were approved by the University of Wisconsin-Madison Health Science Institutional Review Board.

Measurements

Height and weight were measured to calculate body mass index (BMI, kg/m$^2$). Waist and hip circumference were measured to confirm adiposity. Prior to undergoing any study conditions, blood was drawn from an antecubital vein and analyzed for glucose and cholesterol concentration to confirm the presence of MetSyn. Throughout the study visits, subjects were continuously monitored with a pulse oximeter (arterial oxygen saturation, Sp$_{O2}$), automatic sphygmomanometer (blood pressure, MBP), and three-lead electrocardiogram (heart rate, HR) (Duetx-Ohmeda; Helsinki, Finland).

A 2-MHz transcranial Doppler ultrasound probe (Neurovision model 500M, Multigon Industries; Yonkers, NY) was held in place over the temporal window by an adjustable headband to measure middle cerebral artery velocity (MCAv). A linear heated pneumotachometer (model 3813, Hans Rudolph, Shawnee, KS) measured respiratory flow and a GEMINI gas analyzer (CWE, Ardmore, PA) continuously measured inspiratory and expiratory gas concentration.

Protocol

Subjects reported to the laboratory on two separate occasions. The timeline of each visit is illustrated in Fig. 1. Using a double-blind, placebo-controlled design, we tested cerebral vasodilation to graded hypoxia and hypercapnia with acute oral administration of indomethacin to inhibit COX. Subjects were in the semirecumbent position throughout both study visits. After baseline data collection, subjects were given either indomethacin (100 mg) or placebo pills in randomized order. Maalox (20 ml) was administered with both sets of pills to minimize gastrointestinal discomfort. Hemodynamic and respiratory data were collected every 10 min in 5-min intervals following administration of the pharmaceutical. Ninety minutes following oral administration of the pharmaceutical, hypoxia and hypercapnia trials were conducted in randomized order.

Hypoxia. Hypoxia methods have been described earlier (15). After 5 min of baseline, room air breathing, graded isocapnic hypoxia was induced by titrating inspired oxygen (Fi$_{O2}$) to achieve and sustain 5 min of Sp$_{O2} = 90\%$ followed by 5 min of Sp$_{O2} = 80\%$. An Sp$_{O2} = 90\%$ is approximately an arterial Po$_{2} = 60$ mmHg and an Sp$_{O2} = 80\%$ is approximately an arterial Po$_{2} = 43$ mmHg (32). The composition of inspired air was controlled using a gas mixer and a two-way non-rebreathing valve (2700 Series, Hans Rudolph) with the subject wearing a nose clip. Throughout the trial, end-tidal carbon dioxide (PetCO$_{2}$) was maintained at baseline levels by adding CO$_{2}$ to the blended gas to isolate the effects of lowered Sp$_{O2}$.

Hypercapnia. The hypercapnia protocol is identical to previously reported research methods (15). Briefly, subjects breathed through a mouthpiece connected to a three-way sliding valve (model 2870, Hans Rudolph) while nasal airflow was occluded. A meteorological balloon filled with a hyperoxic (Fi$_{O2} = 0.40$), hypercapnic (Fi$_{CO2} = 0.03$) mix of gas was attached to the three-way sliding valve. The balloon was filled to a volume 1 liter above vital capacity as estimated based on sex, height, and age. After 5 min of steady-state room air breathing, subjects were switched to breathing the hyperoxic, hypercapnic mix of gas until PetCO$_{2}$ reached 10 mmHg above baseline values (~2 min). The trial was repeated and results were averaged.

Data Acquisition and Analysis

Continuous MCAv, heart rate, and pulse oximetry were collected along with breath-by-breath gas analysis. Data were digitized, recorded, and stored using PowerLab and LabChart (ADInstruments, Colorado Springs, CO) and analyzed offline. To investigate the effect of oral administration of the drugs over the 90 min of drug wash-in, the last 30 s of baseline and steady-state data were averaged. For hypoxia trials, the last 30 s of baseline and steady-state data were used for analysis. Hypercapnia data analysis included averaging the last 30 s of baseline and the last 10 s of hypercapnia to examine the peak response to CO$_{2}$. Blood pressure was recorded during the last 30 s of each condition.

To account for group differences in perfusion pressure, MCAv was divided by MBP and is presented as cerebrovascular conductance index (CVCi = MCAv / 100 x MBP) (15, 24). The primary analysis was to investigate whether cerebral vasodilation to hypoxia or hypercapnia was different between groups. Cerebral vasodilation was defined as a positive change of CVCi from baseline in response to hypoxia or hypercapnia ($\Delta$CVCi = CVCiCondition – CVCiBaseline). Additionally, analysis was conducted to examine the contribution of COX products to both hypoxia and hypercapnia cerebral vasodilation. The contribution of COX products was quantified as a change in the $\Delta$CVCi to both hypoxia and hypercapnia conditions (Change of $\Delta$CVCi = $\Delta$CVCiIndomethacin – $\Delta$CVCiPlacebo).

Instrumentation

HR, BP, MCAv, and PetCO$_{2}$ continuously monitored

Placebo or Indo

Baseline

90 minute drug wash-in

Serial data collections

S$_{O2}$ 99% 90% 80%

Graded Hypoxia

F$_{CO2}$ 3% rebreathe

Hypercapnia

Fig. 1. Experimental timeline of study visits. Placebo and indomethacin were administered in a double-blind, randomized order. Hypoxia and hypercapnia were performed in randomized order each visit. Indo, Indomethacin; Sp$_{O2}$, pulse oximeter arterial saturation; Fi$_{CO2}$, fraction of inspired CO$_{2}$; HR, heart rate; BP, blood pressure; MCAv, middle cerebral artery velocity; PetCO$_{2}$, end-tidal partial pressure of carbon dioxide.

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Hypoxia-Mediated Cerebral Vasodilation: Role of COX

Table 2. Hemodynamic and gas exchange variables before and 90 min after placebo or indomethacin

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy</th>
<th>MetSyn</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP, mmHg*</td>
<td>Placebo Indo</td>
<td>Placebo Indo</td>
</tr>
<tr>
<td>Baseline</td>
<td>89 ± 2</td>
<td>89 ± 2</td>
</tr>
<tr>
<td>90 min</td>
<td>90 ± 2</td>
<td>103 ± 2</td>
</tr>
<tr>
<td>PETCO₂, mmHg*</td>
<td>37 ± 1</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>Baseline</td>
<td>39 ± 1</td>
<td>37 ± 1</td>
</tr>
<tr>
<td>90 min</td>
<td>38 ± 0</td>
<td>38 ± 1</td>
</tr>
<tr>
<td>SpO₂, %</td>
<td>98 ± 0</td>
<td>98 ± 0</td>
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<tr>
<td>Baseline</td>
<td>98 ± 0</td>
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<tr>
<td>90 min</td>
<td>98 ± 0</td>
<td>97 ± 0</td>
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<tr>
<td>MCAv, cm/s*†‡§</td>
<td>72 ± 3</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>Baseline</td>
<td>73 ± 4</td>
<td>65 ± 4</td>
</tr>
<tr>
<td>90 min</td>
<td>45 ± 2</td>
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<tr>
<td>ΔMCAv</td>
<td>-27 ± 2</td>
<td>-22 ± 2</td>
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<tr>
<td>90 min</td>
<td>-37 ± 2</td>
<td>-34 ± 2</td>
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<td>ΔCVCi*</td>
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<tr>
<td>90 min</td>
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<td>64 ± 4</td>
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<tr>
<td>%ΔCVCi</td>
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<td>-23 ± 2</td>
</tr>
<tr>
<td>90 min</td>
<td>-41 ± 2</td>
<td>-36 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Indomethacin; MBP, mean blood pressure; PETCO₂, end-tidal carbon dioxide; SpO₂, pulse oximeter saturation; MCAv, middle cerebral artery velocity; ΔMCAv, absolute change in MCAv; ΔCVCi, relative change in MCAv; CVCi, cerebrovascular conductance index; ΔCVCi, absolute change in CVCi; %ΔCVCi, relative change in CVCi. *Main effect of group, †main effect of time, ‡main effect of Indo, §Indo × time interaction (P < 0.05).

subjects, but the relative change (%ΔCVCi) was similar between groups (Table 2).

Hypoxia-Mediated Cerebral Vasodilation: Role of COX

Table 3 contains the hemodynamic and gas exchange variables collected during the hypoxia trials. By design, there was a significant main effect of hypoxia as determined by arterial pulse oximeter saturation (SpO₂). Although MetSyn adults had higher MBP and lower end-tidal carbon dioxide (PETCO₂) at baseline, hypoxia did not affect either variable in lean or MetSyn adults (Table 3). With placebo, ΔCVCi was higher in healthy subjects compared with MetSyn (P < 0.05, Fig. 3E).

Indomethacin did not significantly affect PETCO₂, SpO₂, or MBP (Table 3). Indomethacin significantly reduced both MCAv and CVCi (P < 0.05, Table 3). However, there was a group-specific effect on hypoxia-mediated cerebral vasodilation (%ΔCVCi). Indomethacin significantly increased ΔCVCi in adults with MetSyn (P < 0.05, Fig. 3D) along with a trend for decreasing ΔCVCi in healthy controls at SpO₂ = 80% (P = 0.13, Fig. 3C). The directionally different effect of indomethacin on ΔCVCi lead to a significant difference in the magnitude of change in cerebral vasodilation caused by indomethacin (P < 0.05, Fig. 3F).

Hypocapnia-Mediated Cerebral Vasodilation: Role of COX

The hemodynamic and gas exchange variables collected during hypercapnia trials are listed in Table 4. Hypocapnia significantly increased PETCO₂ and SpO₂ in both groups, while
Basal Plasma Concentration of COX Metabolites

The indomethacin-mediated reduction in hypercapnia: MCAv (Table 4) and CVCi (Table 4) as well as the responses to PETCO2, SpO2, or MBP. Indomethacin significantly reduced hypercapnia-mediated cerebral vasodilation in adults with MetSyn. Conversely, these findings indicate younger adults with MetSyn maintain the capacity to dilate the cerebral vasculature to hypercapnia, but a distinct shift in COX signaling restrains cerebral vasodilation in response to hypoxia. This profound change in COX signaling in young adults with MetSyn supports the concept of preclinical vascular impairments developing decades prior to clinical manifestation of cerebrovascular disease, providing an opportunity to intervene and restore vascular function.

Hypoxia

This is the first study to investigate a potential mechanism responsible for decreased hypoxia-mediated cerebral vasodilation in MetSyn humans. Based on previous research, it was hypothesized that adults with MetSyn would display reduced hypoxia-mediated cerebral vasodilation and reduced contribution of vasodilating prostaglandins. Importantly, this study confirms the MetSyn-dependent impairment of hypoxia-mediated cerebral vasodilation in humans (15) and animals (22). Contrary to the hypothesis, the present data indicate prostaglandins do not contribute to hypoxic cerebral vasodilation in healthy controls (Fig. 3). Moreover, the increase in ΔCVCi with indomethacin in MetSyn suggests COX predominately produces a vasoconstrictor product in this population. These findings parallel data from hypertensive adults in whom COX products limit acetylcholine-mediated vasodilation in the forearm (27). We speculate the most likely candidate is hypoxia-induced production of thromboxane A2, a powerful vasoconstrictor and platelet aggregator (9).

Literature indicates the contribution of COX products to hypoxia-mediated cerebral vasodilation is variable and dependent on the severity and duration of hypoxia, indomethacin dosage, measurement technique, and model studied. Animal
data indicate COX is important for hypoxia-mediated increases in cerebral blood flow in newborn piglets (3), as well as dilating isolated rat middle cerebral arteries during severe hypoxia (10).

In contrast, recent evidence in humans indicates indomethacin does not alter hypoxia-mediated cerebral vasodilation (6). To a certain degree, this is in line with our control group where indomethacin tended to decrease vasodilation at SpO2 = 80% (P = 0.13). The present results differ slightly from Fan et al. (6), most likely due to our longer duration of hypoxia exposure. The present study held 5 min of steady-state hypoxia at SpO2 = 80%, whereas Fan et al. (6) terminated the hypoxia trial upon briefly reaching SpO2 = 80%. Although animal data indicate COX is an important contributor to hypoxic cerebral vasodilation (3, 10), human data indicate little (present data) or no role (6) for COX-derived vasodilator prostaglandins in mediating hypoxia-induced cerebral vasodilation in healthy humans, leaving alternate mechanisms to be explored.

We speculate our findings establish inducible thromboxane production as a likely mechanism responsible for impaired hypoxia vasodilation in MetSyn, but the signal which induces thromboxane production was not tested in this study. Correlative analysis (data not shown) indicates the collective accumulation of MetSyn criteria, and not an individual risk factor, is responsible for the decreased hypoxia-mediated cerebral vasodilation. We further speculate the combined impact of cardiovascular risk factors likely induces a low-grade vascular inflammation and oxidative stress in the MetSyn adults (29), which may enhance the production of eicosanoids and modify their effects from vasodilation to vasoconstriction (8).

Although we did not measure inflammation and oxidative stress in the current cohort, MetSyn adults from another study in our lab (18) demonstrate greater levels of C-reactive protein (2.0 mg/dl) compared with healthy controls (0.4 mg/dl), which is consistent with early, subclinical cardiovascular disease. While the mechanistic origin driving this change is not apparent, our data clearly demonstrate COX-derived vasoconstriction limits hypoxic cerebral vasodilation in young adults with MetSyn.

Although it is assumed the main target of indomethacin is endothelial COX, it is possible indomethacin affects vascular smooth muscle. Research conducted in a rat model of MetSyn indicates cerebral vascular smooth muscle produces vasoconstricting prostaglandins (thromboxane) when mitochondrial ATP-sensitive potassium channels are activated (16), which could potentially be activated by hypoxia (28). Thus our findings cannot specify the exact source of vasoconstrictor, but these observations are consistent with the conclusion that hypoxia induced preferential production of vasoconstricting prostanoids in MetSyn.

In summary, current results indicate adults with MetSyn exhibit impaired hypoxia-mediated cerebral vasodilation. While this is consistent with an animal model of MetSyn, novel findings demonstrate the mechanism of impaired hypoxic cerebral vasodilation is different in humans. Rather than a loss of vasodilating COX products, the present findings indicate hypoxia elicits production of the vasoconstrictor thromboxane in MetSyn adults, which limits cerebral vessel dilation.

**Hypercapnia**

MetSyn adults displayed preserved hypercapnia-mediated cerebral vasodilation, confirming previous findings (15) and advancing our understanding that MetSyn adults maintain the ability to utilize vasodilator prostaglandins to mediate ~50% of hypercapnia responses. Impaired hypoxic cerebral vasodilation in the face of preserved hypercapnic vasodilation suggests these younger adults with MetSyn retain the capacity to dilate the cerebral vasculature, but MetSyn negatively impacts the functional vascular responses to hypoxia. Further, the similar reduction in vasodilation to hypercapnia (Fig. 4) with indomethacin indicates COX-mediated vasodilation is preserved, but MetSyn promotes activation of a vasoconstrictor pathway during hypoxia. In other words, both groups retain the enzymatic capacity to activate vasodilator prostaglandins in cerebral vessels, but MetSyn alters endothelial signaling during hypoxia such that a vasoconstrictor prostaglandin signal dominates. Thromboxane is a well-known vasoconstrictor and a potent thrombus activator; thus an increase in inducible thromboxane signaling may help explain the increased risk of ischemic stroke in MetSyn humans.

In summary, COX products have consistently been shown to contribute to hypercapnia-mediated cerebral vasodilation (1, 7, 10, 11, 12).
35) and we show the contribution of COX products is preserved in younger adults with MetSyn. These findings indicate adults with MetSyn retain the capacity to dilate to hypercapnia and the functional decline is specific to hypoxia. These are also the first data demonstrating the role of COX mediating vasodilation is fundamentally different between hypercapnia and hypoxia vasodilation in the same human volunteers.

**Experimental Considerations**

The current research approach has several factors worthy of consideration. Principally, measuring MCAv with TCD is an estimate of blood flow through the MCA. Since the cross-sectional area of the MCA remains constant during the severities of hypoxia and hypercapnia used in this study (23, 33), changes in MCAv accurately reflect changes in cerebral blood flow. Newer evidence suggests the diameter of the MCA may change with more severe alterations in blood gases than used in this study (33). Second, SpO2 and PETCO2 were used as quantitative surrogate measures of arterial blood gas values to minimize invasiveness of the study and should not limit the conclusions as there is no reason to suspect the oxyhemoglobin dissociation curve is altered in MetSyn, and since PETCO2 has been commonly used (1, 15, 35) as an accurate and reliable estimate of arterial CO2 (20). Third, a standard oral dose of indomethacin (100 mg) (6, 7, 34, 35) resulted in healthy subjects receiving a significantly greater relative dose of indomethacin (1.5 mg/kg), raising the possibility that MetSyn subjects were not adequately dosed to inhibit COX (0.9 mg/kg). However, data from the onset of action of indomethacin (Fig. 2), similar decreases in COX metabolites (Table 5), and similar decreases in hypercapnia-mediated responses (Fig. 4) indicate indomethacin adequately inhibited COX to the same extent in both groups. Given the directional effect of indomethacin during hypoxia was opposite to the hypothesis, underdosing in MetSyn may underestimate the contribution of COX restricting hypoxic cerebral vasodilation. Finally, we did not...
Table 4. Hemodynamic and gas exchange variables during hypercapnia

<table>
<thead>
<tr>
<th></th>
<th>Healthy Placebo</th>
<th>Healthy Indo</th>
<th>MetSyn Placebo</th>
<th>MetSyn Indo</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP, mmHg*</td>
<td>Baseline 91 ± 2</td>
<td>94 ± 2</td>
<td>102 ± 3</td>
<td>104 ± 3</td>
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<tr>
<td></td>
<td>Hypercapnia 94 ± 2</td>
<td>95 ± 2</td>
<td>105 ± 3</td>
<td>108 ± 4</td>
</tr>
<tr>
<td>P\textsubscript{ETCO2}, mmHg*†</td>
<td>Baseline 39 ± 0</td>
<td>38 ± 0</td>
<td>38 ± 1</td>
<td>37 ± 1</td>
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<td>48 ± 0</td>
<td>47 ± 1</td>
<td>47 ± 1</td>
</tr>
<tr>
<td>Sp\textsubscript{O2}, %*†</td>
<td>Baseline 98 ± 0</td>
<td>98 ± 0</td>
<td>98 ± 0</td>
<td>98 ± 0</td>
</tr>
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<td>MCAv, cm/s‡‡</td>
<td>Baseline 71 ± 3</td>
<td>48 ± 3</td>
<td>65 ± 4</td>
<td>46 ± 3</td>
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<td></td>
<td>Hypercapnia 87 ± 4</td>
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<td>84 ± 5</td>
<td>56 ± 3</td>
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<tr>
<td>ΔMCAv, cm/s‡‡</td>
<td>Hypercapnia 17 ± 1</td>
<td>8 ± 1</td>
<td>20 ± 2</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Indo Change ΔMCAv</td>
<td>Hypercapnia -9 ± 1</td>
<td>-9 ± 2</td>
<td>-9 ± 2</td>
<td>-9 ± 2</td>
</tr>
<tr>
<td>CVCi, cm\textsuperscript{-1}·mmHg\textsuperscript{-1}±‡‡</td>
<td>Baseline 80 ± 5</td>
<td>51 ± 3</td>
<td>64 ± 4</td>
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<td></td>
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<td>ΔCVCi, cm\textsuperscript{-1}·mmHg\textsuperscript{-1}‡‡</td>
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<td>8 ± 1</td>
<td>16 ± 1</td>
<td>8 ± 1</td>
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<tr>
<td>Indo Change ΔCVCi</td>
<td>Hypercapnia -8 ± 1</td>
<td>-1 ± 1</td>
<td>-9 ± 1</td>
<td>-9 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Main effect of group, †main effect of hypercapnia, ‡main effect of Indo (P < 0.05).

Table 5. Plasma concentration of arachidonic acid metabolites before and 90 min after indomethacin administration

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>MetSyn</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-keto-PGF\textsubscript{1α}, pg/ml*†</td>
<td>Baseline 214 ± 31</td>
<td>372 ± 49</td>
</tr>
<tr>
<td></td>
<td>90 min 109 ± 48</td>
<td>199 ± 64</td>
</tr>
<tr>
<td>2,3-dinor TxB\textsubscript{2}, pg/ml‡</td>
<td>Baseline 323 ± 85</td>
<td>476 ± 74</td>
</tr>
<tr>
<td></td>
<td>90 min 36 ± 8</td>
<td>49 ± 14</td>
</tr>
</tbody>
</table>

Values are means ± SE. For 6-keto-PGF\textsubscript{1α}, we assayed plasma from 12 healthy and 12 MetSyn adults. For 2,3-dinor TxB\textsubscript{2}, we assayed plasma from 11 healthy and 11 MetSyn adults. 6- keto-PGF\textsubscript{1α}, 6-keto prostaglandin F\textsubscript{1α}; 2,3-dinor TxB\textsubscript{2}, 2,3-dinor thromboxane B\textsubscript{2}. *Main effect of group, †main effect of time (P < 0.05).

Collect plasma samples during hypoxia to test for increased thromboxane production during hypoxia. We feel this is a minor limitation because our assay of resting plasma samples verifies robust inhibition of thromboxane synthesis and it seems unlikely systemic concentration of thromboxane would accurately reflect local cerebrovascular production of COX metabolites. Combining the plasma assay results (Table 5) with the directionally opposite hypoxic responses in MetSyn (Fig. 3) supports our conclusion the vasoconstrictor COX product is thromboxane A\textsubscript{2}.

Clinical Implications

The novel mechanistic findings of this study are important for understanding the pathophysiology of cerebrovascular disease closer to its induction. In the context of older MetSyn adults (12), our contrasting findings between hypoxic and hypercapnic vasodilation critically draw attention to the fact that a single vascular function test would fail to identify early functional decline in the cerebral circulation of this young diseased population. When studying early disease processes, it may be prudent to include multiple physiological testing to maximize physiological insight into disease progression at its inception.

Previous studies indicate cerebrovascular responses to CO\textsubscript{2} decline with advancing age (1), particularly in the presence of MetSyn (12). Taken together, evidence indicates aging with MetSyn may be more detrimental to cerebrovascular function than healthy aging. This concept is consistent with the observation that the existence of MetSyn in middle-aged men nearly doubles the risk of stroke (17). Finally, detection of cerebrovascular function in ~30-yr-old MetSyn adults opens a substantial window of opportunity to prevent further functional decline, and the indomethacin results point to a potential target to restore function.

Conclusion

In conclusion, young adults with MetSyn demonstrate marked reductions in hypoxia-mediated cerebral vasodilation. In contrast to our hypothesis, inhibition of COX improved cerebral vasodilation in MetSyn, indicating COX products restrain hypoxia-mediated cerebral vasodilation. In contrast to hypoxia, cerebrovascular reactivity to hypercapnia is preserved in MetSyn, as is the contribution of COX vasodilators. The
collective interpretation is, decades prior to clinical manifestation of cerebrovascular disease, younger adults with MetSyn produce a COX-derived vasoconstrictor signal, likely thromboxane A2, which limits cerebral vasodilation to hypoxia. These mechanistic findings have important implications for understanding the progression of cerebrovascular disease as this rapidly growing clinical population ages, providing an opportunity to intervene and restore vascular function.

ACKNOWLEDGMENTS

We thank all participants. Additionally, we thank P. A. Yanke, G. L. Peloton, C. L. Rousseau, R. E. Johansson, M. K. Crain, G. P. Barton and the Wisconsin National Primate Research Center (insulin assays) for technical assistance.

All experiments were conducted in the Bruno Balke Biodynamics Lab on campus at the University of Wisconsin-Madison.

GRANTS

Funding for this project was provided by the American Heart Association (11PRE730038, J. W. Harrell), National Institutes of Health (HL-105820, W. G. Schrage), and the University of Wisconsin School of Medicine and Public Health Shapiro Summer Research Program.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: J.W.H. and W.G.S. conception and design of research; J.W.H. performed experiments; J.W.H. and W.G.S. interpreted results of experiments; J.W.H. drafted manuscript; J.W.H. and W.G.S. edited and revised manuscript; J.W.H. and W.G.S. approved final version of manuscript.

REFERENCES


