JCBFM

Journal of Cerebral Blood Flow & Metabolism
2022, Vol. 42(4) 559–571

DOI: 10.1177/0271678X211065924
journals.sagepub.com/home/jcbfm

Trans-cerebral HCO_3^- and PCO_2 exchange during acute respiratory acidosis and exercise-induced metabolic acidosis in humans

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Abstract

This study investigated trans-cerebral internal jugular venous-arterial bicarbonate ($[HCO_3^-]$) and carbon dioxide tension (PCO_2) exchange utilizing two separate interventions to induce acidosis: 1) acute respiratory acidosis via elevations in arterial PCO_2 ($PaCO_2$) ($n = 39$); and 2) metabolic acidosis via incremental cycling exercise to exhaustion ($n = 24$). During respiratory acidosis, arterial $[HCO_3^-]$ *increased* by 0.15 ± 0.05 mmol $\cdot$ l^{-1} per mmHg elevation in $PaCO_2$ across a wide physiological range (35 to 60 mmHg $PaCO_2$; $P < 0.001$). The *narrowing* of the venous-arterial $[HCO_3^-]$ and PCO_2 differences with respiratory acidosis were both related to the hypercapnia-induced elevations in cerebral blood flow (CBF) (both $P < 0.001$; subset $n = 27$); thus, trans-cerebral $[HCO_3^-]$ exchange (CBF $\times$ venous-arterial $[HCO_3^-]$ difference) was reduced indicating a shift from net release toward net uptake of $[HCO_3^-]$ ($P = 0.004$). Arterial $[HCO_3^-]$ was *reduced* by -0.48 ± 0.15 mmol $\cdot$ l^{-1} per nmol $\cdot$ l^{-1} increase in arterial $[H^+]$ with exercise-induced acidosis ($P < 0.001$). There was no relationship between the venous-arterial $[HCO_3^-]$ difference and arterial $[H^+]$ with exercise-induced acidosis or CBF; therefore, trans-cerebral $[HCO_3^-]$ exchange was unaltered throughout exercise when indexed against arterial $[H^+]$ or pH ($P = 0.933$ and $P = 0.896$, respectively). These results indicate that increases and decreases in systemic $[HCO_3^-]$ – during acute respiratory/exercise-induced metabolic acidosis, respectively – differentially affect cerebrovascular acid-base balance (via trans-cerebral $[HCO_3^-]$ exchange).

Keywords

Acidosis, bicarbonate, carbon dioxide, exercise, trans-cerebral exchange

Received 28 July 2021; Revised 3 November 2021; Accepted 11 November 2021

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Introduction

The regulation of extracellular pH – which directly affects cells via local changes in intravascular or extravascular/interstitial conditions – is affected by rapid chemical buffering reactions, involving phosphate, glycolysis, and carbon dioxide tension (PCO_2). The cerebral vasculature is exceptionally sensitive to changes in arterial PCO_2 ($PaCO_2$),[1–3] such that cerebral blood flow (CBF) rapidly increases by 6–8% per mmHg increase in $PaCO_2$ (reviewed in: Hoiland et al.[4]) This CBF responsiveness to $PaCO_2/[H^+]$ acts to stabilize CO_2 gradients and thus regulate pH across the blood-brain barrier (BBB).[4–6] As CO_2 travels rapidly across the BBB – indicated by the swift changes in CBF (<15–30 seconds) following stepwise changes in $PaCO_2$[7–10]– perivascular/interstitial fluid (ISF) and intracellular brain tissue pH are tightly related to $PaCO_2$. The Fick principle explains that elevated $PaCO_2$ will be related to reductions in the trans-cerebral venous-arterial PCO_2 difference as: 1) CBF varies directly with $PaCO_2$,[1,3,11] and 2) the cerebral metabolic production of CO_2 ($\dot{V}CO_2$) is unaltered in the physiological range; e.g., $CBF = \dot{V}CO_2/PvCO_2–PaCO_2$.[12,13] Experimental results in several acid-base pathologies indicate the trans-cerebral venous-arterial PCO_2 difference is reduced and increased with acute respiratory acidosis/alkalosis, respectively.[3,5,12,14–18] These regulatory changes in the venous-arterial PCO_2 difference contribute to tight regulation of a narrow range of cerebral interstitial pH[19,20] and are influenced by the responsiveness of CBF to acute alterations in $PaCO_2$; i.e., cerebrovascular CO_2 reactivity.[6]

Exercise-induced metabolic acidosis results when the rate of ATP hydrolysis (H^+ release) eventually exceeds the maximal buffering capacity; the excess CO_2 is then removed via hyperventilation[21,22] and these $PaCO_2$ changes in part explain the CBF kinetics response with exercise.[23–25] With exercise-induced acidosis, arterial $[HCO_3^-]$ is markedly reduced due to excessive buffering of $[H^+]$[21,22] – however, whether this change in arterial $[HCO_3^-]$ is related to alterations in trans-cerebral $[HCO_3^-]$ and PCO_2 exchange as well as *in vivo* buffering capacity has not been experimentally addressed in humans. The cerebral metabolic rate of oxygen (e.g., $CMRO_2 = CBF \times$ arterial-venous oxygen content difference) increases linearly by up to 30% at maximal exercise intensities to support substrate utilization in the face of reductions in CBF.[26–33] At maximal exercise, there is a higher relative contribution of cerebral anaerobic glycolysis and lactate oxidation.[34–36] There is evidence to support that maximal exercise-induced acidosis facilitates trans-cerebral lactate exchange and related increases in lactate oxidation mediated via pH-sensitive transcellular $[H^+]$ gradients[37–39] and increases in systemic lactate availability.[28,32,40–42] Additionally, metabolic acidosis achieved with exhaustive exercise reportedly increases BBB permeability with direct relevance for transcellular CO_2 transport.[38,43] Overall, cerebrovascular acid-base regulation is likely interrelated with cerebral substrate prioritization during exercise via pH-sensitive utilization of lactate.[44,45]

The Henderson-Hasselbalch relationship (equation (2)) explains that acute elevations in arterial $[HCO_3^-]$ would increase arterial buffering capacity; for example, with increases in arterial $[HCO_3^-]$ any given change in $PaCO_2$ will result in a lesser change in arterial $[H^+]$/pH.[13,46] This relationship would theoretically apply with reductions in arterial $[HCO_3^-]$ and therefore be reflective of related decreases in arterial buffering capacity. Pre-clinical experiments in anesthetized and artificially ventilated cats show that rapid/transient $[HCO_3^-]/Cl^-$ exchange occurs within 15 seconds between intravascular and extracellular fluid in response to elevated systemic arterial $[HCO_3^-]$ at maintained $PaCO_2$ [47] – these results indicate that extracellular $[HCO_3^-]$ is partially and transiently altered with changes in arterial $[HCO_3^-]$, however, such changes in extracellular $[HCO_3^-]$ are restored (within 1 hour) and these $[HCO_3^-]/Cl^-$ exchange kinetics are appreciably less influential than persistent and freely diffusible CO_2 transport.[48,49] At rest, cerebrospinal fluid (CSF) PCO_2 – typically indexed via internal jugular venous sampling – is 6 to 11 mmHg higher and pH is 0.05 to 0.08 units lower than that of the arterial blood.[13,50,51] As such, resting trans-cerebral net exchange of $[HCO_3^-]$ and PCO_2 (e.g., CBF × venous-arterial difference) is a positive value reflective of net release. A narrowing of the venous-arterial difference would indicate a shift toward net uptake and can be achieved, for example by: 1) larger increase in arterial relative to venous value; and 2) reduction in venous with a corresponding increase in arterial values. No study to date has investigated whether alterations in the trans-cerebral venous-arterial $[HCO_3^-]$ and PCO_2 differences during acute respiratory/exercise-induced metabolic acidosis are related to CBF and regulatory systemic changes in arterial $[HCO_3^-]$ *in vivo* in humans.

The objective of this study was to investigate acid-base balance via alterations in trans-cerebral internal jugular venous-arterial $[HCO_3^-]$ and PCO_2 exchange utilizing two separate experimental interventions to induce acidosis: Study 1) acute respiratory acidosis via elevations in $PaCO_2$ (range: 35 to 60 mmHg); and Study 2) metabolic acidosis via incremental cycling exercise to exhaustion (range: 7.45 to 7.20). We hypothesized that: 1) acute hypercapnic acidosis would *reduce* the venous-arterial $[HCO_3^-]$ and PCO_2 differences and this reduction would be related to higher CBF, less

trans-cerebral [HCO_3^-] exchange, and elevated arterial [HCO_3^-]; and 2) arterial [HCO_3^-] would progressively decrease with maximal exercise-induced acidosis and – as explained by the non-linear CBF response with incremental exercise – this reduction in systemic [HCO_3^-] would be unrelated to any change in CBF or trans-cerebral venous-arterial [HCO_3^-] and PCO_2 exchange.

Methods

Ethical approval

All participants provided informed written consent before participating in these studies. The original research studies were approved by the University of British Columbia Clinical Review Ethical Board (CREB: H16-01028, H18-01755, H15-00166, H11-03287) and the *Comité d'éthique de la recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec* (CER: 21557) and according to the principles established by the Declaration of Helsinki (except for registration in a database). As the current study included a secondary analysis from previous institutional review board approved studies and the use of de-identified data sharing, this study did not require additional institutional review board review.

Participants

These data were obtained during five separate experimental studies previously conducted at the University of British Columbia, Kelowna, British Columbia, Canada and the Université Laval, Québec City, Québec, Canada. Thirty-nine healthy adults completed the CO_2 trials (n = 34 males and n = 5 females) and twenty-four healthy adults completed the exercise trials (n = 19 males and n = 5 females). Participants had no history of cerebrovascular, cardiovascular, or respiratory disease and were not taking any prescription medication at their time of participation. Participants refrained from alcohol and caffeine consumption as well as vigorous exercise or activity for at least 12 hours prior to testing.

Experimental overview

The experimental questions addressed in this study involved *post-hoc* data analysis; therefore, few of the arterial and venous blood gas data have been reported separately in various context for previously published works from these studies; e.g.,.[36,52,53] Importantly, the current experimental questions involved new data analyses that have not been previously reported.

Study 1: Respiratory acidosis protocols

Participants completed one of the following protocols to elicit progressive stepwise steady-state elevations in $PaCO_2$ via dynamic end-tidal forcing: 1) +3, +6, +9 mmHg $PaCO_2$ (n = 11 males); 2) +4.5 and +9 mmHg $PaCO_2$ (n = 12 males); 3) +8 mmHg $PaCO_2$ (n = 5 females; n = 7 males); or 4) +10 and +20 mmHg $PaCO_2$ (n = 4 males). The alterations in $PaCO_2$ were calculated from the resting eupneic breathing end-tidal values. All measurements were taken after at least 2–3 minutes of steady-state. Previous investigations from our group have shown that cerebrovascular CO_2 reactivity (utilizing the same dynamic end-tidal forcing system) is highly linear in the hypercapnic range up to +20 mmHg.[4,54] Additionally, recent retrospective analysis by our research group[109] has shown that following a rapid stepwise change in $P_{ET}CO_2$ (via the exact same experimental methods/techniques in the current studies), relevant cerebrovascular and cardiorespiratory variables achieve steady-state within 2-minutes of exposure duration to elevated inspired PCO_2. As such, by applying a linear mixed model analysis, there is no statistical or physiological rationale to expect these results to be different whether participants completed the exact same stepwise steady-state elevations in $PaCO_2$ compared to the varied stages utilized in this *post-hoc* analysis.

Study 2: Exercise protocols

Participants completed supine incremental cycling exercise to exhaustion at various relative (0, 20, 40, 60, 80, 100% maximal workload; n = 12 males) and fixed exercise intensities (0, 50, 75, 100 watts; n = 5 females and 0, 75, 100, 125 watts; n = 7 males). Each workload was 3–5 minutes in duration and steady-state blood samples were drawn within the last 20 s of each exercise stage.

Blood sampling

Approximately 1.0 ml of radial arterial and internal jugular venous blood were drawn at the same time into pre-heparinized syringes (SafePICO, Radiometer, Copenhagen, Denmark) and analyzed immediately using a commercial blood gas analyzer (ABL90 FLEX, Radiometer) at each experimental stage (n = 39). This analysis included measurements of $PaCO_2$ and oxygen tension (PaO_2), arterial oxygen saturation (SaO_2), arterial oxygen content (CaO_2), base excess, [H^+], hemoglobin concentration ([Hb]), and pH. Data collected at the Université Laval were analyzed within 10−15 minutes for n = 12 (ABL800 FLEX, ABL825, Radiometer).

Arterial oxygen content (CaO_2) was calculated as:

$$CaO_2(mL \cdot dL^{-1}) = [Hb] \times 1.34 \times [SaO_2(\%)/100] + 0.003 \times PaO_2 \quad (1)$$

Where 1.34 is the O_2 binding capacity of hemoglobin and 0.003 is the solubility of O_2 dissolved in blood.[55,56]

Acid-base balance data analysis

Blood gas analyzers do not typically have the capacity to directly measure $[HCO_3^-]$; instead, it is calculated from measured $PaCO_2$ and pH, by rearranging the Henderson-Hasselbalch equation (equations (2) and (3)).

$$pH = 6.1 + \log \frac{[HCO_3^-]}{0.031 \times PCO_2} \quad (2)$$

$$[HCO_3^-] = 0.031 \times PCO_2 \times 10^{(pH-6.1)} \quad (3)$$

Where 6.1 is the pKa (i.e., −log of the acid dissociation constant) at 37.0°C[57] and 0.031 mEq·l^{-1} per mmHg PCO_2 is the solubility factor for dissolved CO_2 plus carbonic acid (H_2CO_3) at 37.0°C in plasma.

The following calculations were used to quantify an index of *in vivo* buffering capacity termed "extracellular pH defense".[58,59] All *in vivo* buffering capacity calculations were analyzed with the average venous-arterial values of $[HCO_3^-]$, [La], and pH as an estimate of cerebral tissue values. These results were consistent when expressed as arterial or internal jugular venous buffering capacity.

Exercise:

$$\Delta[HCO_3^-] \times \Delta pH^{-1} \quad (4)$$

The reduction in $[HCO_3^-]$ per unit pH is reportedly attributable to hyperventilation and HCO_3^- buffering.[58]

$$-\Delta[La] \times pH^{-1} \quad (5)$$

This index includes lactate ([La]) and is considered total pH defense by hyperventilation, as well as HCO_3^- and non-bicarbonate buffers.[58]

Respiratory acidosis:

$$-\Delta[HCO_3^-] \times \Delta pH^{-1} \quad (6)$$

Equation (4) above has been adapted as $[HCO_3^-]$ *increases* with respiratory acidosis.

Cardiorespiratory

Detailed cardiorespiratory experimental methods and results have been previously reported elsewhere; e.g., literature.[36,52,53] Briefly, the partial pressures of end-tidal CO_2 and O_2 (i.e., $P_{ET}CO_2$ and $P_{ET}O_2$, respectively) were controlled during acute respiratory acidosis using a custom-designed dynamic end-tidal forcing system to effectively regulate end-tidal gases across wide ranges of $P_{ET}CO_2$ and $P_{ET}O_2$,[60,61] independent of ventilation ($\dot{V}_E$).[62] Beat-by-beat arterial blood pressure was continuously acquired via the radial artery pressure transducer positioned at the height of the right atrium (Edwards Lifesciences, TruWave VAMP, CA, USA) and the arterial blood pressure waveform was averaged to calculate MAP.

Cerebrovascular

Detailed cerebrovascular experimental methods and results have been previously reported elsewhere; e.g., literature.[36,52,53] Briefly, extra-cranial blood velocity and diameter of the internal carotid artery (ICA) and vertebral artery (VA) were measured using a 10-MHz multifrequency linear array Duplex ultrasound (Terason t3000; Teratech, Burlington, MA, USA). Pulse-wave mode was used to measure beat-to-beat peak blood velocity and arterial diameter was instantaneously measured via B-mode imaging; data were analyzed using custom edge-detection and wall tracking software (BloodFlow Analysis, version 5.1). The vessel location was decided on an individual basis to allow for reliable image acquisition, with the same location and consistent insonation angle (60°) repeated within participants and between trials.

Blood flow was calculated as:

$$Q\ (mL \cdot min^{-1}) = \frac{\text{peak envelope blood velocity}}{2} \times (\pi(0.5 \times \text{diameter})^2) \times 60 \quad (7)$$

Total cerebral blood flow (CBF) was calculated as:

$$CBF\ (mL \cdot min^{-1}) = 2 \times (Q_{ICA} + Q_{VA}) \quad (8)$$

Net $[HCO_3^-]$ exchange was calculated as:

$$\text{Net } [HCO_3^-] \text{ exchange } (mmol \times min^{-1}) = ([HCO_3^-]_v - [HCO_3^-]_a) \times CBF \quad (9)$$

Net PCO_2 exchange was calculated as:

$$\text{Net } PCO_2 \text{ exchange } (mmHg \cdot min^{-1}) = (PvCO_2 - PaCO_2) \times CBF \quad (10)$$

Where a negative value indicates a net uptake and a positive value indicates a net release.[63]

Statistical analyses

All data are presented in-text as mean ± SD and as individual values in figures. Statistical analyses were performed using SPSS software (IBM statistics, Version 23.0) and Prism (GraphPad Software, Version 9.1.0). Normality was assessed using Shapiro-Wilk tests and by visual inspection of Q-Q plots. Statistical significance was set at $P < 0.05$. Relationships between select variables were analyzed using linear regression. A linear mixed-model analysis with fixed effects of either arterial $[H^+]$, pH, or $PaCO_2$ was used to compare PCO_2, $[HCO_3^-]$, [La], *in vivo* buffering capacity, trans-cerebral exchange, and CBF separately for the respiratory acidosis and exercise interventions. Subjects were included as a random effect.

Results

Study 1: Respiratory acidosis

Total CBF increased by $4 \pm 1\,ml \cdot 100\,g^{-1} \cdot min^{-1}$ per mmHg elevation in $PaCO_2$ across a wide range from 35 to 60 mmHg $PaCO_2$ ($P < 0.001$; Figure 1(a)); this hypercapnia-mediated increase in CBF was related to *narrowing* of the venous-arterial PCO_2 difference ($P < 0.001$; Figure 1(b)) such that trans-cerebral PCO_2 exchange was unaltered with stepwise increases in $PaCO_2$ ($P = 0.057$; Figure 1(c)). Arterial $[HCO_3^-]$ increased by $0.15 \pm 0.05\,mmol \cdot l^{-1}$ per mmHg elevation in $PaCO_2$ ($P < 0.001$; Figure 1(d)). There was a relationship between respiratory acidosis and *narrowing* of the venous-arterial $[HCO_3^-]$ and PCO_2 differences; e.g., $-0.16 \pm 0.11\,mmol \cdot l^{-1}$ and -0.52 ± 0.22 mmHg reduction in venous-arterial $[HCO_3^-]$ and PCO_2 differences per $nmol \cdot l^{-1}$ increase in arterial $[H^+]$, respectively (both $P < 0.001$; Figure 1(e) and (f)). The venous-arterial $[HCO_3^-]$ and PCO_2 differences were each related to hypercapnia-induced increases in CBF (both $P < 0.001$; Figure 1(g)). As such, $[HCO_3^-]$ exchange was reduced by $-0.05 \pm 0.08\,mmol \cdot min^{-1}$ per $nmol \cdot l^{-1}$ increase in arterial $[H^+]$; i.e., less venous $[HCO_3^-]$ efflux/release with respiratory acidosis ($P = 0.004$; Figure 1(h)).

Study 2: Exercise-induced metabolic acidosis

Total CBF was related to $PaCO_2$ throughout progressive cycling exercise to exhaustion corresponding to $1 \pm 1\,ml \cdot 100\,g^{-1} \cdot min^{-1}$ per mmHg change in $PaCO_2$ ($P = 0.004$; Figure 2(a)). There was no relationship between CBF and the venous-arterial PCO_2 difference ($P = 0.177$; Figure 2(b)); therefore, trans-cerebral PCO_2 exchange was unrelated to $PaCO_2$ during exercise ($P = 0.155$; Figure 2(c)). Arterial $[HCO_3^-]$ was reduced by $-0.48 \pm 0.15\,mmol \cdot l^{-1}$ per $nmol \cdot l^{-1}$ increase in arterial $[H^+]$ with exercise-induced acidosis at maximal cycling exercise ($P < 0.001$; Figure 2(d)). There was no relationship between the venous-arterial $[HCO_3^-]$ difference and arterial $[H^+]$ with exercise-induced acidosis ($P = 0.801$; Figure 2(e)). There was, however, a relationship between *widening* of the venous-arterial PCO_2 difference by 0.12 ± 0.20 mmHg per $nmol \cdot l^{-1}$ increase in arterial $[H^+]$ during incremental cycling exercise to exhaustion ($P = 0.006$; Figure 2(f)); i.e., increased PCO_2 uptake reflective of acidosis. The exercise-induced CBF response was unrelated to the venous-arterial $[HCO_3^-]$ difference ($P = 0.682$; Figure 2(g)); as such, there were no changes in trans-cerebral $[HCO_3^-]$ *exchange* during exercise when indexed against arterial $[H^+]$ or pH ($P = 0.933$ and $P = 0.896$, respectively; Figure 2(h)).

In vivo buffering capacity during acute respiratory acidosis and exercise

Linear mixed-model analysis revealed no significant relationship between *in vivo* buffering capacity ($-\Delta [HCO_3^-] \times \Delta\ pH^{-1}$) and the average venous-arterial pH during stepwise respiratory acidosis ($P = 0.078$; Figure 1(i)). Additionally, there were no relationships between *in vivo* buffering capacity ($\Delta\ [HCO_3^-] \times \Delta\ pH^{-1}$ or $-\Delta\ [La] \times \Delta\ pH^{-1}$) and the average venous-arterial pH during incremental cycling exercise to exhaustion ($P = 0.187$ and $P = 0.392$, respectively; Figure 2(i)).

Discussion

The results of this study indicate that trans-cerebral $[HCO_3^-]$ and PCO_2 exchange are differentially regulated in response to acute respiratory and exercise-induced acidosis. This finding is supported by: 1) acute hypercapnic acidosis elevated arterial $[HCO_3^-]$; however, trans-cerebral $[HCO_3^-]$ exchange was *reduced* (i.e., indicating a shift from net release toward net uptake of $[HCO_3^-]$) and this was reflective in *narrower* venous-arterial $[HCO_3^-]$ and PCO_2 differences with higher CBF (Figure 1); and 2) arterial $[HCO_3^-]$ was progressively reduced with maximal exercise-induced acidosis and this was unrelated to venous-arterial $[HCO_3^-]$ and PCO_2 exchange (Figure 2). Additionally, these results show there are no appreciable changes in the *in vivo* buffering capacity across a wide range of respiratory acidosis (up to +20 mmHg

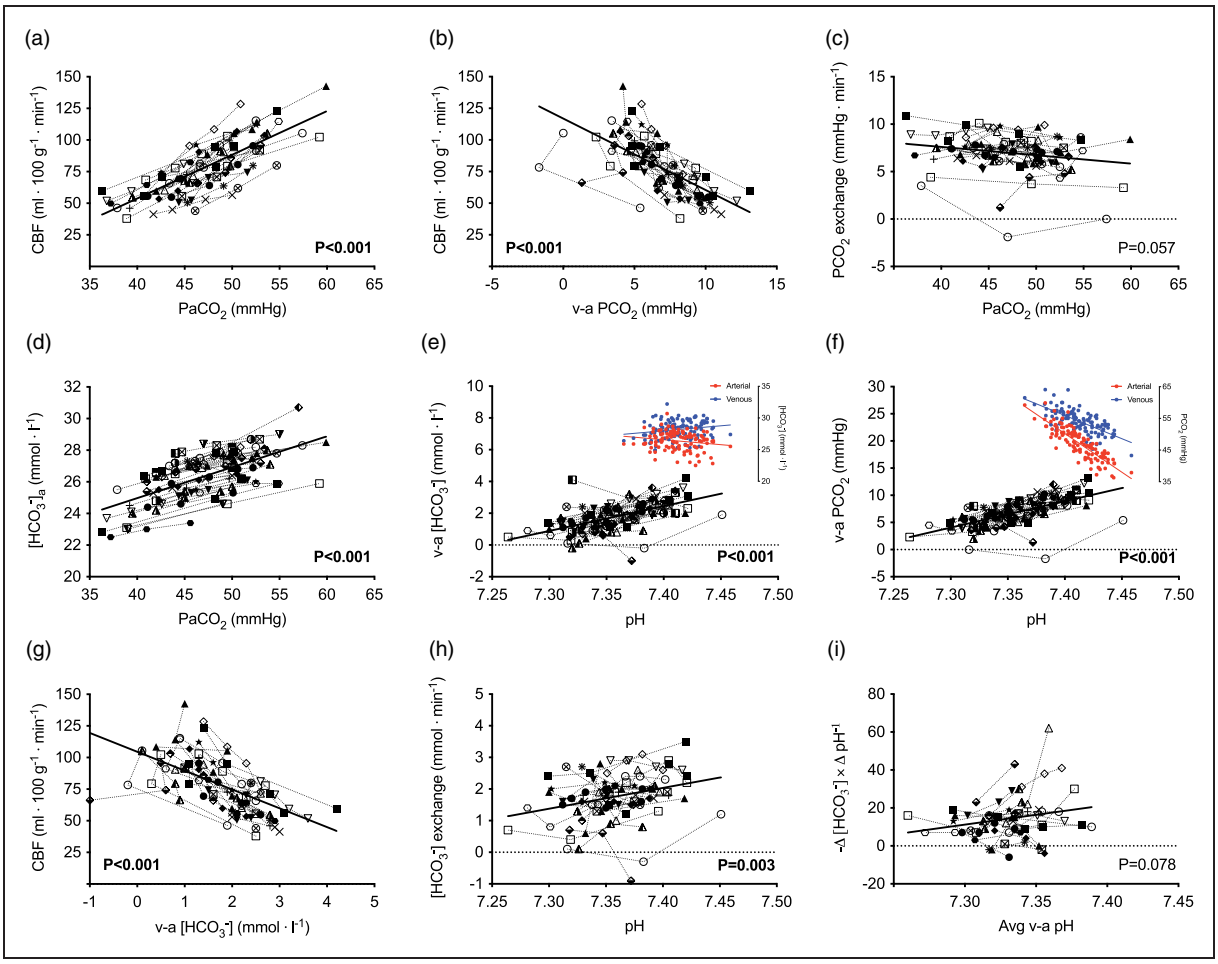

Figure 1. Venous-arterial $[HCO_3^-]$ and PCO_2 exchange with hypercapnic acidosis in humans. Total cerebral blood flow (CBF) increases with stepwise elevations in arterial PCO_2 ($PaCO_2$) (a); this hypercapnia-mediated increase in CBF is related to *narrowing* of the venous-arterial PCO_2 difference (b) such that – explained via the Fick principle – trans-cerebral PCO_2 exchange is unaltered with respiratory acidosis (c). Arterial $[HCO_3^-]$ increases with progressive elevations in $PaCO_2$ (d); this response may theoretically contribute to localized changes in CBF with severe respiratory acidosis – via increases in extravascular $[HCO_3^-]$ – and thus, regulatory changes in $[HCO_3^-]$ may help explain the maximal cerebrovascular vasodilatory reserve to $PaCO_2$. There is a relationship between reductions in arterial pH (e.g., respiratory acidosis) and *narrowing* of the venous-arterial $[HCO_3^-]$ (e) and PCO_2 (f) differences. The reduction in venous-arterial $[HCO_3^-]$ difference is achieved by a decrease in venous $[HCO_3^-]$ and increase in arterial $[HCO_3^-]$; whereas, $PaCO_2$ increases to a larger extent relative to $PvCO_2$ with respiratory acidosis (illustrated by the coloured inlay figures). This narrowing is reflective of less $[HCO_3^-]$ exchange (h) in part attributable to increases in $[HCO_3^-]$ and CO_2 'wash-out' due to higher CBF with hypercapnia (g); i.e., the venous-arterial $[HCO_3^-]$ and PCO_2 differences were each related to increases in CBF (both $P < 0.001$; subset n=27). There was no relationship between the average venous-arterial *in vivo* buffering capacity ($-\Delta\,[HCO_3^-] \times \Delta\,pH^{-1}$) with stepwise respiratory acidosis when indexed against average venous-arterial pH (i). Data are individual values across stepwise progressive increases in $PaCO_2$ for n = 27 (a, b, c, g, h, i) and n=39 (d, e, f) participants via dynamic end-tidal forcing.

$PaCO_2$) or incremental cycling exercise to exhaustion in humans (Figures 1(i) and 2(i)).

Respiratory versus metabolic acidosis – influence of $[HCO_3^-]$ exchange

The two experimental interventions of acute respiratory acidosis and exercise-induced metabolic acidosis each provoke equivalent reductions in pH with a markedly disparate arterial $[HCO_3^-]$ response. For example, the reductions in arterial $[HCO_3^-]$ during exercise are reflective of a 3-fold larger rate of change in $[HCO_3^-]$ versus the small increases in $[HCO_3^-]$ observed with respiratory acidosis (e.g., approx. -0.48 vs. $+0.15\,mmol \cdot l^{-1}$ $[HCO_3^-]$ per nmol $\cdot$ l^{-1} increase in $[H^+]$). Additionally, it is noteworthy to consider how alterations in $CMRO_2$ with exercise and respiratory acidosis will affect $\dot{V}CO_2$ and, therefore, $PvCO_2$.[25,35,64–66]

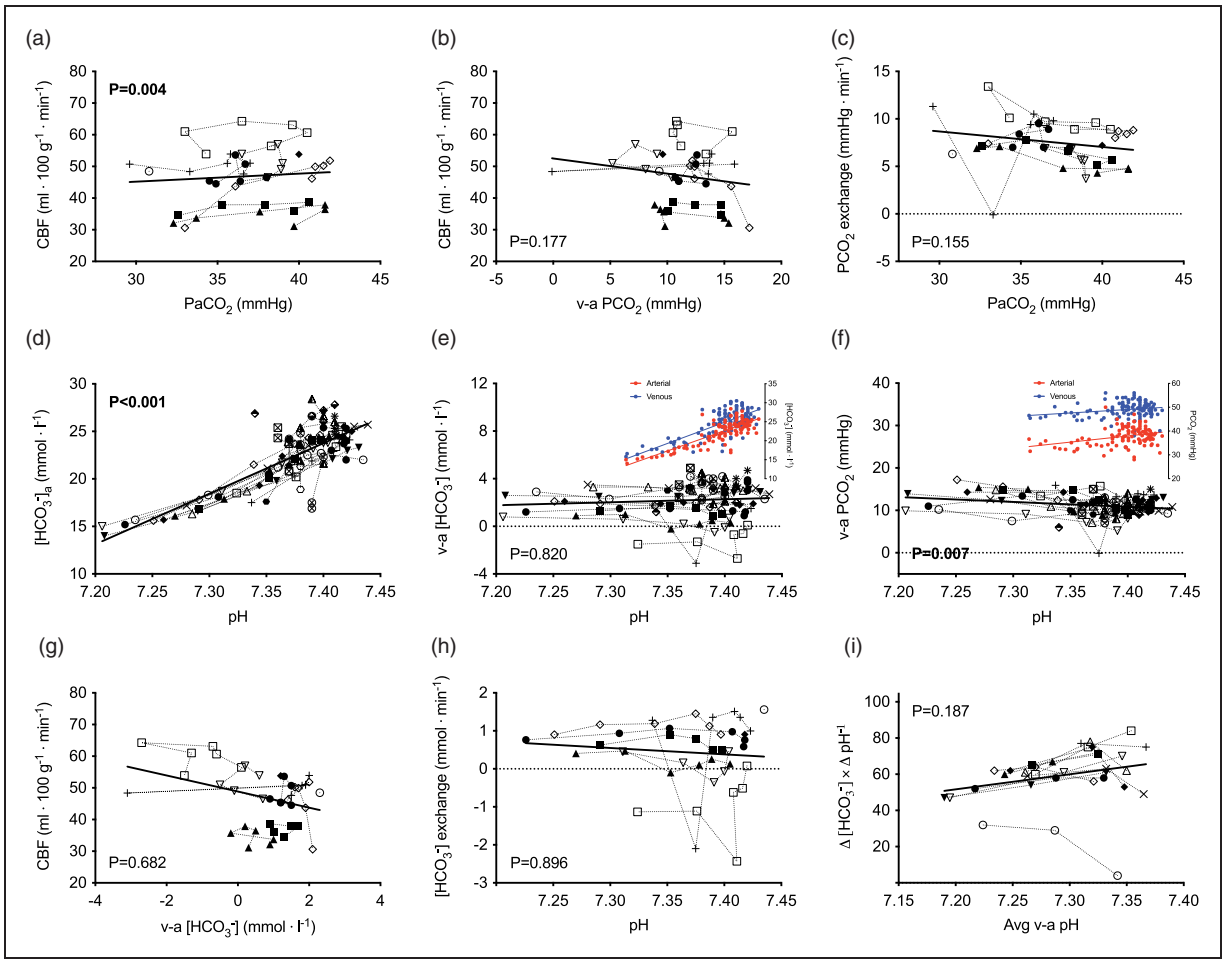

Figure 2. Venous-arterial $[HCO_3^-]$ and PCO_2 exchange with progressive submaximal to maximal cycling exercise. Total cerebral blood flow (CBF) is positively related to arterial PCO_2 ($PaCO_2$) throughout progressive cycling exercise to exhaustion (a); however, there is no relationship between CBF and the venous-arterial PCO_2 difference (b), therefore, trans-cerebral PCO_2 exchange is unrelated to $PaCO_2$ during exercise (c). Arterial $[HCO_3^-]$ is reduced with exercise-induced acidosis at maximal exercise (d). There is no relationship between the venous-arterial $[HCO_3^-]$ difference and arterial pH (e) – as indicated by the equivalent reduction in arterial and venous $[HCO_3^-]$ – however, there is *widening* of the venous-arterial PCO_2 difference with exercise-induced acidosis (f) as indicated by a larger relative reduction in $PaCO_2$ versus $PvCO_2$ (illustrated by the coloured inlay figures). The exercise-induced CBF response was unrelated to the venous-arterial $[HCO_3^-]$ difference (g); as such, there were no changes in trans-cerebral $[HCO_3^-]$ *exchange* during exercise when indexed against arterial pH (h). There are no relationships between the average venous-arterial *in vivo* buffering capacity ($\Delta\ [HCO_3^-] \times \Delta\ pH^{-1}$ or $-\Delta\ [La] \times \Delta\ pH^{-1}$) during incremental cycling exercise to exhaustion (i). These data are reflective of the linear relationship between pH, reductions in $[HCO_3^-]$, and increases in [La] with exercise-induced acidosis and indicate an unaltered *in vivo* buffering capacity at maximal cycling exercise. Data are individual values during supine incremental cycling exercise to exhaustion for n = 12 (a, b, c, g, h, i) and n=24 (d, e, f). Exercise stages included various relative (0, 20, 40, 60, 80, 100% maximal workload; n=12 males) and fixed exercise intensities (0, 50, 75, 100 watts; n = 5 females and 0, 75, 100, 125 watts; n=7 males). The *in vivo* buffering capacity was calculated at 60, 80, 100% maximal workload (I).

Increases in $PaCO_2$ facilitate rapid passive diffusion of CO_2 across the BBB via reduced CSF driving pressure leading to increases in intra- and extracellular $[H^+]$, thus provoking acidosis through acid-base re-equilibration[67,68] in accordance with Le Chatelier's Principle (rightward shift in equation (11)).[69] Overall, CO_2 transport is the sum of the diffusion of 1) dissolved CO_2; and 2) CO_2 bound as HCO_3^- (i.e., "facilitated CO_2 diffusion") – this process involves a flux of H^+ equivalent to that of HCO_3^- as per equation (11).[70–72]

$$CO_2 + H_2OCAH_2CO_3 \leftrightarrow HCO_3^- + H^+ \qquad (11)$$

Acute respiratory acidosis increases arterial $[HCO_3^-]$ via continuous conversion of CO_2 – rapidly catalyzed by carbonic anhydrase (CA) – shifting the

equilibrium relationship toward the [HCO_3^-] buffer reaction. This is reflective of rapid increases in extracellular [HCO_3^-] due to intracellular [HCO_3^-] exchange with Cl^- to buffer the [H^+] produced via CO_2 hydration.[73] Importantly, however, the ratio of arterial [HCO_3^-] to $PaCO_2$ is lower such that pH is reduced (as per equation (2)).

At rest, the relative contributions of dissolved CO_2, chemically bound to hemoglobin and protein carbamate, and HCO_3^- to total CO_2 exchange are approximately 5%, 10% and 85%, respectively. During severe exercise, HCO_3^- facilitated CO_2 diffusion is reduced to approximately 2/3 of total CO_2 exchange (as the contribution of dissolved CO_2 increases sevenfold with exercise-induced acidosis).[71,74] With maximal exercise-induced acidosis, arterial [HCO_3^-] is markedly reduced due to compensatory buffering of [H^+] leading to production of CO_2 and H_2O (leftward shift in equation (11)); the excess CO_2 is then removed via hyperventilation.[21,22]

Acute elevations in cerebral blood flow stabilize [HCO_3^-] and PCO_2 gradients

Acute respiratory acidosis provokes three key regulatory responses: 1) arterial [HCO_3^-] increases (via interconversion of CO_2) in response to elevated $PaCO_2$ (Figure 1(d)); 2) there is rapid/transient exchange of HCO_3^- and Cl^- across the BBB and between brain tissue and extracellular fluid;[47,73] and 3) total CBF increases thus reducing the difference between arterial and extravascular PCO_2, thereby limiting the rise in intracellular tissue PCO_2 (Figure 1(b)). The increase in CBF in response to $PaCO_2$ is restricted by the maximal vasodilatory response to hypercapnia *in vivo* in humans; e.g., +15–20 mmHg $PaCO_2$ equates to 150% increase in CBF.[54] Elevations in arterial [HCO_3^-] may theoretically contribute to localized changes in CBF with severe respiratory acidosis – via increases in extravascular [HCO_3^-] – and thus, regulatory changes in [HCO_3^-] may help explain the maximal cerebrovascular vasodilatory reserve to $PaCO_2$. The internal jugular venous-arterial [HCO_3^-] difference is reduced with acute severe hypercapnic acidosis (Figure 1(e)) – a response likely explained by increased extravascular HCO_3^- 'wash-out' as the reduction in trans-cerebral [HCO_3^-] difference is related to the hypercapnic CBF response ($P < 0.001$; Figure 1(g)). Arvidsson and colleagues (1981) showed that intravenous infusion of $NaHCO_3$ in hypercapnic dogs causes a 50–70% reduction from the $PaCO_2$-induced higher CBF. Additionally, cerebrospinal fluid [HCO_3^-] ([HCO_3^-]$_{CSF}$) was appreciably higher following $NaHCO_3$; these data indicate that acutely elevated $PaCO_2$ may facilitate the transport of exogenous HCO_3^- across the BBB (via higher CBF) resulting in cerebral vasoconstriction due to increased extravascular pH.[75] The present results support that the CBF response to acute respiratory acidosis contributes to tight regulation of cerebrovascular pH (via narrowing of the trans-cerebral [HCO_3^-] and PCO_2 differences) *in vivo* in humans.

Regulation of cerebrovascular acid-base balance during exercise

Trans-cerebral venous-arterial [HCO_3^-] exchange was unaffected by maximal exercise-induced acidosis (e.g., pH 7.20; Figure 2(e)) and there was a *widening* of the venous-arterial PCO_2 difference during incremental cycling exercise to exhaustion (Figure 2(f)). Total CBF increases steadily by 10−20% up to intensities of approximately 60–70% of maximal oxygen uptake ($\dot{V}O_{2max}$) to regulate cerebral substrate delivery,[32,76] and is mediated via relative alveolar hypoventilation (i.e., elevations in $PaCO_2$) and increases in $CMRO_2$.[25,36,77] With progressive increases in cycling exercise intensity, the relatively linear $CMRO_2$ response is coupled to cerebral oxygen delivery ($CBF \times CaO_2$) and oxygen extraction ($CaO_2 - CvO_2/CaO_2) \times 100\%$)) rather than CBF *per se*. At maximal exercise, hyperventilatory-induced reductions in CBF – together with marked acidosis – would conceivably adversely affect intracellular/extravascular [HCO_3^-] and CO_2 'wash-out'. Previous work by Bisgard and colleagues (1978) reported unaltered [HCO_3^-]$_{CSF}$ and $PaCO_2$-mediated *increases* in CSF pH (via hyperventilatory response) with severe near-maximal exercise in ponies[78] – these data emphasize the importance of the ventilatory response on cerebrovascular PCO_2/[HCO_3^-] regulation during exercise.[6] Additionally, albeit during resting conditions, several studies report that sustained changes in CSF/extracellular [HCO_3^-] respond slowly (e.g., several hours) and only partially to systemic changes in arterial [HCO_3^-],[50,79–84] thus explaining the steady [HCO_3^-]$_{CSF}$ during acute exercise (<10 minutes). Taken together with the remarkably consistent trans-cerebral [HCO_3^-] exchange (Figure 2(h)) and *in vivo* [HCO_3^-] and [La] buffering capacity throughout exercise (Figure 2(i)), these data indicate that the CBF response to exercise *per se* likely plays a lesser role in cerebrovascular acid-base regulation (versus respiratory acidosis, for example).

Experimental considerations

A key strength of this study was the invasive direct Kety-Schmidt technique to quantify trans-cerebral venous-arterial exchange of [HCO_3^-], PCO_2, and [H^+] *in vivo* in healthy humans; we recognize that this

relies on the assumption that these sampling sites are reflective of *trans-cerebral exchange* due to practical and ethical experimental constraints.[85] Additionally, the Duplex ultrasound derived indexes of extracranial blood flow utilized in the current experiment are assumed to indicate cerebral tissue nutritive flow. Although we verified $PaCO_2$ values with blood gas sampling, without temperature correcting to adjust for higher blood temperature during exercise, we may have underestimated $PaCO_2$ by 1–2 mmHg (due to reduced CO_2 solubility at higher temperatures).[86,87] Additionally, the influences of exercise[88–90] and temperature[91–95] may conceivably have a small effect on PCO_2, H^+, and HCO_3^- via alterations in carbonic anhydrase activity in the exercise trial[96,97] – however, this is unlikely to differentially affect the trans-cerebral venous-arterial exchange values *within* participants during this trial.

A subset of female participants completed the +8 mmHg $PaCO_2$ respiratory acidosis protocol as well as the fixed intensity submaximal exercise protocol ($n = 5$ females and $n = 7$ males). Previous literature is equivocal, with studies reporting that cerebrovascular CO_2 reactivity is higher in females,[98–100] higher in males,[101–103] or not different between sexes.[104,105] Whether sex-related differences in cerebrovascular CO_2 reactivity exist between females and males in response to a fixed targeted elevation in $PaCO_2$, this is unlikely to affect our results as we saw no change in the *in vivo* buffering capacity ($\Delta [HCO_3^-] \times \Delta\ pH^{-1}$) when indexed against the average venous-arterial pH during respiratory acidosis. These results indicate that any within-subject change in the sensitivity of CBF to $PaCO_2$ would be reflected in an equivalent pH response (with which we indexed against our dependent variables). Additionally, recent studies have shown no difference in the CBF response to moderate-intensity exercise between females and males[106,107] with sex-related differences in CBF regulation only apparent at severe intensity exercise (80–100% maximal workload).[108] As such, we do not expect that the inclusion of 5 female participants in the fixed intensity submaximal exercise protocol (0, 50, 75, 100 watts) would affect the variability of our results. Future investigations are merited to address any sex-related differences in cerebrovascular acid-base regulation in response to respiratory and exercise-induced metabolic acidosis.

Conclusion

Taken together, these results indicate that increases and decreases in systemic arterial $[HCO_3^-]$ – during acute respiratory/exercise-induced metabolic acidosis, respectively – differentially contribute to cerebrovascular acid-base balance. The previously recognized tight regulation of cerebral interstitial pH during acute hypercapnic acidosis[19,20] is likely attributable to: 1) narrower internal jugular venous-arterial $[HCO_3^-]$ and PCO_2 differences; 2) higher CBF; and 3) reductions in trans-cerebral $[HCO_3^-]$ exchange indicating less venous $[HCO_3^-]$ efflux/release. These results are supportive of unaltered trans-cerebral $[HCO_3^-]$ exchange with exercise-induced acidosis – consistent with the unchanged *in vivo* buffering capacity – which was unrelated to the CBF response throughout progressive cycling exercise to exhaustion.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Philip N. Ainslie was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) and a Canada Research Chair. Hannah G. Caldwell was funded by a NSERC PGS-Doctoral Scholarship. Patrice Brassard was funded by the Foundation of the Institut universitaire de cardiologie et de pneumologie de Québec and a NSERC Discovery Grant. Damian M. Bailey was supported by a Royal Society Wolfson Research Fellowship (#WM170007) and Higher Education Funding Council for Wales (Postdoctoral Fellowship for Benjamin S. Stacey).

Acknowledgements

We would like to extend our thanks to the volunteers who participated in these studies. Additionally, we would like to thank past and present members of the Centre for Heart, Lung and Vascular Health at UBCO for their contribution to this series of studies by our group.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' contributions

Study design: RLH, KJS, PB, ARB, DMB, PNA. Data collection: HGC, RLH, KJS, PB, ARB, MMT, CAH, JMJRC, BSS, DMB, AD, MSS, DBM, PNA. Data analysis: HGC, RLH, KJS, PB, ARB. Data interpretation: HGC, RLH, JMJRC, DMB, PNA. Drafted manuscript: HGC. Critically reviewed manuscript: HGC, RLH, KJS, PB, ARB, MMT, CAH, JMJRC, BSS, DMB, AD, MSS, DBM, PNA. Approved final version: HGC, RLH, KJS, PB, ARB, MMT, CAH, JMJRC, BSS, DMB, AD, MSS, DBM, PNA.

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