Indomethacin markedly blunts cerebral perfusion and reactivity, with little cognitive consequence in healthy young and older adults

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Indomethacin markedly blunts cerebral perfusion and reactivity, with little cognitive consequence in healthy young and older adults.

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Running Title: Cerebral blood flow, cognition, and aging

Key Points Summary

- Cognitive function depends on adequate cerebrovascular perfusion and control. However, it is unknown if acutely-reduced cerebral blood flow (CBF) impairs cognition in healthy adults.
- In this study we used a placebo-controlled, single-blinded, randomised cross-over design to test the hypothesis that acutely-reduced CBF (using a pharmacological aid; indomethacin) would impair cognition in young and older healthy adults.
- At baseline, older adults had lower cognitive performance and CBF, but similar cerebrovascular reactivity to CO_2 and dynamic cerebral autoregulation compared to young adults.
- In both young and older adults, cognitive performance on a mental switching task was slightly (7%) reduced after indomethacin, but not significantly associated with reductions in CBF (~31%).
- These results indicate that cognitive performance is broadly resilient against a ~31% reduction in CBF *per se* in healthy young and older adults.

Abstract

Cognitive function depends on adequate cerebrovascular perfusion and control. However, it is unknown if acutely-reduced cerebral blood flow (CBF) impairs cognition in healthy adults. Using a placebo-controlled, single-blinded, randomised cross-over design, we tested the hypothesis that acutely-reduced CBF (using indomethacin [1.2 mg/kg oral dose]) would impair cognition in young (n=13; 25±4 y) and older (n=12; 58±6 y) healthy adults. CBF and cerebrovascular control were measured using middle cerebral artery blood velocity ($MCAv_{mean}$) and its reactivity to hypercapnia (CVR_{HYPER}) and hypocapnia (CVR_{HYPO}), respectively. Cognitive function was assessed using a computerised battery including response time tasks. Baseline comparisons revealed that older adults had 14% lower $MCAv_{mean}$ and 15% lower cognitive performance (all $p \leq 0.048$) but not lower $CVR_{HYPER/HYPO}$ ($p \geq 0.26$). Linear and rank-based mixed models revealed that indomethacin decreased $MCAv_{mean}$ by 31% [95% CI:-35,-26], CVR_{HYPER} by 68% [IQR:-94,-44] and CVR_{HYPO} by 50% [IQR:-83,-33] (treatment-effect; all $p<0.01$), regardless of age. Baseline $CVR_{HYPER/HYPO}$ were strongly associated with their indomethacin-induced reductions ($r=0.70$ to 0.89, $p<0.01$). Mental switching performance was impaired 7% [IQR:0,19] after indomethacin ($p=0.04$), but not significantly associated with reductions in $MCAv_{mean}$ (Young: rho=-0.31, p=0.30; Older: rho=0.06, p=0.86). Conclusion: indomethacin reduced $MCAv_{mean}$ and impaired cognition slightly, however no

clear association was evident in younger or older adults. Older adults had poorer cognition and lower $MCAv_{mean}$ but similar $CVR_{HYPER/HYPO}$.

Keywords: aging, cognition, cerebral blood flow, hypercapnia, indomethacin

Introduction

Cognitive function has an immediate and critical reliance on adequate cerebrovascular control, perfusion, and metabolism (Barnes *et al.*, 2013). While an acute increase in perfusion does not measurably improve cognitive performance (Shoemaker *et al.*, 2019b; Shoemaker *et al.*, 2020), the extent to which cognition is resilient against *lower* cerebral perfusion is unknown. To our knowledge, no one has imposed a (reversible) reduction of cerebral blood flow (CBF) in healthy individuals to test if an *acute* reduction in CBF or cerebrovascular responsiveness impairs cognition.

It is possible to acutely reduce CBF and cerebrovascular reactivity to CO_2 (CVR_{CO2}; i.e., modulation of vascular tone in response to increases and/or decreases in CO_2) in young and older healthy adults pharmacologically. Indomethacin, a non-steroidal anti-inflammatory drug, inhibits the enzyme cyclooxygenase (COX) and thus prostaglandin synthesis. While similar to other COXinhibitors (e.g., naproxen, ibuprofen), only indomethacin reduces CBF in healthy humans, without changing cerebral metabolic rate (Hohimer *et al.*, 1985; Kraaier *et al.*, 1992) or plasma catecholamine concentrations (Wennmalm *et al.*, 1983; Staessen *et al.*, 1984; Green *et al.*, 1987). Indomethacin reduces basal CBF by 19 - 42% and CVR_{CO2} by 50 - 65% in both young and older adults (Eriksson *et al.*, 1983; Wennmalm *et al.*, 1983; Jensen *et al.*, 1993; Markus *et al.*, 1994; Kastrup *et al.*, 1999; Bruhn *et al.*, 2001; St. Lawrence *et al.*, 2002; Xie *et al.*, 2006; Ivancev *et al.*, 2009; Xie *et al.*, 2009; Barnes *et al.*, 2012a; Hoiland *et al.*, 2015; Peltonen *et al.*, 2015; Hoiland *et al.*, 2016; Peltonen *et al.*, 2016), and alters dynamic cerebral autoregulation (in young males; (Smirl *et al.*, 2014)).

Aging is associated with independent reductions in cerebral perfusion and some aspects of cognition, so older adults may be more cognitively susceptible to acute reductions in CBF (i.e., lower reserve). A brief reduction in perfusion impairs cognition in patients with cardiovascular disease (Marshall *et al.*, 2001) and with end-stage kidney disease during haemodialysis (Findlay *et al.*, 2019). Yet, the impact of acute reductions in cerebral perfusion on cognition has not been addressed in healthy adults. Young adults, with higher cognition, CBF and perhaps CVR_{CO2} (Lucas *et al.*, 2012; Bailey *et al.*, 2013), may be able to cognitively tolerate acute reductions in cerebral perfusion better than older counterparts (i.e., have a higher reserve).

Indomethacin reduces cerebral perfusion to a similar extent as occurs with healthy aging [i.e., ~30% (Ainslie *et al.*, 2008)]. Indomethacin therefore provides a means to acutely eliminate age-related

differences in resting CBF and the possible differences inCVR_{CO2}, and their potential impact on cognitive function. Therefore, the primary aim of this study was to test whether and to what extent an acute reduction in CBF and cerebrovascular control would impair cognition in young and older adults. A secondary aim was to elucidate the extent to which age-related reductions in cognition at baseline are modulated by the usually-observed impairment in CBF and cerebrovascular control. Expecting that older adults would show lower baseline CBF (~30%) and lower cognitive performance in both response time and working memory tasks, we hypothesised that an acute reduction of CBF (i.e., with indomethacin) *per se* would impair cognition, but more so in older adults.

Methods

This study was approved by the New Zealand Central Health and Disability Ethics Committee (18/CEN/142) in accordance with the standards set by the Declaration of Helsinki. This study was prospectively registered in the Australian New Zealand Clinical Trials Registry on 27/09/2018 (ACTRN12618001603202). All participants gave written informed consent prior to data collection.

Participants

Prospective participants were invited to take part if they were aged 18-35 or 50-75 y, with no sign of cognitive impairment and were not smokers. Exclusion criteria were known cardiovascular, cerebrovascular, neurological, metabolic, respiratory, renal, or haematological disease or condition, or current usage of medication such as cardiac glycosides, aminoglycosides, diuretics, anticoagulants, antihypertensive, aspirin or corticosteroids. The use of nonsteroidal anti-inflammatory drugs was restricted for a minimum of 7 d prior to the initial study visit and continued through the final experimental visit. Older females were recruited only if they reported being postmenopausal or having experienced amenorrhea for a minimum of 12 mo. Young females were in luteal menstrual phase or on oral contraception (active pill phase) for all familiarisation and experimental visits. All participants were screened for cognitive impairment using the Montreal Cognitive Assessment (MoCA©). A score of 25 or higher was required for participation, as a score below 25 represents abnormally low cognitive performance (Nasreddine *et al.*, 2005).

Twenty-nine participants were recruited and undertook familiarisation. One subsequently moved to a different city, two were excluded due to insonation difficulties of the MCA and PCA from poor temporal windows, and one was excluded due to a low MoCA© score. Therefore, 25 participants

were subsequently randomised after familiarisation, using a computer-generated and counter-balanced allocation. The allocation sequence was concealed until the moment of assignment.

Participants reported to the laboratory having abstained from caffeine and food for a minimum of 2 h, and from strenuous exercise for 12 h. Diet and activity were kept consistent for the 24 h prior to each visit.

Experimental Procedures

This study used a placebo-controlled, single-blinded, randomised cross-over design, in which two groups (young vs. older) each completed two conditions in cross-over fashion (indomethacin vs. placebo). Seven of thirteen young adults completed the indomethacin condition first, while the twelve older adults were counter-balanced. Participant information is in Table 1. Participants were blinded to the treatment. Measures of cognitive function and cerebrovascular control were assessed before and after ingestion of indomethacin and a placebo (Figure 1). Conditions were undertaken at least 10 d after a familiarisation visit and separated by at least 72 h. Testing was completed between 7:00 AM and 9:00 PM, with time-of-day consistent within participants (within 30 min).

Familiarisation

During the familiarisation visit participants completed the MoCA©. After full instrumentation, participants completed familiarisation rounds of cerebrovascular reactivity to hypercapnia (CVR_{HYPER}) and hypocapnia (CVR_{HYPO}). In addition, they sufficiently practiced (as evidenced by a plateau in performance) the cognitive battery (i.e., Pro, Anti, Pro/Anti and Backward Digit Span) to minimise future concern of practice effects. Pro, Anti, Pro/Anti was practiced three times, while the Backward Digit Span was practiced once.

After the experimental conditions, below, participants undertook a fourth visit for estimating their peak rate of oxygen consumption ($\dot{V}O_{2peak}$) on the treadmill. A standard submaximal paradigm was used; 3 stages of four minutes, each with mildly increasing intensities (+10-15% of calculated heart rate reserve (Karvonen, 1957)) (Golding *et al.*, 1989; Akalan *et al.*, 2008). Heart rate (HR) and $\dot{V}O_2$ were averaged across the fourth minute of each stage, and $\dot{V}O_{2peak}$ subsequently estimated from a linear regression using predicted maximal HR (i.e., 220 minus age (Fox, 1973)).

Experimental Conditions

Participants attended the laboratory for the placebo and indomethacin conditions, where they sat comfortably in a semi-recumbent chair throughout (i.e., ~3.5 h), except when undertaking dynamic cerebral autoregulation (CA) tests (~8 min). After instrumentation and collection of baseline data, participants completed the cognitive battery twice and tests of dynamic CA, CVR_{HYPER} and CVR_{HYPO}. Measures of CVR_{CO2} (CVR_{HYPER} followed by CVR_{HYPO}) were completed after dynamic CA as hypocapnia can profoundly affect cerebrovascular control (Ide *et al.*, 2003). Dynamic CA and $CVR_{HYPER/HYPO}$ were measured to characterise cerebrovascular control mechanisms alongside changes in cerebral blood velocity in anterior and posterior regions of the brain, in response to indomethacin in young and older adults. Participants then received 100 mg of an anti-nausea medication, Simethicone (De-Gas®, Pfizer Australia Pty Limited) and 1.2 mg/kg (rounded to the nearest 25 mg) of either placebo or indomethacin. Simethicone has previously been used (Barnes *et al.*, 2012b) to prevent GI upset, a potential side-effect of indomethacin.

After a minimum of 60 min of rest (during which participants read or watched Netflix, consistent between conditions), cognitive function and cerebrovascular control measures were repeated in the same sequence as at baseline.

Measurements

<u>Cerebrovascular and Cardiovascular measures</u>

Cerebral blood velocity was measured in both the middle and posterior cerebral arteries using Transcranial Doppler Ultrasound (Spencer Doppler, Sterling VA, USA). Depth was controlled between 45-60 mm for the middle cerebral artery (MCA) and 60-70 mm for the posterior cerebral artery (PCA). Carotid compression was used to differentiate the MCA from the PCA. The probe was then secured in place with a headband device (Marc 600 Headframe) to maintain insonation angle and position.

Heart rate and its rhythm were measured continuously using a standard lead-II electrocardiogram (ADInstruments, Dunedin, NZ). Blood pressure was measured on a beat-by-beat basis using finger photoplethysmography (Finometer®Pro, Finapres Medical Systems, Enschede, The Netherlands), calibrated against manual blood pressure measurements. Mean MCA velocity ($MCAv_{mean}$) and mean arterial pressure (MAP) were first calculated as the mean time integrals and then divided by cardiac period.

Participants breathed through a leak-free respiratory mask (Hans-Rudolf 8980, Kansas City, MO) attached to a turbine and two-way valve, as described in Shoemaker *et al.* (2019a). Partial pressure of end-tidal CO_2 ($PETCO_2$) was measured continuously using an online gas analyser (Model CD-3A Carbon Dioxide Analyser, AEI Technologies, Pittsburgh, USA). The $PETCO_2$ was controlled using a customised dynamic end-tidal clamping system (C.E.T. Gas Clamp, School of Physical Education, Sport and Exercise Sciences, University of Otago, Dunedin, NZ). Hypercapnia was achieved by titrating CO_2 into room air using solenoid-valve control, resulting in +5 and +10 mm Hg steps in $PETCO_2$ from baseline. A single step for hypocapnia (-10 mm Hg) was achieved by increasing ventilation with verbal coaching and visual feedback.

All data were sampled continuously (1 kHz) using an analogue-to-digital converter (Powerlab /16SP ML795; ADInstruments, Dunedin, NZ) and stored for later analysis on Labchart software (version 7, ADInstruments).

Cerebrovascular Control

For both CVR_{HYPER} and CVR_{HYPO}, data were averaged across 30 s after a minimum of 2 min from the onset of $PETCO_2$ manipulation, in agreement with the CVR_{HYPER} recommendations for lower error and individual variability (Burley *et al.*, 2020). The $CVR_{HYPER/HYPO}$ were calculated using linear regression as the change in $MCAv_{mean}$ divided by the change in mean $PETCO_2$ (cm/s/mm Hg). The CVR_{HYPER} was determined as the slope of two +5 mm Hg hypercapnia steps. Likewise, CVR_{HYPO} was determined as the slope of the change from baseline to -10 mm Hg. Exceptions to these procedures were as follows: 3 of 25 participants had one CVR_{HYPER} discarded and 8 of 25 had one CVR_{HYPO} discarded due to validity problems in baseline or elevated/reduced $PETCO_2$, and were thus excluded from inferential analyses of this measure; 6 of 25 participants had CVR_{HYPER} calculated from one CO_2 step, due to insufficient clamping of $PETCO_2$; 9 participants had one CO_2 epoch beginning 75 - 120 s after hypercapnia onset, for reasons of data integrity (e.g., prioritising signal measurement quality or stability), and in all cases these were time matched as closely as possible with their corresponding intra-trial CVR_{HYPER}.

Dynamic CA was determined using a sit-to-stand protocol, performed for 5 min at a frequency of 0.05 Hz. Transfer function analysis for pressure-flow relations was computed using commercially-available software (Ensemble version 1.0.0.14, Elucimed Ltd, Wellington, NZ) as described in Tzeng *et al.* (2012). Briefly, Ensemble uses the R-R interval from the electrocardiogram to obtain precise beat-to-beat BP and MCAv signals, which are spline interpolated and resampled at 4 Hz for

spectral analysis and TFA based on the Welch algorithm. Recordings are sub-divided into overlapping (50%) windows which are then linearly detrended and passed through a Hanning window prior to fast Fourier transform analysis. The cross-spectrum between the two signals (BP and MCAv) was divided by the auto spectrum of the input signal (BP) to derive transfer function values of coherence, absolute gain, and phase. These values were selectively analysed at the point estimate of the 0.05 Hz driven frequency. All coherence values included in analysis were above the statistically-calculated threshold (0.63). The Ensemble algorithms for TFA have been cross-validated in Meel-van den Abeelen *et al.* (2014) and Tzeng *et al.* (2012).

Cognitive Function

Choice response time, inhibition, and mental switching were tested using a response time battery consisting of Pro, Anti, and Pro/Anti tasks, respectively (described in Shoemaker *et al.* (2019b) and based on (Guiney *et al.*, 2015). Participants were instructed to respond to a green (Pro) or red (Anti) visual stimulus by pressing a button on the corresponding (Pro) or opposite (Anti) side to which the stimulus appeared. Response time (ms) and accuracy were recorded for all trials. Accuracy-adjusted response time (aRT) was used to account for any speed-accuracy trade-off, and was calculated as:

aRT = Median response time of correct responses / (1-error rate).

This measure can be interpreted like unadjusted response time and has been used previously in peer-reviewed research (Guiney *et al.*, 2019) with high within-day reliability following multiple practise trials (Shoemaker *et al.*, 2020).

Working memory was assessed using the Backward Digit Span task. This task involves participants recalling a list of numbers in *reverse* order. The task started with 3 digits and continued up to 9. Each level had two trials, whereby the same number of digits was presented verbally (e.g., 4-8-1 and 5-3-8). An unsuccessful trial was where the digits were not recalled in perfect reverse order. The task was stopped when two unsuccessful sequential trials of the same length occurred. The highest recalled digit span was recorded.

Lastly, participants were asked to report their “feeling” (overall feeling state) pre- and post- placebo and indomethacin, using the 11-point Affect Feeling Scale (Giblin, 2011). Participants also reported any gastrointestinal upset and tiredness on a 7-point Likert scale.

Statistical Analysis

A priori power calculations with an expected moderate ($\eta p^2 = 0.06$) effect size revealed that a sample of 24 participants was needed to obtain 80% power with 2 groups and 4 measurements ($\alpha = 0.05$). Data were analysed using R (R Development Core Team, 2008) and graphed with GraphPad Prism (Prism Version 8, GraphPad Software, CA, USA). An alpha of 0.05 was used for each analysis.

Independent two-tailed t-tests were performed to compare baseline measures of cerebrovascular and cognitive characteristics between groups. Primary and secondary outcome variables were assessed for homogeneity of variances with Levene's test. Linearity and approximate normal distribution of model and individual residuals were assessed qualitatively using visual inspection of histograms and Q-Q plots and formally tested with Shapiro-Wilk's Test. If the assumptions of a parametric test were met (i.e., homogeneity of variance, linearity, normality, and independence), then the data were analysed using a linear mixed-effect model for repeated measures. Only $MCAv_{mean}$, $PCAv_{mean}$, MAP, CVC, and Phase (from CA measures) met these assumptions and were tested using the parametric approach. Variance-covariance structure, model inclusion of random and fixed factors, and weighting of model errors was assessed using Akaike's Information Criteria (AIC). At minimum, condition (2 levels: indomethacin, placebo), time (2 levels: pre, post), age (continuous variable), and their interactions were treated as fixed factors. Sex and fitness were included according to AIC values. Fitness did not have a significant fit to any model. Participant was included as a random effect. Post-hoc testing for significant interactions of parametric testing was completed with Tukey's HSD. All results from the linear mixed-effect models are reported using mean ± SD or 95% confidence intervals [CI: lower limit, upper limit].

Non-parametric rank-based analysis method was used for $CVR_{HYPER/HYPO}$, Gain and Coherence [from CA measures], heart rate, $PETCO_2$, and cognitive data using the R package developed by Noguchi *et al.* (2012), to assess the three-way interaction of age-by-condition-by-time. Due to the package allowing for only three factors, effects of sex were tested using an additional analysis, of condition-by-time-by-sex. P-values are reported from the ANOVA-type statistic. Multiple comparisons for significant interactions were completed with Bonferroni's post-hoc adjustment. All results from the nonparametric rank-based methods are reported as median and interquartile ranges [IQR: quartile 1, quartile 3]. Lastly, correlation data involving cognition were analysed using Spearman's rho correlation coefficient (*rho*) while $CVR_{HYPER/HYPO}$ were analysed using Pearson correlation coefficients (*r*).

Reliability was assessed using intraclass correlation (ICC) estimates and their 95% confidence intervals, calculated based on a mean-rating, absolute agreement, 2-way mixed-effects (test-retest) model. Consecutive pairwise comparisons of reliability from 2 trials for both between-day (i.e., placebo and indomethacin) and within-day (e.g., pre- and post-placebo), as calculated by ICC, show excellent (>0.9), good (0.76-0.9), moderate (0.5-0.75), and poor (<0.5) reliability (Table 2, as described by Koo and Li (2016)). Reliability is presented also as the coefficient of variation (SD/mean*100).

Cognitive, cerebro- and cardio-vascular data are reported in the results from all 25 participants. However, $PCAv_{mean}$ was accessible for the entire protocol in only 20 participants (n = 13 young and n = 7 older). Measures of CA are reported from 24 participants due to a poor beat-to-beat blood pressure recording from one older adult (n = 13 young and n = 11 older). The CVR_{CO2} measures are reported as described above.

In text and Figure 6, all means with 95% confidence intervals and medians with interquartile ranges are calculated from the difference between the change from indomethacin (i.e., 'post' minus 'pre') and the change from placebo, and thus represent the treatment effect.

Results

Reliability of measures

Resting measures of $MCAv_{mean}$ and $PCAv_{mean}$ (Table 2) showed good reliability across days (i.e., baseline measures) and excellent reliability within one day (placebo condition). aRT measures show good-to-excellent reliability within a single day and good reliability between days. The functional CBF measures of $CVR_{HYPER/HYPO}$ show good-to-excellent reliability within days and poor reliability between days.

Cerebro- and Cardio-vascular Responses

Baseline comparisons between groups revealed that $MCAv_{mean}$ was 14% lower in older adults ($p = 0.048$), whereas $PCAv_{mean}$, CVR_{HYPER} and CVR_{HYPO} were not significantly lower ($p \geq 0.263$). Older adults had lower heart rate (13%; $p = 0.049$) and CVC (17%; $p = 0.043$), whereas MAP ($p = 0.405$) and $PETCO_2$ ($p = 0.179$) were similar between groups. Older adults had lower aerobic fitness (estimated $\dot{V}O_{2peak}$) than young adults. However, according to a new $\dot{V}O_{2peak}$ calculation that accounts for age, sex, height, weight and exercise mode (de Souza e Silva *et al.*, 2018), both groups

were equivalently (Table 1; two-tailed T-test, $p = 0.308$) *more* fit than would be expected for their respective wider populations.

Older adults did not have greater cerebro- or cardio-vascular sensitivity to indomethacin, as all age-by-condition-by-time interactions were non-significant ($p \geq 0.249$). Furthermore, there were no significant main effects or interactions with sex for any cerebro- or cardio-vascular variable (all $p \geq 0.174$; Figure 2).

Indomethacin reduced $MCAv_{mean}$, CVR_{HYPER}, and CVR_{HYPO} (all interaction effects and subsequent pairwise comparisons $p < 0.001$ vs. pre-indomethacin and post-placebo). Specifically, $MCAv_{mean}$ declined by 31% [CI: -35, -26], CVR_{HYPER} by 68% [IQR: -94,-44] and CVR_{HYPO} by 50% [IQR: -83,-33], irrespective of age. Baseline CVR_{HYPER} (young: $r = 0.81$, older: $r = 0.89$) and CVR_{HYPO} (young: $r = 0.70$, older: $r = 0.89$) were strongly associated (all $p \leq 0.02$) with their indomethacin-induced change (Figure 3). Specifically, participants who had the largest CVR_{HYPER} and CVR_{HYPO} at baseline had the largest decrease post-indomethacin (Figure 3), in both young and older groups. Regardless of age, indomethacin increased MAP (8 ± 12%) and decreased HR (7 ± 8%), CVC (34 ± 17%) and $PCAv_{mean}$ (26 ± 14%; all time-by-condition interaction: $p \leq 0.001$).

Cerebral Autoregulation (Figure 4)

In all conditions, coherence was above the statistically-calculated threshold (i.e., > 0.63, Figure 4A), allowing for the interpretation of gain and phase. Age groups were not different for coherence, gain and phase (all $p \geq 0.08$) at 0.05 Hz. Indomethacin dampened both BP and $MCAv_{mean}$ power ($p < 0.046$ vs. pre-indomethacin, $p < 0.007$ vs. post-placebo). It reduced gain by 30% ([IQR: -46, 1]; Figure 4B) and increased phase by 57% ([CI: 4,110]; all $p < 0.001$ vs. pre-indomethacin and post-placebo). Coherence was lowered by 4% ([IQR: -11, 4]; condition-by-time interaction $p = 0.006$; $p < 0.001$ vs. pre-indomethacin and post-placebo). Coherence was 3% higher in males than females (main effect of sex: $p = 0.001$).

Cognitive Performance (Figure 5)

At baseline, older adults had 14-15% lower performance than young adults (all $p < 0.001$) in Pro (14%), Anti (15%) and Pro/Anti (15%) tasks, but not lower working memory ($p = 0.663$; Figure 5D). Furthermore, there were no significant main effects or interactions involving sex for any cognitive outcome measures (all $p \geq 0.377$).

Older adults' cognitive performance was not more affected by indomethacin than that of young adults' (age-by-condition-by-time interaction: all $p \geq 0.377$). Pro/Anti performance was 7% [IQR: 0, 19] worse post-indomethacin ($p \leq 0.042$ vs. pre-indomethacin and post-placebo), however Pro (4% [IQR: 0,11]) and Anti (4% [IQR: -4,17]) performance were not measurably affected (time-by-condition interaction; $p = 0.061$ and $p = 0.181$ respectively) but showed the same pattern as Pro/Anti.

Working memory improved during the placebo condition (1 AU [IQR: 0,1]; $p = 0.001$ vs pre-placebo and post-indomethacin) but not during the indomethacin condition (0 AU [IQR: -1,0]; $p = 0.219$ vs. pre-indomethacin). Participants reported slightly but significantly worse overall feelings after indomethacin than after placebo (-1 ± 1 vs 0 ± 1 AU, respectively; $p = 0.038$), but not more gastrointestinal upset or tiredness (both $p \geq 0.120$).

Associations between Changes in Cerebrovascular and Cognitive Function (Figure 6)

The indomethacin-induced reduction in $MCAv_{mean}$ was not associated with an acute change in aRT within either age group (all rho ≤ 0.34; $p \geq 0.249$; Figure 6). Additionally, changes in CVR_{HYPER} and CVR_{HYPO} were not reliably associated with Pro (rho = 0.22 and rho = -0.18, respectively), Anti (rho = -0.11 and rho = -0.06, respectively), or Pro/Anti performance (rho = -0.29 and rho = 0.22, respectively; all $p \geq 0.167$).

Discussion

The novel findings of the current study were that executive cognitive function (mental switching and short-term memory) was broadly resilient to a moderate (31%) acute reduction in $MCAv_{mean}$ in healthy young and older adults. Other findings are valuable because they have seldom been shown and/or are equivocal in the literature. Specifically, the current results show that: (i) those who had the largest cerebrovascular reactivities to CO_2 at baseline had the largest decrease post-indomethacin; (ii) healthy older adults had lower $MCAv_{mean}$ and cognitive performance than healthy younger adults did, which is consistently reported, but (iii) they did not have lower CVR_{HYPER}, CVR_{HYPO}, or CA, which is equivocal in the literature.

The effects of indomethacin on cognitive function and cerebral perfusion.

The current findings only partially supported our hypothesis that an acute reduction in $MCAv_{mean}$ (i.e., 31% reduction with indomethacin) *per se* would impair cognition function and would be more evident in older adults. The cognitive function domain of mental switching ability (i.e., Pro/Anti)

was impaired by ~7% in young and older adults post-indomethacin, and showed a small to moderate effect size (Cohen's dz = 0.42) that was beyond the within-day coefficient of variation (3.8%). There was no evidence that the acute reductions in mental switching ability and $MCAv_{mean}$ were associated, but this is difficult to identify and characterise within a single-dose study.

Prior to the current study, the impact of acute reductions in CBF on cognitive performance had been addressed only in clinical cohorts. Marshall *et al.* (2001) concluded that a 23 - 54% reduction in perfusion from an acute internal carotid artery balloon test occlusion (30 min) resulted in transient (and reversible) reductions of sustained attention (response time) in patients with inoperable peri-cavernous aneurysms or head and neck tumours, despite markedly varied cognitive responses between patients. Findlay et al. (2019) showed in end-stage kidney disease patients that $MCAv_{mean}$ decreased significantly by 10% during at least 2 h of haemodialysis (a pro-inflammatory state), which moderately (Spearman's rho -0.32) correlated with the intradialytic decline in executive function (trail making tasks; 13.5 s slower). Clinical populations with particular susceptibility to cerebral hypoperfusion during head-up tilt (i.e., postural tachycardia and chronic fatigue syndromes) have also shown simultaneous cognitive impairment on working memory and attention tasks (n-back) (Stewart *et al.*, 2012; Medow *et al.*, 2014). Interestingly, phenylephrine restored both the head-up tilt-induced cerebral hypoperfusion and the impaired cognitive performance in chronic fatigue syndrome patients (Medow *et al.*, 2014). Although we acutely reduced cerebral perfusion substantively (~31%) in *healthy* young and older adults, we did not find evidence to support a similar cognitive impairment related to the decreased flow. Healthy individuals with greater fitness, such as those recruited for this study, can buffer physiological strain - such as inflammation (Hamer & Steptoe, 2007) and oxidative stress (Radak *et al.*, 2005; Radak *et al.*, 2008) - to a higher degree. Higher fitness additionally provides higher cerebral perfusion chronically (Ainslie et al., 2008). Collectively, such attributes of fitness may act as a buffer against functional and cognitive impairments caused by short-term reductions in perfusion. Although seemingly unrelated to concurrent reductions in cerebral perfusion, cognition was impaired after ingestion of indomethacin. In an acute sense, indomethacin is an *anti*-inflammatory drug that does not appear to alter cerebral metabolism (Pickard & MacKenzie, 1973; Sakabe & Siesjö, 1979; Dahlgren *et al.*, 1981; Wennmalm *et al.*, 1981; Jensen *et al.*, 1991). However, indomethacin is reported to promote oxidative stress in the small intestine and kidney of rodents by virtue of drug-induced generation of reactive oxygen species and decreased level of anti-oxidants and oxygen uptake (Basivireddy *et al.*, 2002; Varghese *et al.*, 2009; Tomita *et al.*, 2014). The brain is particularly vulnerable to oxidative

stress, but the effect of indomethacin on acute cerebral oxidative stress (and cognition) has not been addressed. Therefore, the mechanism by which cognition is acutely impaired with ingestion of indomethacin remains unclear. One possibility is that participants were distracted by overall feelings of (mild) discomfort, leading to worse performance. But if this occurred, a reduction in cognitive performance might be expected across all measures. Future research should consider implementing a fatigue and gastrointestinal upset control such as a visually draining or vertigo-inducing stimulus.

Indomethacin inhibits vasodilating prostaglandin synthesis, leading to cerebral vasoconstriction increasing resistance and reducing cerebral perfusion. As expected, and in agreement with previous literature (Eriksson *et al.*, 1983; Wennmalm *et al.*, 1983; Jensen *et al.*, 1993; Markus *et al.*, 1994; Kastrup *et al.*, 1999; Bruhn *et al.*, 2001; St. Lawrence *et al.*, 2002; Xie *et al.*, 2006; Ivancev *et al.*, 2009; Xie *et al.*, 2009; Barnes *et al.*, 2012a; Hoiland *et al.*, 2015; Peltonen *et al.*, 2015; Hoiland *et al.*, 2016; Peltonen *et al.*, 2016), we also observed decreased CVR_{HYPER} and CVR_{HYPO} across young and older groups. These reductions (68% and 50%, respectively) were beyond the day-to-day and within-day variability of CVR measures (Table 2: 11 – 24%) and may appear greater than what is typically observed following indomethacin ingestion (i.e., ~30-65%). One reason may be that these reactivities represent the indomethacin treatment-effect, a reduction greater than the effect of time (i.e., controlling for within-day variability). Our mean ± standard deviation values for indomethacin-related reductions (pre vs. post indomethacin) are similar to those typically reported for both CVR_{HYPO} (Young: 51 ± 24%; Older: 59 ± 21%) and CVR_{HYPER} (Young: 67 ± 20%; Older: 74 ± 13%). Although our data (Figure 3) appear to support a role of prostaglandin synthesis in CVR_{CO2}, it is important to consider that indomethacin is the only COX-inhibitor to reduce both basal cerebral blood flow and reactivity to CO_2 (Eriksson *et al.*, 1983; Wennmalm *et al.*, 1984; Markus *et al.*, 1994; Hoiland *et al.*, 2016), despite other potent COX-inhibitors (e.g., aspirin and naproxen) having similar inhibition of the cerebrovascular production of prostaglandins (Chemtob *et al.*, 1991). Therefore, it is possible that indomethacin reduces CBF via a mechanism(s) independent of prostaglandin synthesis inhibition. Indeed, indomethacin has numerous inhibitory and rapid-acting enzyme and cellular actions (Flower, 1974; Chemtob *et al.*, 1991) that are likely to cause systemic vasoconstriction independent of COX inhibition. One such action is via cyclic AMP-dependent protein kinase inhibition, as discussed by Hoiland *et al.* (2016) and Hoiland and Ainslie (2017). Briefly, cyclic AMP is involved in the regulation of vascular smooth muscle tone (Adelstein *et al.*, 1978) and indomethacin has been shown to inhibit cAMP-dependent protein

kinase (Kantor & Hampton, 1978; Goueli & Ahmed, 1980). However, our finding that individuals with heightened CVR_{CO2} at rest experience greater indomethacin-related reductions (Figure 3) is in line with previous literature. Specifically, Kastrup *et al.* (1997) showed that the indomethacin-induced decrease in CVR_{HYPER} is linearly correlated with initial baseline CVR_{HYPER} (r = 0.74), and we have extended this finding to show the same relation with CVR_{HYPO}, as well as for older adults (r = 0.87). Given the dominant role of CVR_{CO2} in cerebrovascular control, this has numerous implications for lifestyle and pharmacological interventions that impact cerebrovascular tone, and on assessment of cerebrovascular health (Burley *et al.*, 2016).

Although $CVR_{HYPER/HYPO}$ was *reduced* after indomethacin, it is likely by the same drug-induced vasoconstrictive-effect that CA appears to be *enhanced*. Dynamic CA offers mechanistic insight into the indomethacin-related reductions in cerebral perfusion, and how indomethacin may alter the pressure-flow relation in young and older adults. Decreases in perfusion and increases in vascular resistance intensify the signal power for blood pressure and weaken signal power for blood flow. These power changes result in significant decreases in gain and increases in phase after indomethacin, in agreement with existing literature in young men (Smirl *et al.*, 2014), new born and fetal lambs (Van Bel *et al.*, 1993; Van Bel *et al.*, 1995), and head-injured humans (Puppo *et al.*, 2007). A decrease in gain indicates that less flow is transmitted per unit (i.e. mm Hg) of pressure, i.e., blood pressure is having less influence on $MCAv_{mean}$. Changes in blood pressure normally trigger a rapid downstream vasoconstrictive response. However, in this scenario indomethacin has pre-emptively caused systemic vasoconstriction, facilitating the CA response. Similarly, an increase in phase indicates the flow response to a pressure-pulse is lengthened. This likely results from increased vascular resistance. Therefore, we are careful to interpret “enhanced” CA as anything but increased vascular tone – which is typically a sign of vascular dysfunction. Importantly, our data extend the findings of Smirl *et al.* (2014), such that indomethacin alters the cerebral pressure-flow relation not only in young men, but also in young women and healthy older adults, with no apparent sex-differences.

Baseline Group and Cerebrovascular Characteristics

The CBF in the middle cerebral artery declines by ~5% every ten years (Grolimund & Seiler, 1988), or 28 - 50% between ages 30 and 70 y (Heo *et al.*, 2010; Ogoh *et al.*, 2014). The prefrontal cortex – which is often associated with cognitive function - also has volumetric declines of ~5% per decade after age 20 y (Raz *et al.*, 2004). In addition to other structural changes (Bhogal *et al.*, 2016), the

age-related decrease in CBF may be partly attributed to a loss of prostaglandin function (Barnes *et al.*, 2012b). The current study shows a 14% difference in $MCAv_{mean}$ between young and older adults (Figure 2A), despite the older group having high levels of fitness and being sampled from an academic population. The reduction in anterior cerebral perfusion is likely due to a degree of vascular dysfunction, as evident from reduced CVC in older adults (Figure 2C). Lower CVC reflects less vasculature, higher vascular resistance (Tarumi & Zhang, 2018), or both. However, older adults did *not* show a significant difference in posterior cerebral blood velocity compared to young adults (Figure 2B). Although $PCAv_{mean}$ has been reported to decline ~3.7% every 10 years (Grolumund & Seiler, 1988), more recent literature reports that older (age-range: 40-73 y) adults have similar posterior perfusion to young (20-30 y) adults (Krejza *et al.*, 1999; Sorond *et al.*, 2005; Sorond *et al.*, 2008). The current study has limited power (n=20) and age range (25 vs 58 y) to address this issue but provides data for future meta-analytic study.

Older adults also did not have a measurable impairment in cerebrovascular control at rest, as determined by CVR_{HYPO}, CVR_{HYPER}, and dynamic CA. The finding that CVR_{CO2} was not impacted by age agrees with Braz *et al.* (2017), who also found no difference between young and older groups of trained and sedentary adults. At this time, there is no clear evidence whether CVR_{CO2} is impacted by healthy aging, or not (see review by Hoiland *et al.* (2019)). It is possible that any differences between age groups were missed due to CVR_{CO2} being a variable measure, despite using a reliable measure of CO_2 control (i.e., computerised clampling) and following the current recommendations for lower intra-individual variability (Burley *et al.*, 2020). That being said, we are confident our findings are accurate as the reliability is similar to or better than what has been reported elsewhere, particularly in studies using TCD (Wilson *et al.*, 2010; McDonnell *et al.*, 2013). Cerebrovascular reactivity to CO_2 is a strong perturbation used to assess cerebrovascular control. The cerebrovascular response to CO_2 is highly integrated and affects many interrelated physiological systems that have direct and indirect impacts on cerebrovascular control. Thus, the consistently reported variability in this measure might be attributable to the numerous physiological systems involved. Furthermore, environmental influences (e.g., stress, prolonged sitting, fasting, circadian rhythms) affect these systems and may cause the appearance of changed cerebrovascular control by virtue of CVR_{CO2}.Cognitive functions, such as executive functioning, also decline with age (Li *et al.*, 2001; Colcombe & Kramer, 2003; Kramer *et al.*, 2003; Brown *et al.*, 2010). The older adults in the current study were, on average, only 15% worse with response time tasks than their younger counterparts were (Figure 5). This may be because the older adults recruited for this study

were exceptionally healthy, aerobically fit, and cognitively active. For instance, 75% of the older group were current or recently-retired academic staff of the University. Thus, this older group may underestimate the cognitive and cerebrovascular change expected for an "older" population, even if healthy.

One consideration is that an acute reduction in CBF was used to partially inform chronic age-related effects. In an acute sense, cognition was not evidently impaired by virtue of decreased CBF *per se*. This does not mean that a *chronic* loss of, or reductions in, CBF or cerebrovascular control would not lead to cognitive impairment. In fact, meta-analyses reveal that early-stage cognitive decline is associated with abnormal cerebral haemodynamics, including reductions in perfusion and CO_2-reactivity (Beishon *et al.*, 2017). Wolters *et al.* (2017) have also demonstrated that cerebral hypoperfusion was a risk factor for cognitive impairment after a 6-y follow-up with 4759 adults. Additionally, a decrease in CBF over 3 y correlated to a decrease in global cognition (mini-mental state exam; r = 0.59) of 27 hypertensive and cognitively-sound older adults (Kitagawa *et al.*, 2009). Thus, there may be a case for chronic reductions in cerebral perfusion impacting cognition.

Limitations

Due to the nature of a correlation analysis, it is possible that the true effect of reduced perfusion on cognition was missed due to the consistent reduction in $MCAv_{mean}$ between participants, limiting the spread of data. Future research could administer graded doses of indomethacin across multiple days to measure any cognitive reactivity to reductions in perfusion within participants. It is also possible that chronic reductions in perfusion impair cognition (as occurs with aging). One avenue to investigate this notion may be via long-term use of indomethacin. However, Eriksson *et al.* (1983) found that cerebral perfusion normalises after one week of oral indomethacin intake (1.5 mg/kg). Moreover, this study was designed to measure any change in cognition that occurs with an *acute* reduction in perfusion, and not to simulate the changes that occur with aging.

As normal for studies using a cross-sectional design, the young group cannot be interpreted as younger versions of the older group. Although both groups were healthy and had above normal fitness, it is not certain that each younger adult will maintain this status across their lifespan. The results may therefore underestimate any age-related effects on cerebrovascular or cognitive function, due to the above-normal fitness and cognitive status in our older cohort. This is encouraging to an aging population, such that a healthy lifestyle may protect against functional (cognitive) effects of acutely reduced cerebral perfusion, which may occur during surgery,

dehydration, orthostasis, and heat stress. As such, indomethacin is only one model to acutely reduce CBF. Importantly, indomethacin reduces cerebral perfusion without manipulating the local tissue metabolism. Maintaining cerebral metabolism was paramount to addressing the question of whether acute reductions in blood flow *per se* negatively affect cognitive performance. Therefore, although using indomethacin to reduce CBF may not be generalisable to a "real-world" context of acute CBF reductions, it was the best option to limit physiological and psychological confounding factors.

The attending researchers were not blinded due to the obvious nature of CBF decline with indomethacin. For example, quality recordings and participant welfare were ensured by keeping a close watch on all physiological variables during the testing protocol; thus, the researcher would be immediately aware of a drop in $MCAv_{mean}$ caused by indomethacin. However, the research team took great care to ensure participants were blinded to each condition and to ensure the same monitoring of participants, and minimal verbal engagement, regardless of treatment, to reduce potential confounding conditions.

The current study also relied on the usual assumption that changes in CO_2 increase flow by dilation of downstream vessels without a meaningful change in MCA (or PCA) vessel diameter. This has been extensively disproven with MRI, wherein the MCA may dilate up to 7% (Coverdale *et al.*, 2014; Verbree *et al.*, 2014; Al-Khazraji *et al.*, 2018). However, any vessel dilation would result in an overall underestimation of changes in flow. Furthermore, by using a drug to purposefully reduce flow and increase vascular resistance, we may be underestimating the total reduction in flow. Indeed, Kellawan *et al.* (2020) demonstrated that COX inhibition via indomethacin reduces MCA cross-sectional area by 0.2 mm. Lastly, although sex was included as a potential confounding factor within the statistical design, we did not power the study to test for sex differences.

Conclusion

Cognitive performance on a mental switching task was slightly (~7%) worse after an oral dose of indomethacin. However, we did not find evidence of an association between the reduction in performance and the 31% reduction in cerebral perfusion, even in older adults. Although older adults had lower $MCAv_{mean}$ and worse cognitive performance at baseline, both groups experienced a similar reduction in cerebral perfusion and cognition after indomethacin. Therefore, cognitive performance may not be influenced by a ~31% reduction in CBF *per* se in healthy young and older adults. These findings are encouraging to a healthy aging population, as both young and older individuals appear broadly resilient to acute reductions in cerebral perfusion up to ~31%.

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Author Contribution

All authors contributed to the study conception and design and interpretation of results. Data collection and analysis were performed by LNS, LCW and JDC. The first draft of the manuscript was written by LNS and all authors commented on previous versions of the manuscript. RW provided clinical oversight related to the safety of indomethacin in the study design and medical clearance for older participants. All authors read and approved the final manuscript.

Disclosures

The authors declare that there is no conflict of interest.

Data Availability Statement

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

Figure Legends

Figure 1. Timeline summary of experimental protocol, which took ~3.5 h (including instrumentation) and was undertaken twice. Time was allocated after cognitive tasks and before CA to allow for recovery of blood pressure and heart rate. *Abbreviations:* BL, baseline; CA, cerebrovascular autoregulation; $CVR_{CO2,}$ cerebral blood velocity hypercapnic (CVR_{HYPER}) and hypocapnic (CVR_{HYPO}) reactivity. Green/Red boxes represent a cognitive battery measuring visuomotor processing speed (Pro), inhibitory control (Anti) and mental switching (Pro/Anti) performance.

Figure 2. Cardio- and cerebro-vascular outcomes pre- and post- placebo and indomethacin for young (black bars, n = 13) and older (white bars; n = 12) adults were analysed using linear (Panels A, B, C, D) and nonparametric (Panels E, F, G, H) mixed-models and are therefore represented with mean or median (*) bars, respectively. *Abbreviations:* $MCAv_{mean}$, mean middle cerebral artery blood velocity; $PCAv_{mean}$, mean posterior cerebral artery blood velocity (Young: n = 13; Older: n = 7 CVC, cerebrovascular conductance; MAP, mean arterial pressure; HR, heart rate; $PETCO_2$, pressures of end-tidal carbon dioxide; CVR_{HYPO} (Young: n = 11; Older: n = 6), cerebrovascular reactivity to hypocapnia; CVR_{HYPER}(Young: n = 13; Older: n = 9), cerebrovascular reactivity to hypercapnia. $^{a}p \leq 0.010$ vs young (main effect of age); $^{b}p < 0.001$ vs pre-indomethacin, regardless of age (condition-by-time interaction); $^{c}p < 0.001$ vs post-placebo, regardless of age (condition-by-time interaction).

Figure 3. Cerebrovascular reactivity (CVR_{CO2}) to hypercapnia (grey; n = 22) and hypocapnia (black; n = 17) at rest (x-axis) is strongly associated with the indomethacin-induced reduction (y-axis) in both young (closed circles) and older (open circles) adults. i.e., those with the greatest CVR_{CO2} at rest experienced the greatest reduction in CVR_{CO2} after a dose of indomethacin.

Figure 4. Transfer function analysis of dynamic cerebral autoregulation during a sit-stand protocol (0.05 Hz) in young (black bars; n = 13) and older (white bars; n = 7) adults pre- and post-indomethacin. Both pre- and post-placebo (not shown) and indomethacin data were included in each mixed-model analysis. Bars represent median (*, Panels A and B) and mean (Panel C). $^{b}p \leq 0.033$ vs pre-indomethacin, regardless of group; $^{c}p \leq 0.038$ vs post-placebo, regardless of group.

Figure 5. Young (black bars;, n = 13) and older (white bars; n = 12) adults' cognitive performance as determined by accuracy-adjusted response time (aRT) for Pro, Anti, and Pro/Anti batteries

(panels A-C) and working memory score (panel D) pre- and post-placebo and indomethacin using nonparametric mixed-models (bars represent median). [a]$p < 0.001$ vs young (main effect of age); [b]$p \leq 0.040$ vs pre-indomethacin, regardless of age; [c]$p \leq 0.042$ vs post-placebo, regardless of age.

Figure 6. Spearman's rho correlations between absolute (Panels A and B) and relative (Panels C and D) changes in accuracy-adjusted response time (aRT) and mean middle cerebral artery blood velocity ($MCAv_{mean}$). All change scores are calculated as the difference between the change from baseline with indomethacin and the change from baseline withplacebo for young adults (panel A and C; closed circles, n = 13) and older adults (panel B and D; open circles, n = 12) during Pro (Green), Anti (Red), and Pro/Anti (Black) tasks.

Tables

Table 1. Participant Characteristics

	Young n = 13 (6 female)	**Older** n = 12 (6 female)
Age (y)	25 ± 4	58 ± 6*
Mass (kg)	78 ± 18	73 ± 13
Height (cm)	175 ± 10	169 ± 10
Estimated $\dot{V}O_{2peak}$ (mL/min/kg)	52 ± 8	42 ± 11*
% of Predicted $\dot{V}O_{2peak}$	131 ± 22	130 ± 26
MoCA© Score (/30)	29 ± 1	28 ± 1
Resting Systolic BP (mm Hg)	107 ± 10	115 ± 8
Resting Diastolic BP (mm Hg)	68 ± 7	69 ± 6

Note. Baseline measures are reported as mean ± standard deviation from familiarisation and pre-placebo conditions. Abbreviations: $\dot{V}O_{2peak}$, peak rate of oxygen consumption (mL/min/kg); MoCA©, Montreal Cognitive Assessment – any score above 25 is considered "normal". % of Predicted $\dot{V}O_{2peak}$ was calculated as the percent difference between estimated $\dot{V}O_{2peak}$ (treadmill) and the predicted $\dot{V}O_{2peak}$ using the equation from de Souza e Silva *et al.* (2018) *$p \leq 0.048$ vs. Young, using Student's independent samples T-test.

Table 2. Reliability of dependent variables, shown as coefficients of variation (CV) and intraclass correlations (ICC).

		CV (%)	**ICC**	**[95% CI]**
A. Across 2 Days (Baseline)	$MCAv_{mean}$	5.4%	0.89	[0.75 - 0.95]
	$PCAv_{mean}$	9.4%	0.85	[0.62 - 0.94]
	CVR_{HYPER}	15.5%	0.48	[-0.21- 0.78]
	CVR_{HYPO}	23.6%	0.49	[-0.15 - 0.78]

	Pro aRT	5.4%	0.79	[0.53 - 0.91]
	Anti aRT	6.1%	0.75	[0.44 - 0.89]
	Pro/Anti aRT	4.5%	0.84	[0.63 - 0.93]
B.	$MCAv_{mean}$	4.3%	0.96	[0.91 - 0.98]
Within 1 Day	$PCAv_{mean}$	5.9%	0.96	[0.90 - 0.98]
(Two measures, 2	CVR_{HYPER}	12.9%	0.88	[0.71 - 0.95]
h apart during the	CVR_{HYPO}	10.9%	0.91	[0.77 - 0.97]
placebo	Pro aRT	3.9%	0.92	[0.81 - 0.96]
condition)	Anti aRT	5.2%	0.86	[0.67 - 0.94]
	Pro/Anti aRT	3.8%	0.92	[0.82 - 0.96]

Abbreviations: CI, confidence interval; $MCAv_{mean}$, mean middle cerebral artery blood velocity; $PCAv_{mean}$, mean posterior cerebral artery blood velocity; CVR_{HYPER}, cerebrovascular hypercapnic reactivity; CVR_{HYPO}, cerebrovascular hypocapnic reactivity; aRT, accuracy-adjusted response time. Reliabilities represent data from 25 participants for $MCAv_{mean}$, $PCAv_{mean}$, Pro, Anti, and Pro/Anti (A and B), 23 participants for CVR_{HYPER} (A and B), 21 for CVR_{HYPO} (A), and 18 for CVR_{HYPO} (B).

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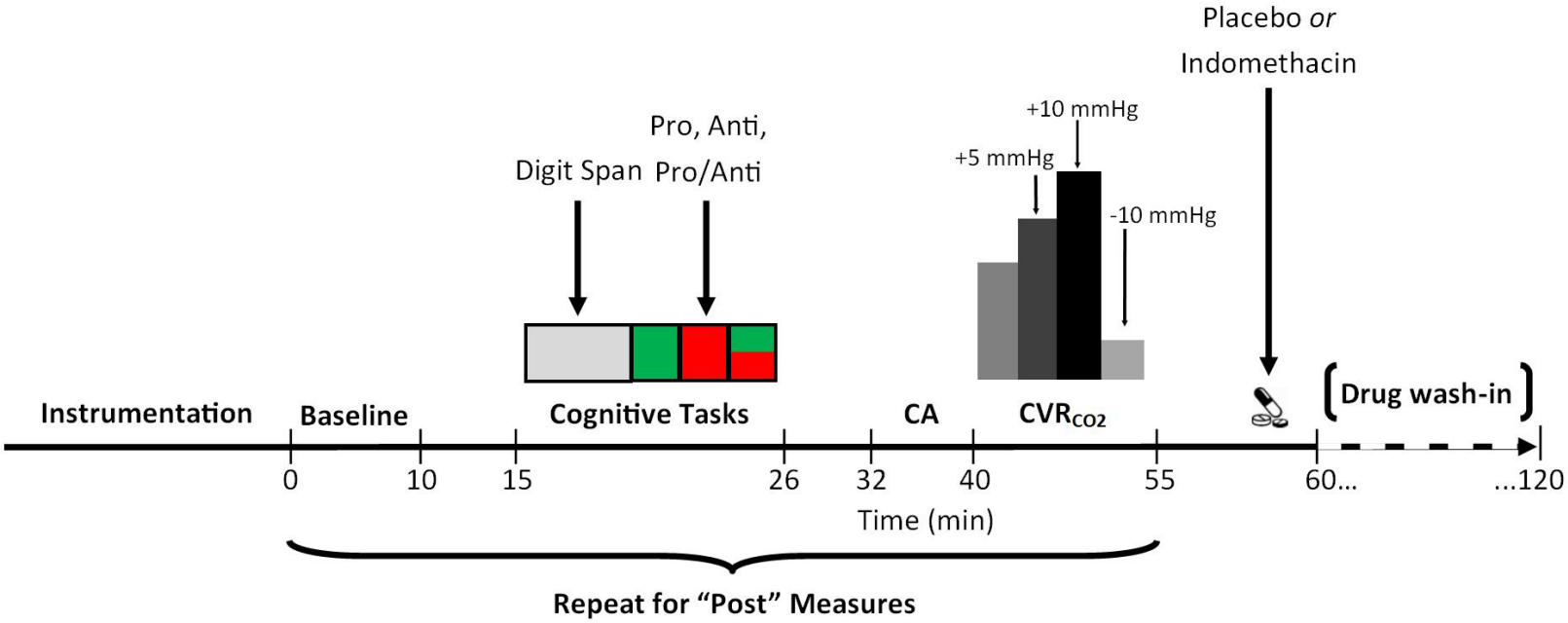
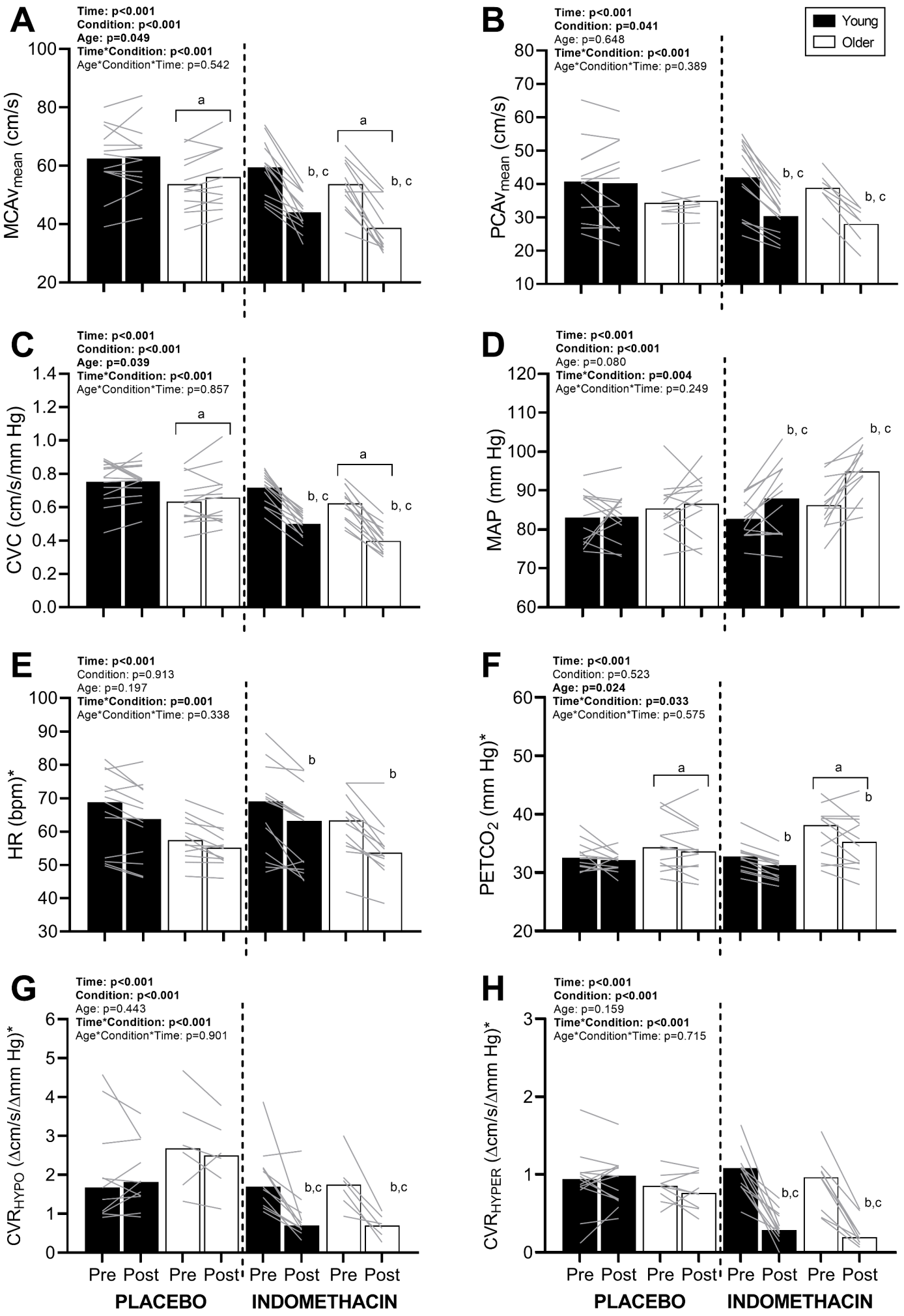
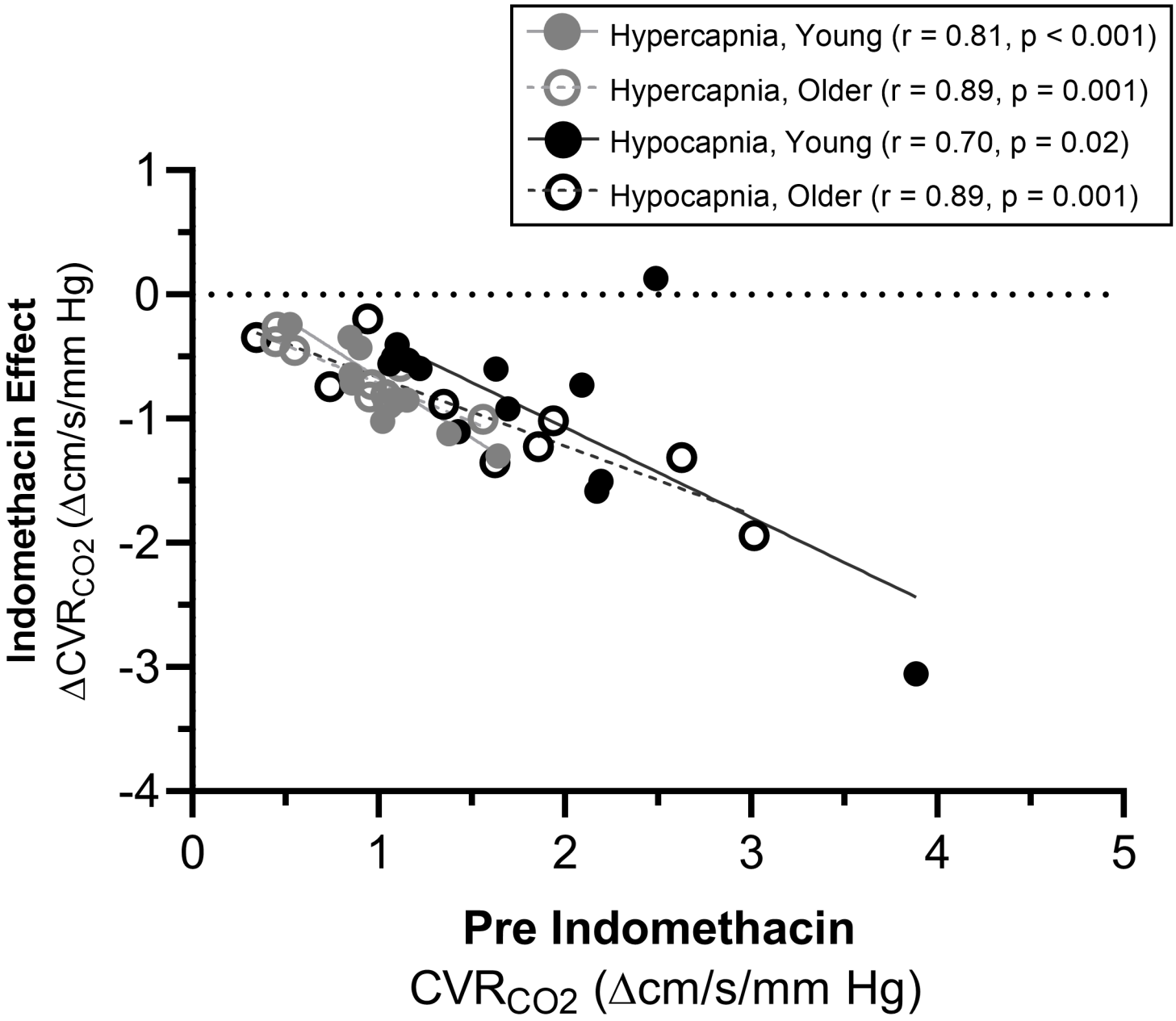
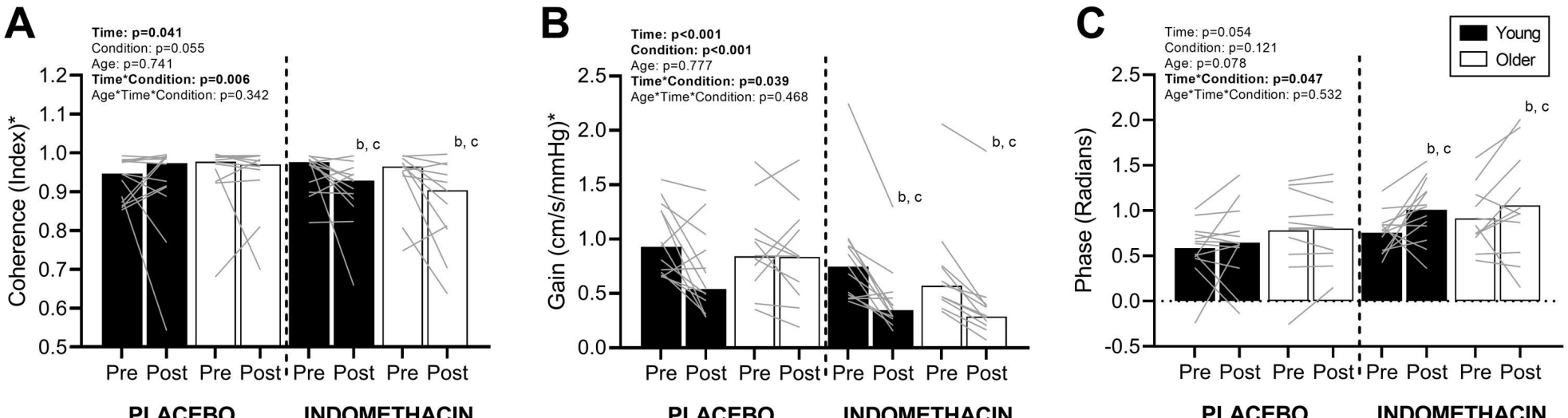
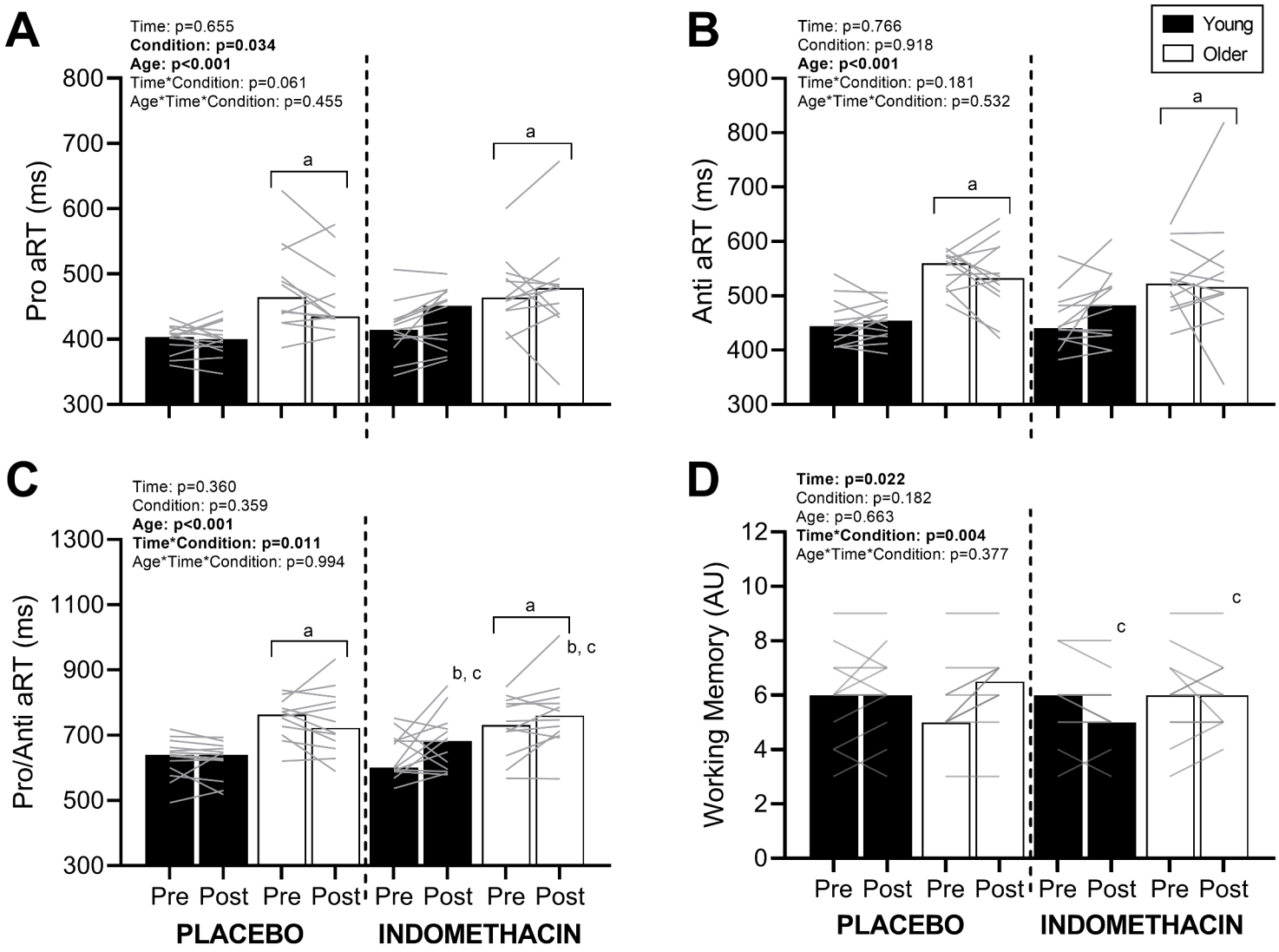

Placebo *or*
Indomethacin
+10 mmHg
+5 mmHg
-10 mmHg
Pro, Anti,
Digit Span Pro/Anti
Instrumentation
Baseline
Cognitive Tasks
CA
CVR$_{CO2}$
Drug wash-in
0
10
15
26
32
40
55
60...
...120
Time (min)
Repeat for "Post" Measures


A
Time: p<0.001
Condition: p<0.001
Age: p=0.049
Time*Condition: p<0.001
Age*Condition*Time: p=0.542
MCAv$_{mean}$ (cm/s)
a
a
b, c
b, c
B
Time: p<0.001
Condition: p=0.041
Age: p=0.648
Time*Condition: p<0.001
Age*Condition*Time: p=0.389
Young
Older
PCAv$_{mean}$ (cm/s)
b, c
b, c
C
Time: p<0.001
Condition: p<0.001
Age: p=0.039
Time*Condition: p<0.001
Age*Condition*Time: p=0.857
CVC (cm/s/mm Hg)
a
a
b, c
b, c
D
Time: p<0.001
Condition: p<0.001
Age: p=0.080
Time*Condition: p=0.004
Age*Condition*Time: p=0.249
MAP (mm Hg)
b, c
b, c
E
Time: p<0.001
Condition: p=0.913
Age: p=0.197
Time*Condition: p=0.001
Age*Condition*Time: p=0.338
HR (bpm)*
b
b
F
Time: p<0.001
Condition: p=0.523
Age: p=0.024
Time*Condition: p=0.033
Age*Condition*Time: p=0.575
PETCO$_2$ (mm Hg)*
a
a
b
b
G
Time: p<0.001
Condition: p<0.001
Age: p=0.443
Time*Condition: p<0.001
Age*Condition*Time: p=0.901
CVR$_{HYPO}$ (Δcm/s/Δmm Hg)*
b,c
b,c
H
Time: p<0.001
Condition: p<0.001
Age: p=0.159
Time*Condition: p<0.001
Age*Condition*Time: p=0.715
CVR$_{HYPER}$ (Δcm/s/Δmm Hg)*
b,c
b,c
Pre Post Pre Post Pre Post Pre Post
PLACEBO
INDOMETHACIN


Hypercapnia, Young (r = 0.81, p < 0.001)
Hypercapnia, Older (r = 0.89, p = 0.001)
Hypocapnia, Young (r = 0.70, p = 0.02)
Hypocapnia, Older (r = 0.89, p = 0.001)
Indomethacin Effect
ΔCVRCO2 (Δcm/s/mm Hg)
1
0
-1
-2
-3
-4
0
1
2
3
4
5
Pre Indomethacin
CVRCO2 (Δcm/s/mm Hg)


A
Time: p=0.041
Condition: p=0.055
Age: p=0.741
Time*Condition: p=0.006
Age*Time*Condition: p=0.342
Coherence (Index)*
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
b, c
b, c
Pre Post Pre Post Pre Post Pre Post
PLACEBO
INDOMETHACIN
B
Time: p<0.001
Condition: p<0.001
Age: p=0.777
Time*Condition: p=0.039
Age*Time*Condition: p=0.468
Gain (cm/s/mmHg)*
2.5
2.0
1.5
1.0
0.5
0.0
b, c
b, c
Pre Post Pre Post Pre Post Pre Post
PLACEBO
INDOMETHACIN
C
Time: p=0.054
Condition: p=0.121
Age: p=0.078
Time*Condition: p=0.047
Age*Time*Condition: p=0.532
Young
Older
Phase (Radians)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
b, c
b, c
Pre Post Pre Post Pre Post Pre Post
PLACEBO
INDOMETHACIN


A
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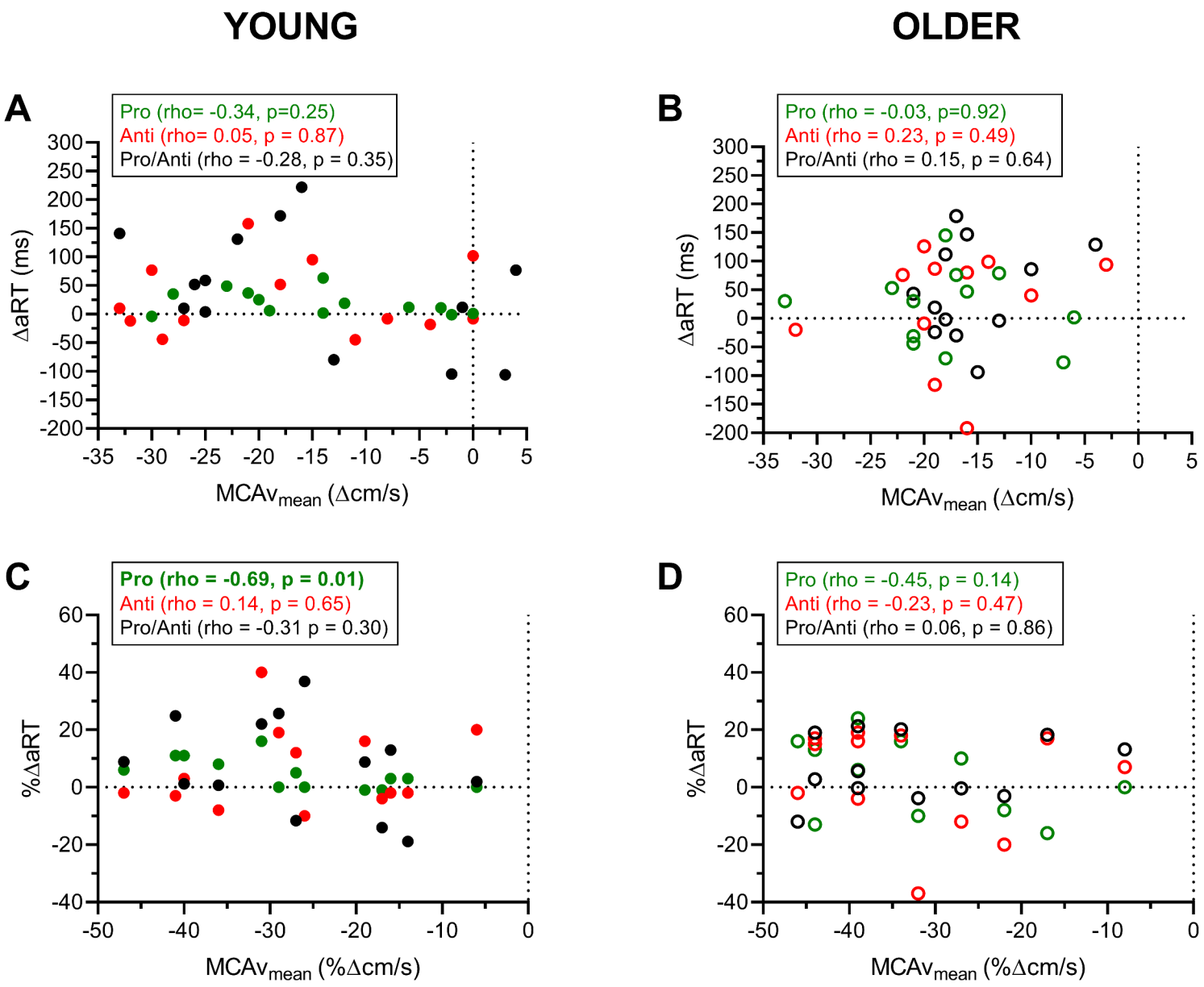
Age: p<0.001
Time*Condition: p=0.061
Age*Time*Condition: p=0.455
Pro aRT (ms)
800
700
600
500
400
300
a
a
B
Time: p=0.766
Condition: p=0.918
Age: p<0.001
Time*Condition: p=0.181
Age*Time*Condition: p=0.532
Young
Older
Anti aRT (ms)
900
800
700
600
500
400
300
a
a
C
Time: p=0.360
Condition: p=0.359
Age: p<0.001
Time*Condition: p=0.011
Age*Time*Condition: p=0.994
Pro/Anti aRT (ms)
1300
1100
900
700
500
300
a
a
b, c
b, c
D
Time: p=0.022
Condition: p=0.182
Age: p=0.663
Time*Condition: p=0.004
Age*Time*Condition: p=0.377
Working Memory (AU)
12
10
8
6
4
2
0
c
c
Pre Post Pre Post Pre Post Pre Post
PLACEBO
INDOMETHACIN


YOUNG
OLDER
A
Pro (rho= -0.34, p=0.25)
Anti (rho= 0.05, p = 0.87)
Pro/Anti (rho = -0.28, p = 0.35)
ΔaRT (ms)
MCAv$_{mean}$ (Δcm/s)
B
Pro (rho = -0.03, p=0.92)
Anti (rho = 0.23, p = 0.49)
Pro/Anti (rho = 0.15, p = 0.64)
ΔaRT (ms)
MCAv$_{mean}$ (Δcm/s)
C
Pro (rho = -0.69, p = 0.01)
Anti (rho = 0.14, p = 0.65)
Pro/Anti (rho = -0.31 p = 0.30)
%ΔaRT
MCAv$_{mean}$ (%Δcm/s)
D
Pro (rho = -0.45, p = 0.14)
Anti (rho = -0.23, p = 0.47)
Pro/Anti (rho = 0.06, p = 0.86)
%ΔaRT
MCAv$_{mean}$ (%Δcm/s)