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Talbot, Jack S. ORCID logoORCID: <https://orcid.org/0000-0003-0234-1426>, Perkins, Dean R. ORCID logoORCID: <https://orcid.org/0000-0002-9044-8508>, Dawkins, Tony G. ORCID logoORCID: <https://orcid.org/0000-0001-5203-135X>, Douglas, Andrew J. M. ORCID logoORCID: <https://orcid.org/0000-0003-2965-6081>, Griffiths, Thomas D. ORCID logoORCID: <https://orcid.org/0000-0003-3104-4332>, Richards, Cory T ORCID logoORCID: <https://orcid.org/0000-0003-3605-7044>, Owen, Kerry, Lord, Rachel N. ORCID logoORCID: <https://orcid.org/0000-0002-5385-7548>, Pugh, Christopher J. A. ORCID logoORCID: <https://orcid.org/0000-0002-5932-4793>, Oliver, Jon L., Lloyd, Rhodri S., Ainslie, Philip N., McManus, Ali M. ORCID logoORCID: <https://orcid.org/0000-0002-7817-230X> and Stemberge, Mike ORCID logoORCID: <https://orcid.org/0000-0003-0818-6420> (2023) Neurovascular coupling and cerebrovascular hemodynamics are modified by exercise training status at different stages of maturation during youth. *American Journal of Physiology-Heart and Circulatory Physiology*, 325 (3). H510-H521. doi:10.1152/ajpheart.00302.2023

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Official URL: <https://doi.org/10.1152/ajpheart.00302.2023>
DOI: <http://dx.doi.org/10.1152/ajpheart.00302.2023>
EPrint URI: <https://eprints.glos.ac.uk/id/eprint/15540>

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RESEARCH ARTICLE

Exercise, Physical Activity, and Cardiovascular Health

Neurovascular coupling and cerebrovascular hemodynamics are modified by exercise training status at different stages of maturation during youth

Jack S. Talbot,^{1,2} Dean R. Perkins,³ Tony G. Dawkins,⁴ Andrew J. M. Douglas,^{1,2}
Thomas D. Griffiths,^{1,2} Cory T. Richards,^{1,2} Kerry Owen,^{1,5} Rachel N. Lord,^{1,2}
Christopher J. A. Pugh,^{1,2} Jon L. Oliver,^{6,7} Rhodri S. Lloyd,^{6,7,8} Philip N. Ainslie,⁴ Ali M. McManus,⁴ and
Mike Stembridge^{1,2}

¹Cardiff School of Sport and Health Sciences, Cardiff Metropolitan University, Cardiff, United Kingdom; ²Centre for Health, Activity and Wellbeing Research, Cardiff Metropolitan University, Cardiff, United Kingdom; ³Department of Sport Science, University of Innsbruck, Innsbruck, Austria; ⁴Centre for Heart, Lung and Vascular Health, School of Health and Exercise Sciences, University of British Columbia Okanagan, Kelowna, British Columbia, Canada; ⁵Windsor Clive Primary School, Cardiff, United Kingdom; ⁶Youth Physical Development Centre, Cardiff Metropolitan University, Cardiff, United Kingdom; ⁷Sports Performance Research Institute New Zealand, AUT University, Auckland, New Zealand; and ⁸Centre for Sport Science and Human Performance, Waikato Institute of Technology, Waikato, New Zealand

Abstract

Neurovascular coupling (NVC) is mediated via nitric oxide signaling, which is independently influenced by sex hormones and exercise training. Whether exercise training differentially modifies NVC pre- versus postpuberty, where levels of circulating sex hormones will differ greatly within and between sexes, remains to be determined. Therefore, we investigated the influence of exercise training status on resting intracranial hemodynamics and NVC at different stages of maturation. Posterior and middle cerebral artery velocities (PCA_v and MCA_v) and pulsatility index (PCA_{PI} and MCA_{PI}) were assessed via transcranial Doppler ultrasound at rest and during visual NVC stimuli. *N* = 121 exercise-trained (males, *n* = 32; females, *n* = 32) and untrained (males, *n* = 28; females, *n* = 29) participants were characterized as pre (males, *n* = 33; females, *n* = 29)- or post (males, *n* = 27; females, *n* = 32)-peak height velocity (PHV). Exercise-trained youth demonstrated higher resting MCA_v (*P* = 0.010). Maturity and training status did not affect the Δ PCA_v and Δ MCA_v during NVC. However, pre-PHV untrained males (19.4 ± 13.5 vs. 6.8 ± 6.0%; *P* ≤ 0.001) and females (19.3 ± 10.8 vs. 6.4 ± 7.1%; *P* ≤ 0.001) had a higher Δ PCA_{PI} during NVC than post-PHV untrained counterparts, whereas the Δ PCA_{PI} was similar in pre- and post-PHV trained youth. Pre-PHV untrained males (19.4 ± 13.5 vs. 7.9 ± 6.0%; *P* ≤ 0.001) and females (19.3 ± 10.8 vs. 11.1 ± 7.3%; *P* = 0.016) also had a larger Δ PCA_{PI} than their pre-PHV trained counterparts during NVC, but the Δ PCA_{PI} was similar in trained and untrained post-PHV youth. Collectively, our data indicate that exercise training elevates regional cerebral blood velocities during youth, but training-mediated adaptations in NVC are only attainable during early stages of adolescence. Therefore, childhood provides a unique opportunity for exercise-mediated adaptations in NVC.

NEW & NOTEWORTHY We report that the change in cerebral blood velocity during a neurovascular coupling task (NVC) is similar in pre- and postpubertal youth, regardless of exercise-training status. However, prepubertal untrained youth demonstrated a greater increase in cerebral blood pulsatility during the NVC task when compared with their trained counterparts. Our findings highlight that childhood represents a unique opportunity for exercise-mediated adaptations in cerebrovascular hemodynamics during NVC, which may confer long-term benefits in cerebrovascular function.

adolescence; exercise; neurovascular coupling

INTRODUCTION

The matching of cerebral perfusion to fluctuations in neural activity, termed neurovascular coupling (NVC), is essential for supporting cerebral metabolism and neurocognitive

health (1). Childhood and adolescence represent critical periods of neurocognitive, neurovascular, and cardiovascular development (2–6). Accordingly, optimizing NVC during childhood and adolescence benefits long-term cardiovascular, neurocognitive, and mental health (7–9), but it is unclear



Correspondence: M. Stembridge (mstembridge@cardiffmet.ac.uk).
Submitted 23 May 2023 / Revised 10 July 2023 / Accepted 10 July 2023



what factors modify cerebral perfusion for a given cognitive task during adolescence (10, 11). When expressed as the change in cerebral perfusion during spontaneous fluctuations in resting neuronal activity, NVC declines across adolescence (10). The natural decline may be underpinned by axon myelination and synaptic pruning that aim to make neural connections more efficient (3, 6), so that the same neural impulse will place a lower metabolic demand in an adolescent teenager when compared with a child. However, it is not clear whether the NVC response to a cognitive task, rather than just during the resting state, is modified during pubertal maturation, partly due to the difficulty in selecting an age-appropriate test across the adolescent spectrum (11).

Adolescence is a period of youth characterized by marked, but sex specific, changes in insulin-like growth factor 1 (IGF-1) and sex hormones, which are known to mediate cerebral angiogenesis, endothelial function, and cerebrovascular tone during physiological stimuli in adults (12–16). Indeed, the developmental trajectory of resting cerebral perfusion is modified by biological sex across puberty (5, 12). Furthermore, reductions in nitric oxide availability and IGF-1 in older adults contribute to the progression of neurocognitive disease via cerebrovascular endothelial dysfunction (17, 18). Estrogen acts to increase neuronal excitability via glutaminergic neurotransmissions (15), while also improving endothelial function via increased expression of endothelial nitric oxide (NO) synthase (16). On the other hand, in males, influxes in androgens can impair endothelial function (17) by reducing NO availability (18). Thus, one may expect a sex-specific change in NVC across the pubertal period.

The process of long-term cerebrovascular and neurocognitive decline may begin early in life, as the combination of low cardiorespiratory fitness ($\dot{V}O_{2\max}$) and low cognitive performance at 18 years of age has been associated with a hyperadditive risk of neurocognitive disease in males with advancing age (7). Endurance exercise training has consistently been shown to increase NO availability (19–21) and promote cerebral angiogenesis (14, 22). Therefore, critical periods of neural development during adolescence may represent a window of opportunity for external stimuli such as exercise to further stimulate the development of NVC.

Despite clear mechanistic evidence demonstrating the role of maturation and endurance training in modifying cerebrovascular function, it remains to be determined whether exercise training across the adolescent spectrum modifies NVC. Therefore, the aim of this study was to investigate the impact of exercise training status on intracranial hemodynamics and the NVC response in males and females at different stages of adolescence. We hypothesized that 1) post-PHV endurance-trained youth would demonstrate elevated intracranial blood velocities compared with their untrained counterparts, whereas there would be no training-related differences in pre-PHV participants; and 2) pre-PHV and post-PHV endurance-trained youth would demonstrate a larger NVC response than untrained counterparts during the NVC task.

MATERIALS AND METHODS

Ethical Approval

Ethical approval was granted by Cardiff Metropolitan University's School of Sport and Health Sciences Research

Ethics Committee (PGR-1339), and the study conformed to the Declaration of Helsinki (2013), except for registration in a database. Detailed, age-appropriate summaries of the methods and study design were given verbally and in writing to each participant before providing written assent. Furthermore, a legal guardian of each participant was given a verbal and written explanation of the methods and study design before providing written informed consent.

Experimental Design

A group of youths ($n = 163$) volunteered to participate in the study. Participants were excluded if they failed to attend all laboratory visits ($n = 3$) or failed to meet our cohort health or physical activity criterion ($n = 6$). Based on self- and parental-reported physical activity, $n = 154$ participants were categorized as either endurance-trained (males, $n = 42$, age = 9.0–17.1 yr; females, $n = 45$, age = 8.2–17.0 yr) or untrained (males, $n = 31$, age = 8.0–17.7 yr; females, $n = 36$, age = 8.0–17.6 yr). “Trained” youths had completed ≥ 3 structured endurance exercise training sessions per week for ≥ 12 mo and were recruited from local-endurance sport clubs (see Table 1 for training volume data). “Untrained” youths were recruited from local schools and community clubs and were not taking part in regular endurance exercise training or meeting UK Chief Medical Officers' Physical Activity Guidelines for children and young people (23). Following eligibility screening, participants attended the laboratory at Cardiff Metropolitan University on one occasion. Per technical guidelines for the assessment of cerebral blood flow (24), participants refrained from vigorous exercise, caffeine, and alcohol for ≥ 12 h before the data collection. Similar to comparable pediatric studies, participants attended the laboratory having fasted for ≥ 4 h (25, 26).

Data collection was conducted in a quiet, temperature-controlled room with great care to minimize any external sensory stimulation during cerebrovascular measures. Upon arrival, participants completed a series of questionnaires quantifying their weekly exercise training or physical activity levels which were corroborated with parents, before completing anthropometric measurements. Participants were then instructed to lie down in the supine position, where they were fitted with instrumentation for the acquisition of cerebrovascular and cardiorespiratory data. Following baseline measures, we assessed NVC during a visual searching task previously used in pediatric cohorts (27). Thirty minutes after the NVC assessment, cardiorespiratory fitness ($\dot{V}O_{2\max}$) was then determined via an incremental exercise test to volitional exhaustion and confirmed via a supramaximal verification of $\dot{V}O_{2\max}$ on the same cycle ergometer as recommended for pediatric exercise testing (28, 29).

Anthropometrics and Estimated Maturity Status

Body mass (kg) was measured using electronic scales, and stature (cm) and sitting height (cm) using a stadiometer, with participants barefoot and wearing light clothing. Anthropometrics, chronological age, and sex were entered into sex-specific regression equations to calculate maturity offset (predicted age from PHV), an estimate of somatic maturation (30). Participants were classified into pre- and post-PHV groups using ≥ 0.5 yr before and post-

Table 1. Anthropometric and training status-related characteristics of participants

	Pre-PHV				Post-PHV				P Value (Interaction)						
	Untrained males	Trained males	Untrained females	Trained females	Untrained males	Trained males	Untrained females	Trained females	Maturation status	Sex	Training status	Maturation x sex	Maturation x training	Sex x training	Maturation x sex x training
<i>n</i>	15	18	13	16	13	14	16	17							
Maturation offset, yr	-2.7 (1.1)†	-2.4 (1.1)	-1.9 (0.7)	-1.6 (0.9)†	2.2 (0.8)*	1.8 (0.9)*	1.6 (0.9)*	2.1 (1.1)*	≤ 0.001	0.127	0.430	0.013	0.531	0.356	0.148
Age, yr	10.6 (1.6)	11.2 (1.7)	9.9 (1.2)	10.1 (1.2)	16.4 (1.0)	15.5 (0.9)	13.8 (1.6)	14.3 (1.4)	≤ 0.001	≤ 0.001	0.496	0.067	0.130	0.528	0.055
Weight, kg	38.9 (9.6)	36.2 (7.8)	33.7 (6.1)	3.25 (6.1)	65.0 (9.7)	62.7 (9.9)	51.3 (8.1)	55.4 (8.3)	≤ 0.001	≤ 0.001	0.728	0.052	0.342	0.187	0.427
Height, cm	145.7 (10.4)	145.5 (9.9)	137.7 (7.1)†	139.8 (8.8)†	179.3 (8.2)*	176.5 (8.9)*	161.1 (5.3)†	165.4 (6.9)†	≤ 0.001	≤ 0.001	0.570	0.012	0.955	0.129	0.437
Lean body mass, kg	29.8 (5.2)	31.0 (6.1)	25.5 (3.0)†	26.6 (4.6)†	53.5 (5.5)*	54.6 (6.9)*	39.3 (5.4)†	44.3 (6.3)†	≤ 0.001	≤ 0.001	0.040	≤ 0.001	0.366	0.360	0.318
MAP, mmHg	76 (6)	74 (5)	76 (5)	75 (7)	81 (7)	81 (5)	78 (5)	80 (6)	≤ 0.001	0.33	0.845	0.239	0.319	0.347	0.920
Training volume, h/wk	1.2 (0.9)	6.9 (2.3)†	1.0 (1.0)	6.2 (2.0)†	0.8 (0.8)	10.8 (2.9)†	0.6 (0.9)	9.3 (3.0)†	≤ 0.001	0.104	≤ 0.001	0.636	≤ 0.001	0.231	0.712
$\dot{V}O_{2max}$, mL·min ⁻¹ ·kg LBM ^{0.93}	46.7 (7.2)	54.3 (6.3)†	44.2 (4.3)	49.3 (5.0)†	45.5 (5.7)	56.1 (5.0)†	40.3 (4.0)†	50.5 (3.9)†	0.580	≤ 0.001	≤ 0.001	0.428	0.044	0.496	0.600

Values are means (SD); n, number of participants. LBM, lean body mass; MAP, mean arterial blood pressure; PHV, peak height velocity; $\dot{V}O_{2max}$, maximal oxygen consumption. Group differences were assessed via three-way ANOVA. *Significant difference between pre- and post-PHV youth. †Significant difference between males and females. #Significant difference between trained and untrained youth. Boldface indicates significant values at $P \leq 0.05$.

PHV, respectively, to account for the reported measurement error in the prediction equation. Skin fold thickness (skin fold callipers, Harpenden, Baty International, Burgess Hill, West Sussex, UK) was assessed at the triceps and subscapular for the estimation of lean body mass (LBM) as previously described (31, 32).

Resting Cerebrovascular and Cardiorespiratory Measures

Resting measurements were acquired following ≥ 15 min of supine rest. During this time, participants wore a bilateral headset with 2-MHz TCD ultrasound probes (Spencer Technologies, Seattle, WA) placed over the temporal acoustic windows and adjusted to obtain MCA_v and PCA_v. The M-1 segment of MCA was insonated on the right side of the head, whereas the P-1 segment of the PCA was insonated on the left side. MCA_v and PCA_v were identified and optimized according to their signal depth and waveform, and subsequently confirmed with a visual stimulation test as per the recommended TCD technique guidelines (33). The 2-MHz TCD ultrasound probes were secured and fastened in place once suitable MCA_v and PCA_v signals were confirmed.

All cardiorespiratory variables were sampled continuously at 1 kHz via an analog-to-digital converter (Powerlab 16/30, AD Instruments, Oxford, UK). Mean arterial blood pressure (MAP) and heart rate (HR) were measured by finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, The Netherlands). Both PET_{CO₂} and PET_{O₂} and PET_{O₂} were sampled via insertion of a sample line into a mouthpiece worn by the participant that connected in series to a bacteriological filter and a calibrated gas analyzer (ML206, AD Instruments Ltd, Oxford, UK). All data were interfaced with LabChart (v. 8) for subsequent off-line data analysis.

Cerebral blood velocities were also used to calculate the pulsatility index of the MCA (MCA_{PI}) and PCA (PCA_{PI}) at rest and during the NVC stimulus. Cerebral blood pulsatility index provides an index of downstream cerebrovascular resistance, which increases during older adulthood and may be related to a decline in cerebral metabolism and cerebrovascular dysfunction (34–36). Pulsatility index increases when the relative contribution of the peak systolic portion of the waveform to mean blood velocity increases, or when the contribution of the end-diastolic portion of the waveform decreases. As such, MCA_{PI} and PCA_{PI} were calculated as follows:

(Peak systolic velocity – minimum diastolic velocity)/mean velocity

Furthermore, cerebrovascular conductance of the MCA (MCA_{CVC}) and PCA (PCA_{CVC}) was calculated to account for the expected maturity-related differences in MAP across pre- and post-PHV youth (37), and to ensure group differences in intracranial velocities were not due to differences in cerebral perfusion pressure (38). Both MCA_{CVC} and PCA_{CVC} were calculated as follows:

Mean velocity/mean arterial blood pressure

Neurovascular Coupling

Following resting measurements, participants remained in the supine position and a visual task was used to activate the visual cortex. Bilateral TCD was used to insonate the left PCA and right MCA through the transtemporal windows, allowing

for selective increases in PCA_v , and a regional comparison via the MCA_v . Baseline PCA_v , MCA_v , HR, MAP, PET_{CO_2} , and PET_{O_2} were acquired during a 2-min eyes-open and a 2-min eyes-closed trial before the visual stimulus. A portable device (iPad Air, Apple Distribution International, Hollyhill, Republic of Ireland) with a 19 cm × 15 cm visual field was then held 30–35 cm directly above the participant's face throughout the NVC assessment. PCA_v , MCA_v , HR, blood pressure, PET_{CO_2} , and PET_{O_2} were recorded continuously across five cycles of 30 s of eyes closed and 30 s of eyes open, consistent with other NVC research (39, 40).

The visual stimulus used in this experiment required the participant to search for an on-screen object (“Waldo”) that was hidden in a field of distractors consisting of similarly shaped characters in a variety of colors (41). The visual stimulus was chosen because of its previous use in assessing NVC in adults (41–43) and its use in vision research in youth (27). Furthermore, this visual stimulus may be particularly useful for the assessment of NVC in pediatric cohorts due to its better NVC signal-to-noise ratio compared with other paradigms (41).

Data Processing

The interpolated PCA_v , MCA_v , HR, MAP, PET_{CO_2} , and PET_{O_2} signals were visually inspected for artefacts or noise and corrected by cubic spline interpolation and downsampled to 10 Hz. Acceptable PCA_v and MCA_v waveforms were exported on a breath-by-breath and beat-by-beat basis and time aligned to HR, blood pressure, PET_{CO_2} , and PET_{O_2} data, which were cubic spline interpolated at 5 Hz using a custom-built MATLAB code (The MathWorks, Natick, MA) (44). Data from each trial were aligned to stimulus onset (eyes open), and then averaged to generate one response per participant. The percent change in PCA_v and MCA_v , as well as PCA_{PI} and MCA_{PI} , was calculated from the average of the 5-s preceding initiation (i.e., the last 5 s of “eyes closed”) to the peak response during the subsequent “eyes open” to control for group differences in baseline PCA_v and MCA_v , as well as the unknown insonation angle of the TCD probes (45).

Data Exclusion

Of the 154 participants recruited to the study, 21 participants were classified as “circa PHV” (between −0.5 and 0.5 yr from PHV) and were excluded from the data analysis to address the study hypothesis. We were unable to insonate one of either the right MCA or left PCA concurrently in 12 participants during resting measures. Therefore, 121 participants were included in the analysis of resting cerebrovascular measures. Furthermore, 12 participants were unable to complete at least five cycles of the visual stimuli with all cerebrovascular and cardiorespiratory measures (because of either TCD headset/probe discomfort, mouthpiece discomfort, disturbance of finger photoplethysmography signal, or insufficient motivation to keep eyes closed between visual stimuli). A further three participants were excluded from the data analysis because of unacceptable PCA_v or MCA_v waveforms during the NVC assessment. Therefore, 106 participants were included in the final NVC analysis.

Cardiorespiratory Fitness

Cardiorespiratory fitness was assessed via an incremental exercise test on an electronically braked cycle ergometer

(Excalibur Sport, Lode, Gronigen, The Netherlands) to volitional exhaustion. Adjustments were made to the saddle and handlebars of the ergometer for each participant to ensure a comfortable cycling position. HR (RS400, Polar Electro, Kempe, Finland) and oxygen consumption ($\dot{V}O_2$) were assessed at rest and continuously throughout the exercise protocol (Oxycon Pro, Jaeger, Hoechberg, Germany). The exercise test used a ramp incremental protocol where workload increments were determined by participant stature and training status (46). Participants were encouraged to maintain a cadence of 75–85 rpm throughout the protocol. The test was ended once the participant failed to maintain a cadence ≥ 70 rpm for ≥ 5 consecutive seconds. Following 15 min of rest, participants completed a constant-load supra-maximal verification test at 105% of power output achieved at peak $\dot{V}O_2$ during the incremental ramp test to confirm attainment of $\dot{V}O_{2max}$, as recommended for cardiorespiratory fitness testing in pediatric cohorts (28, 47). Individual $\dot{V}O_{2max}$ values were then allometrically scaled to lean body mass using a cohort determined exponent ($LBM^{0.93}$) to account for developmental changes in lean body mass across adolescence (48).

Statistical Analysis

Power analyses for data presented in this manuscript were conducted a priori by sampling pilot data assessing MCA_v via transcranial Doppler ultrasound in a similar cohort of pre ($n = 12$; $74.3 \pm 7.1 \text{ cm}\cdot\text{s}^{-1}$) and post ($n = 12$; $68.4 \pm 5.8 \text{ cm}\cdot\text{s}^{-1}$) PHV youth. The minimum required sample size for a statistically significant main effect of training status was $n = 12$ per group based on 90% power at a two-sided 0.05 significance level where $f^2 = 0.47$. As such, we aimed to recruit at least 12 participants in each group to achieve statistical power for main effects of training status for baseline intracranial velocities, as well as allowing for data dropout during the NVC assessment. Statistical analysis was conducted on SPSS statistical software package (version 23.0, Chicago, IL). Normal distribution of outcome variables was confirmed via Shapiro–Wilk statistical tests and visual inspection of p-p plots. All data are presented as group means (SD) with statistical significance set to $P < 0.05$ unless otherwise stated. A three-factor analysis of variance (ANOVA) was used to determine the main effects of maturity status, biological sex, and training status, as well as the interaction effects of these variables on intracranial velocities and NVC. Post hoc comparisons with Bonferroni corrections were conducted to identify significant differences among groups when significant main or interaction effects were observed. The main aim of this study was to understand the influence of training status on intracranial velocities and NVC during different stages of maturity. As such, the reporting of post hoc comparisons will focus on the effect of training status on intracranial hemodynamics and NVC.

RESULTS

Descriptive Characteristics

Post-PHV youth had a higher maturity offset, chronological age, stature, body mass, LBM, and MAP than their pre-PHV counterparts (all; $P \leq 0.001$, Table 1). In addition, post-

PHV youth demonstrated greater training volumes compared with their pre-PHV counterparts ($P \leq 0.001$), but $\dot{V}O_{2\max}$ was similar in pre- and post-PHV youth ($P = 0.580$, Table 1). Endurance-trained youth had a higher training volume and $\dot{V}O_{2\max}$ compared with untrained youth (both; $P \leq 0.001$, Table 1).

The Influence of Training Status on Baseline Intracranial Hemodynamics

Endurance-trained youth demonstrated a higher resting MCA_v ($P = 0.010$) and MCA_{CVC} ($P = 0.015$) when compared with untrained counterparts (Table 2 and Fig. 1). However, post hoc comparisons revealed no differences in MCA_v and MCA_{CVC} between trained and untrained pre-PHV males ($P = 0.443$ and $P = 0.198$) and females ($P = 0.217$ and $P = 0.186$) or post-PHV males ($P = 0.111$ and $P = 0.182$) and females ($P = 0.117$ and $P = 0.331$). There was also a significant main effect for maturity status and biological sex on several metrics of resting cerebrovascular hemodynamics. Baseline MCA_v ($P \leq 0.001$), PCA_v ($P = 0.009$), MCA_{CVC} ($P \leq 0.001$), and PCA_{CVC} ($P \leq 0.001$) were all lower in post-PHV youth when compared with pre-PHV counterparts (Table 1 and Fig. 1). Similarly, baseline MCA_v ($P \leq 0.001$), PCA_v ($P = 0.039$), MCA_{CVC} ($P \leq 0.001$), and PCA_{CVC} ($P = 0.037$) were lower in males when compared with females.

The Influence of Training Status on Neurovascular Coupling

There was no effect of training status on the relative ΔHR [10.5 (9.2) vs. 12.2 (6.6) %; $P = 0.312$], ΔMAP [3.6 (2.6) vs. 3.5 (2.9) %; $P = 0.789$], or ΔPET_{CO_2} [10.7 (7.2) vs. 9.6 (7.0) %; $P = 0.528$] during the NVC assessment. Likewise, the relative ΔMCA_v and ΔPCA_v during the NVC assessment were not influenced by training status ($P = 0.370$ and $P = 0.987$; Table 3 and Fig. 2). The relative ΔMCA_{PI} during NVC was similar in trained and untrained youth during the NVC assessment ($P = 0.717$, Table 3), but the relative ΔPCA_{PI} was lower in trained youth when compared with untrained counterparts ($P = 0.017$, Table 3 and Fig. 3). Indeed, post hoc comparisons revealed that the relative ΔPCA_{PI} during the NVC assessment was lower in pre-PHV trained males ($P \leq 0.001$) and females ($P = 0.016$) when compared with their untrained counterparts, but there were no training-related differences in post-PHV youth ($P = 0.784$ and $P = 0.334$). Furthermore, the relative ΔPCA_{PI} during the NVC assessment was lower in post-PHV untrained males and females compared with their pre-PHV counterparts (both; $P \leq 0.001$), but there were no maturity-related differences in trained youth ($P = 0.956$ and $P = 0.577$). In addition, the relative ΔMCA_{CVC} during the NVC assessment was lower in trained youth compared with untrained youth ($P = 0.037$), whereas there was a significant maturity- and training-status interaction effect on the ΔPCA_{CVC} ($P = 0.035$). However, post hoc comparisons revealed no differences in the ΔMCA_{CVC} or ΔPCA_{CVC} between trained and untrained pre-PHV males ($P = 0.442$ and $P = 0.197$) and females ($P = 0.490$ and $P = 0.152$) or post-PHV males ($P = 0.064$ and $P = 0.248$) and females ($P = 0.377$ and $P = 0.765$) during the NVC assessment.

There was no effect of maturity status or biological sex on the peak relative ΔHR [12.1 (6.9) vs. 10.8 (8.8) %; $P = 0.401$

Table 2. Baseline cerebrovascular hemodynamics and cardiorespiratory variables across all groups

	Pre-PHV						Post-PHV						P Value (Interaction)			
	Untrained			Trained			Untrained			Trained			Sex	Training status	Maturational status	Sex $\times$ training
	males	females	n	males	females	n	males	females	n	males	females	n				
<i>n</i>	15	13	16	13	13	14	16	16	17							
MCA_v , $cm \cdot s^{-1}$	73.3 (12.2)	80.3 (10.1)	84.3 (8.0)	61.4 (5.7)	66.8 (6.8)	75.9 (5.0)	71.0 (10.6)	75.9 (5.0)	75.9 (5.0)	0.010	0.010	0.010	≤ 0.001	0.010	0.010	0.010
MCA_{PI} , AU	0.74 (0.12)	0.75 (0.13)	0.82 (0.12)	0.89 (0.12)*	0.83 (0.17)	0.74 (0.08)†	0.79 (0.08)†	0.74 (0.08)†	0.74 (0.08)†	0.915	0.915	0.915	0.076	0.915	0.915	0.915
MCA_{CVC} , $cm \cdot s \cdot mmHg^{-1}$	0.97 (0.18)	1.02 (0.13)	1.13 (0.12)	0.76 (0.11)	0.83 (0.09)	0.96 (0.08)	0.91 (0.14)	0.96 (0.08)	0.96 (0.08)	0.015	0.015	0.015	≤ 0.001	0.015	0.015	0.015
PCA_v , $cm \cdot s \cdot mmHg^{-1}$	38.6 (6.4)	41.9 (6.3)	45.7 (9.8)	37.4 (9.4)	35.7 (7.4)	42.4 (8.9)	40.2 (8.0)	42.4 (8.9)	42.4 (8.9)	0.178	0.178	0.178	0.027	0.178	0.178	0.178
PCA_{PI} , AU	0.78 (0.15)	0.74 (0.09)	0.84 (0.15)††	0.84 (0.15)	0.89 (0.15)*	0.77 (0.11)†	0.73 (0.07)†	0.77 (0.11)†	0.77 (0.11)†	0.080	0.080	0.080	0.049	0.080	0.080	0.080
PCA_{CVC} , $cm \cdot s \cdot mmHg^{-1}$	0.59 (0.09)	0.57 (0.12)	0.64 (0.15)	0.52 (0.14)	0.52 (0.12)	0.53 (0.14)	0.54 (0.13)	0.53 (0.14)	0.53 (0.14)	0.438	0.438	0.438	0.037	0.438	0.438	0.438
RHR, beats/min	73 (9)	63 (7)†	72 (9)	64 (9)	57 (7)	63 (8)	74 (6)	63 (8)	63 (8)	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	0.534	0.678
MAP, mmHg	76 (6)	74 (5)	75 (7)	81 (7)	81 (5)	80 (6)	78 (5)	80 (6)	80 (6)	0.845	0.845	0.845	0.333	0.845	0.319	0.920
PET_{CO_2} , mmHg	38.9 (2.8)	40.2 (3.2)	39.8 (2.5)	41.2 (2.8)	41.0 (2.3)	41.2 (3.2)	39.7 (2.5)	41.2 (3.2)	41.2 (3.2)	0.422	0.422	0.422	0.422	0.570	0.562	0.523
PET_{O_2} , mmHg	104.7	103.7	104.6	104.9	103.2	101.2	103.1	101.2	101.2	0.157	0.157	0.157	0.055	0.157	0.063	0.356

Values are means (SD); *n*, number of participants. MAP, mean arterial blood pressure; MCA_{CVC} , middle cerebral artery conductance; MCA_{PI} , middle cerebral artery pulsatility index; MCA_v , middle cerebral artery blood velocity; PCA_{CVC} , posterior cerebral artery conductance; PCA_{PI} , posterior cerebral artery pulsatility index; PCA_v , posterior cerebral artery blood velocity; PHV, peak height velocity; PET_{CO_2} , end-tidal carbon dioxide; PET_{O_2} , end-tidal oxygen; RHR, resting heart rate. Group differences were assessed via three-way ANOVA. *Significant difference between pre- and post-PHV youth. †Significant difference between males and females. ‡Significant difference between trained and untrained youth. Boldface indicates significant values at $P \leq 0.05$.

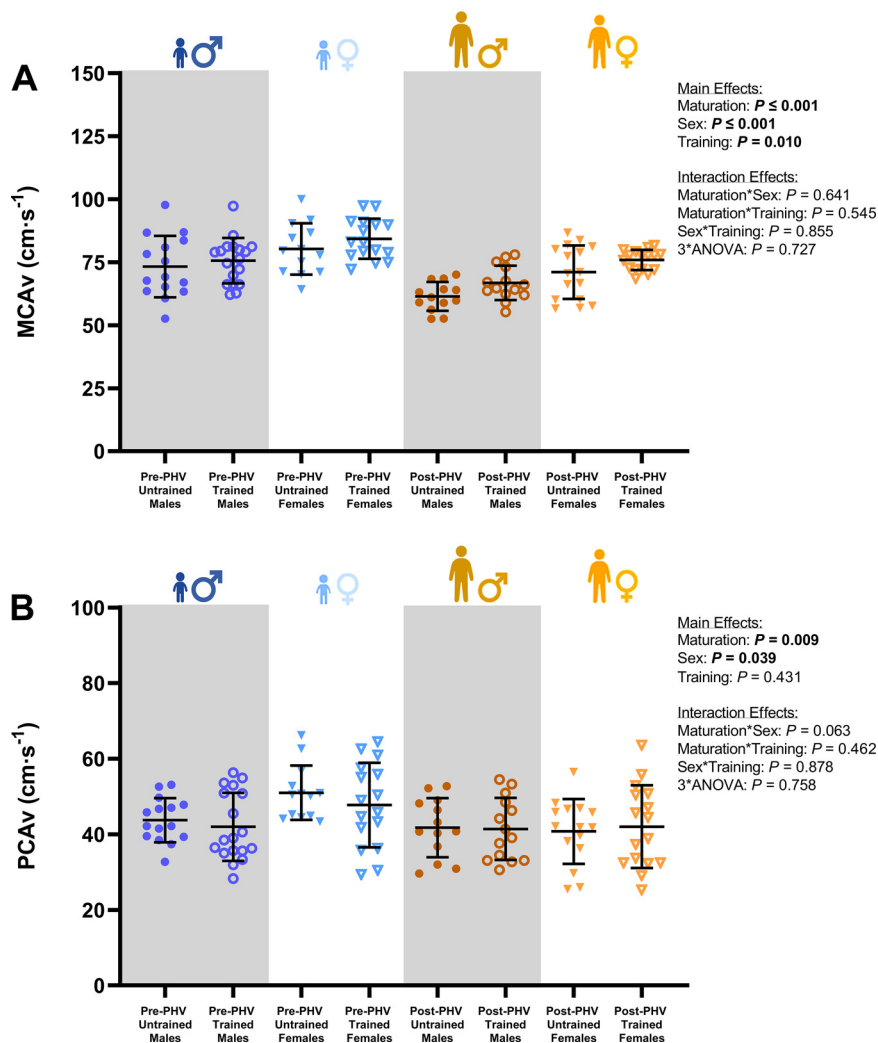


Figure 1. Group means for resting middle cerebral artery blood velocity (MCA_v; A) and posterior cerebral artery blood velocity (PCA_v; B) in males [pre-peak height velocity (PHV) untrained, $n = 15$; pre-PHV trained, $n = 18$; post-PHV untrained, $n = 13$; and post-PHV trained, $n = 14$] and females (pre-PHV untrained, $n = 13$; pre-PHV trained, $n = 16$; post-PHV untrained, $n = 16$; and post-PHV trained, $n = 17$) assessed via a three-way ANOVA. Error bars indicate group means (SD). P values outside of the figure plot indicate where significant main and interaction effects of maturity status, biological sex, and training status occurred. ANOVA, analysis of variance.

and 11.4 (9.7) vs. 11.4 (6.1) %; $P = 0.886$], Δ MAP [4.1 (3.0) vs. 3.1 (2.4) %; $P = 0.055$ and 3.8 (2.8) vs. 3.3 (2.7) %; $P = 0.436$], or Δ PETCO₂ [10.0 (7.1) vs. 10.1 (7.1) %; $P = 0.970$ and 9.8 (6.0) vs. 10.3 (7.9) %; $P = 0.774$] during the NVC assessment. Likewise, the peak relative Δ MCA_v and Δ PCA_v during the NVC assessment were not influenced by maturity status ($P = 0.492$ and $P = 0.426$) or biological sex ($P = 0.662$ and $P = 0.218$; Table 1 and Fig. 2). However, the relative Δ MCA_{PI} ($P = 0.023$) and Δ PCA_{PI} ($P \leq 0.001$) were lower in post-PHV youth when compared with pre-PHV counterparts.

DISCUSSION

The aim of this study was to investigate the impact of exercise training status on resting intracranial cerebrovascular hemodynamics and their response to a visual NVC task at different stages of maturity. For the first time, we report that 1) endurance-trained youth demonstrate higher MCA_v but similar PCA_v to untrained counterparts, 2) the magnitude of the relative Δ MCA_v and Δ PCA_v during a visual NVC task was similar in trained and untrained youth, and 3) the Δ PCA_{PI} during NVC was lower in pre-PHV trained youths when compared with untrained counterparts. Collectively, our data indicate that exercise training elevates regional cerebral

blood velocity during youth, but training-mediated adaptations in NVC are only attainable before the onset of somatic maturation, highlighting the importance of exercise training on cerebrovascular function throughout youth.

Training-Status Modulates Middle Cerebral Artery Hemodynamics during Youth

Endurance-trained youth demonstrated elevated MCA_v, but not PCA_v, when compared with their untrained counterparts, independent of potential differences in cerebral perfusion pressure. Thus, it seems likely that exercise-mediated adaptations in cerebral perfusion (14) are particularly feasible in the anterior region of the adolescent brain and supported by a reduction in downstream cerebrovascular resistance that elevates intracranial blood velocities. Indeed, several anterior regions of the brain are associated with a protracted neural development in adolescent humans (49), which may provide a greater window of opportunity for their exposure to pubertal spikes in IGF-1 and brain-derived neurotrophic factor for further exercise-mediated adaptations in neurogenesis (22, 50) and angiogenesis (14). Interestingly, resting MCA pulsatility was elevated in endurance-trained pre-PHV youth compared with untrained counterparts, but resting MCA pulsatility was lower in endurance-trained youth post-PHV when

Table 3. Absolute and relative changes in cerebrovascular hemodynamics during neurovascular coupling task

	Pre-PHV						Post-PHV				P Value (Interaction)			
	Untrained males			Trained males			Untrained females		Trained females		Training status	Maturation × sex	Maturation × training	Sex × training
	12	13	12	13	12	13	12	13	14	17				
<i>n</i>	12	13	12	13	12	13	12	13	14	17				
ΔMCA_v														
Absolute, $\text{cm}\cdot\text{s}^{-1}$	10.3 (5.9)	9.1 (7.3)	12.0 (7.0)	12.0 (6.7)	9.0 (3.1)	5.9 (3.2)	8.8 (4.2)	7.7 (4.8)	0.012	0.177	0.244	0.527	0.512	0.868
Relative, %	13.6 (6.8)	11.0 (6.0)	13.5 (5.1)	16.1 (7.8)	14.9 (7.1)	11.5 (8.2)	12.7 (4.6)	11.1 (5.6)	0.492	0.662	0.370	0.178	0.372	0.558
ΔMCA_{PI}														
Absolute, AU	0.14 (0.11)	0.09 (0.08)	0.12 (0.15)	0.10 (0.07)	0.05 (0.05)*	0.07 (0.06)	0.07 (0.05)	0.11 (0.05)	0.028	0.375	0.832	0.357	0.050	0.933
Relative, %	19.8 (11.7)	15.3 (8.7)	15.0 (8.2)	13.3 (9.0)	7.8 (5.7)*	9.7 (7.1)	10.8 (9.4)	17.8 (8.0)†	0.023	0.556	0.717	0.018	0.046	0.768
ΔMCA_{CVC}														
Absolute, $\text{cm}\cdot\text{s}\cdot\text{mmHg}^{-1}$	0.13 (0.07)	0.13 (0.06)	0.18 (0.11)	0.18 (0.06)	0.15 (0.13)	0.09 (0.08)	0.14 (0.06)	0.12 (0.06)	0.109	0.138	0.271	0.288	0.395	0.760
Relative, %	14.9 (7.6)	12.1 (4.0)	19.0 (10.3)	16.8 (5.7)	19.5 (10.5)	13.1 (8.1)	16.0 (6.7)	13.3 (6.4)	0.886	0.406	0.037	0.074	0.541	0.650
ΔPCA_v														
Absolute, $\text{cm}\cdot\text{s}^{-1}$	12.1 (4.9)	14.4 (5.7)	13.1 (4.0)	14.5 (4.4)	12.4 (2.4)	12.2 (4.2)	12.4 (5.2)	13.2 (4.5)	0.265	0.576	0.242	0.985	0.409	0.615
Relative, %	33.6 (8.1)	32.6 (9.5)	30.2 (8.0)	33.1 (9.3)	35.8 (8.9)	34.5 (8.9)	32.7 (9.3)	32.1 (8.0)	0.426	0.218	0.987	0.710	0.582	0.642
ΔPCA_{PI}														
Absolute, AU	0.13 (0.13)	0.04 (0.04)	0.12 (0.11)	0.10 (0.08)	0.05 (0.06)	0.06 (0.07)	0.05 (0.04)	0.06 (0.05)	0.003	0.403	0.185	0.423	0.053	0.274
Relative, %	19.4 (13.5)	7.9 (6.0)†	19.3 (10.8)	11.1 (7.3)†	6.8 (6.0)*	7.7 (7.6)	6.4 (7.1)*	9.4 (7.0)	≤0.001	0.448	0.017	0.782	≤0.001	0.847
ΔPCA_{CVC}														
Absolute, $\text{cm}\cdot\text{s}\cdot\text{mmHg}^{-1}$	0.16 (0.07)	0.20 (0.06)	0.17 (0.06)	0.21 (0.08)	0.15 (0.05)	0.17 (0.08)	0.18 (0.07)	0.17 (0.06)	0.249	0.314	0.081	0.840	0.272	0.694
Relative, %	30.8 (8.8)	35.6 (7.5)	27.7 (8.7)	34.6 (8.2)	39.4 (12.0)*	35.8 (8.0)	35.0 (10.0)	34.0 (9.8)	0.039	0.157	0.503	0.628	0.035	0.703

Values are means (SD); *n*, number of participants. MCA_{CVC} , middle cerebral artery conductance; MCA_{PI} , middle cerebral artery pulsatility index; MCA_v , middle cerebral artery blood velocity; PCA_{CVC} , posterior cerebral artery conductance; PCA_{PI} , posterior cerebral artery pulsatility index; PCA_v , posterior cerebral artery blood velocity; PHV, peak height velocity. Group differences were assessed via three-way ANOVA. *Significant difference between pre- and post-PHV youth. †Significant difference between males and females. #Significant difference between trained and untrained youth. Boldface indicates significant values at $P \leq 0.05$.

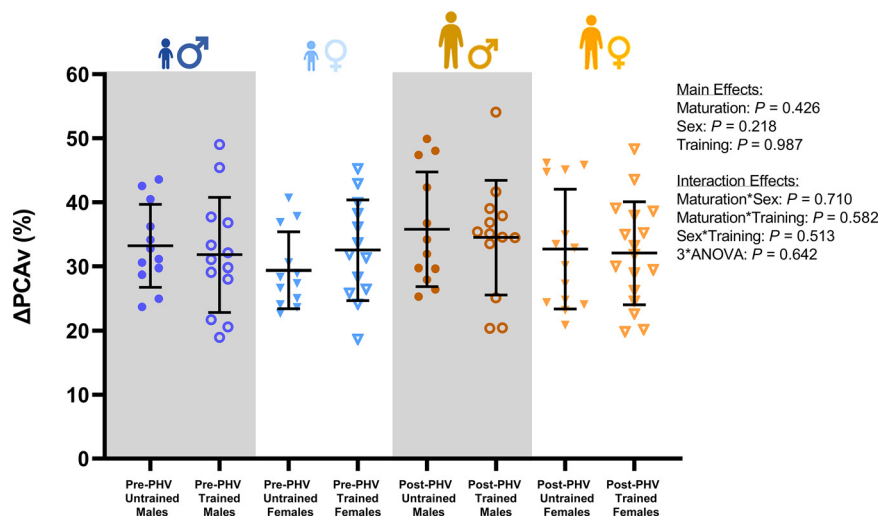


Figure 2. Group means for the percent change in posterior cerebral artery blood velocity (ΔPCA_v) during the neurovascular coupling task in males [pre-peak height velocity (PHV) untrained, $n = 12$; pre-PHV trained, $n = 13$; post-PHV untrained, $n = 12$; and post-PHV trained, $n = 13$] and females (pre-PHV untrained, $n = 12$; pre-PHV trained, $n = 13$; post-PHV untrained, $n = 14$; and post-PHV trained, $n = 17$) assessed via a three-way ANOVA. Error bars indicate the group means (SD). P values outside of the figure plot indicate where significant main and interaction effects of maturity status, biological sex, and training status occurred. ANOVA, analysis of variance.

compared with untrained counterparts. This observation indicates that a threshold of circulating IGF-1 achieved during puberty, which is not present during childhood, is needed to facilitate further exercise-mediated cerebral angiogenesis and reduce downstream cerebrovascular resistance. Thus, adolescents may experience structural remodeling of the cerebrovascular network in response to exercise-mediated pulsatile flow that subsequently dampens the pulsatility of cerebral perfusion. However, whether training-mediated adaptations in cerebrovascular hemodynamics result in remodeling of the larger upstream cerebrovascular arteries during childhood and adolescence requires further investigation.

We have also demonstrated that MCA_v , but not PCA_v , is lower in post-PHV youth when compared with their pre-PHV counterparts, whereas MCA_v was lower in males when compared with females, independent of changes in cerebral perfusion pressure. These findings support the assertion that cerebral perfusion, particularly in the anterior regions, declines across adolescence in a sex-specific manner, corresponding to structural and metabolic neural development across adolescence (5, 51). Furthermore, MCA pulsatility index, a metric of downstream cerebrovascular resistance,

was lower in post-PHV females compared with males, but similar in pre-PHV males and females. Thus, cerebrovascular resistance at the level of the MCA appears to only diverge in males and females during adolescence despite sex-related differences in cerebral blood velocity that are present across childhood and adolescence. Accordingly, the divergence in pulsatility index in males and females during adolescence may be a consequence of vasoactive sex hormones (52) modulating basal cerebrovascular tone during adolescence without altering cerebral perfusion.

Exercise-Trained Children Demonstrate Smaller Increases in Cerebrovascular Pulsatility during Neurovascular Coupling Assessments

Although the relative change in PCA_v and MCA_v was similar across groups during the NVC task, the change in PCA pulsatility was greater in pre-PHV untrained youths compared with their post-PHV untrained counterparts, as well as their pre-PHV-trained counterparts. Therefore, despite similar cerebral blood velocities and pulsatility at rest, alongside similar changes in cerebral perfusion during the visual stimulus, the pulsatility of blood flow during the NVC task is dampened by exercise training in pre-PHV youth. Indeed,

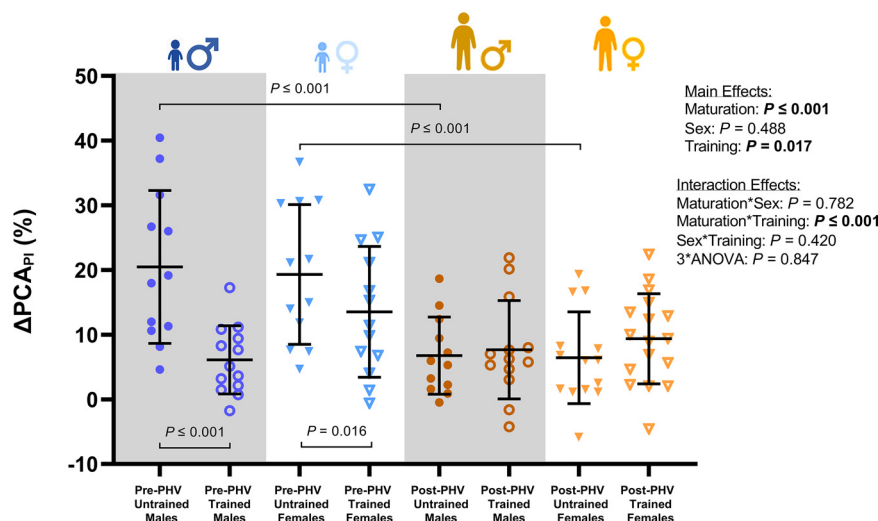


Figure 3. Group means for the percent change in posterior cerebral artery blood pulsatility index (ΔPCA_{PI}) during the neurovascular coupling task in males [pre-peak height velocity (PHV) untrained, $n = 12$; pre-PHV trained, $n = 13$; post-PHV untrained, $n = 12$; and post-PHV trained, $n = 13$] and females (pre-PHV untrained, $n = 12$; pre-PHV trained, $n = 13$; post-PHV untrained, $n = 14$; and post-PHV trained, $n = 17$) assessed via a three-way ANOVA. Error bars indicate group means (SD). P values outside of the figure plot indicate where significant main and interaction effects of maturity status, biological sex, and training status occurred. P values within the figure plot indicate a significant difference between groups during post hoc comparisons. ANOVA, analysis of variance.

evidence from adults indicates that the increase in PCA_v during NVC tasks is driven by the change in end-diastolic velocity rather than the peak systolic velocity (43). An increase in pulsatility occurs when there is a greater contribution of peak systolic velocity to the mean velocity of the waveform. Therefore, the peak systolic velocity has a greater contribution to the change in PCA_v during NVC tasks in children when compared with adolescents and adults. This transition from peak systolic- to end diastolic-mediated changes in PCA_v appears to be hastened by exercise training during childhood. We speculate that a higher resting cerebral perfusion and larger increases in cerebrovascular pulsatility during NVC in children relate to lower vascular tone at rest and an attenuated decline in cerebrovascular resistance during metabolic stimuli, when compared with adolescents and adults. This observation would highlight the transition from childhood to adolescence, or puberty, as a key period in the development of cerebrovascular function.

Given the evidence that cardiorespiratory fitness at 18 yr of age is associated with reduced incidence of neurocognitive disease later in life (7), it is likely that elevated cardiorespiratory fitness during adolescence promotes a healthy NVC phenotype across adulthood. However, it is important to consider that our cohort of trained adolescent youth had been exercise training for several years (i.e., since they were children) and had likely already experienced similar training-mediated adaptations in NVC as their younger counterparts. It is an intriguing possibility that functional adaptations in NVC during childhood are accompanied by complimentary structural adaptations in cerebrovascular remodeling or angiogenesis with continued endurance training into adolescence (14, 53) that make further functional changes in NVC at this early stage of life redundant. Indeed, cerebrovascular angiogenesis can be stimulated by exercise training in rodents (14) and humans (54). Moreover, exercised-mediated angiogenesis is dependent on the presence of IGF-1 *in vivo* (14), which is relatively low during childhood before peaking during adolescence (52). Therefore, exercise training during adolescence may result in further positive cerebrovascular adaptations that modulate aspects of NVC beyond the scope of this study. Regardless of the uncertainty of how exercised-mediated improvements in NVC translate from childhood to adolescence, it appears that exercise training has a positive impact on NVC during childhood. The exercised-mediated adaptations in NVC during childhood highlight the importance of physical activity for neurocognitive health during the formative developmental years, far earlier than the pathogenesis of neurocognitive disease. Accordingly, exercise training across childhood and adolescence may be vital for optimizing cerebrovascular function across the lifespan and delay the onset of neurocognitive disease.

There were no maturity or biological sex-related differences in the PCA_v response or the change in HR, MAP, PET_{CO_2} , and PET_{O_2} during the NVC task. As such, our findings are not confounded by group differences in cerebral perfusion pressure or metabolic feedback mechanisms of cerebral blood flow regulation. The lack of maturity- or biological sex-related difference in the change in cerebral artery blood velocities during the NVC task conflicts with previous findings (10, 11), and is

somewhat surprising given the contribution of NO-mediated vasodilation to NVC in adults (55) and the potential sex differences in the development of endothelial function across puberty (15, 26, 56). However, the conflicting findings are likely to be related to methodological differences in the NVC stimulus. Unlike previous reports (10, 11), the current study utilized a selective visual stimulus that is unlikely to be influenced by age- or learning-related differences across groups, while also invoking a large change in cerebral perfusion across all groups. Although it should be noted that the oxygen-carrying capacity of the blood increases across adolescence (2), the similar relative change in PCA_v in children and adolescents during the NVC task was present despite higher resting cerebral perfusion in children (5). Thus, our data show that children experience a larger absolute change in cerebral perfusion during the NVC task that counteracts the higher oxygen-carrying capacity in adolescent youth.

Limitations

There are a few limitations that must be considered in the current study. First, Pa_{CO_2} was indirectly monitored via PET_{CO_2} . However, PET_{CO_2} is commonly used as a suitable surrogate for Pa_{CO_2} during assessments of NVC (39, 40). Likewise, PET_{CO_2} has a strong relationship with Pa_{CO_2} in nonventilated children (57, 58), and the lack of change in PET_{CO_2} during the NVC task in the current study was similar across groups. Therefore, we are confident that our data provide novel insight into the influence of maturity and training status on NVC during youth. Second, we used PCA_v via TCD ultrasound to infer changes in cerebral perfusion during the NVC task. The larger proximal portions of the PCA (as insonated in the current study) appear to only dilate $\approx 0.9 \pm 2.2\%$ in response to similar visual stimuli (59). Therefore, we feel it is appropriate to use PCA_v as an index of cerebral perfusion during NVC stimuli because of the excellent temporal resolution of TCD (33) and the relatively small vasodilation of the P1 segment of the PCA. Finally, recent work has highlighted the important contribution of NO signaling to NVC in adults (55, 60). Likewise, endogenous sex hormones may play an important role in NO signaling and vascular function in adults (61, 62). We were unable to acquire suitable measurements of endothelial NO activity or sex hormones in the current study because of the ethical considerations associated with invasive venous blood sampling and pharmaceutical blockades in a nonclinical pediatric cohort.

Conclusions

Our novel findings demonstrate that the change in cerebral perfusion during visual stimuli is unaffected by maturity or training status during youth. However, endurance-trained children demonstrated attenuated increases in cerebral blood flow pulsatility when compared with their untrained counterparts, whereas no training-mediated differences were present during the NVC task in adolescent youth. Therefore, exercise training during childhood, or before somatic maturation, modifies neurovascular coupling and may provide a foundation for additional cerebrovascular remodeling during adolescence that benefits long-term neurocognitive function.

DATA AVAILABILITY

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

ACKNOWLEDGMENTS

We appreciate and thank all participants and their parents/guardians for being a part of this study. Thanks also goes to Jo Phillips for assistance during the recruitment process. The authors are also grateful for the assistance provided by Dr. Mike Tymko, Dr. Ryan Hoiland, Bryony Curry, Katie Noteman, and Zavia Penn.

GRANTS

This study was supported by The Waterloo Foundation (1129/4226; to M.S. and J.S.T.) and a FIFA Research Scholarship (to M.S. and J.S.T.).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.S.T., J.L.O., R.S.L., P.N.A., A.M.M., and M.S. conceived and designed research; J.S.T., D.R.P., T.G.D., A.J.M.D., T.D.G., C.T.R., K.O., and R.N.L. performed experiments; J.S.T. analyzed data; J.S.T., C.J.A.P., J.L.O., R.S.L., P.N.A., A.M.M., and M.S. interpreted results of experiments; J.S.T. prepared figures; J.S.T. drafted manuscript; J.S.T., D.R.P., T.G.D., A.J.M.D., T.D.G., C.T.R., K.O., R.N.L., C.J.A.P., J.L.O., R.S.L., P.N.A., A.M.M., and M.S. edited and revised manuscript; J.S.T., D.R.P., T.G.D., A.J.M.D., T.D.G., C.T.R., K.O., R.N.L., C.J.A.P., J.L.O., R.S.L., P.N.A., A.M.M., and M.S. approved final version of manuscript.

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