UBC-Nepal Expedition: Markedly lower cerebral blood flow in high altitude Sherpa children compared to children residing at sea-level

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Running title: Cerebral hemodynamics in Sherpa and Lowlander children

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Abstract

Developmental cerebral hemodynamic adaptations to chronic high altitude exposure, such as in the Sherpa population, are largely unknown. To examine hemodynamic adaptations in the developing human brain, we assessed common carotid (CCA), internal carotid (ICA) and vertebral artery (VA) flow and middle cerebral artery (MCA) velocity in 25 (9.6±1.0 y, 129±9 cm, 27±8 kg, 14 girls) Sherpa children (3800m, Nepal) and 25 (9.9±0.7 y, 143±7 cm, 34±6 kg, 14 girls) age-matched sea-level children (344m, Canada) during supine rest. Resting gas exchange, blood pressure, oxygen saturation and heart rate were assessed. Despite comparable age, height and weight were lower (both $P<0.01$) in Sherpa compared to sea-level children. Mean arterial pressure, heart rate and ventilation were similar, whereas oxygen saturation (95±2 vs. 99±1%, $P<0.01$) and end-tidal PCO_2 (24±3 vs 36±3 mmHg, $P<0.01$) were lower in Sherpa children. Global cerebral blood flow was ~30% lower in Sherpa compared to sea-level children. This was reflected in a lower ICA flow (283±108 vs. 333±56 ml/min, $P=0.05$), VA flow (78±26 vs 118±35 ml/min, $P<0.05$) and MCA velocity (72±14 vs 88±14 cm/s, $P<0.01$). CCA flow was similar between Sherpa and sea-level children (425±92 vs. 441±81 ml/min, $P=0.52$). Scaling flow and oxygen uptake for differences in vessel diameter and body size respectively, led to the same findings. A lower cerebral blood flow in Sherpa children may reflect specific cerebral hemodynamic adaptations to chronic hypoxia.

New & Noteworthy

Cerebral blood flow is lower in Sherpa children compared to children residing at sea-level; this may reflect a cerebral hemodynamic pattern, potentially due to adaptation to a hypoxic environment.

Keywords: brain blood flow, high altitude, preadolescents, hypoxia

Introduction

The human brain is a highly demanding organ using 15% of the total cardiac output and ~ 20% of the oxygen taken up by a human at rest (29). Furthermore, because of the limited substrate storage, an adequate delivery of oxygen and nutrients to the brain is essential (11). Limited oxygen availability, as experienced during hypoxia, challenges brain blood flow regulation to meet its high energy requirements (4, 6). Some animals have evolved to tolerate extreme limitations of oxygen by lowering energy expenditure and utilization in the brain to allow survival despite prolonged hypoxemia or anoxia (16). Tibetans and Sherpas who have migrated from the Tibetan plateau, have adapted to their low-oxygen environment [reviewed: (14)]. Although other native populations to high altitude exist (e.g., Andeans and Ethiopians), the Tibetan population have resided at altitudes between 3500 – 4500 m above sea level for the longest [~25000 years, (8)] and thus serve as a unique model to study long-term adaptation to hypobaric hypoxia.

Acute exposure to hypoxia in sea-level residents (Lowlanders) results in an elevation in cerebral blood flow (CBF) which serves to offset reductions in arterial oxygen content and thus maintain cerebral oxygen delivery. Over time at a given altitude, when oxygen content improves, CBF returns towards baseline [reviewed in: (5, 21)]. In Tibetans (and Sherpas) CBF has been reported to be the same (26) or lower (30) compared to populations residing at sea-level. Existing data are sparse and mostly based on adults. Exposure to limited oxygen availability during development can result in reduced birth weight and cardio-pulmonary pathologies, but also negatively affect neurodevelopment (38). Developmental cerebral hemodynamic adaptations to chronic high altitude exposure are, however, largely unknown.

A previous study exposing children (n=9; 9±2 y, 5 boys) normally residing at sea-level to acute (24 h at 3500 m after a 5 day trek) hypobaric hypoxia (e.g., altitude) resulted in a 29% increase in the middle cerebral artery (MCA) velocity (13). Evidence from Andean children demonstrates MCA velocity in those living at 500 m (n=18, 8±2 y, 8 boys) compared to those living at 3700 m (n=19, 8±1 y, 10 boys) was not different (19). In contrast, the authors reported that velocity in the basilar artery was lower in those living at high altitude (19). Both studies in children (13, 19) as well as studies in adults (25, 26) have used transcranial Doppler ultrasound to assess velocity in the intracranial arteries (MCA or basilar artery). A limitation of this approach is the assumption that vessel diameter is unchanged, however, hypoxia can lead to changes (dilation) in the diameter of cerebral arteries (53); which would result in an underestimation of CBF (1, 20).

Accordingly, the primary aim of the present study was to compare regional and global CBF in high altitude Sherpa children and age-matched children residing at sea-level. This aim was achieved by assessing volumetric flow in the common (CCA) and internal carotid (ICA) arteries and the vertebral artery (VA) and employing a novel edge detection software approach (43). We hypothesized that both regional and global CBF would be lower in the Sherpa children compared to age-matched children residing at sea level.

Materials and Methods

Ethical Approval

All experimental protocols and procedures were approved by the clinical research ethics board at the University of British Columbia and the Nepal Health Medical Research Council and

conformed to the Declaration of Helsinki. This independent project was part of the UBC expedition to Nepal 2016 which included a larger series of experiments. Prior to participation a detailed verbal and written explanation of the measurements was provided and each participant and their parent/guardian completed written informed consent. All documents were provided in the first language of the participant. The research team consisted of a Nepali and a Sherpa language translator (SN) to facilitate on-site communication with the high altitude participants.

Participants

Twenty-five Sherpa children (9.6±1.0 y, 14 girls, Thame and Khunde, Nepal, 3800 m) and 25 age-matched children residing at sea level (9.9±0.7 y, 14 girls, Kelowna, Canada, 340 m) took part in the study. As determined by an oral screening questionnaire of the Sherpa children by a Nepalese physician (SN) and the child's parent/guardian, and a written screening questionnaire in the children from sea-level completed by their parent/guardian, none of the children were reported to have any cardiovascular, cerebrovascular or respiratory disease. None of the children were on prescription or over-the-counter medications. As some of the Sherpa children and their parents only knew their birthdate in the Nepali calendar, our Nepalese physician (SN) and Sherpa guide converted these to the Gregorian calendar in order to calculate the specific birthdate.

Experimental measures

Body mass was measured with electronic scales with subjects barefoot and dressed in light clothing. Stature and sitting height were measured barefoot with a Harpenden stadiometer. Body mass index (BMI) was calculated from body mass (kg) divided by stature (m^2). Predicted age at peak height velocity (aPHV) was estimated (35) and used to classify the children as pre-adolescent or adolescent. Heart rate (HR) was assessed using a 3-lead electrocardiogram (ECG;

ADI BioAmp ML132), and blood pressure using an automated cuff (Tango+; SunTech, Morrisville, NC). Oxygen saturation (SpO_2) was measured using a pulse oximeter (Rad-5®, Masimo SET, Irvine CA, USA) with child-size adhesive sensors (LNCS® Neo, Masimo SET, Irvine CA, USA). Pulmonary gas exchange [oxygen uptake (VO_2), CO_2 production (VCO_2), partial pressure of end-tidal CO_2 ($PetCO_2$) and O_2 ($PetO_2$)], tidal volume (V_T), ventilation (VE) and breathing frequency (BF) were recorded breath by breath using a metabolic cart (Oxycon Pro, Carefusion, USA), interpolated to 1-s intervals. Children were breathing through a mouthpiece with their nose occluded using a nose clip. The gas analyzer was calibrated daily using gases of known concentrations before the first test, and volume was calibrated before each test in Kelowna and in Nepal. Alveolar ventilation (V_A) was estimated according the following equation: $PaCO_2 = VCO_2 \cdot K / V_A$, where $PaCO_2$ is the arterial partial pressure of CO_2 and K is 0.863 (49). An estimation of $PaCO_2$ was derived from $PetCO_2$ (33). In one child residing at sea-level respiratory data is missing, as such all respiratory data in children residing at sea-level are based on n=24. Transcranial Doppler Ultrasound (2MHz, TCD, Spencer Technologies, Seattle, WA) was used to assess middle cerebral artery mean velocity ($MCAv_{mean}$). To secure the probe in place the TCD probes were attached to a headpiece (child-sized adjusted model M600 bilateral head frame, Spencer Technologies). The MCA was insonated through the middle trans-temporal window, using previously described locations and standardization techniques (50). Due to insufficient imaging quality in one of the children residing at sea-level, $MCAv_{mean}$ data is based on n=24 in the sea-level group. Blood velocity and vessel diameter of the left CCA, left ICA and left VA were measured using a 10 MHz multi-frequency linear array vascular ultrasound (Terason T3200, Teratech, Burlington, MA). B-mode imaging was used to measure arterial diameter, while pulse-wave mode was used to simultaneously measure peak blood velocity. Extracranial blood flow measurements were made in accordance with recent technical

recommendations (43). All CCA, ICA and VA recordings were screen captured and stored as video files for offline analysis (55). A minimum of 20 consecutive cardiac cycles were used to determine extracranial blood flow measurements. Due to insufficient imaging quality in one of the children residing at sea-level, VA data is based on n=24 in the sea-level group. Volumetric blood flow was calculated using the following formula:

$$\text{CCA, ICA or VA flow} = \frac{\text{CCA, ICA or VA Peak Envelope Velocity}}{2} \cdot [\pi\ (0.5\ \cdot \text{Diameter})^2]$$

Global cerebral blood flow (gCBF) was calculated using the following formula:

$$\text{gCBF} = 2\ (\ \text{ICA flow} + \text{VA flow})$$

ECG and $MCAv_{mean}$ were sampled continuously at 1000 Hz using an analogue-to-digital converter (Powerlab, 16/30; ADInstruments, Colorado Springs, CO, USA) and data were interfaced with LabChart (Version 7), and analyzed offline.

Study protocol

Sherpa children were either tested in Thame (3800 m, Thame Community Health Clinic, Thame, Nepal) or in Khunde (3840 m, Khunde Hospital, Khunde, Nepal). Children from sea-level were tested in Kelowna (344 m, Kelowna, Canada). Before any measurements were taken, children were rested in a supine position for at least 10 min. Thereafter, measurements were taken supine over a 15 min period while volumetric flow measurements of the CCA, ICA and VA were assessed consecutively.

Data and statistical analysis

Values were taken as an average over at least 1 minute. Using a two-way ANOVA all outcome measures were tested for a sex effect; however, since the results revealed an absence of

difference between girls and boys, data were therefore pooled together. Comparison of the main outcome variables between Sherpa children and children residing at sea-level were made using a Student's t-test. Data are given as mean±SD unless otherwise indicated. To adjust for size differences in respiratory parameters (adjusted for body mass) and blood flow (vessel size) between Sherpa children and children from sea-level we applied allometric scaling using a log linear ANCOVA. Covariate adjusted means (±SE) were obtained from this model. To confirm whether the allometric adjustment using body mass adequately accounted for differences in size between the two groups of children, a subset analysis of 8 Sherpa children and 8 size-matched children residing at sea-level was conducted. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using SAS Enterprise Guide (4.3, SAS Institute, Cary, NC).

Results

Sherpa children were smaller (129±9 vs. 143±7 cm, $P<0.01$) and weighed less (27±8 vs. 34±6 kg, $P<0.01$) compared to the age-matched children residing at sea-level. Both groups, however, had a similar BMI (16±2 vs 17±2 kg/m^2, $P=0.13$). Calculation of predicted aPHV showed both groups were preadolescent; however, Sherpa children show a younger biological age despite similar chronological age in comparison to children residing at sea-level, with aPHV -3±1 y in Sherpa children vs -2±1 y in children residing at sea-level ($P=0.01$). Table 1 reports the cardiovascular and respiratory parameters of Sherpa children and children residing at sea-level. Briefly, there was no difference in mean arterial pressure (MAP), HR, V_E, V_A and V_T. In contrast, SpO_2, $P_{ET}CO_2$, $P_{ET}O_2$, VO_2 and VCO_2 were lower in the Sherpa children, whereas BF was higher in Sherpa children compared to children residing at sea-level. Scaling VO_2 (242±1 vs. 325±1

177 $ml \cdot l^{-1}$, $P<0.01$), VCO_2 (186±1 vs. 250±1 $ml \cdot l^{-1}$, $P<0.01$) and V_E (8.1±1 vs. 8.6±1 $l \cdot min^{-1}$, $P=0.34$)
178 for body mass did not diminish the difference between Sherpa children and children residing at
179 sea-level.

180 *Cerebrovascular variables*: Sherpa children had a lower $MCAv_{mean}$ (71.5±13.6 vs. 87.5±14.3
181 $cm \cdot s^{-1}$, $P<0.01$) compared to children residing at sea-level. Figure 1 illustrates the mean velocity,
182 diameter and mean flow in the CCA, ICA and VA. There was no difference in mean flow in the
183 CCA between the two groups. In contrast, ICA and VA flow were lower in the Sherpa children
184 compared to children residing at sea-level. As such, gCBF in Sherpa children was ~ 30% lower
185 compared to children residing at sea level (Figure 2). Furthermore, VA flow contributed less to
186 the gCBF in Sherpa children compared to children residing at sea level (22±7% vs. 26±6%,
187 $P=0.03$, Figure 2). Adjusting CCA (432±1 vs. 411±1 $ml \cdot min^{-1}$, p=0.33, mean±SE), ICA (270±1
188 vs. 317±1 $ml \cdot min^{-1}$, $P<0.01$, mean±SE) and VA (82±1 vs. 97±1 $ml \cdot min^{-1}$, $P<0.01$, mean±SE)
189 flow for differences in diameter size between Sherpa children and children residing at sea-level
190 did not affect the above-mentioned findings. Furthermore, these findings (gCBF, CCA flow, ICA
191 flow, VA flow and $MCAv_{mean}$) in Sherpa children versus children residing at sea level persisted
192 when expressed as cerebrovascular conductance (8.4±1.7 vs. 11.5±2.5 $ml \cdot min^{-1} \cdot mmHg^{-1}$,
193 $P<0.01$; 5.1±1.1 vs. 5.6±1.2 $ml \cdot min^{-1} \cdot mmHg^{-1}$, $P=0.14$; 3.3±0.7 vs. 4.3±1.0 $ml \cdot min^{-1} \cdot mmHg^{-1}$,
194 $P<0.01$; 0.9±0.3 vs. 1.5±0.4 $ml \cdot min^{-1} \cdot mmHg^{-1}$, $P<0.01$ and 0.9±0.2 vs. 1.1±0.3 $cm \cdot s^{-1} \cdot mmHg^{-1}$,
195 $P<0.01$, respectively).

196 *Size-matched comparisons:* The size-matched comparison resulted in no difference between the 8
197 Sherpa children and children residing at sea-level in weight and stature (Table 2). Predicted
198 aPHV showed the same biological age for Sherpa children and the children residing at sea-level
199 (-2.4±1.3 vs. -2.6±1.0, $P=0.83$). Similar to the age-matched comparison, Sherpa children showed

a lower ICA flow, VA flow, gCBF and $MCAv_{mean}$ (P<0.01, Table 2) compared to the children residing at sea-level.

Discussion

In this study, for the first time, we assessed global and regional CBF in pre-adolescent Sherpa children and compared them to age-matched children residing at sea-level. Our main finding was a 30 % lower gCBF in Sherpa children compared to children residing at sea-level. In contrast, there was no difference observed in CCA flow, indicating a possible redistribution to the external circulation. The following discussion considers the evidence, methodological assumptions and the relevance underlying the findings of this study.

Comparisons with previous studies: Our results are broadly consistent with a recent study reporting a lower gCBF in Tibetans born and raised at 3200-4500 m compared to Tibetans living at sea-level for two years (30). The difference in gCBF is somewhat smaller compared to the present study (~15% vs. ~ 30%) – that may be explained due to (i) the inclusion of data of adults, (ii) that the comparison was performed between the same ethnicity or (iii) differences in diameter evident in our dataset. Estimating gCBF using the diameter adjusted ICA and VA mean flows, attenuates the difference in gCBF between the Sherpa children and sea-level children to ~ 15%. Similarly, an unpublished dataset comparing adults residing at sea level and adult Sherpas at high altitude confirms the lower gCBF in the adult Sherpa population (22). Andean children residing at 3700 m have also been shown to have a reduced basilar artery velocity compared to those living at sea level (19). A previous study (26), that failed to show a difference in CBF in Sherpa and Tibetan adults compared to those residing at sea-level, relied on $MCAv_{mean}$ as a measure for

CBF and thus did not take differences in diameter into account. Assessing only velocity can lead to an under- or overestimation of flow as in accordance with Poiseuille's Law, even the smallest changes in diameter have a major effect on flow [e.g., flow ~ $(\text{diameter}/2)^4$]. In a further study (23) ICA flow was assessed in Tibetans versus Han Chinese adults and no difference was observed between the two groups; however, the Han Chinese had been living in Lhasa for the last 1-15 years and thus it is not a true comparison between sea-level natives and those native to high altitude.

Maturation, CBF and hypoxia: Although the regulation of CBF in adults is relatively well studied, the influence of maturation is poorly understood in the pediatric brain. Growth and maturation occur alongside critical surges in neural development, myelination, metabolic demands and increased cerebral utilization of glucose (3). It is known that global CBF at birth is, on average, 50 ml (100 g)/ min, increasing after birth to a maximum of 70–80 ml (100 g)/ min at 5–7 years and then decreasing to reach adult levels after 19 years (42). This temporal pattern of change is probably a consequence of brain development and subsequent 'shaping' of neurons, synapses and pathways that occurs with maturation (3). In our study cohort the Sherpa children are biologically younger compared to the children residing at sea-level and are smaller, thus might have a lower brain volume. We also found a smaller arterial diameter in the Sherpa children compared to those residing at sea-level. It is worth noting that Murray's law (37) suggests flow varies with vessel diameter (D^3) in arteriolar beds where the relationship between blood volume and vascular resistance is important for the maintenance and regulation of regional blood flow. This may provide the most efficient transport of oxygen to the relatively smaller, less mature and/or high-altitude adapted brain, which may have a relatively lower oxygen uptake and/or utilization. Nevertheless, the temporal pattern of maturation as well as neurodevelopment

could, potentially be altered due to exposure to hypoxia (45). Furthermore, cognitive impairments have been reported in high altitude natives [reviewed in (47, 57)]. Moreover, a recent study has also shown cognitive impairment in school children residing at high altitude compared to children going to school at low altitude (48). Whether limited oxygen availability is the only underlying cause is difficult to define as high altitude natives also face malnutrition, underdeveloped living infrastructure, as well as poor access to medical care [reviewed in (38, 47)].

Putative mechanism(s) of action: A lower CBF in Sherpa children highlights a cerebral hemodynamic pattern that may reflect a long term adaptation to chronic hypoxia. A potential underlying mechanism mediating the lower CBF could be a lower cerebral metabolism as reported in hypoxia-tolerant animals (16) and Quechua high altitude residents (at sea level) (17). In Sherpas, cerebral glucose metabolism did not differ in Sherpas, at least when examined at sea level, in comparison to sea-level residents (18). Data on cerebral metabolism in adult or child Sherpas at altitude does not exist. We found a markedly lower resting VO_2 in the Sherpa children which was weakly related with gCBF ($r^2 = 0.15$, $p < 0.01$). Data on the relationship between VO_2 and cerebral metabolism in children is sparse. Evidence in children with traumatic brain injury shows no relationship between whole body and cerebral metabolism (32), and corroborates our findings that whole body VO_2 plays a minimal role in the variability in gCBF.

At sea-level, changes in arterial PCO_2 is well-known to be the major regulator of CBF (27, 51). Thus, the observed hypocapnia in the Sherpa children at high altitude could be the cause of vasoconstriction and hence reductions in CBF. We feel this is an unlikely explanation since it is the extra-cellular pH rather than arterial PCO_2 that acts to influence cerebrovascular tone [(28) and reviewed in: (52)]. In the context of a sojourn at given altitude, excretion of HCO_3 occurs in order to provide metabolic compensation of the respiratory alkalosis [reviewed in (2)]. Over 2-3

weeks at 4000m, at least in adults, it is likely that pH would be nearly corrected [reviewed in (2)]; therefore, this normal hypocapnic / alkalotic stimulus to attenuate CBF is removed (5).

Moreover, as the Sherpa children had a lower biological age and potentially experience lower growth rate, brain volume might play a role. Brain volume has been shown to increase up to about 18 years of age. In contrast, gCBF peaks between 4 to 6 years of age, declining thereafter and as such brain volume and gCBF were not correlated (56). Our findings of a lower gCBF in the Sherpa children remained when we matched a subgroup by body size, lending support to our scaling approach.

A further factor affecting blood flow is blood viscosity, as increased blood viscosity can lead to reduced CBF (15, 24, 25, 34). Exposure to a hypoxic environment stimulates red blood cell production and thus can result in an increased hematocrit and blood viscosity (40). Hemoglobin concentrations in high altitude residents were reported to be higher in Andeans than in Tibetans (and Sherpas), whereas hemoglobin in the latter diverges less from sea-level residents (54). In a recent study of prepubertal (6-13 years) Tibetan children residing at 4300m (12), hemoglobin concentrations approximate the 95^{th} percentile for the sea-level US population; these hemoglobin concentrations thus do not exceed the normal range seen in this age group at sea level. Hemoglobin concentration was not acquired in the present data, thus we can only speculate possible differences in hemoglobin concentrations in the Sherpa children compared to children residing at sea-level. Furthermore, as an inverse relationship exists between CBF and hemoglobin to match oxygen content [reviewed in (21)], a higher hemoglobin concentration in Sherpa children would support the lower CBF compared to children residing at sea-level. Nevertheless, it seems differences in hemoglobin and blood viscosity between high altitude dwelling Tibetans

290 and sea-level residents are small and likely only contribute to a minor extent to the decreased
291 gCBF in Sherpa children.

292 *Ventilation and saturation*: Interestingly, there was no difference in V_E and V_A between Sherpa
293 children and children from sea-level, even when scaled for differences in body mass. Previously,
294 elevated V_E has been shown in Sherpas compared to Andeans and sea-level residents (9). An
295 increased V_E appears to facilitate a higher VO_2, compensating for the low oxygen availability
296 [reviewed in: (8)]. We found rather high SpO_2 values when compared to existing data in the same
297 age group (7, 46). In a more recent study (30) in Tibetan adolescents at 3658 m, the SpO_2 values
298 were similar to those we report a 92%. Furthermore SpO2 peaks at 10-19 years and decreases
299 during adulthood (7), supporting our higher values in comparison to adult data. Additionally,
300 higher SpO_2 values could be a consequence of a possible left shift of the oxygen dissociation
301 curve which favors a better pulmonary oxygen uptake in a hypoxic environment (36). This would
302 support the low $PetCO_2$ values we observed in the Sherpa child and thus potential elevations in
303 pH (i.e. respiratory alkalosis), promoting a leftward shift in the oxygen dissociation curve.

304 *Regional cerebral blood flow distribution:* The left and right CCA and VA supply the head and
305 brain with blood. The CCA branches into the ICA and the external carotid artery (ECA) at the
306 carotid bulb and whereas the ICA and VA supply the brain, the ECA supplies the face with
307 blood. Unlike the ICA and VA, CCA flow was comparable between Sherpa children and children
308 living at sea-level. Thus, given a similar flow is going to the head in both groups, it would appear
309 that the lower ICA flow in the Sherpa children would suggest greater flow is distributed towards
310 the ECA. The distribution towards the ECA could be driven by the "Plateau Red Face"
311 phenomenon - an expansion of small blood vessels in the face, as observed in high altitude
312 residents (31). Furthermore, during exercise in the heat, an increase in ECA flow for

thermoregulation has been observed in adults (39, 41). Whether the likely redistribution of flow to the ECA in Sherpa children is driven by a small blood vessel expansion or a form of thermoregulatory adaptation to the high altitude environment remains to be determined. Likewise, similar to unpublished data on adult Sherpas (22), Sherpa children show a lower percentage of gCBF distributed to the posterior circulation (Figure 2). Whether the unequal distribution of ICA, ECA and VA flow in Sherpa children serves as an adaptive mechanism to long term exposure to high altitude remains speculative.

Methodological considerations: There are a number of methodological considerations that underlie our findings. The current study contains data from Sherpa children which were collected in two villages (Thame and Khunde) in Nepal. The sample size and the assessment of CBF for this study cohort are some of the largest to date. However, field studies, as well as studies in children, yield limitations such as the lack of blood samples or poor control of pre-testing guidelines. Additionally, we have attempted to examine two different population groups that are matched for chronological age; however, stature, mass, maturation and the living environment diverge between the Sherpa children and children residing at sea-level. Despite allometric scaling for differences in size, it is impossible to fully overcome this limitation. To partially consider this influence, in a subset of the study cohort, we matched children for size. The subset analyses which was independent of size differences supported the lower CBF in Sherpa children compared to children residing at sea-level. Calculating predicted aPHV to estimate the biological age in the Sherpa children yields an additional limitation as a slower growth rate has been reported for this population (44). Thus our biological age calculation most likely has overestimated the biological age of the Sherpa children. A further consideration is that both groups of children were tested at the altitude where they were born and raised. For future studies, comparing these children at the

same altitude, via acclimatizing sea-level residents to high altitude, or by deacclimatizing high altitude residents to sea-level, would lead to further insight to long term adaptation to a hypoxic environment in children.

Clinical implications: In many childhood diseases there is a lack of oxygen either due to limited oxygen uptake, transport or utilization. Thus knowledge describing how children adapt to an environment where oxygen availability is limited may help to establish optimal mechanisms that can be incorporated into the development of improved treatments and therapies to help these children [reviewed in (10)]. Furthermore, there is increasing evidence for children living at high altitude experiencing cognitive impairment (48). Oxygen conditioning, a technique that enriches rooms with oxygen (48), could potentially help to improve cognitive abilities in these children. Whether or not daily oxygen enrichment might enhance cognitive function via a CBF or direct central nervous system mechanism is unclear.

In conclusion, Sherpa children have a reduced gCBF in comparison to sex and age-matched children from sea-level. A lower CBF in Sherpa children highlights a cerebral hemodynamic pattern that may reflect a long term adaptation to chronic exposure to a hypoxic environment.

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Disclosures

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Table 1. Cardiovascular and respiratory parameters of age-matched Sherpa children and children residing at sea-level.

	Sherpa children	Sea-level children	*P* - value
	n = 25	n = 25	
MAP (mmHg)	82±10	80±11	0.487
HR (bpm)	87±15	81±9	0.095
SpO_2 (%)	**94.6±1.8**	**99.6±0.7**	**<0.001**
V_E (l min^{-1})	8.38±1.66	8.67±2.04	0.594
V_A (l min^{-1})	6.66±1.75	6.53±1.52	0.782
BF (min^{-1})	**23.3±6.1**	**20.3±3.6**	**0.047**
V_T (ml)	381.3±109.8	434.4±113.5	0.103
$P_{ET}O_2$ (mmHg)	**99.9±6.2**	**61.3±3.7**	**<0.001**
$P_{ET}CO_2$ (mmHg)	**23.6±2.6**	**36.0±2.7**	**<0.001**
VO_2 (ml)	**233.7±42.8**	**349.5±65.6**	**<0.001**
VCO_2 (ml)	**178.0±36.1**	**275.1±65.0**	**<0.001**
RER	0.77±0.06	0.76±0.07	0.709

Values are mean±SD. MAP, mean arterial pressure; HR, heart rate; SpO_2, oxygen saturation; V_E, ventilation; V_A, alveolar ventilation BF, breathing frequency; V_T, tidal volume; $PetCO_2$, end-tidal partial pressure of carbon dioxide; VO_2, oxygen uptake; VCO_2, carbon dioxide production; RER, respiratory exchange ratio. *P* - values represent student's t-test results.

370 Table 2. Cardiovascular, respiratory and cerebrovascular parameters of size-matched Sherpa
371 children and children residing at sea-level.

	Sherpa children	Sea-level children	*P* - value
	n = 8	n = 8	
Age (y)	10.2±1.2	9.9±0.7	0.444
Weight (kg)	32.3±10.8	31.1±6.5	0.793
Height (cm)	137.7±8.6	138.2±67.6	0.904
Sitting height (cm)	70.7±4.3	71.3±3.8	0.753
BMI (kg m^{-2})	16.7±3.9	16.1±2.0	0.701
MAP (mmHg)	83±6	81±9	0.535
HR (bpm)	85±11	82±11	0.482
SpO_2 (%)	**93.8±1.0**	**99.6±0.5**	**<0.001**
V_E (l min^{-1})	8.18±1.87	7.98±1.58	0.814
V_A (l min^{-1})	6.02±1.48	6.52±1.70	0.542
BF (min^{-1})	21.2±5.0	19.1±3.2	0.339
$PetCO_2$ (mmHg)	**25.1±2.1**	**35.2±3.2**	**<0.001**
VO_2 (ml)	**241.9±43.9**	**312.2±56.6**	**0.016**
VCO_2 (ml)	**187.1±41.7**	**242.5±45.9**	**0.024**
RER	0.78±0.06	0.77±0.07	0.838
CCA velocity (cm s^{-1})	51.5±5.3	50.0±6.2	0.594
CCA diameter (cm)	0.58±0.03	0.61±0.05	0.139
CCA flow (ml min^{-1})	417.3±47.1	447.5±80.8	0.297
ICA velocity (cm s^{-1})	**49.2±9.2**	**60.4±8.0**	**0.021**
ICA diameter (cm)	0.46±0.03	0.49±0.05	0.257
ICA flow (ml min^{-1})	**251.3±51.7**	**338.5±37.5**	**0.002**
VA velocity (cm s^{-1})	**23.5±1.4**	**33.4±4.7**	**<0.001**
VA diameter (cm)	0.35±0.03	0.37±0.03	0.174
VA flow (ml min^{-1})	**67.3±10.0**	**107.0±6.2**	**<0.001**
MCA velocity (cm s^{-1})	**65.3±11.6**	**93.9±10.7**	**<0.001**
gCBF (ml min^{-1})	**637.2±93.1**	**895.7±70.5**	**0.003**

372 Values are mean±SD. BMI, body mass index; MAP, mean arterial pressure; HR, heart rate;
373 SpO_2, oxygen saturation; V_E, ventilation; V_A, alveolar ventilation; BF, breathing frequency; V_T,
374 tidal volume; $PetCO_2$, end-tidal partial pressure of carbon dioxide; VO_2, oxygen uptake; VCO_2,
375 carbon dioxide production; RER, respiratory exchange ratio; CCA, common carotid artery;
376 ICA, internal carotid artery; VA, vertebral artery; MCA, middle cerebral artery; gCBF, global
377 cerebral blood flow. *P* - values represent student's t-test results.
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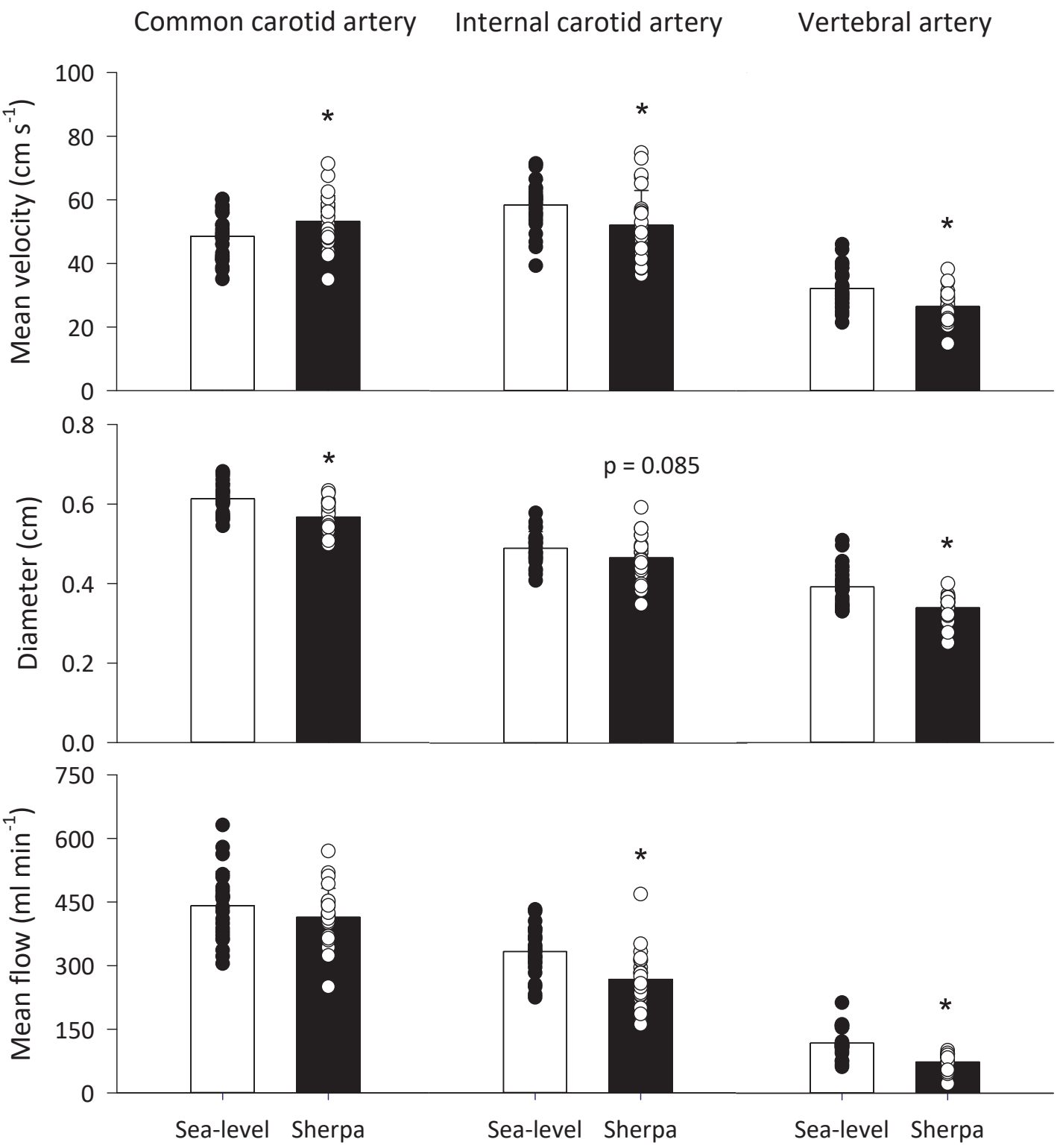
Figure captions

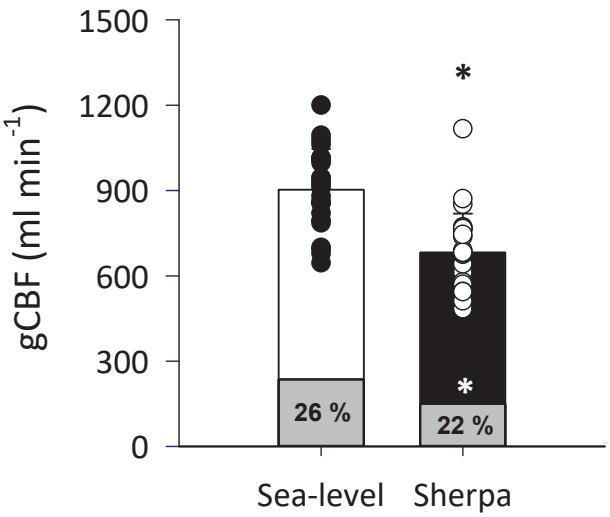
Figure 1

Mean velocity, diameter and mean blood flow of the common carotid, internal carotid and vertebral artery presented as mean and individual data in Sherpa children (black bars and open circles) and children from sea-level (open bars and black circles). *P* - values represent student's t-test results. * $P<0.05$ compared to children from sea-level.

Figure 2

Global cerebral blood flow in Sherpa children (black bars and open circles) and children from sea-level (open bars and black circles). Grey bars represent percentage of vertebral artery flow. * $P<0.05$ compared to children from sea-level.


Common carotid artery
Internal carotid artery
Vertebral artery
Mean velocity (cm s-1)
100
80
60
40
20
0
Diameter (cm)
0.8
0.6
0.4
0.2
0.0
p = 0.085
Mean flow (ml min-1)
750
600
450
300
150
0
*
Sea-level
Sherpa
Sea-level
Sherpa
Sea-level
Sherpa


1500
1200
900
600
300
0
gCBF (ml min-1)
26 %
22 %
Sea-level
Sherpa