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WM-S25: High Altitude Reduces Circulating Concentrations of Notch1 and VEGF-A, Independent of Acute Changes in Shear Rate

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Notch1 and VEGF-A at High Altitude

Abstract

Notch1 and vascular endothelial growth factor (VEGF-A) are known to be involved in endothelial responses to mechanical and hypoxic stimuli; however, their responses to mechanical stimuli (shear stress) following sustained hypoxic exposure, and their contribution to high altitude (HA) vascular physiology, remain poorly understood. Eighteen (9F) young, healthy lowlanders were tested at sea level (SL; ~340m) and on days 4-7 at HA (~3800m). Brachial arterial and antecubital venous blood samples were collected at baseline, following 20 min of axial (40 mmHg) and wrist (220 mmHg) blood pressure cuff inflation (low shear), and 1 min (high shear) and 15 min following wrist cuff deflation (recovery). Blood gases, Notch1 extracellular domain (NECD), and VEGF-A were measured during each shear phase. Brachial shear rate was quantified using Duplex ultrasound. Linear mixed models assessed NECD, VEGF-A, and net exchange (blood flow x a-v difference) across condition, shear phase, and sampling site, as appropriate. Circulating NECD and VEGF-A were significantly lower at HA (both $p < 0.001$); however, there were no effects of shear phase (NECD: $p=0.149$; VEGF-A: $p=0.444$) or sampling site (NECD: $p=0.683$; VEGF-A: $p=0.191$), and no condition x shear phase interactions (NECD: $p=0.872$; VEGF-A: $p=0.932$). The arterial-venous difference and exchange of NECD and VEGF-A did not differ between condition (NECD: $p=0.584$; VEGF-A: $p=0.094$) or shear phase (NECD: 0.304; VEGF-A: 0.141), with no condition x shear phase interactions (NECD: $p=0.658$; VEGF-A: $p=0.066$).

These findings indicate that 4-7 days of HA exposure at 3800m decreases NECD and VEGF-A concentrations, independent of acute changes in shear rate.

Keywords: Notch1, VEGF-A, hypobaric hypoxia, mechanosensors, shear stress.

Introduction

The vascular endothelium is highly sensitive to changes in blood flow patterns and oxygen availability (Davis et al. 2023; Tóth and Jeney 2025). During prolonged exposure to high altitude (HA), low blood oxygen (i.e., hypoxemia) is accompanied by alterations in vascular hemodynamics and endothelial shear stress (Hainsworth and Drinkhill 2007; Luks et al. 2017; Theunissen et al. 2022). Together, these responses create a unique mechanical and metabolic vascular milieu that can influence endothelial signalling pathways involved in vascular remodelling, angiogenesis, and the maintenance of endothelial function (Imray et al. 2010; Iglesias et al. 2015; O'Brien et al. 2022). Among the numerous molecules involved in these processes (e.g. nitric oxide, endothelin-1, reactive oxygen species) (Ni et al. 1998; Modesti et al. 2006; Stone et al. 2022), Notch1 and its interactions with vascular endothelial growth factor-A (VEGF-A) are of particular interest given their complementary roles in hypoxic and shear-mediated endothelial signalling (Dong et al. 2011; dela Paz et al. 2012; Mack et al. 2017). However, how Notch1 and VEGF-A respond to the combined influences of sustained hypoxia and vascular shear stress remains poorly understood.

The molecular signalling for chronic hypoxia-induced vascular adaptations (e.g., angiogenesis), is in part, mediated by the upregulation of hypoxia-inducible factor 1α (HIF-1α) (Ziello et al. 2007), which itself is a key regulator of vascular endothelial growth factor (VEGF-

A) (Forsythe et al. 1996). In turn, VEGF-A has been shown to increase with acute HA exposure in some (Walter et al. 2001; Dorward et al. 2007; Nourkami-Tutdibi et al. 2023), but not all studies (Fan et al. 2020). VEGF-A is a primary mediator of angiogenic signalling, which involves a complex, coordinated interplay between VEGF-A receptors (e.g., VEGFR), the endothelial mechanosensor Notch1, and Notch1 ligands (Benedito et al. 2009). In addition to its role in angiogenesis, Notch1 is imperative for maintaining endothelial function, as it is necessary for processes such as endothelial cell elongation, alignment with flow direction, and suppression of cell proliferation (Mack et al. 2017). Importantly, Notch1 signalling can be approximated from plasma samples by utilizing the Notch1 extracellular domain (NECD) as a surrogate, which is cleaved from Notch1 upon activation by increased shear stress (Mack et al. 2017; Badour et al. 2022).

Given the extensive crosstalk between Notch1 and VEGF-A, it is possible that Notch1 signalling may be altered at HA, in part through VEGF-A activity. Supporting the role of Notch1 in hypoxic vascular adaptation, the Notch1 gene and pathway regions are positively selected in Indigenous HA populations and have been implicated in vascular remodelling and acclimatization (O'Brien et al. 2022). However, acute hypoxia may reduce Notch1 and its primary ligands (Li et al. 2017). Taken together, the effects of acute (≤ 1 week) HA exposure on Notch1 are unclear, and it is unknown whether hypoxia influences shear-mediated responses. In addition, circulating NECD and VEGF-A have primarily been assessed in venous blood, leaving their arterial concentrations and responses to shear and hypoxia poorly understood. The present study addresses this gap by measuring both arterial and venous NECD and VEGF-A concentrations at SL and HA, providing further insight into their responses to sustained hypoxic exposure.

Accordingly, the primary purposes of this exploratory study were to: 1) characterize arterial and venous NECD and VEGF-A concentrations at SL and following 4-7 days of HA exposure; and 2) determine whether HA exposure modifies arterial and venous NECD and VEGF-A responses to an acute increase in vascular shear stress. These findings may provide new insight into how sustained hypoxia influences vascular responses to mechanical stimuli, with implications for understanding the mechanistic targets for endothelial adaptation to HA.

Methods

Participants

Eighteen (nine female) healthy lowlanders (21-35 years of age) participated in this study, as part of the White Mountain Summer 2025 expedition (WMS25). Participants were normotensive, non-obese (BMI: 25 ± 3 kg/m^2), and free from cardiovascular, respiratory, renal, hematological (e.g., severe anemia), and cerebrovascular diseases. All participants refrained from HA exposure (> 1500m) for at least three months prior to experimentation. The Research Ethics Board at the University of Guelph (REB no. 1487) and the University of Windsor (REB no. 25-049) approved all experimental procedures and protocols in adherence to the principles of the Tri-Council Policy Statement and the *Declaration of Helsinki,* aside from registration in a clinical database. Both written and verbal consent were obtained following a briefing of the protocol.

Experimental Design

1. Sea Level: All testing occurred in Guelph, Ontario (334 m). On the day of testing, participants arrived at the lab following an overnight fast and abstained from caffeine, alcohol, and

strenuous physical activity for a minimum of 12 hours. Venous (antecubital) and arterial (brachial) catheters were inserted into the participants' right arm, while in the supine position. Thereafter, pneumatic cuffs were secured around the right arm: one cuff on the wrist, inflated to 220 mmHg, and one on the upper arm below the axilla, inflated to 40 mmHg. The lower cuff was used to alter shear-stress patterns, while the upper cuff was used to 'trap' circulating proteins in the arm and avoid systemic dilution (Jenkins et al. 2013). This protocol is shown in Fig. 1, and we have previously provided evidence that the inclusion of the upper arm cuff alone does not alter NECD concentrations (Badour et al. 2022). Briefly, the wrist and upper cuffs were inflated simultaneously for 20 minutes, then the wrist cuff was deflated while the upper cuff remained inflated for another 15 minutes. Blood samples and ultrasound recordings for shear rates were taken at baseline (prior to cuff inflation), at the end of the 20-minute cuff inflation (low shear), 1-2 minutes following the rapid wrist cuff deflation (high shear), and 15 minutes following the wrist cuff deflation (recovery).

2. High Altitude: The SL protocol was repeated at Barcroft Research Station on White Mountain in California, USA (3800m), approximately two months following SL testing. Participants rapidly ascended to the research station by vehicle (~5-6 hours) from Las Vegas and refrained from prophylactic use of HA medication. Testing was conducted on days 4-7 to minimize variability from initial acclimatization, and AMS symptoms resolved for all participants by day 4.

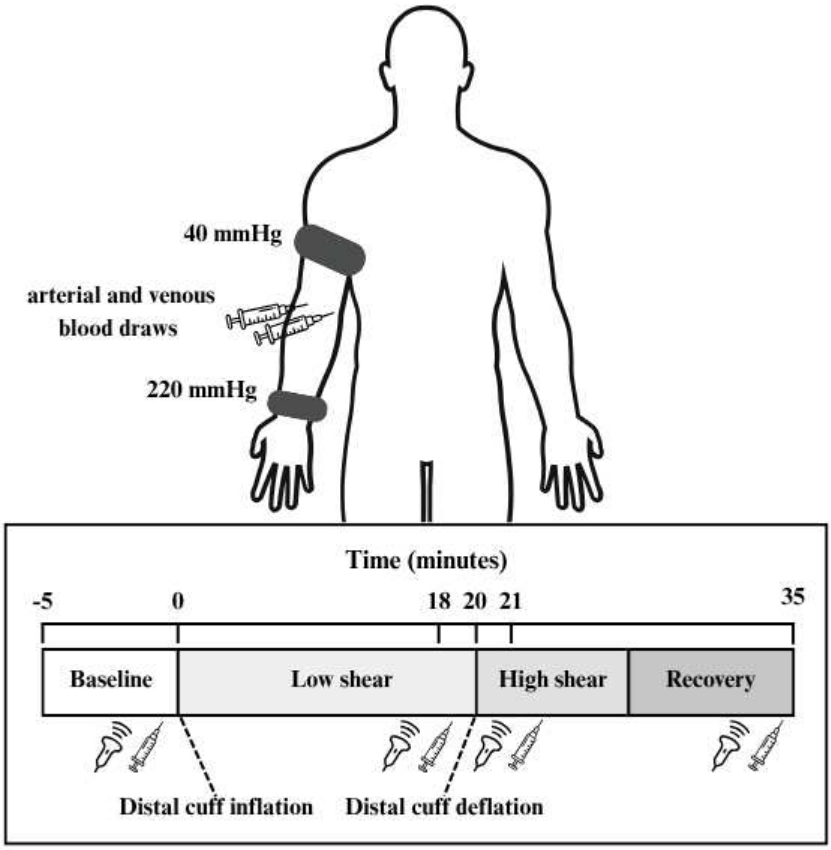


Fig. 1 Experimental design. The upper cuff was inflated to 40 mmHg to 'trap' circulating NECD and VEGF-A. The wrist cuff was inflated to 220 mmHg to reduce antegrade shear stress (i.e., low shear). The wrist cuff was then released to acutely increase antegrade shear (i.e., high shear). The vacutainers denote when blood samples were taken, and the ultrasound probes denote when the recordings occurred.

Quantification of NECD and VEGF-A

Whole blood was collected in sodium citrate tubes (Vacutainer, cat. No. 363080, BD Biosciences, San Jose, CA, USA). The blood was centrifuged for 10min at 1600-1800g, and at SL, plasma was extracted and immediately stored at -80°C. At HA, plasma was pipetted into 2mL

cryovials and then snap-frozen in liquid nitrogen (~-196°C) prior to being shipped to the University of Windsor, Canada for batch analysis.

Plasma samples were used to determine the concentrations of NECD using commercially available immunosorbent assays (RayBio, cat. no. ELH-NOTCH1, RayBiotech Life, Peachtree Corners, GA, USA), with an intra- and inter-assay coefficient of variation of <10% and <12%, respectively. The minimal detectable dose is 20 pg/mL. VEGF-A was also quantified using commercially available immunosorbent assays (Bio-Techne, cat. No. DVE00, USA R&D Systems Inc., Minneapolis, MN, USA), with an intra- and inter-assay coefficient of variation of < 7% and < 9%, respectively. The minimal detectable dose is < 9.0 pg/mL. All samples were read according to each manual on a microplate reader (BioTek Synergy HT, BioTek Instruments, Winooski, VT, USA) using Gen5 software (Version 1.11, BioTek Instruments).

Quantification of Forearm Shear Rates

Alterations in forearm shear rates were confirmed using Duplex vascular ultrasound (uSmart 3300, Terason, Burlington, MA, USA) of the brachial artery for ~1 minute before and after each blood draw. Ultrasound recordings were captured using screen recording software (Camtasia®) and analyzed offline using custom-designed edge-detection software (Woodman et al. 2001). Shear rate was calculated as 4 × the ratio between mean blood velocity (in cm/s) and artery diameter (in cm) [Shear Rate = 4 × (mean velocity/diameter)].

Quantification of Blood Gas and Metabolic Variables

Arterial blood samples were immediately analyzed for the partial pressure of arterial oxygen (PaO_2), partial pressure of arterial carbon dioxide ($PaCO_2$), SaO_2, pH, total hemoglobin (tHb), glucose, and lactate using a radiometer (ABL90 FLEX, Radiometer®, Copenhagen, Denmark).

Calculation of Exchange

The exchange rate of NECD and VEGF-A was calculated at baseline and during high shear by multiplying the arterial-venous concentration difference by brachial blood flow (mean envelope flow) and expressed as net mass exchange rate (pg/min). Accordingly, positive exchange values indicate net release across the forearm circulation, whereas negative values indicate net uptake.

Statistical Analyses

Data are presented as means ± standard deviation (SD). An a priori power calculation using G*Power (v.3.1.9.7), based on our previous research (Badour et al. 2022), indicated that a total sample size of 12 participants would provide > 80% power to detect increases in Notch1 following elevated shear stress, at an effect size of 0.40 (Cohen's *f*) with an alpha level of 0.05. Linear mixed models were used to examine brachial artery diameter, envelope velocity, mean envelope flow, and mean shear rate across condition (SL vs. HA) and shear phase (baseline, low shear, high shear, recovery). Linear mixed models were also used to examine circulating NECD and VEGF-A across condition, shear phase, and sampling site (arterial vs. venous), as well as the exchange of NECD and VEGF-A across condition and shear phase. For all models, condition x shear phase interactions were included as fixed effects, and participant ID was included as a random intercept to account for repeated measures within individuals. Two-tailed correlations were determined using Pearson's correlations. Baseline arterial blood gas and hemodynamic variables were compared between SL and HA using two-tailed Student's t-tests. Normality was assessed using the Shapiro-Wilk test. Statistical outliers of the raw data were identified as values exceeding twice the SD from the mean and were Winsorized to preserve sufficient statistical power (Ette and Onyiah 2002; Sharma and Chatterjee 2021; Yang et al. 2024). Statistical analyses were performed using JASP (JASP Team, Amsterdam, Netherlands) and PRISM (GraphPad, Boston, MA, USA).

Results

Baseline arterial blood gases and hemodynamic variables at SL and HA are presented in Table 1. Baseline PaO_2, $PaCO_2$, and SaO_2 were significantly reduced from SL to HA (all $p < 0.001$), whereas HR and DBP were significantly elevated ($p = 0.003$ and $p = 0.005$, respectively). SBP did not differ significantly between SL and HA ($p = 0.126$).

Table 1 Baseline arterial blood gases and hemodynamic variables at sea level and high altitude. Data are presented as mean ± SD (n = 17 except for HR, where n = 16). PaO_2 = partial pressure of arterial oxygen; $PaCO_2$ = partial pressure of arterial carbon dioxide; SaO_2 = arterial oxygen saturation; HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure. Significant values ($p<0.05$) are bolded.

Variable	Sea Level	High Altitude	*P* Value
PaO_2 (mmHg)	98.15 ± 10.22	54.66 ± 5.25	**< 0.001**
$PaCO_2$ (mmHg)	37.22 ± 2.48	29.05 ± 2.23	**< 0.001**
SaO_2 (%)	98.37 ± 0.66	88.65 ± 2.91	**< 0.001**
HR (bpm)	66 ± 6	74 ± 9	**0.003**
SBP (mmHg)	121 ± 8	125 ± 12	0.126
DBP (mmHg)	71 ± 8	78 ± 6	**0.005**

Brachial Artery Responses to Shear Manipulation at Sea Level and High Altitude

The linear mixed model results demonstrate a significant main effect of condition for mean brachial artery diameter [$F(1, 109.26) = 9.40$, $p = 0.003$], with a smaller blood vessel diameter at HA compared to SL. However, there was no main effect of shear phase ($p = 0.225$), and no

significant condition x shear phase interaction ($p = 0.578$). In contrast, there were significant main effects of shear phase for mean envelope blood velocity [$F(3, 101.18) = 254.87$, $p < 0.001$], mean envelope blood flow [$F(3, 102.22) = 140.56$, $p < 0.001$], and mean shear rate [$F(3, 97.31) = 203.39$, $p < 0.001$], with all variables decreasing during low shear, increasing during high shear compared to baseline, and partially returning toward baseline during recovery. However, there was no main effect of condition for mean envelope velocity ($p = 0.804$), mean envelope flow ($p = 0.322$), or mean shear rate ($p = 0.832$). There was also no significant condition x shear phase interaction for mean envelope velocity ($p = 0.329$), mean envelope flow ($p = 0.172$), or mean shear rate ($p = 0.792$).

NECD Responses to Varying Shear Rates at Sea Level and High Altitude

Results from the linear mixed model revealed a significant main effect of condition for NECD [$F(1, 227.81) = 21.89$, $p < 0.001$], with concentrations lower at HA than at SL (Fig. 2). However, there was no main effect of sampling site (arterial vs. venous) [$F(1, 228.48) = 0.17$, $p = 0.683$] or shear phase [$F(3, 227.02) = 1.79$, $p = 0.149$], and no significant condition x shear phase interaction was observed [$F(3, 227.02) = 0.23$, $p = 0.872$]. Individual responses across condition and shear phase in the arterial and venous circulation are illustrated in Fig. 2.

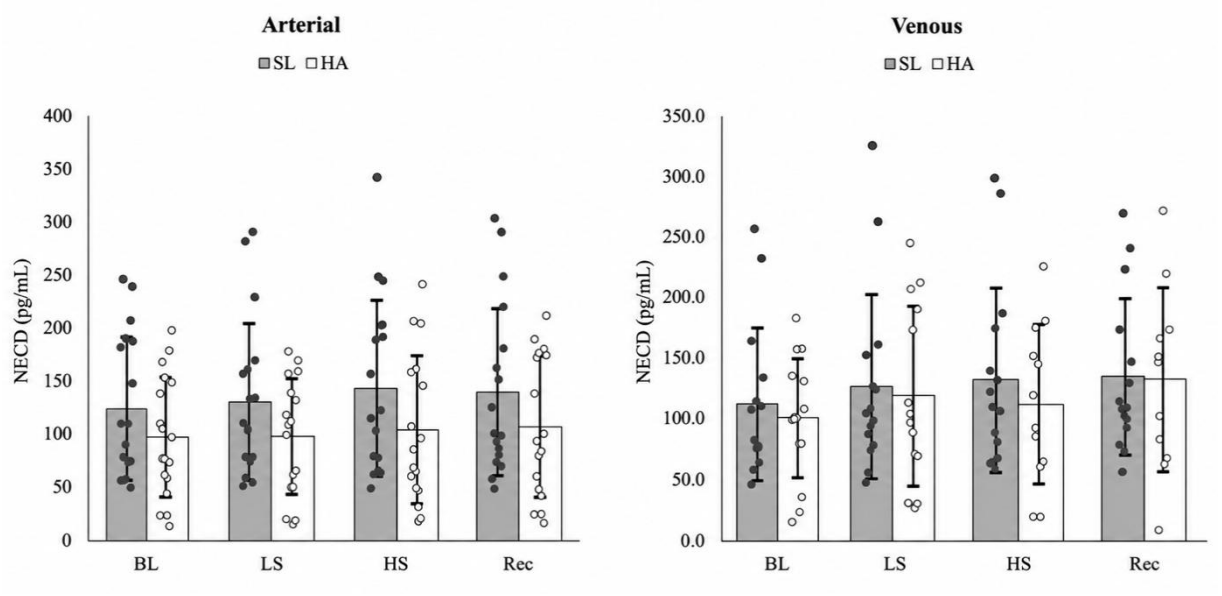
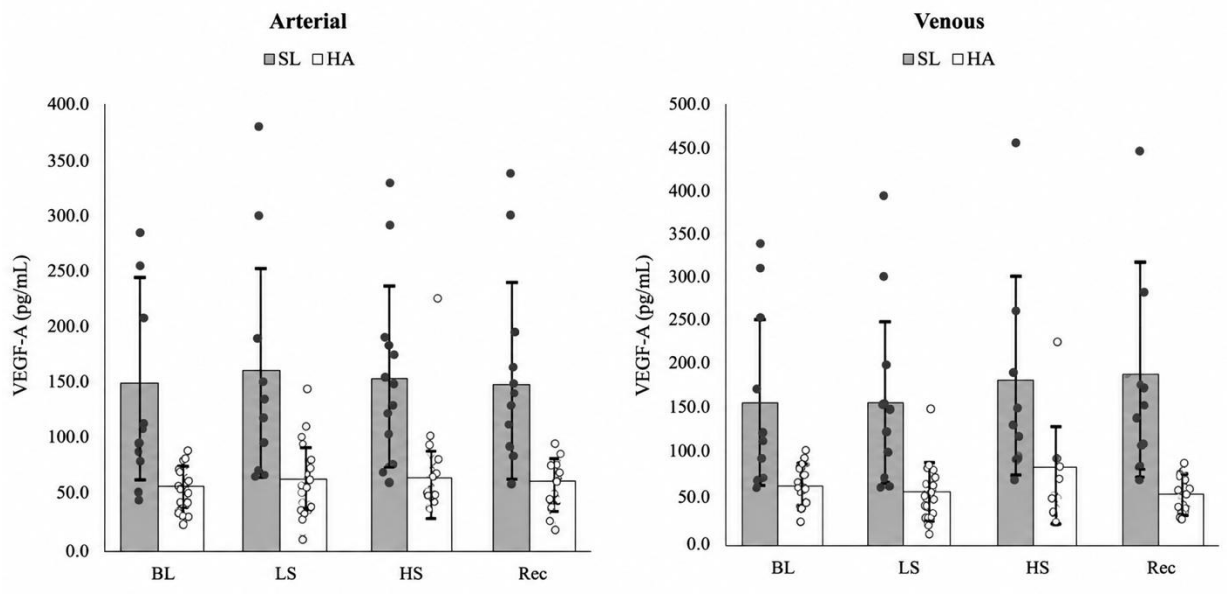


Fig. 2 Arterial (left) and venous (right) NECD concentrations during baseline (BL), low shear (LS), high shear (HS), and recovery (Rec) at sea level (SL) and high altitude (HA). Data are presented as mean ± SD with individual participant values. A linear mixed model revealed a significant condition effect (SL vs. HA; $p < 0.001$).

VEGF-A Responses to Varying Shear Rates at Sea Level and High Altitude

The linear mixed model revealed a significant main effect of condition for VEGF-A [$F(1, 228.34) = 171.14$, $p < 0.001$], with concentrations lower at HA than at SL (Fig. 3). However, there was no main effect of sampling site (arterial vs. venous) [$F(1, 229.43) = 1.72$, $p = 0.191$], or shear phase [$F(3, 226.99) = 0.90$, $p = 0.444$], and no significant condition x shear phase interaction was observed [$F(3, 226.99) = 0.147$, $p = 0.932$]. Individual responses across condition and shear phase in the arterial and venous circulation are shown in Fig. 3.



240

241 **Fig. 3** Arterial (left) and venous (right) VEGF-A concentrations during baseline (BL), low shear
242 (LS), high shear (HS), and recovery (Rec) at sea level (SL) and high altitude (HA). Data are
243 presented as mean ± SD with individual participant values. A linear mixed model revealed a
244 significant condition effect (SL vs. HA; $p < 0.001$).

245 ***Exchange of NECD and VEGF-A at Sea Level and High Altitude***

246 The linear mixed model revealed no significant main effect of condition for NECD
247 exchange [$F(1, 37.89) = 0.31$, $p = 0.584$] or shear phase [$F(1, 32.37) = 1.09$, $p = 0.304$], and no
248 condition x shear phase interaction was observed [$F(1, 34.51) = 0.20$, $p = 0.658$]. Individual NECD
249 exchange responses during baseline and high shear at SL and HA are presented in Fig. 4.

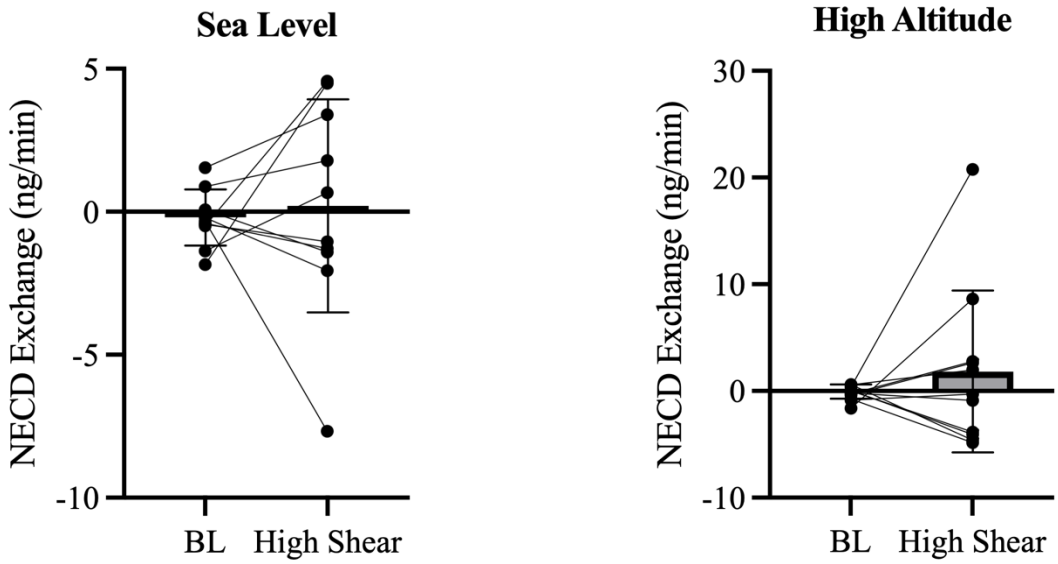


Fig. 4 The exchange of NECD during baseline (BL) and high shear at sea level (left; n = 10, 2 outliers removed) and high altitude (right; n = 11, 1 outlier removed). Individual participants are connected by lines. Bars represent mean ± SD. Positive values denote net release, whereas negative values denote net uptake. A linear mixed model demonstrated no significant condition (Sea level vs. high altitude; $p = 0.584$) or shear phase (BL vs. high shear; $p = 0.304$) effects.

Results from the linear mixed model revealed no significant main effect of condition for VEGF-A exchange [$F(1, 37.27) = 2.95$, $p = 0.094$], or shear phase [$F(1, 33.04) = 2.27$, $p = 0.141$], and no significant condition x shear phase interaction was observed [$F(1, 31.94) = 3.63$, $p = 0.066$]. Individual VEGF-A exchange responses during baseline and high shear at SL and HA are presented in Fig. 5.

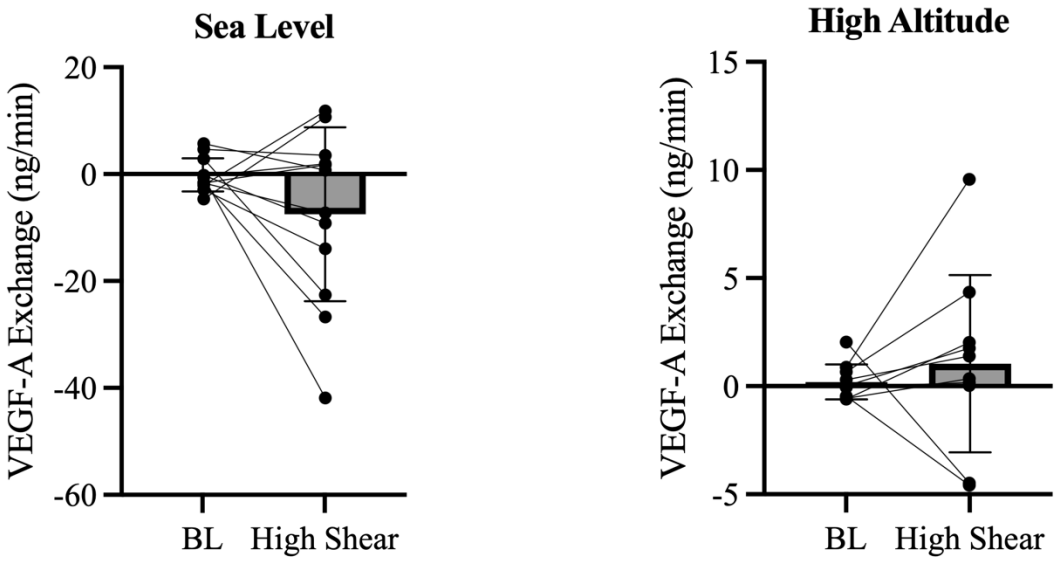


Fig. 5 The exchange of VEGF-A during baseline (BL) and high shear at sea level (left; n = 12, 1 outlier removed) and high altitude (right; n = 10, 2 outliers removed). Individual participants are connected by lines. Bars represent mean ± SD. Positive values denote net release, whereas negative values denote net uptake. A linear mixed model demonstrated no significant condition (Sea level vs. high altitude; p = 0.094) or shear phase (BL vs. high shear; p = 0.141) effects.

Exploratory Associations with SaO_2

Individual SaO_2 was not related to the percentage change in baseline NECD from SL to HA in the arterial circulation (p = 0.951, r = -0.017) or venous circulation (p = 0.277, r = -0.342). Similarly, SaO_2 was not related to the percentage change in baseline VEGF-A from SL to HA in the arterial circulation (p = 0.281, r = 0.278) or venous circulation (p = 0.868, r = -0.054).

Discussion

The purpose of this study was to investigate the effects of HA on arterial and venous forearm circulating concentrations of NECD and VEGF-A in response to acute bouts of increased shear rates The primary findings were that: 1) concentrations of both arterial and venous NECD and VEGF-A were significantly lower at HA, but were unaffected by low and high shear rates, and 2) the arterial-venous exchange of NECD and VEGF-A were not impacted by shear rate or altitude. Collectively, these findings indicate that acute HA exposure reduces circulating concentrations of NECD and VEGF-A, but does not alter their peripheral exchange.

NECD Responses to High-Altitude Exposure

To our knowledge, this is the first study to examine the *in vivo* response of NECD to 4-7 days of HA exposure in humans. The marked decrease in baseline NECD at HA compared to SL (arterial: ~33% reduction; venous: ~13% reduction) indicates that the vascular Notch1 pathway is particularly sensitive to hypobaric hypoxic conditions. In support of our findings, human umbilical vein endothelial cells (HUVECs) exposed to hypoxia demonstrated reduced Notch1 and its primary ligands: delta-like ligand 4 (Dll4) and Jagged1 (Li et al. 2017). Additionally, VEGF has been shown to promote Dll4 in endothelial cells (Patel et al. 2005), whereas reduced VEGF leads to reduced Dll4 expression (Lobov et al. 2007). Therefore, it is plausible that the reduction in circulating VEGF-A at HA shown in the present study may have contributed to reduced Dll4, potentially decreasing Notch1 activation and, as a result, NECD. That said, it is important to note that this remains speculative, as we did not measure ligand availability, receptor density, or receptor-ligand affinity. Interestingly, the positive selection involving *NOTCH1* identified in HA natives (O'Brien et al. 2022), combined with the reduced baseline circulating NECD observed in the present study, suggests that the Notch1 pathway may be relevant to vascular responses during both acute HA exposure and long-term evolutionary adaptations to HA. However, the functional

consequences of the positively selected variants and their precise implications for hypoxic vascular adaptations remain to be determined.

In contrast to the condition effect, NECD concentrations did not significantly differ between arterial and venous samples. There was also no main effect of shear phase, despite significantly altered shear rate throughout the protocol. While this finding diverges from Badour et al. (2022), who demonstrated significantly altered NECD in response to changes in shear, exploratory paired Student's t-tests revealed significant differences between baseline and high shear NECD at SL (arterial: $p = 0.030$; venous: $p = 0.010$), consistent with Badour et al. (2022), whereas this was not demonstrated at HA (arterial: $p = 0.222$; venous: $p = 0.239$). Therefore, the absence of an overall shear phase effect in the linear mixed model may reflect the inclusion of both the SL and HA conditions.

VEGF-A Responses to High-Altitude Exposure

Similar to NECD, overall circulating VEGF-A concentrations were reduced by altitude but not impacted by shear phase, and concentrations of VEGF-A were not significantly different between the arterial and venous samples. Our findings are aligned with other studies demonstrating significant reductions in VEGF-A at HA (Fan et al. 2020) and acute (30-minute) hypoxic exposure in a simulation chamber (Oltmanns et al. 2006). However, these findings are not universal. Other reports indicate that VEGF-A concentrations increase with acute HA exposure (Walter et al. 2001; Dorward et al. 2007; Nourkami-Tutdibi et al. 2023). Discrepancies in the response of VEGF-A to HA across studies may be partly explained by the level of exertion and rate of ascent. In the present study, participants rapidly ascended by vehicle, whereas participants in studies demonstrating increases in VEGF-A actively ascended (i.e., trekked) for several hours to days before reaching their destination (Walter et al. 2001; Dorward et al. 2007; Nourkami-Tutdibi et al. 2023).

Therefore, the increased oxygen demands associated with physical activity (prolonged trekking) (Dominelli et al. 2021) paired with reduced oxygen tension at HA may have stimulated the VEGF-A pathway, potentially through HIF-1α (Zoll et al. 2006). Moreover, the difference may be due to circulating levels of soluble VEGF receptor, otherwise known as soluble Fms-like tyrosine kinase 1 (sFlt-1), which is upregulated by hypoxia (Gerber et al. 1997). Circulating VEGF-A binds to sFlt-1, thereby reducing the ligand's bioavailability and subsequent angiogenic signalling (Kendall et al. 1996). Plasma sFlt-1 has been shown to increase significantly during acute HA exposure compared to SL and corresponds to altered VEGF-A levels, whereby participants with elevated sFlt-1 experienced reduced free circulating VEGF-A compared to those with lower sFlt-1 (Tissot van Patot et al. 2005).

The significant condition effect, despite the absence of acute changes in response to varying forms of shear rates, may reflect delayed VEGF-A responsiveness, as peak concentrations have been reported several days following stimulation (Slevin et al. 2000). Circulating VEGF-A, at least in vivo in humans, may also be more sensitive to sustained environmental stimuli, such as hypoxia (Tammela et al. 2005; Pramkratok et al. 2022), than to relatively brief mechanical stimuli, such as acutely altered shear stress. This is consistent with our previous studies demonstrating no significant changes in venous VEGF-A in response to varying forms of shear stimuli (Shepley et al. 2025, 2026).

Peripheral Exchange of NECD and VEGF-A

Although Notch1 is expressed in both arteries and veins, the primary canonical ligand, Dll4, is predominantly expressed in arteries, contributing to greater arterial Notch1 activity than in veins (Jahnsen et al. 2015; Mack et al. 2017). Therefore, arterial endothelium may be an

important source of circulating NECD. Accordingly, simultaneous arterial and venous measurements allow for the assessment of local NECD dynamics across the forearm vascular bed.

Despite the influence of altitude on absolute circulating NECD and VEGF-A, arterial and venous concentrations were relatively similar, and peripheral exchange of NECD and VEGF-A did not differ significantly between SL and HA. In other words, the net uptake or release of NECD and VEGF-A did not differ by condition and was not altered across varying shear rates. Thus, the reductions in NECD and VEGF-A do not appear to be a result of altered local forearm exchange. These findings suggest that processes outside of the forearm contribute to the clearance of circulating NECD and VEGF-A. For example, rat liver sinusoidal endothelial cells have been shown to endocytose and degrade VEGF-A (Mousavi et al. 2019). Moreover, hepatic blood flow has been shown to increase at HA (Kalson et al. 2010); therefore, hepatic clearance may contribute to circulating VEGF-A concentrations. However, systemic clearance and degradation of circulating NECD remains unexplored. The lack of a shear phase effect on peripheral exchange may also reflect the relatively small changes in circulating NECD and VEGF-A across shear phases. Moreover, the null findings may be partly attributable to the limited sample size resulting from several missing venous samples, primarily at HA, in addition to interindividual variability in exchange responses.

Nevertheless, the absence of a significant difference between arterial and venous NECD and VEGF-A concentrations, in conjunction with no difference in peripheral exchange, may have methodological implications for future research, as our findings suggest that venous sampling may yield results similar to arterial sampling when invasive arterial catheterization is not feasible. However, this would require dedicated agreement analyses, which were not conducted in the present study.

Negligible Influence of SaO_2 on NECD and VEGF-A Activity

As an exploratory analysis, we found that changes in NECD and VEGF-A were unrelated to SaO_2 between SL and HA, suggesting factors beyond the degree of hypoxemia alone may contribute to alterations in NECD and VEGF-A concentrations. One potential variable that may account for the baseline reductions in NECD and VEGF-A from SL to HA is altered inflammatory signalling. Specifically, HA is associated with activation of inflammatory pathways, including those involving tumour necrosis factor-alpha (Iglesias et al. 2015; Nourkami-Tutdibi et al. 2023; Cai et al. 2026), which interact with the Notch1 (Tirziu et al. 2012; Shang et al. 2016; Fazio and Ricciardiello 2016; Wang et al. 2017) and VEGF-A (Fujita et al. 2002; Failla et al. 2024) pathways. In addition to inflammation, exposure to HA increases oxidative stress (Mrakic-Sposta et al. 2021; Zhang et al. 2021), which impairs endothelial function (Stone et al. 2022), and may therefore influence endothelial Notch1 and VEGF-A. Indeed, oxidative stress has been shown to modulate Notch1 signalling in HUVECs (Yang et al., 2013) and is associated with serum VEGF-A in patients with breast cancer (Pande et al. 2011). However, the present study did not measure inflammatory biomarkers; therefore, it remains unclear whether inflammation or oxidative stress contributed to changes in circulating NECD and VEGF-A. Additionally, although shear rate remained relatively stable from SL to HA, NECD and VEGF-A may have been influenced by other mechanical stimuli. The significantly reduced brachial diameter, combined with elevated diastolic blood pressure at HA, may have altered mechanical forces experienced by the endothelium, including radial stress (Davis et al. 2023). However, this warrants further investigation, as radial stress was not measured in the present study.

Study Considerations

A few considerations should be noted. First, this study was conducted on days 4-7 at HA. Therefore, obtaining additional blood samples upon arrival (e.g., days 1-2) would be beneficial for gaining a more complete understanding of the effects of HA on NECD and VEGF-A during the acute acclimatization period. Second, due to the study being conducted over three days, participants may have experienced varying degrees of acclimatization (e.g., days 4 vs. 7). However, this was the only feasible way to schedule participants, given the nature of the expedition, which involved several simultaneous studies. Moreover, our sample size was relatively small for venous samples collected at HA, where venous collapse during blood collection reduced the final sample size, which limited the exchange comparisons (n = 10-12). Therefore, future research should consider larger sample sizes with more diverse populations to improve the generalizability of these findings. We were unable to acquire viscosity data at HA due to equipment malfunctions; therefore, we used shear rate instead of shear stress. Changes in plasma volume were also not assessed, which may have influenced circulating NECD and VEGF-A concentrations at HA. Additionally, due to the limited settings of the available centrifuge at HA, the speed varied by ~200 x g compared with SL; however, this is unlikely to significantly affect NECD and VEGF-A concentrations (Sjöbom et al. 2022). Lastly, while NECD is an indirect measure of Notch1 activity, it is the only feasible, minimally invasive method to assess Notch1 activation in vivo in humans.

Conclusion

In conclusion, circulating concentrations of NECD and VEGF-A were significantly lower at HA than at SL; however, the exchange kinetics of NECD and VEGF-A did not differ at HA compared with SL. Moreover, changes in baseline NECD and VEGF-A from SL to HA were unrelated to SaO_2. Together, these findings suggest that circulating NECD and VEGF-A are acutely

sensitive to HA exposure, but the magnitude of these responses may not be solely explained by arterial oxygen saturation. This research provides novel insight into vascular endothelial mechanosignalling during 4-7 days of HA exposure and may inform understanding of these pathways in other hypoxic environments.

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Conflict of Interest

No conflicts of interest are declared by the authors.

Author Contributions

ARB conceived the study. BRS and ARB analyzed the data and drafted the study. All authors contributed to data collection and revised the final version of the manuscript.

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