Plasma Vascular Endothelial Growth Factor in Acute Mountain Sickness*

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Study objectives: To investigate the hypothesis that an increase in circulating vascular endothelial growth factor (VEGF) occurs in mountaineers at high altitude, particularly in association with acute mountain sickness (AMS) and/or low hemoglobin oxygen saturation.

Design: Collection of medical histories, AMS scores, plasma samples, and arterial oxygen saturation (SaO₂) measurements from mountaineers at 1,500 feet (sea level) and at 14,200 feet.

Setting: Mount McKinley (“Denali”), AK.

Participants: Sixty-six mountaineers.

Interventions: None.

Measurements and results: Plasma VEGF at 14,200 feet was not increased in any group. In fact, plasma VEGF was significantly lower in subjects who did not develop AMS (53 ± 7.9 pg/mL; mean ± SEM; n = 47) compared to control subjects at sea level (98.4 ± 14.3 pg/mL; n = 7; p = 0.005). Plasma VEGF at 14,200 feet for subjects with AMS (62 ± 12 pg/mL; n = 15) did not differ significantly from subjects at 14,200 feet without AMS, or from control subjects at sea level.

Of a small number of subjects with paired specimens at sea level and at base camp (n = 5), subjects who exhibited a decrease in plasma VEGF at 14,200 feet were those who did not develop AMS. Neither SaO₂, prior AMS, AMS symptom scores, or acetazolamide use were correlated with plasma VEGF.

Conclusions: Subjects at high altitude who do not develop AMS have lower plasma VEGF levels compared to control subjects at sea level. Plasma VEGF at high altitude is not elevated in association with AMS or hypoxia. Sustained plasma VEGF at altitude may reflect a phenotype more susceptible to AMS.

Key words: acute mountain sickness; endothelial permeability; high-altitude pulmonary edema; vascular endothelial growth factor

Abbreviations: AMS = acute mountain sickness; HAPE = high-altitude pulmonary edema; SaO₂ = arterial oxygen saturation; VEGF = vascular endothelial growth factor

Rapid altitude ascent can lead to acute mountain sickness (AMS). This syndrome of fatigue, anorexia, insomnia, nausea, and headache is common among those who travel to mountain heights. The pathophysiologic features of AMS may include increased systemic capillary permeability, although this is controversial. Other mechanisms proposed to explain the pathology of AMS include inflammatory mediator release at altitude based on urine and blood indexes, effects of hypoxia on the gene expression of vasoactive mediators, endothelial activation/injury, and fluid retention. The symptom complex of AMS and the related syndrome of high-altitude cerebral edema suggest a central role for brain abnormalities, particularly edema, in the genesis of AMS. Characteristically, AMS improves with oxygen (and descent), suggesting a central role for alveolar hypoxia.

Vascular endothelial growth factor (VEGF) is an endothelial-specific mitogen with potent endothelial permeability enhancing properties and is expressed prominently in the lung, choroid plexus of the brain, and in platelets. The potent endothelial permeability effects of VEGF, attributable to receptor-mediated guanylate cyclase activation, nitric oxide synthase induction, and subsequent nitric oxide generation are demonstrable both in vivo, where
VEGF has a potency 10,000 times that of histamine, and induces endothelial fenestration, and in vitro, where VEGF promotes leak in endothelial monolayers at picomolar concentrations, including in brain microvascular endothelial cells. Hypoxia increases VEGF gene expression and protein content in animal models. Interestingly, ischemia increases brain expression and content of VEGF protein and hypoxia increases brain expression of VEGF protein and receptors in animal models. In human brain tumors, VEGF expression parallels the degree of surrounding edema. Of note, corticosteroids block hypoxic upregulation of VEGF protein in vitro, and are effective in the prophylaxis and treatment of AMS.

We hypothesized that plasma VEGF levels would be elevated in mountaineers at high altitude following increased VEGF gene expression and increased tissue VEGF release, a postulate supported by the studies above and evidence of increased circulating VEGF protein and receptors in animal models. We postulated studies above and evidence of increased circulating VEGF release, a postulate supported by the increased VEGF gene expression and increased tissue content of VEGF protein and hypoxia increases brain expression of VEGF protein and receptors in animal models. In human brain tumors, VEGF expression parallels the degree of surrounding edema. Of note, corticosteroids block hypoxic upregulation of VEGF protein in vitro, and are effective in the prophylaxis and treatment of AMS.

We hypothesized that plasma VEGF levels would be elevated in mountaineers at high altitude following increased VEGF gene expression and increased tissue VEGF release, a postulate supported by the studies above and evidence of increased circulating VEGF in hypoxic animal models. We postulated that plasma VEGF elevations would be particularly evident in those subjects with AMS and lower oxygen saturations. Two of us (D.W., T.D.) obtained medical histories, plasma samples, and simultaneous pulse oximetry readings from mountaineers at sea level, and later at a high base camp (14,200 feet) on Mt. McKinley, AK. We correlated plasma VEGF levels to oximetry readings, the presence of AMS or high-altitude pulmonary edema (HAPE), prior history of altitude illness, acetazolamide use, and AMS self-reported symptom scores.

### Materials and Methods

#### Setting and Participants

Prior study approval was obtained through the Stanford University Institutional Review Board. We studied mountaineers after informed consent during the 1996 climbing season on Mt. McKinley ("Denali"; summit altitude, 6,150 m). Plasma was first collected from mountaineers at low altitude (Talkeetna, AK; 1,500 feet; n = 7) before ascent; these subjects were designated as “sea level” control subjects. Subsequently, samples were collected in a medical hut at a high base camp (14,200 feet; 4,200 m) below the west buttress approach of Mt. McKinley during a rest period before summit attempts (only two subjects were enrolled after summit descent). Symptomatic mountaineers were approached after they sought shelter and aid at the medical hut, and asymptomatic mountaineers were approached throughout the high base camp. The subjects at 14,200 feet were classified as those with AMS (n = 15), those without AMS ("no AMS"; n = 47), and those with HAPE (n = 2). The two subjects with HAPE were not included as part of the AMS group. No subject had high-altitude cerebral edema diagnosed. At least 1 day of rest passed before phlebotomy after the climb from the airstrip embarkation site (1,500 feet) to high base camp was completed. Pulse oximetry readings (simultaneous with phlebotomy) were obtained from mountaineers at 14,200 feet (but not at sea level) with a portable oximeter after hand warming. Paired specimens were available for five subjects both at sea level and at 14,200 feet (two of the seven sea level subjects were not subsequently located at base camp). All subjects denied use of dexamethasone. There were five smokers. Acetazolamide use and other group characteristics are shown in Table 1.

#### Definition of Illness

The diagnoses of AMS and HAPE were made based on the definitions endorsed by the 1990 Lake Louise Consensus Committee guidelines. Headache and the presence of one other symptom (GI symptoms, fatigue and/or weakness, dizziness or lightheadedness, or sleep disturbance) on the AMS self-report score was required for AMS case definition. Each of these symptoms was graded on a 0- to 3-point scale and summed. To meet the definition of HAPE, subjects had to have at least two symptoms (dyspnea at rest, cough, decrement in prior exertional tolerance, chest tightness) and two signs (rales, resting heart rate > 100 beats/min, resting respiratory rate > 24 breaths/min, or arterial oxygen saturation (SaO₂) < 80%, consistent with HAPE. Radiography was not performed.

#### Sample Handling and Analysis

Blood was drawn by venipuncture into citrate-containing tubes and allowed to stand 20 to 60 min before centrifugation in a clinical centrifuge (powered by solar converter) at 2,000g for 15 min. The plasma supernatant was kept frozen until the time of assay. After shipment of frozen specimens on ice to Talkeetna, AK, the specimens were then placed on dry ice and transported by air to Palo Alto, CA. Samples were thereafter frozen at −70°C.

### Table 1—Demographics

<table>
<thead>
<tr>
<th>Variables</th>
<th>AMS</th>
<th>No AMS</th>
<th>HAPE</th>
<th>Sea Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, No.</td>
<td>15</td>
<td>47</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Mean age, yr</td>
<td>37</td>
<td>35</td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>Female, No. (%)</td>
<td>2 (13)</td>
<td>4 (9)</td>
<td>0</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Prior AMS, No. (%)</td>
<td>4 (27)†</td>
<td>20 (42)†</td>
<td>0</td>
<td>3 (43)</td>
</tr>
<tr>
<td>Acetazolamide use, No.</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dexamethasone use, No.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean ascent to camp, d</td>
<td>6.3†</td>
<td>7.5†</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mean time at camp, d</td>
<td>1.8†</td>
<td>2.9†</td>
<td>4.8</td>
<td>NA</td>
</tr>
</tbody>
</table>

*NA = not available.
†p < 0.05 between AMS and No AMS groups.
until shipment on dry ice to Denver, CO, for final analysis. Plasma use was selected, as serum VEGF levels predominantly reflect \textit{ex vivo} platelet VEGF release during clotting of serum.\textsuperscript{25} Determination of plasma VEGF levels was by commercial enzyme-linked immunosorbent assay (R&D Systems; Minneapolis, MN). Samples were assayed in duplicate with a Titertek Multiskan MC plate reader (Flow Laboratories; Helsinki, Finland). VEGF concentration was determined by linear regression from a standard curve using GraphPad software (GraphPad; San Diego, CA).

\textbf{Statistical Analysis}

The data for groups are expressed as mean \pm SEM. Samples were paired for only five of the seven individual mountaineers sampled at both sea level and base camp; this data group was too small for meaningful statistics. Comparison between groups was performed using SAS software (SAS Institute; Cary, NC) and GraphPad software (GraphPad) with nonparametric testing by Kruskal-Wallis analysis. Correlation coefficients by Spearman analysis were used to examine relationships between plasma VEGF levels and simultaneous \(\text{Sa}_\text{o}_2\) readings and AMS self-report scores. Comparisons between only AMS and no-AMS groups were by Mann-Whitney \(U\) test. Statistical significance was defined at the \(p < 0.05\) level.

\textbf{Results}

We enrolled mountaineers at sea level and at 14,200 feet before further ascent. Subjects at 14,200 feet were grouped into those with AMS (\(n = 15\)), those without AMS (\(n = 47\)), and those with HAPE (\(n = 2\)). Demographics are shown in Table 1. Five subjects had paired specimens collected at both sea level and later at 14,200 feet. As only two HAPE subjects were enrolled, their data is shown graphically, but was not further analyzed.

\textit{Plasma VEGF Levels}

The group of mountaineers at 14,200 feet who did not develop AMS had significantly lower plasma VEGF levels (53 \(\pm 7.9\) pg/mL; range, 0 to 281 pg/mL; \(n = 47\); mean \(\pm\) SEM) than those of the control group at sea level (98.4 \(\pm 14.3\) pg/mL; range 54 to 156 pg/mL; \(n = 7\); \(p = 0.005\); Fig 1). Plasma VEGF levels did not differ significantly between mountaineers at 14,200 feet with AMS (62 \(\pm 12\) pg/mL; range, 4 to 170 pg/mL; \(n = 15\)) compared to those without AMS (\(p = 0.28\)). VEGF levels were not significantly different between the group that developed AMS as compared to sea level control subjects, although VEGF levels were decreased as a trend (\(p = 0.07\)). When the highest VEGF level in the AMS group was eliminated as an outlier, the AMS group remained not significantly different than the sea level group (\(p > 0.05\)). One of the two subjects with HAPE had a plasma VEGF level that was the highest of all subjects (302 pg/mL), while the other subject had a plasma VEGF level of 41 pg/mL.

Five mountaineers had paired samples from sea level and later at base camp. Of these five subjects, the two who developed AMS had absolute increases in their plasma VEGF at base camp compared to sea level. The three subjects who did not develop AMS all had absolute decreases in plasma VEGF at base camp compared to sea level (Fig 2). Of all the subjects enrolled at base camp, two had already returned from a summit climb of Mt. McKinley; both had undetectable VEGF levels and neither had AMS.

\textbf{Figure 1.} Scattergraph of plasma VEGF levels expressed in picograms per milliliter for mountaineers at sea level (SEA, \(n = 7\)); and at 14,200 feet with AMS (\(n = 15\)), without AMS (no-AMS; \(n = 47\)), and with HAPE (\(n = 2\)). The bar indicates the median of each group. Plasma VEGF at 14,200 feet was significantly lower in subjects who did not develop AMS compared to sea level control subjects. Other groups did not differ statistically in comparisons.

\textbf{Figure 2.} Paired collections for plasma VEGF at sea level (sea; squares) and subsequently at base camp (basecamp; triangles) for five individuals. The three subjects who did not develop AMS all had individual decreases in plasma VEGF at altitude vs sea level (broken lines). The two subjects who developed AMS both had individual increases in plasma VEGF at altitude vs sea level (solid lines). Number too small for statistics.
Correlation of Plasma VEGF With Other Variables

Sao2 by simultaneous finger oximetry was not correlated with plasma VEGF levels (all groups; Fig 3). This was also true when the subjects who developed AMS and those who did not were analyzed separately. Those subjects who used prophylactic acetazolamide did not have different VEGF levels than those who did not use this drug (acetazolamide group, 65 ± 18 pg/ml; range 32 to 151 pg/ml; n = 6; p = 0.49). Subjects with a prior history of AMS did not have VEGF levels that differed from the subjects without a history of altitude illness: prior history AMS (55 ± 7 pg/ml; range, 0 to 151 pg/ml; n = 22) vs no prior history AMS (63 ± 11 pg/ml; range, 0 to 302 pg/ml; n = 42; p = 0.52). VEGF levels were also not correlated with the AMS self-report score (r² = 0.05), or days at 14,200 feet (r² = 0.09). Plasma VEGF was not different in the subjects who smoked (n = 5), who ingested aspirin/anti-inflammatory drugs (n = 7), or in women (n = 6; data not shown). There was a significantly higher prevalence of a prior AMS event in the group who did not develop AMS in this study (Table 1). Days at 14,200 feet and days of ascent were also significantly less in the AMS group, compared to the group without AMS (Table 1).

Discussion

We found that plasma VEGF at 14,200 feet was lower in mountaineers who did not develop AMS when compared to sea level control subjects. While plasma VEGF was lower as a trend overall at altitude, a significant decrease was only seen in the group who did not develop AMS. Plasma VEGF was not different between groups with or without AMS (Fig 1). Interestingly, of five subjects with paired plasma collections, those who developed AMS had an increase in plasma VEGF at altitude, while those without AMS had decreased plasma VEGF at altitude (Fig 2). Plasma VEGF was not correlated with oxygen desaturation (Fig 3) nor time at base camp. Only two subjects developed HAPE, a number that precluded further analysis. The finding that times of ascent to base camp and times at base camp were less in the AMS group is consistent with prior reports of AMS susceptibility.26 More of those without AMS in our study had prior AMS events, and ascended more slowly for this climb than the AMS group (Table 1).

This is the first report of circulating VEGF levels at high altitude in humans. Prior reports on the effects of high altitude on circulating VEGF levels in humans are limited to one study reporting decreased serum VEGF in eight elite athletes after running over mountain passes.27 Overall, our data suggest that mountaineers with sustained circulating VEGF levels at high altitude are more susceptible to AMS. While the VEGF concentrations we report are less than those needed to elicit capillary leak in normoxic states,15 this is not inconsistent with a role for VEGF in AMS. Blood VEGF may be an index that parallels much higher tissue VEGF levels, as appears to be true in cancer patients, where circulating VEGF is correlated with tumor burden,28 and in rheumatoid arthritis29 where circulating VEGF parallels indexes of disease activity. Furthermore, VEGF receptors increase in many organs, including the brain, during hypoxia; persistence of normal blood and tissue VEGF concentrations in this setting may reflect a phenotype more prone to AMS. Decreased plasma VEGF in mountaineers without AMS may in part be due to enhanced renal VEGF clearance, a postulate supported by prior descriptions of renal VEGF clearance30 and of fluid retention in subjects more susceptible to AMS.26 Little is known of other clearance mechanisms for VEGF in humans. Importantly, the effects of prolonged hypoxia on VEGF tissue expression (or blood levels) in humans have not been reported. The apparent predilection for capillary leak in brain during altitude illness suggests that brain edema may be a result of rapid induction of leak mediators such as VEGF.10 If this is true, the known steroid-mediated downregulation of VEGF gene expression in hypoxic environments in vitro could help explain the efficacy of dexamethasone in the prophylaxis and treatment of AMS.

Our study has limitations. The mean plasma VEGF of our control subjects was higher than prior reports for healthy subjects at 5,280 feet13 or at sea level.32 Nonetheless, our collection procedures were

![Correlation of plasma VEGF levels in picograms per milliliter and simultaneous pulse oximetry readings (Sao2%) for all 47 subjects at 14,200 feet, regardless of symptoms. No linear correlation exists.](http://publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21949/)

**Figure 3.** Correlation of plasma VEGF levels in picograms per milliliter and simultaneous pulse oximetry readings (Sao2%) for all 47 subjects at 14,200 feet, regardless of symptoms. No linear correlation exists.
uniformly applied to prevent intergroup artifacts. Our control group was small, and the collection of a larger number of paired samples would have aided our analysis. We also did not collect urine samples, which could have resolved whether VEGF undergoes enhanced renal clearance in subjects who do not develop AMS. Finally, while we did not seek paired collections of serum, these may have yielded complementary data for our study.

CONCLUSION

We conclude that sustained plasma VEGF at high altitude is a feature of subjects more prone to AMS. Decreased plasma VEGF may also be a feature of high altitude ascent in general, but that would require a study larger than ours to confirm. We speculate that enhanced renal clearance and/or decreased tissue secretion of VEGF at altitude are mechanisms for our findings. Teleologically, if plasma VEGF is an index of tissue VEGF, a response associated with a decrease in circulating VEGF in hypoxic environments may indicate protection against the development of VEGF-mediated capillary leak in organs such as the brain. This may be particularly relevant given that a normal response to hypoxia is to increase endothelial receptors for VEGF in the brain and other organs. Thus, while elevation in plasma VEGF is not a feature of hypobaric hypoxia or of AMS in our study, perhaps in hypoxic environments failure to increase clearance of VEGF or to dampen inducible VEGF production may predispose an individual to AMS. Further study of plasma VEGF levels and urinary VEGF clearance at high altitude and in altitude-related illness is warranted to advance our understanding of edema mechanisms at altitude.

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