Soluble Endothelial-Derived Adhesion Molecules ICAM-1, VCAM-1 and E-Selectin After Hyperbaric Decompression in Divers versus Non-Divers

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Abstract

BACKGROUND: The vascular endothelium, positioned at the interface between the components of the blood and the interstitial milieu, plays a strategic role in the control of vascular physiology and maintenance of body homeostasis. It plays a key role in inflammation by regulating vascular permeability and tone, hemostasis and cytokine production. Endothelial adhesion molecules orchestrate the recruitment and binding of inflammatory cells to endothelium. With endothelial activation, dysfunction or vascular injury, levels of soluble vascular cell adhesion molecule (sVCAM)-1, intercellular cell adhesion molecule (sICAM)-1 and E-selectin increase. **MATERIALS AND METHODS:** To investigate the possible role of endothelial activation and/or dysfunction in hyperbaric decompression stress (HDS), a population of asymptomatic non-divers (n=10) and experienced divers (n=12) were exposed for 30-min to progressive levels of hyperbaric stress (18, 30, and 45 msw), separated by 1-week intervals. Serum concentrations (ng/mL) of endothelial-derived adhesion molecules were measured pre-dive, 20- and 60-min post-dive by ELISA. Statistical differences (p<0.05) by ANOVA. **RESULTS:** Consistent with a link between severe HDS and endothelial activation/dysfunction, significantly higher 60-min post-dive concentrations of sVCAM-1 (976±82 *vs.* 777±47), sICAM-1 (275±14 *vs.* 236±13) and E-selectin (46±2 *vs.* 37±3) were detected in experienced divers compared to non-divers, respectively, following decompression from 45 msw. Exposure to lower levels of HDS (i.e., 18 and 30 msw) did not significantly alter levels of soluble endothelial adhesion molecule in either group. Upon entry to the study, basal levels of the three soluble markers were not significantly different between experienced divers and non-divers, despite trends toward higher levels in divers. **CONCLUSIONS:** These data suggest that compared to non-divers, exposure to a relatively high degree of HDS in experienced divers elicits acute endothelial cell activation with enhanced expression and subsequent shedding of sVCAM-1, sICAM-1 and sE-selectin into the circulation. Elevated levels of these soluble adhesion molecules may be indicative of a subclinical inflammatory response and/or prognostic risk of endothelial dysfunction in individuals undergoing routine HDS.

Introduction

Diving exposes humans to hyperoxia and hyperbaria, which result in a range of physiological effects and potentially pathophysiological sequelae. During a compressed air dive, inert gas is taken-up as a function of depth and time until it saturates the surrounding tissues. As a diver ascends, dissolved gas diffuses from the tissues into the blood and is eliminated via the lungs. As the partial pressure of gas in physical solution exceeds ambient pressure, stable inert gas bubbles form and are released from the tissues, resulting in the clinical syndrome of decompression sickness (DCS).

It is commonly accepted that the formation of bubbles is the primary agent initiating the pathogenic cascade of events leading to DCS, but understanding of the pathobiologic mechanisms by which bubbles trigger the diverse features of DCS is incomplete. Bubbles evolve from pre-existing gas-filled micronuclei adherent to hydrophobic surfaces along the endothelium of blood vessels that subsequently seed the circulation as they expand (***Figure 1***).

DCS is linked to both mechanical and biological actions of inert gas bubbles in soft tissues and the vascular system. Bubbles exert pathophysiological effects by mechanically distorting tissues and/or obstructing blood flow, causing ischemia-reperfusion injury in the microvasculature. Intravascular bubbles are also capable of eliciting biological responses via mechanical-molecular surface interactions with the endothelial lining of blood vessels and circulating leukocytes. Circulating proteins can also absorb to the surface of bubbles through hydrophobic interactions precipitating immunological interactions. Once in the bloodstream, detached bubbles may alter microvascular rheology or physically damage the blood vessel luminal surfactant layer and/or endothelial cell (EC) surface glycocalyx. This reduces the integrity of the vessels and causes endothelial activation/dysfunction that compromises vascular barrier integrity, increases permeability, and initiates local inflammation and coagulation cascades.

Cellular adhesion molecules (CAMs) expressed on the surface of ECs play a crucial role in endothelial activation and inflammation by mediating the binding and recruitment of circulating leukocytes to the blood vessel wall and their subsequent migration into the subendothelial spaces (***Figure 2***). Among the identified CAMs, the expression and biological properties of intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1) and endothelial-leukocyte adhesion molecule (E-selectin), are well characterized; selectins mediate initial rolling of leukocytes along the endothelium and VCAM-1 and ICAM-1 play important roles in the firm attachment and transendothelial migration of leukocytes. Soluble (s) forms of E-selectin, VCAM-1 and ICAM-1 derived from proteolytic shedding from ECs, are present in the circulation and their blood levels are elevated during inflammatory conditions.

In response to a variety of noxious stimuli, the endothelium undergoes a phenotypic modulation to a nonadaptive state, commonly termed endothelial dysfunction, characterized by loss or dysregulation of homeostatic mechanisms operative in healthy endothelial cells. Limited experimental studies suggest that decompression-induced bubble-mediated endothelial insult causes in EC activation/dysfunction and elicits the induction of CAM expression inassociation with DCS.

Purpose

The present study was designed to test the hypothesis that circulating levels of sE-selectin, sVCAM-1, and sICAM-1 may be useful markers of diving-related decompression stress in healthy subjects exposed to progressive levels of hyperbaria. We also tested whether the baseline or stress-induced levels of circulating CAMs were different between experienced divers versus non-divers.

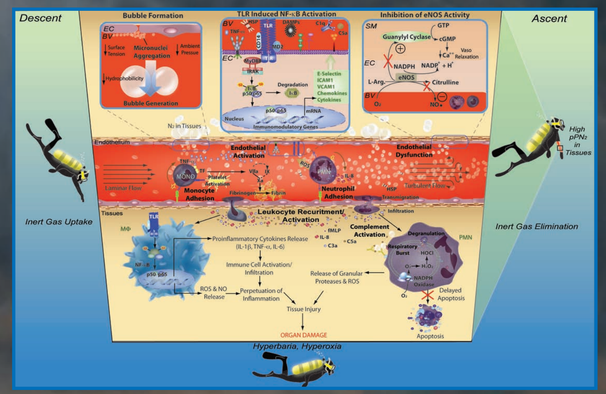


Figure 1

Pathogenic immuno-inflammatory mechanisms leading to DCS.

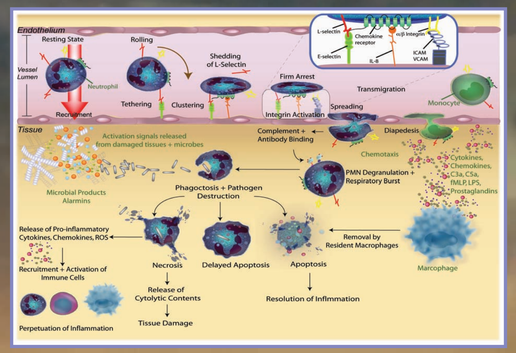


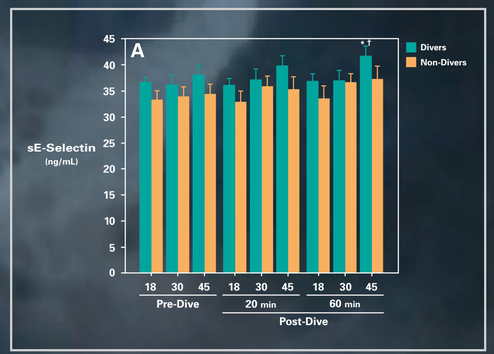
Figure 2

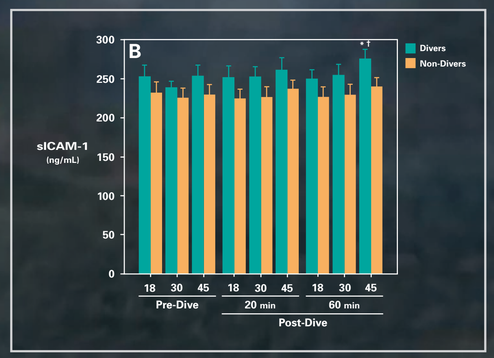
Leukocyte-endothelial cell activation, adhesion and tissue infiltration cascade.

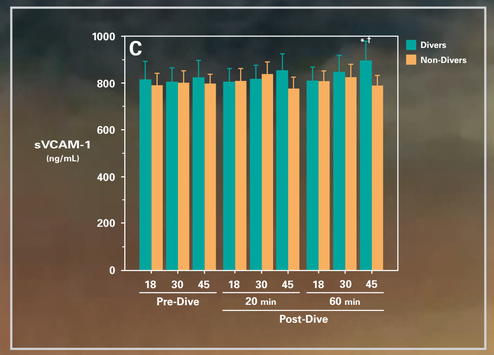
Results

Figure 3A, B and ***C*** shows the circulating concentrations of sE-Selectin, sICAM-1, and sVCAM-1, respectively. Consistent with a link between severe hyperbaric decompression stress and endothelial activation/dysfunction, significantly higher 60-min post-dive concentrations of sE-selectin (46±2 *vs.* 37±3), sVCAM-1 (976±82 *vs.* 777±47), and sICAM-1 (275±14 *vs.* 236±13) were detected in experienced divers compared to non-divers, respectively, following decompression from 45 msw. Exposure to lower levels of decompression stress (i.e., 18 and 30 msw) did not significantly alter levels of sCAMs in either group. Upon entry to the study, basal levels of the three markers were not significantly different between experienced divers and non-divers, despite trends toward higher levels in divers.

Figure 3







Materials and Methods

Subjects

Twenty males volunteered to participate in the study. Respective mean values and SD for age, height and weight were 38.9 ± 8.4 y, 1.76 ± 0.07 m and 83.0 ± 13.2 kg. Subjects were recruited and classified as being either non-divers (n=9) having never experienced hyperbaric stress or experienced divers (n=11) having been exposed at least once per month during the previous 6 months to hyperbaric stress. There were no differences between groups in the descriptive characteristics listed above.

Experimental Design

A minimum of 14 days prior to the first experimental session subjects completed a familiarization exposure to 90 kPa for 15 min experiencing all of the instrumentation and testing described below. The 3 experimental sessions involved 30 min of exposure to 180 (18 msw), 300 (30 msw), or 450 kPa (45 msw), which were defined as low, moderate or high level of hyperbaric stress, respectively. Pressurization rates were 18 kPa/min and decompression rates were in accordance with established dive tables and represented one, 15 and 55 min for the low, moderate and high levels of hyperbaric stress. Experimental sessions were separated by at least 6-7 days with the order of presentation standard for all subjects from the lowest through the highest level of stress.

Quantification of Serum Soluble Adhesion Molecule Concentrations

Serial venous blood samples were collected immediately pre-dive, 20 and 60-min post-dive into non-additive tubes, allowed to clot at room temperature and then centrifuged at 1000 *x g* for 15 min. The serum was frozen at -80˚C until batch analysis. Samples were subsequently thawed and assayed in duplicate for circulating concentrations of sICAM-1, sVCAM-1, and sE-selectin using commercially available quantitative ELISA kits according to the according to the manufacturer's protocol (Quantikine®, R&D systems). Absorbance was read at 450 nm in an automated microplate photometer. Assay sensitivities for sICAM-1, sVCAM-1, and sE-selectin were 0.096, 0.6, and 0.009 ng/mL, respectively.

Statistical Analyses

Data are shown as mean ± SE. Differences in sCAM levels over time and between groups were analyzed by two-way repeated measures ANOVA. Statistical significance was set at p<.05.

Summary and Conclusions

- These data suggest that compared to non-divers, exposure to a relatively high degree of decompression stress in experienced divers elicits acute endothelial cell activation with enhanced surface expression and subsequent shedding of sE-selectin sICAM-1, and sVCAM-1 into the circulation.
- The potential physiological/ pathophysiological significance of these elevated sCAMs is not fully understood, but may be indicative of a subclinical inflammatory response and/or prognostic risk of endothelial dysfunction in individuals undergoing routine diving and decompression.

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