Endothelial Function and Stress Response After Simulated Dives to 18 msw Breathing Air or Oxygen

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Introduction: Decompression sickness is caused by gas bubbles released upon decompression. These bubbles have the potential to occlude blood vessels and damage the vascular endothelium. The aim of this study was to quantify damage to the vascular endothelium resulting from decompression by measuring endothelial microparticles (MP) and endothelial function. ***Methods:*** Five healthy male volunteers undertook a simulated (hyperbaric chamber) air dive and 1 wk later a second dive breathing 100% oxygen at 283 kPa (18 msw) for 60 min bottom time, decompressed with 5-min stops at 161 kPa (6 msw) and 131 kPa (3 msw). Endothelial function was tested pre- and postdive by reactive hyperemia peripheral artery tonometry (RH-PAT) and CD105 (Endoglin) positive MP were quantified by flow cytometry. Plasma E- and P-selectin, interleukin-6, and serum cortisol were also quantified. ***Results:*** RH-PAT showed a significantly decreased endothelial function post-decompression after breathing air when compared to oxygen (-0.33 ± 0.27 vs. $+0.18 \pm 0.14$). CD105 MP pre- and postdive showed no change on the oxygen dive (460 ± 370 to 360 ± 163), however, they increased after breathing air (440 ± 70 to 1306 ± 359). There was no change in expression of CD105 on MP. Furthermore no changes were observed in plasma E- or P-selectin, IL-6, or serum cortisol. ***Conclusion:*** From the data, at least in the time frame involved, there appears to be no detectable physiological/stress response to decompression, rather decompression from breathing air probably caused mechanical damage to the endothelium, resulting in both MP release and a reduction in endothelial function.

Keywords: decompression sickness, microparticles, hyperbaric oxygen, reactive hyperemia.

DECOMPRESSION sickness (DCS) is an inherent risk in a growing population of professional and recreational divers. The Professional Association of Diving Instructors (PADI) consistently issued over 500,000 entry-level certificates annually during the period 2002-2008 (30). The symptoms of DCS range from joint pain to pulmonary edema and barotrauma, often involving the central nervous system, and can ultimately lead to death if treatment cannot be administered quickly. The pathophysiology of DCS is presumed to stem from gas bubble formation and release into the circulation upon decompression. Gas bubbles can occlude blood vessels and cause vascular endothelial cell stripping through mechanical interaction with the endothelium (2). Various predive preconditioning strategies have been employed, for example hyperbaric oxygen prebreathing (7), physical exercise (36), and heat exposure (4). The measure of efficacy in these studies was a reduction in bubble formation upon decompression. However, as previously shown, Doppler imaging of bubble scores has limitations (13), although it has also been reported that there is an increased risk of DCS with increasing bubble scores (27). A quantifiable biological response would therefore be useful in evaluation of decompression stress.

It has previously been reported that a single air dive results in a decrease in endothelial function postdecompression (6). Antioxidants (28,29) and exercise (3,12) preconditioning were shown to prevent this decrease. The endothelium is known to shed microparticles (MP) upon activation or remodeling due to damage (15). MP are cellular membrane fragments and typically carry the antigens characteristic of the parent cell. Elevated numbers of endothelial MP within the circulation are observed within diseases characterized by endothelial dysfunction (16) such as in acute coronary syndromes (22), multiple sclerosis (25), arteriosclerosis (33), diabetes (9,10), and hypertension (31,32). Furthermore, endothelial MP have been shown to correlate with endothelial function (35). It seems likely that bubble formation and transit within the circulation have the potential to interact with the endothelium.

Previously we have observed an increase in vascular cell adhesion molecule-1 positive MP following a simulated (hyperbaric chamber) dive after breathing air at depth (34) and suggested this may be a sign of endothelial activation. This observation was not seen when hyperbaric oxygen (HBO) was used instead of air and therefore may be bubble related. We have also hypothesized that there may be endothelial dysfunction at depth which may play a role in the initiation and consequences of DCS (20).

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The aim of this study was to quantify damage to the vascular endothelium resulting from decompression using endothelial microparticles and endothelial function. Endothelial MP expressing endoglin were quantified by flow cytometry and endothelial function was tested by reactive hyperemia peripheral artery tonometry (RH-PAT). RH-PAT is a noninvasive technique that measures digital pulse volume during reactive hyperemia and is partially nitric oxide (NO) dependent (24). This technique has been reported as useful in identifying patients with early atherosclerosis (5).

Endoglin, also known as CD105, is a constitutively expressed endothelial membrane protein that has been previously described (17) and was investigated in this study as a biological marker of decompression stress. Markers of stress, serum cortisol, interleukin-6 (IL-6), and serum endothelial adhesion molecules (E- and P-selectin) were also determined in order to differentiate between a systemic stress response and a nonstress response.

METHODS

Simulated Dive

Five healthy male subjects naïve to pressure diving were recruited to participate in the study. Subjects fasted for 6 h predive so as not to influence measurement of endothelial function. The subjects reported to the Hull and East Riding Hospital 2 h prior to the dive. Within the hour predive endothelial function was measured. The dive consisted of breathing compressed air at 283 kPa (18 msw) for 60 min bottom time. One week later the same subjects returned for an identical dive profile, however breathing 100% oxygen (20 min O_2/5min air) instead of air. Subjects were decompressed with 5 min stops at 161 kPa (6 msw) and 131 kPa (3 msw). Venous blood was collected immediately pre- and postdive into potassium EDTA, sodium citrate, and serum separator blood tubes (Vacuette, BD Biosciences, Cowley, UK). All subjects provided written informed consent in accordance with the departmental and university ethical procedures and following the principles outlined in the Declaration of Helsinki.

Endothelial Function Measurement

RH-PAT was measured by a trained technician using an EndoPat device (Itamar Medical, Caesarea, Israel). This technique provides a noninvasive measurement of endothelial function that is partially dependent on NO and has the advantage that the results are operator-independent. RH-PAT measurements were made immediately pre- and postdive. A blood pressure cuff was placed on one arm, with the other acting as a control. Probes were placed on the index finger of each arm. The PAT signal is based upon finger arterial pulsatile volume changes obtained from modified plethysmographic biosensors. These biosensors apply pressure uniformly to the distal two-thirds of the fingertips, which prevents veno-arteriolar vasoconstriction and minimizes movement artifacts.

Subjects were maintained in a comfortable position for 10 min in a temperature (22°C) controlled room and baseline readings taken for 5 min. A cuff was then inflated to a suprasystolic pressure on one (experimental) arm for 5 min, after which the cuff was deflated and the RH response was measured for 5 min. Data were gathered throughout the procedure and the RH-PAT was calculated relative to the control arm. There is a significant postprandial decrease in endothelial function in healthy humans, which subsequently returns to baseline after 6 h (23). Therefore subjects in this study were asked to fast for 6 h prior to arrival at the hyperbaric chamber.

MP Quantification

MP were quantified using a BD FACSCalibur flow cytometer (BD Biosciences, Cowley, UK) as previously described (34). Forward and side scatter were set as triggers as determined by the scatter properties of megamix beads (Biocytex, Marseille, France). Anti-CD105:FITC (4 μl, AbD Serotec, Oxford, UK) were incubated with platelet poor citrated plasma (25 μl) for 30 min prior to addition of Caltag counting beads (25 μl) (Caltag Medsystems, Buckingham, UK) and subsequent analysis using CellQuest software (BD Biosciences, Cowley, UK). CD105 MP were quantified as an absolute count per microliter platelet poor plasma (PPP).

Determination of Plasma Markers

E-selectin, P-selectin, and IL-6 were determined by quantitative ELISA (Bender Medsystems, Vienna, Austria) on EDTA plasma according to the manufacturer's instructions. Concentrations were determined from a 4-parameter standard curve generated on a Bio-Tek Synergy HT running KC4 software (LabTech, UK).

Determination of Serum Cortisol

Cortisol was quantified from serum samples by chemiluminescence immunoassay (Immulite 1000 cortisol kit, Immulite 1000 analyzer (Siemens, UK).

Statistical Analyses

All statistical analyses were performed using PASW statistics 17.0 (SPSS Inc., Chicago, IL). The effect of condition (compressed air and hyperbaric oxygen) on the change in blood markers and endothelial function were analyzed using linear mixed models. The postdive values were used as the dependent variable and the predive values as a covariate. Condition was modeled as a fixed effect repeated measures fixed factor. Various covariance structures were assumed and the one that minimized the Hurvich and Tsai's criterion (AICC) value was chosen for the final model for each dependent variable. Changes in blood markers and endothelial function within each condition were analyzed using paired samples *t*-tests. The family type I error rate was controlled using Sidak-adjusted *P*-values. Two-tailed statistical significance was accepted as $P < 0.05$.

RESULTS

None of the subjects showed any symptoms of DCS following decompression after either simulated dive.

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Following the dive on air, a reduction in endothelial function (mean change = -0.33 ± 0.27) as measured by a decrease in reactive hyperaemic index (RHI) was observed. Furthermore, RHI was shown to improve (mean change = +0.18 ± 0.14) after breathing O_2.

The decrease in endothelial function pre- to postdive, as indicated by the RHI, in the compressed air condition was not significant ($P = 0.31$), nor was the increase in RHI in the O_2 condition ($P = 0.29$). However, the change in RHI pre- to postdive was significantly different between conditions ($F = 59.2$, $P = 0.016$).

Table I shows the mean (SEM) blood marker concentrations pre- and postdive for the air and O_2 conditions. Flow cytometry profiles showing the MP gate and CD105 MP are shown in **Fig. 1**.

After decompression, CD105 MP numbers were seen to significantly increase from 440 ± 70 to 1306 ± 359 (per μl PPP) after breathing air ($P = 0.016$), whereas there was no significant difference (460 ± 370 to 360 ± 163) following the O_2 dive ($P = 0.87$). The difference in the change in CD105 MP between conditions was significant ($F = 28.6$, $P = 0.002$). No significant difference was observed in the median fluorescence intensity of CD105 MP from pre- to postdive in either the air ($P = 0.92$) or O_2 ($P = 0.94$) conditions, and there was no difference between conditions in the amount of change pre- to postdive ($F = 2.1$, $P = 0.19$).

No significant changes were observed pre- to postdive for any of the other blood markers within either of the two conditions. E-selectin ($ng \cdot ml^{-1}$) measurements on air (30.9 ± 11.8 to 33.8 ± 11.8) and O_2 (35.8 ± 11.7 to 41.1 ± 8.5), P-selectin ($ng \cdot ml^{-1}$) on air (129 ± 30 to 153 ± 66), and O_2 (118 ± 20 to 127 ± 29) or IL-6 ($pg \cdot ml^{-1}$) on air (1.35 ± 0.35 to 1.22 ± 0.30) and O_2 (0.95 ± 0.23 to 1.29 ± 0.17) did not change significantly within conditions. Furthermore, there were no significant differences between conditions in the amount of change pre- to postdive for cortisol ($F = 0.06$, $P = 0.81$) (Table I) or E-selectin ($F = 0.3$, p 0.62), P-selectin ($F = 0.04$, $P = 0.85$), or IL-6 ($F = 1.7$, $P = 0.23$).

DISCUSSION

This study showed that endothelial dysfunction occurs following a dive with air in comparison to O_2. Our data are in accord with the endothelial dysfunction observed by Brubakk et al. (6). In that study measurements were taken by flow-mediated dilation postdecompression and showed a significant reduction in endothelial function.

CD105 MP were seen to increase significantly postdecompression after breathing air. Endoglin is a constitutive endothelial marker and the observation that CD105 MP only changed in their quantified number, rather than antigen expression (as determined by fluorescence intensity) suggests that the increase postdecompression is due to physical damage to the endothelium rather than a physiological change resulting in MP release. The increase in CD105 MP immediately post decompression from an air dive is consistent with the current model of DCS. Bubbles released into the circulation from saturated tissue interact with the endothelium causing physical damage resulting in MP release. This observation suggests that CD105 MP could be used as a biological marker of decompression stress and may prove a useful outcome measure of preconditioning strategies.

No significant change in CD105 MP was seen after the O_2 dive and this, coupled with the improvement in RHI, may be a result of NO induction via a free radical mechanism (14). Furthermore NO donors have previously been used as a preconditioning tool in diving and shown a reduction in bubble formation upon decompression (11,26).

Breathing normobaric oxygen before a dive was found to significantly reduce bubble formation postdecompression in a mixed sex study of 21 people (8). A single hyperbaric oxygen pretreatment has also been found to reduce bubble formation postdecompression in humans (19) and the incidence of DCS in animal studies (7,18,21). Furthermore, gas bubble size was found to be reduced in prawns after predive oxygen saturation (1). These studies suggest that predive conditioning using oxygen may be useful in reducing bubble formation, possibly via an NO-dependent mechanism, and subsequent endothelial damage.

No changes in the systemic stress markers IL-6 or serum cortisol were observed, suggesting that the damage caused was local or peripheral and not generalized. These data further add to the conclusion that DCS is most probably a local mechanical/physical process, due to bubble transit within the peripheral vasculature leading to endothelial cell dysfunction. This dysfunction could then accumulate to cause an inflammatory response consistent with the symptoms of DCS that can manifest up to 24 h after decompression.

TABLE I. MEAN (SEM) BLOOD MARKER CONCENTRATIONS PRE- AND POSTDIVE FOR THE COMPRESSED AIR AND OXYGEN CONDITIONS.

Blood Marker	Condition	Predive	SEM	Postdive	SEM	Difference*	95% CI	*P*-Value
Reactive hyperemic index	Air	2.07	0.37	1.74	0.20	−0.33	−1.190, 0.527	0.52
	O_2	1.55	0.09	1.74	0.22	0.18‡	−0.273, 0.640	0.50
CD105 MP†	Air	440	31	1306	161	866	424, 1307	0.016
	O_2	460	167	360	73	−100‡	−512, 311	0.87
Cortisol ($\mu g \cdot dl^{-1}$)	Air	12.3	1.3	12.0	2.4	−0.3	−5.1, 4.5	0.98
	O_2	10.4	1.2	9.5	1.1	−0.9	−3.1, 1.3	0.57

* Any discrepancies between this column and the difference between the previous two columns are due to rounding errors.
† Absolute count per microliter PPP.
‡ Significantly different from the compressed air condition.

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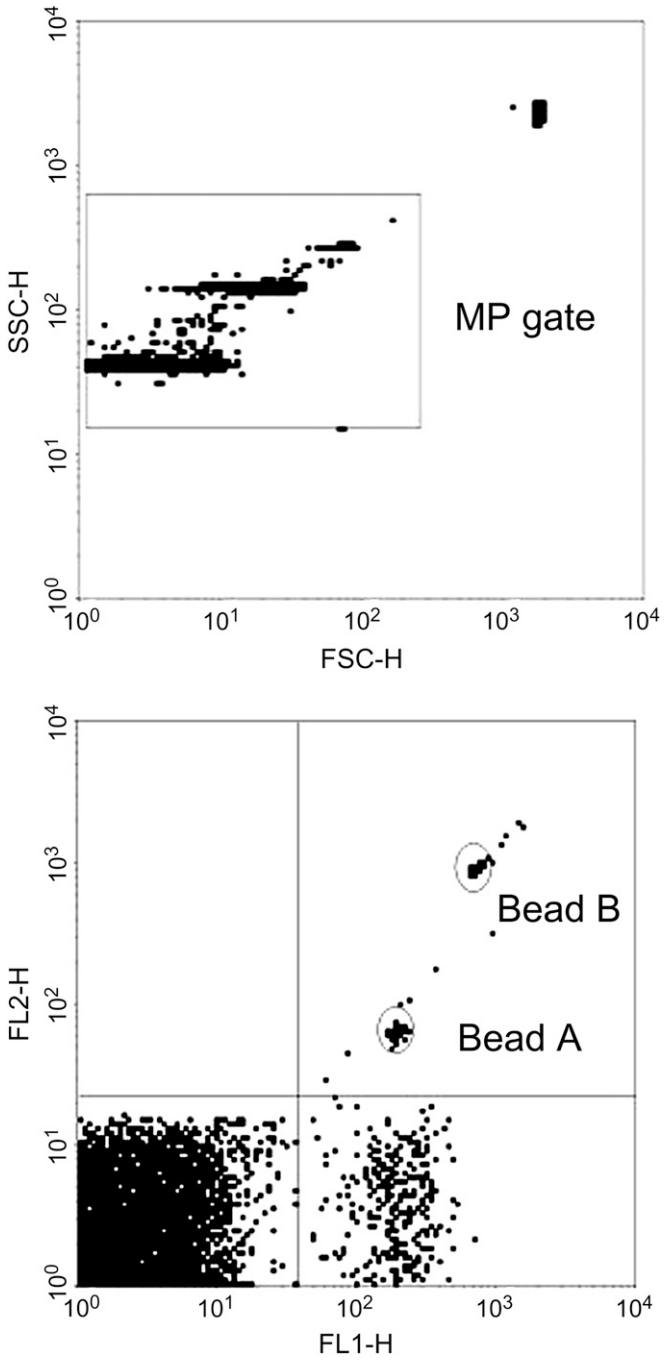

Fig. 1. Flow cytometry profiles showing the (upper) microparticle gate set using megamix beads of 0.5 and 0.9 μm; and (lower) typical CD105 staining of MP showing positive events in the lower right quadrant, negative events in the lower left quadrant, and counting beads in the upper right quadrant.

The small subject pool is an obvious limitation of our study. Measurement of bubbles was not undertaken as part of the experimental protocol but, as previously noted, Doppler does have limitations, and bubbles, despite being a causative agent, have limited diagnostic or prognostic value in relation to DCS. In the time frame involved there seems little doubt that the CD105 MP released must be from damaged rather than activated endothelium, however, there may be other as yet unknown mechanisms behind this rapid MP release. Active, biological membrane remodeling leading to MP release could not occur within the time from reaching depth to the endothelial function test postdecompression. Pressure per se may affect endothelial function due to differences in blood oxygen content (20). However it would be experimentally difficult to determine as measurements would have to taken at depth.

In conclusion, the data presented here are consistent with potential bubble formation upon decompression from depth breathing air and subsequent transit through the vasculature, causing measurable physical damage to the endothelium. This is manifested as both a reduction in endothelial function and an increase in the numbers of circulating endothelial MP. Breathing O_2 resulted in an improvement in endothelial function coupled with no discernable MP release from the endothelium. These findings have potential importance both in the progression of DCS and the therapeutic value of hyperbaric oxygen treatment where treatment aimed at improving endothelial function may have value for diseases characterized by endothelial dysfunction and this warrants further investigation.

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