

Short communication

Effects of a single hyperbaric oxygen exposure on haematocrit, prothrombin time, serum calcium, and platelet count

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Key words

Hyperbaric oxygenation, oxygen, platelets, coagulation, haematology, research

Abstract

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We investigated whether administration of hyperbaric oxygen (HBO) affects platelet counts and some components of the haemostatic system. Ten test subjects were treated with 100% hyperbaric oxygenation at 253 kPa (2.5 ATA) for 60 minutes. A comparison was made between pre-exposure and post-exposure measurements of haematocrit, prothrombin time (PT), serum calcium concentration, and platelet counts. While no significant changes were detected in haematocrit, PT, or serum calcium concentration, platelet levels demonstrated significant decreases with a mean pre-HBO platelet count of $283 \times 10^3 \text{ mm}^3 \pm 32$ compared to a mean post-HBO platelet count of $255 \times 10^3 \text{ mm}^3 \pm 33$ ($P = 0.001$). This is consistent with previous studies modelling coagulation pathways and suggests that HBO may activate components of the haemostatic system. Characterisation of the mechanisms associated with this decrease in platelet count by future studies may provide insight into platelet adhesion and aggregation properties during exposure to high oxygen tensions, and may find applicability towards the vascular component of disorders in which oxidant stress and coagulation are prominent, such as hypertension, diabetes, and coronary artery disease.

Introduction

The effects of hyperbaric oxygen (HBO) upon the haemostatic system have not been entirely determined although previous studies indicate several haemostatic factors are affected by HBO. Olszanski et al found a decrease in platelet count and factors I, X, and XII after air, but not helium, diving.¹ Ersoz et al reported that, in New Zealand rabbits, HBO results in decreased collagen-induced platelet aggregation.² Yamami et al found differences in human fibrinolytic activity after HBO.³ More recently, studies by Thom et al and Puthuchery et al demonstrate respectively unaltered platelet function and decreased exhaled nitric oxide (NO) after HBO.^{4,5} This study was designed to test the null hypotheses that HBO would have no effect upon: 1) haematocrit, 2) prothrombin time (PT), 3) serum calcium concentration, or 4) platelet count.¹

Subjects and methods

The study design was a non-randomised, before-and-after study that was evaluated by the Saba University School of Medicine (SUSOM) Ethics Committee, Human Subjects Review Board. Informed consent was obtained from all subjects. All testing was completed within 90 minutes for each subject, eliminating possible diurnal variations. Baseline laboratory values for each subject were ascertained before HBO and served as the control measurements for post-HBO exposure.

Inclusion criteria were: 1) at least 18 years of age, 2) no bleeding disorders, 3) no prescription medications, and 4) no contra-indications for HBO. Eight male and two female volunteers were recruited from SUSOM, aged between 24 and 50 years. Subjects were instructed to avoid products containing aspirin for at least four days, and to fast for four hours prior to the study. Subjects were then compressed in pairs in a Dräger 5.19 m³ multiplace hyperbaric chamber to 253 kPa (2.5 ATA) for 60 minutes, breathing 100% oxygen continuously through a tight-fitting oxygen delivery mask that covered the nose and mouth. As the risks for oxygen toxicity and decompression illness were minimal, there were no oxygen breaks and both compression and decompression were accomplished within five minutes.

Blood samples were drawn from the right median antecubital vein immediately prior to entering the chamber and from the left median antecubital vein within 15 minutes of exiting the chamber. Each set of blood samples was examined to measure haematocrit, PT, serum calcium and platelet count.⁶ Blood was collected into a 4.5 mL EDTA tube (haematocrit and platelet count), 4.5 mL sodium citrate tube (serum calcium concentration), and 5 mL SST tube with gel and clot activator (PT).

Haematocrit was measured using a standard capillary tube centrifugation technique and expressed as a percentage of the total column height.⁶ PT was performed using a manual technique. Following centrifugation and separation, the

plasma was incubated for three minutes at 37 +/- 0.5°C. Dade Thromboplastin C plus was reconstituted with 10 ml distilled water and incubated in the same manner as the plasma. Three parts of thromboplastin were added to one part plasma. The time to achieve visible coagulation in two aliquots was recorded and averaged.⁶ Platelet count was measured using Unopipette; one drop was pipetted onto each side of a haemocytometer and allowed to stand for at least three minutes before the platelet count was read.⁶ Serum samples for calcium concentrations were separated, frozen and analysed six days later. Serum was added to a pretreated slide and allowed to stand for at least three minutes. After the incubation period, total calcium was measured using an automated spectrophotometer.⁶

Data were evaluated using a paired T-test with the statistical software Minitab version 15; a P-value less than 0.05 was considered significant. A pre-study power analysis was not calculated.

Results

There were no significant differences in haematocrit, PT and serum calcium following HBO compared with before exposure (variation < 1%, Table 1).

Platelet counts (Table 1 and Figure 1) demonstrated significant changes post-HBO. All subjects demonstrated a decreased platelet count with a mean pre-HBO platelet count of 283 x 10³ mm³ ± 32 and mean post-HBO platelet count of 255 x 10³ mm³ ± 33 (P = 0.001).

Discussion

The responses of healthy volunteers may not represent those of patients undergoing HBO therapy. No external control group was used because there is no evidence that platelet counts or the other parameters typically change over 90 minutes in healthy individuals. Large numbers of subjects would have been needed to improve the power of the study for parameters other than the platelet count. There was no measurement of a dose/response relationship since only one exposure of HBO was administered. There were no follow-up samples collected to measure the duration of any changes observed.

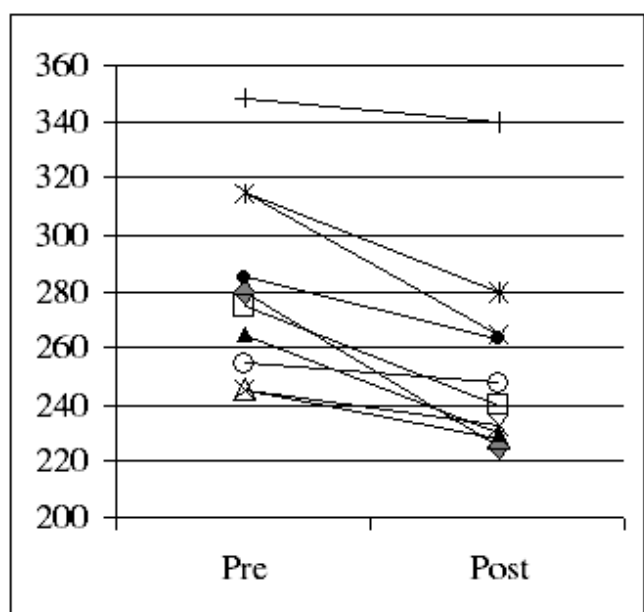
The data reveal a decrease in platelet count soon after a single exposure to HBO. This decrease could be attributed to the modulating effects of reactive oxygen species (ROS) inhibiting and scavenging NO production and bioavailability during HBO. Jaimes et al suggest that ROS significantly impair endothelial nitric oxide synthase (eNOS) activity, while Krotz et al suggest that superoxide anion may induce platelet aggregation.^{7,8} Clutton et al suggest that platelet-derived NO contributes to an inhibition of platelet activation.⁹

Table 1
Haematocrit, prothrombin time, total calcium and platelet count pre- and post-hyperbaric exposure to 253 kPa pressure oxygen for 60 mins; mean ± SD; *P = 0.001

Sample	Pre-HBOT	Post-HBOT	Percentage difference
Haematocrit (Per cent %)	42%	42%	0%
Prothrombin (time, seconds)	12.1 ± 0.4	12.2 ± 0.4	< 1%
Total calcium (mm.L ⁻¹)	2.45 ± 0.14	2.46 ± 0.05	< 1%
Platelet count (x100,000 mm ³)	283 ± 32	255 ± 33*	~ 10%

There has been recent research that suggests inflammatory reactions may modulate NO levels by up-regulating inducible nitric oxide synthase (iNOS). Puthuchery et al reported no changes in platelet counts for healthy attendants breathing air at 243 kPa or breathing oxygen at 101.3 kPa.⁵ What Puthuchery et al suggest is an up-regulation of iNOS in patients with inflammatory reactions that becomes significantly reduced after HBO. Interestingly, the inflammatory up-regulation of iNOS may explain the conclusion of Thom et al that HBO had no effect upon platelet count, especially since the subjects in that study

Figure 1
Platelet counts pre- and post-exposure to hyperbaric oxygen at 253 kPa pressure for 60 mins



were undergoing prophylactic HBO for osteoradionecrosis and potentially had disease processes that promoted inflammatory responses.⁴ This could explain the difference in results as compared with the present study, which utilised healthy volunteers.

Is the platelet reduction revealed in this study a temporary manifestation or a longer-lasting degradation? One proposed mechanism suggests that temporary platelet adhesion to the endothelium lasts only a few hours after HBO as levels of ROS become reduced and normal levels of NO become replenished by eNOS. However, a second possible mechanism proposes that micro-aggregates form within the vasculature and eventually become sequestered and degraded by the spleen, which would result in a longer duration of decreased platelet count. Additionally, if micro-aggregates are formed, this poses a potentially dangerous side-effect for patients with increased risk factors for thrombus formation, such as those with vascular disease and diabetes. The data from this study cannot reliably suggest which mechanism may be responsible for the observed decrease in platelet count.

Conclusion

A single exposure to HBO at 253 kPa for 60 minutes was shown to lower platelet levels in healthy individuals. The exact mechanism by which this is accomplished is unknown; however, the possibilities include permanent degradation of platelets by the spleen or simply a temporary binding of platelets to the endothelium.

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\$200,000 fine for diving death

A Melbourne diving company was fined \$200,000 after failing to ensure the safety of an inexperienced diver who drowned after experiencing problems with his rented diving equipment during a dive out of Portsea in January 2004. Found guilty by a jury of failing to ensure the victim's care under the terms of the Occupational Health and Safety Act, the judge condemned the company's breach of duties as 'profound' and said that the fatality could have easily been avoided had the company and its employees followed good safety procedures. The judge also said there were many warnings and indications that the victim lacked the skills and experience to dive in Victorian waters. In fining the company an unprecedented \$200,000, the judge said that while the company was now in receivership and the fine would, therefore, not be paid, it was important to send a clear message to the diving industry about the responsibility operators had towards their customers.