RESEARCH ARTICLE

Decrease in dysbaric osteonecrosis severity as a result of 45-minute oxygen pre-breathe

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ABSTRACT

Sudden decompression can result in bubble formation as the result of nitrogen gas (N_2) dissolved in tissue during disabled submarine escape (DISSUB). This may cause dysbaric osteonecrosis (DON), a condition in long bones where bubbles in fatty marrow result in ischemia and necrosis. Previous research has shown that oxygen (O_2) pre-breathe of two hours resulted in a reduction of DON; however, effects of shorter O_2 pre-breathe remain uncertain.

This study's aim was to understand the effect of shorter lengths of O_2 pre-breathe. Eight adult Suffolk ewes (89.5± 11.5 kg) were exposed to 33 feet of seawater (fsw) for 24 hours. They were placed randomly into four groups and exposed to either 45, 30 or 15 minutes of O_2 (91-88%) pre-breathe; the controls received none.

They were then rapidly decompressed. Alizarin complexone was later injected intravenously to visualize the extent of DON in the right and left long bones (radii, tibiae, femur and humeri). The 30- and 15-minute pre-breathe groups saw the greatest deposition. There was significant decrease of variance in the 45-minute group when compared with all other treatments, suggesting that 45 minutes of O_2 pre-breathe is required to effectively increase confidence in the reduction of DON. Similar confidence was not reflected in the 30-minute and 15-minute groups: 45 minutes of pre-breathe was the minimum amount needed to effectively prevent against DON in DISSUB escape at 33 fsw. However, future research is needed to determine how to calculate effective dosages of O_2 pre-breathe to prevent DON in any given scenario.

INTRODUCTION

Dysbaric osteonecrosis (DON) is the formation of necrotic lesions in the long bone fatty marrow as a result of a sudden drop in barometric or hydrostatic pressure, causing bubble formation in the intramedullary space. DON can arise after a single exposure to a sudden decrease in environmental pressure [1] but it is more common in individuals who experience multiple exposures, including compressed-air workers [2,3] diving fishermen [4,5] and divers [6].

Rapid evacuation during a DISSUB scenario has also been shown to lead to DON [7]. Clinically, DON may manifest as chronic pain in the hips, knees and shoulders, and in extreme cases, total joint failure [8].

Among groups regularly exposed to pressures required for the formation of DON lesions, military divers experience the lowest rates of DON [9-11]. The highest rates of DON were documented in Turkish sponge divers and Hawaiian fishermen, with prevalence rates of 71% and 65% respectively [4,5]. These values mirror other diving fisherman communities that are at increased risk because of a lack of education about, and resources for, the prevention and treatment of DON [4,5,9]. Compressed-air workers are also at risk of developing DON, with prevalence rates of around 17% [12,13]. The true prevalence of DON may be inaccurate for some populations because detection and reporting are often expensive and challenging [10,13].

The main risk factors that predispose a person to the development of DON are multiple exposures to radical drop in pressure and/or greater maximal diving depth [13,14]. Other potential risk factors for DON are higher levels of plasminogen activator inhibitor-1, fat embolisms and hyperlipidemia [14,15]. Like many other forms of osteonecrosis the underlying mechanism for DON is not entirely understood. This provides certain

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challenges when trying to determine what pre-existing habits and conditions can increase the chances of developing these lesions [8].

The University of Wisconsin-Madison Sheep Model of decompression injury developed by Lehner, et al. (1997) is a reliable surrogate for DON in humans [16]. The ability to produce accelerated decompression conditions in adult sheep provides an effective human surrogate, as these animals have similar bone size, structure, and fatty marrow distribution to humans [16]. The sheep have similar body weights as well as metabolic and tissue perfusion rates which are factors that control the amount of dissolved gas. This means the likelihood of bubble formation and radiographic and histological findings of DON are remarkably similar to those of humans [16].

When exposed to high ambient pressure, nitrogen (N_2) dissolves in body tissue relative to the partial pressure [17]. Factors such as time of exposure, tissue perfusion and solubility of gas in tissue play vital roles in determining the N_2 load [17]. When studying DON it becomes clear that tissue perfusion rates and solubility of N_2 in fatty tissue play an important role in developing lesions. Fatty marrow in long bones has a low perfusion rate, and N_2 is five times more soluble in fat than in aqueous tissue. This dissolved N_2 can form bubbles on active hydrophobic surfaces when ambient pressure is reduced [18]. One widely accepted theory for the formation of these lesions is that extravascular bubbles lead to an increase in intramedullary pressure [13,17, 19]. Blood vessels then experience vascular stasis as a result of the Starling resistor mechanism [19]. If the intramedullary pressure is not reduced, and the bubbles do not disappear, fatty marrow and bone begin to experience ischemia, which can lead to bone necrosis [19].

It has also been proposed that intravascular bubbles may block the sinusoids of the fatty bone marrow, leading to ischemia and necrosis [13]. Another hypothesis is that DON occurs because N_2 bubbles can lead to the release of liquid fat and thromboplastin [15]. This can cause an increase in blood coagulation in intravascular regions, causing vessel blockage [15]. Although the exact mechanism for necrosis that produces DON lesions is not completely understood, it is known that increased environmental pressure causes an increase in N_2 saturation [17,19]. This leads to increased saturation of N_2 in fatty marrow tissue, which results in the formation of gas bubbles when pressure is rapidly decreased [15,17,18]. These bubbles are then responsible for the formation of DON lesions [15, 17-19].

We hypothesize that O_2 washout treatment in the form of O_2 pre-breathe is an effective form of prevention for DON. The aim of this study is to determine the effect of shorter durations of O_2 pre-breathe on DON at 33 fsw.

METHODS

The Institutional Animal Care and Use Committee of the University of Wisconsin reviewed and approved this protocol to perform DISSUB sheep O_2 pre-breathe trials for Naval Sea System Command that followed guidelines for medical research in relation to animals set forth by the Helsinki Animal Committee.

Subjects

Eight adult female Suffolk ewes (89.5±11.5 kg) were exposed to 33 feet of seawater (fsw) for 24 hours in a high-pressure chamber at the University of Wisconsin Biotron Laboratory. In order to avoid acclimatization sheep selected for this study had no prior exposure to a hyperbaric environment. Each animal was randomized to one of four groups (n = 2):

- Group 1 received 45 minutes of O_2 (91-88%) pre-breathe;
- Group 2 received 30 minutes of O_2 (91-88%) pre-breathe;
- Group 3 received 15 minutes of O_2 (91-88%) pre-breathe; and
- Group 4 controls received only compressed air before dropout decompression.

Diving procedures

Sheep enter the hyperbaric chamber while experiencing normal surface pressure. The chamber is then compressed at a rate of 30 feet per minute to a final pressure of 33 fsw. The sheep then remain in the chamber for 24 hours. After 24 hours, each group is given its respective treatment. The sheep then undergo decompression at the same rate that they were compressed, reaching surface pressure in 60-90 seconds.

Analysis

Alizarin complexone, a fluorochemical used to visualize areas of bone remodeling [20], was injected into sheep one week prior to euthanasia. MRI images of long bones prior to exposure and then five weeks post-treatment were used to confirm that areas of alizarin complexone deposition were indeed DON lesions. The tibia, humerus, femur, and radius were removed during autopsy and segmented longitudinally to observe areas of DON

lesions. The long bones were preserved through fixation with a 10% solution of neutral buffered formalin to preserve the bone and alizarin color. Digital images were taken to analyze the gross pathology of long bones. Raw images were processed with ImageJ Scion Image software (Scion Corporation, Frederick, Maryland) and the software extension WEKA segmentation (University of Waikato, Verena Kaynig, Johannes Schindelin). The images were then used to calculate the area of alizarin complexone deposition and the total bone area as shown in Figure 1. These values were used to calculate total bone percent alizarin complexone deposition, as well as percent deposition values for the proximal, distal, and middle thirds of each bone.

Statistical analysis

All statistical work was done in Prism 9 (GraphPad Software) and R (The R Foundation). Percent alizarin deposition was compared using a T-test with Wilcoxon correction to check for statistically differences between groups for each of the bones studied. F-test was used to determine significance of variation between groups in each of the four bones. Descriptive statistics were used to determine mean, standard deviations and variation values.

RESULTS

When looking across the total bone at alizarin complexone deposition a trend was evident in the 15-minute and 30-minute oxygen pre-breathe groups: Both saw an increase in the percent deposition when compared to the 45-minute group (Figure 2). This, however, is not a statistically significant increase, with no bone showing significant increases between groups when compared by t-test. But when looking at Figure 3, the radius and humerus show a significant decrease in variance of DON occurrence when comparing the 45-minute group to the 30-minute, 15-minute and controls ($p < 0.0001$, $p < 0.05$) Both the femur and tibia saw significant decreases in variance when comparing the 45-minute group to the 30-minute and 15-minute groups, ($p < 0.0005$), but no significant differences were observed between the 45-minute group and the controls. In all bones the 15-minute and 30-minute groups showed no significant differences in variance of deposition. The radius and tibia saw the highest rates of lesions. The radius mean depositions were 0.05 ± 0.07 in the 45-minute group, 4.86 ± 5.00 in the 30-minute group, 13.15 ± 14.79 in the 15-minute group and 6.377 ± 12.36 for the controls.

Figure 1: initial bone image on the left and WEKA segmented selection of alizarin complexone (white) on the right

The tibia mean depositions were 0.13 ± 0.18 in the 45-minute group, 4.75 ± 5.99 in the 30-minute group, 6.78 ± 11.23 in the 15-minute group and 0.61 ± 0.4 for the controls. There was, however, no significant increase in deposition between any of the bones.

When evaluating the alizarin complexone deposition in the proximal third, a similar trend was observed. Figure 4 shows what looks like a trend toward an increase in deposition in the 30- and 15-minute groups, which is not significant. When comparing the 45-minute to the 15-minute, 30-minute and the controls the only bone that showed significant decreases in the variance in this third was the humerus ($p < 0.05$). In all other bones both the 45-minute group and the controls saw a significant decrease in variance of deposition when compared to the 15-minute and 30-minute groups. The bones that saw

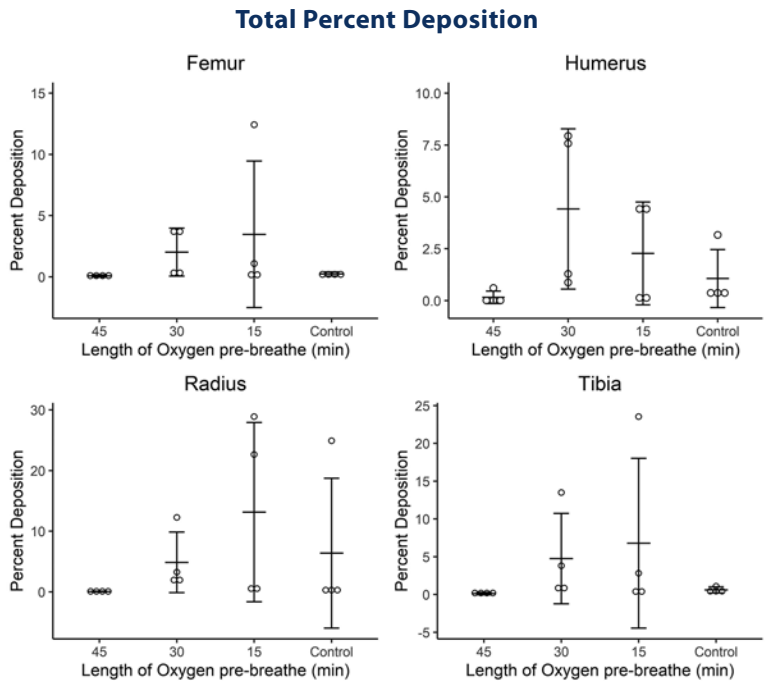

Figure 2: Percent deposition across the entire exent of the femur, humerus, radius and tibia. Treatment was 33 fsw for every sheep with one of four durations of pre-breathe.The groups were as follows: 45 minutes (n=2); 30 minutes (n=2); 15 minutes (n=2); and the controls (n=2).

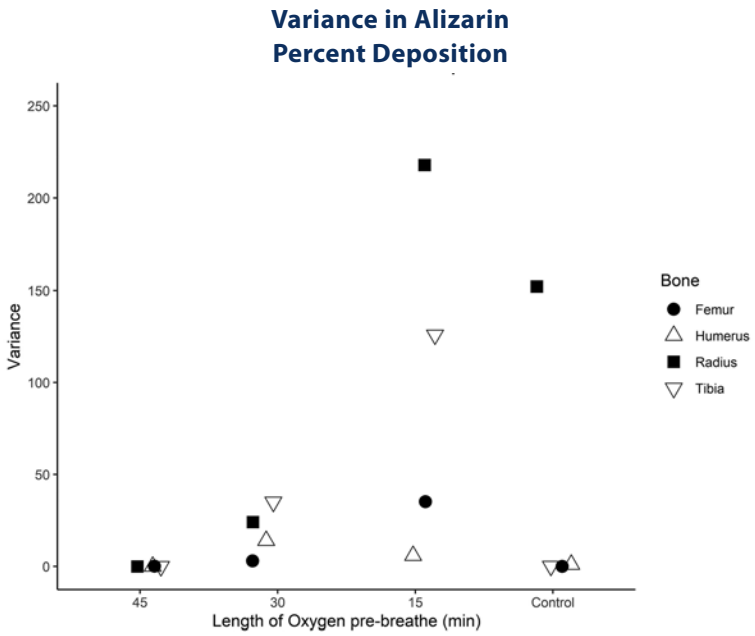

Figure 3: Variance of percent deposition across the entire extent of the femur, humerus, radius and tibia. Treatment was 33 fsw for every sheep with one of four durations of pre-breathe.

the most DON in the proximal third was the humerus and tibia. The humerus saw mean percent deposition rates of 0.322 ± 0.631 in the 45-minute group, 8.91 ± 7.69 in the 30-minute group, 4.06 ± 4.78 in the 15-minute group and 2.13 ± 2.87 in the control group. In the tibia the mean percent deposition rates were 0.0441 ± 0.0874 in the 45-minute group, 5.55 ± 6.63 in the 30-minute group, 7.82 ± 12.5 in the 15-minute group and 0.708 ± 0.932 in the control group. The humerus saw a significant increase in deposition in this third when compared to the femur ($p < 0.05$). However, all other differences in deposition between bones in this third were not significant.

Figure 5 shows that when compared to the distal and proximal thirds, the middle saw the least deposition in all four treatment groups. The humerus and tibia remained almost completely unaffected in this area, with the highest percent depositions occurring in the tibia of the 30-minute and 15-minute groups at rates of 1.71 ± 2.58 and 1.6 ± 1.99, respectively. The femur saw deposition at rates of 4.5 ± 8.98 in the 15-minute group, but all other groups remained unaffected. Of the four bones, the radius was the only bone to show significant signs of deposition, but these differences were not statistically significant. The 45-minute group showed significant decrease in variance of deposition when compared with the 15-minute, 30-minute and control groups ($p<0.0005$). The 30-minute group also saw a significant decrease in variance when compared with the 15-minute and the control groups ($p<0.05$) The 15-minute and the

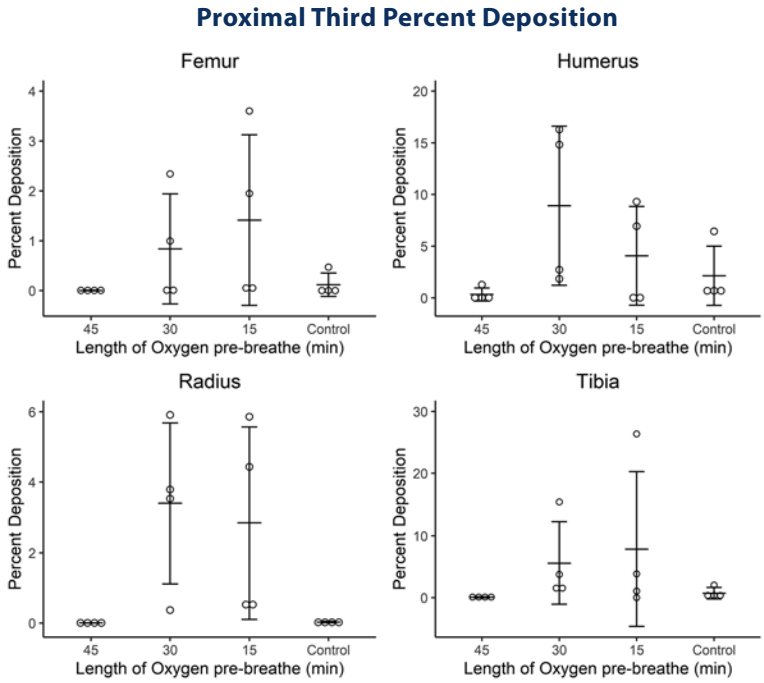

Figure 4: Percent deposition for the proximal third of the femur, humerus, radius and tibia. Treatment was 33 fsw for every sheep with one of four durations of pre-breathe. The groups were as follows: 45 minutes (n=2); 30 minutes (n=2); 15 minutes (n=2); and the controls (n=2).

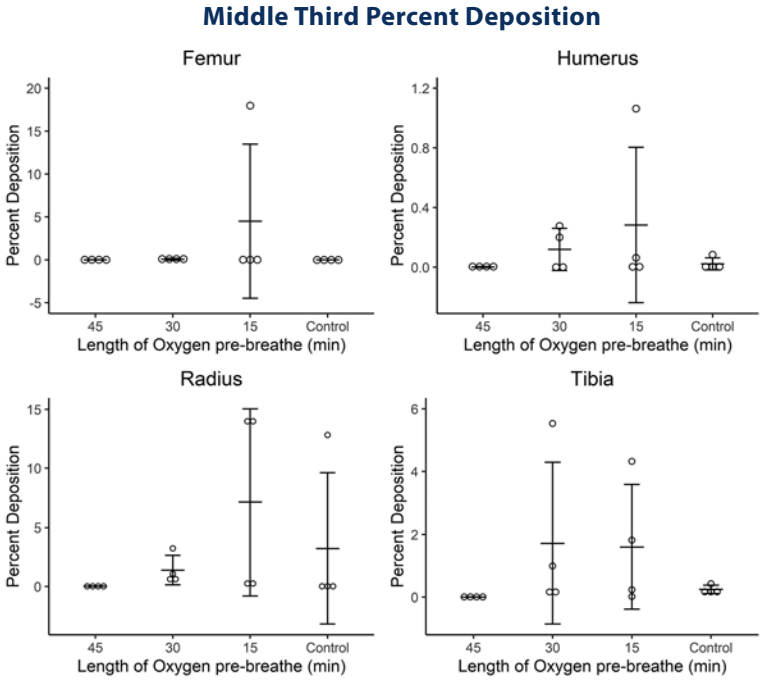

Figure 5: Percent deposition for the middle third of the femur, humerus, radius and tibia. Treatment was 33 fsw for every sheep with one of four durations of pre-breathe. The groups were as follows: 45 minutes (n=2); 30 minutes (n=2); 15 minutes (n=2); and the controls (n=2).

controls did not show significant differences in variances. In the radius the mean percent deposition rates were 0.0166 ± 0.0164 in the 45-minute group, 1.39 ± 1.26 in the 30-minute group, 7.12 ± 7.93 in the 15-minute group and 3.22 ± 6.4 in the control group. However, most of the deposition in radius in this third occurred near the distal region.

The trend observed in the proximal third and when looking at the total bone was also seen in the distal region as well (Figure 6). The 45-minute group saw the lowest amounts of deposition when compared to the other treatment groups. These differences were not significant. The 45-minute group saw significantly lower variance when compared to the 15-minute, 30-minute and control groups in the radius and humerus ($p < 0.005$). In the femur and tibia, the 45-minute group saw significantly lower variance when compared to the 15-minute and the 30-minute groups ($p < 0.005$). However, in theses bones the 45-minutes variance was not significantly lower than in the control groups. The two most affected bones in this third were the radius and tibia. In the radius mean percent deposition rates were 0.11 ± 0.169 in the 45-minute group, 8.61 ± 14.6 in the 30-minute group, 25.4 ± 30.2 in the 15-minute group and 12.6 ± 24.3 in the control group. As for the tibia the mean percent deposition rates were 0.405 ± 0.729 in the 45-minute group, 6.8 ± 11.2 in the 30-minute group, 9.57 ± 17.3 in the 15-minute group and 0.696 ± 0.75 in the control group. These differences between bones were not significant.

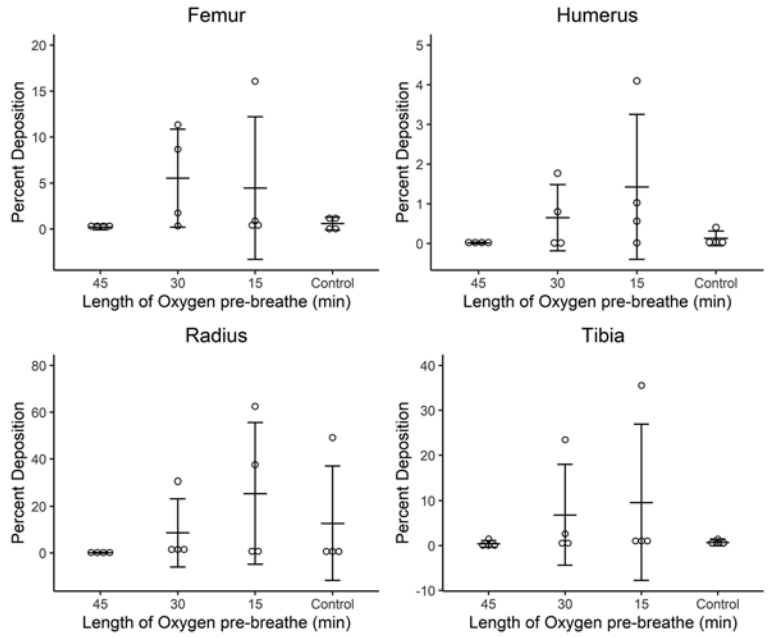

Figure 6: Percent deposition for the distal third of the femur, humerus, radius and tibia. Treatment was 33 fsw for every sheep with one of four durations of pre-breathe. The groups were as follows: 45 minutes (n=2); 30 minutes (n=2); 15 minutes (n=2); and the controls (n=2).

DISCUSSION

The 30-minute, 15-minute and control groups all showed large amounts of deposition in at least one of the bones studied. However, because of the small n value for these groups and the sporadic nature of DON lesion formation these differences did not appear to be significant. In spite of this fact, significantly high variability in DON lesions in at least one of the bones studied for all groups other than the 45-minute suggests there is confidence in the preventative capabilities of 45-minutes of O_2 pre-breathe under this study's conditions. We hypothesize that the variance observed in the 30-minute, 15-minute and control groups is indicative of an ineffective duration of O_2 pre-breathe which allowed for varying rates of DON based on a multitude of factors; these include body weight, bone composition, duration of dive and time at depth. The 45-minute pre-breathe was, however, enough at this study's depth and time to overcome these factors and increase the confidence in the reduction of DON. The fact that the 15-minute, 30-minute, and controls did not see a similar reduction in variance means that confidence in their preventative capabilities is not justified.

The increased N_2 partial pressure, high solubility and low perfusion rates in fatty marrow means that N_2 does not rapidly diffuse out of marrow. Because bubble formation is the main factor determining the development of DON, removing this dissolved N_2 may be an effective form of prevention. According to Dalton's law, raising the partial pressure of O_2 in blood through O_2 pre-breathe will lower the partial pressure of N_2. This lowering of the partial pressure of N_2 means it can diffuse more rapidly from highly saturated fatty marrow. The lower concentration of N_2 should therefore decrease the probability of bubble formation. A previous study done in the UW Sheep Model showed that two hours of O_2 pre-breathe greatly reduced the formation of DON lesions.

In that study, using similar methods, sheep were subjected to 15 minutes, one hour and two hours of O_2 pre-breathe at a depth of 60 fsw, while the sheep in our study were subjected to 33 fsw. (The 15-minute and one-hour groups were ineffective at preventing the formation of DON lesions.) However two hours saw a significant reduction in DON lesions. The discrepancy in the time of pre-breathe needed is most likely the result of the difference in pressure. As pressure increases during a dive the amount of dissolved gas in the tissue and blood also increases. The increased pressure in the previous study would have resulted in a greater amount of dissolved N_2. The exact amount of time required for N_2 to be flushed out completely remains uncertain and most likely varies between individuals because of factors that control the amount of dissolved N_2 and the rate at which N_2 can be washed out. In spite of this our results suggest that greater dive depth and therefore greater dissolved N_2 mean a greater length of pre-breathe is required. However, without further research into the exact relationship between these two factors this remains uncertain.

Confounding variables appear to have an impact on our results, most notably in the control group. Both the tibia and femur showed

no significant difference in variance between the 45-minute group and the controls. The fact that the control group saw a lower extent of DON lesions compared to the 15-minute and 30-minute groups appears to contradict the claim that 45-minute pre-breathe is causing the reduction of variance. However, DON does not uniformly affect those who experience rapid drops in ambient pressure. For example, when comparing DON to decompression sickness – another disease caused by bubble formation as the result of a rapid reduction in pressure – it is generally accepted that not all those who experience decompression sickness also experience DON [24]. In spite of this the UW Sheep model has been shown to accurately recreate DON lesions like those seen in humans [16]. This leads us to believe that the control results are a product of our small sample size and not indicative of larger relationship where both the no oxygen pre-breathe and 45-minute pre-breathe see similar preventative qualities in our study environment. On the contrary, the lower percent deposition in the controls is most likely the cause of their lower weights compared to the other treatment groups. As previously mentioned, the mechanism for DON is not entirely understood, and a complete list of risk factors remains uncertain. Variables such as higher body mass index (BMI), fat mass, concentration of lipids in blood and plasminogen activator inhibitor-1 have all been linked to increases in dysbaric disease [14,25-27]. However without further study the effect of these factors remains uncertain. More research is needed to better understand the effects of BMI and bone composition along with the previously mentioned effects of pressure and depth.

CONCLUSIONS

This study's finding of low rates of DON lesion formation in the 45-minute pre-breathe group as well as a significant reduction in variance of DON lesions compared to controls and other pre-breathe groups suggests the possible preventative capability of O_2 pre-breathe. However, a larger sample size is required to determine the efficacy of O_2 pre-breathe treatment. Moreover, in order to effectively determine the amount of O_2 pre-breathe needed to prevent DON lesions for a specific individual, a better understanding of the relationship between factors including but not limited to BMI, bone composition, dive depth and time at depth is needed.

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