Normobaric oxygen can enhance protein captation by the lymphatic system in healthy humans.

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INTRODUCTION

The use of normobaric oxygen (NBO) as a first aid tool for decompression sickness (DCS) has been advocated for a long time. Several beneficial effects of NBO have been demonstrated, one of which is the faster elimination of tissue nitrogen bubbles.

During DCS occurrence, a cascade of intravascular reactions has been demonstrated. These inflammatory reactions occur within minutes of the onset of DCS, and involve the precipitation of proteins on the gas-bubble interface, causing a stabilization of the intravascular and tissue bubbles. Little is known about the elimination of the protein-coated bubbles except that they are smaller than the obstructive ones, thus allowing them to pass through the circulation and probably enter the interstitium during the extravasation phase, subsequent to hypoxia.

Also known is that the proteins can denature. This may cause the accumulation of free fat globules found during decompression sickness (1, 2). Fat emboli have been observed in several studies of decompression sickness and can contribute to central nervous system damage(3). As interstitial proteins are evacuated by the lymphatic circulation, we wanted to investigate if NBO enhances lymphatic activity and thus, protein elimination (4, 5).

The rationale relations between edema and hypoxia are clear if we think of the augmented distance between capillaries and the presence of "oxidative burst" during inflammation involving bacterial activity (6). Furthermore, the presence of a large number of mitochondria in the lymphatic endothelium shows a marked oxidative metabolism for the activation of lymphatic contractile properties. It has been shown that reactive nitrogen species can reduce the contractile properties of the lymphatic vessel. Hence, denitrogenation has been proposed as a positive action on lymphatic contraction (7).

Oxygen, it seems, is involved in positive effects on lymphatic vessel metabolism and edema reduction. The potential interest in clinical use and a more in-depth understanding of NBO during in-field decompression first aid lead us to test the beneficial link between oxygen and lymphatic protein captation (8, 9).

METHODS

Seven healthy subjects (five males and two females) received an injection of 0.2 ml of Tc99-marked human albumin (HSA Nanocoll), diluted with 2.3ml of S.S.P.P., into the first interdigital space. This injection produced moderate subcutaneous edema. The saline preparation of the injected proteins had a protein size range between 50 and 100 nm, allowing the solution to be exclusively reabsorbed through the lymphatic bed. The proteins were first phagocyted by macrophages, then drained via the lymphatics (10). The lymphoscintigraphic technique was chosen because it is the usual clinical investigation for lymphatic activity, particularly on the upper limb for breast cancer patients.

Experimental lymphoscintigraphy sessions were performed while subjects (age range 19-27 years) were recumbent. Initial studies were performed while breathing air. On a separate day, the procedure was repeated, but the subjects breathed NBO from oronasal demand regulators (Life Support Product, Ltd.) immediately after injection and continued breathing for thirty minutes. The dynamics of the isotopic activity at the axillary ganglia was recorded to assess the speed and quantity of the lymphatic protein drainage. In parallel, $TcPO_2$ in the edema region was constantly monitored.

Exclusion criteria were diabetes, pregnancy, vascular disease, upper limb traumatic lesion, and some sports practice, such as volleyball and martial arts, since such activities can induce lesions of the lymphatic system.

RESULTS

Qualitative analysis

Since lymphoscintigraphy (**Figure 1**) is basically qualitative, we began with the classical approach. In all subjects, NBO produced a marked increase in the isotopic activity at the axillary level after one hour, starting thirty minutes after the beginning of oxygen breathing (the first thirty minutes were kept as the baseline level).

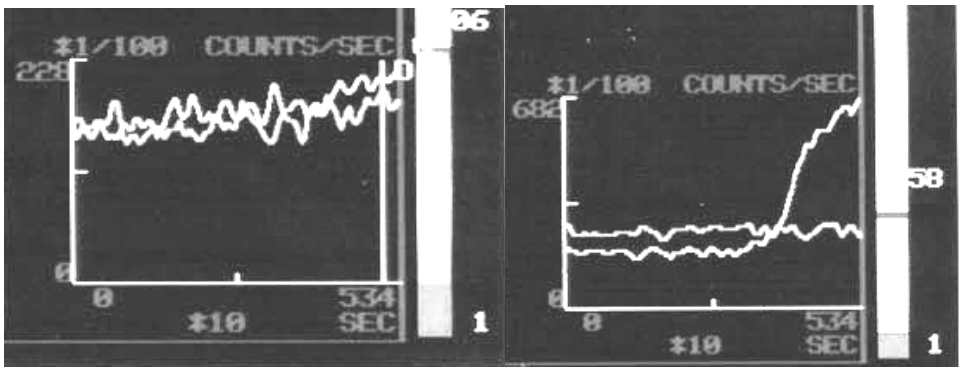

Figure 1. The figure (left side) shows a typical lymphoscintigraphy without oxygen breathing. The pertinent trace is the white one because the other represents the other arm, without experimental edema. During oxygen breathing (right side), the trace of the injected arm rises drastically in all subjects. The Y-axis expresses the number of isotopic impacts per second; the X-axis expresses the number of frames stored. Each frame equals ten seconds.

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In these subjects, $PTcO_2$ levels at the site of edema showed a marked increase during the first ten minutes. A plateau phase is then observed during the complete oxygen breathing time, followed by a rapid return to baseline after oxygen breathing cessation.

Quantitative analysis

This analysis is uncommon in lymphoscintigraphy. We analyzed the area under the trace (arbitrary units) to compare lymphatic protein elimination with and without oxygen breathing. The statistical analysis was accomplished using paired t-test after passing normality by the Kolmogorov-Smirnov test. The area under the lymphoscintigraphic trace was significantly greater after oxygen breathing. The total amount of proteins eliminated was greater after 30 minutes of 100% oxygen breathing (P=0.0226).

The second quantitative analysis was the angular variation of the trace. We looked at the parameter that could best express the evacuation speed of labeled proteins. We chose the angular variation of the lymphoscintigraphic trace because the trace's slope expresses the speed of elimination. **Figure 2** shows the mean angular variation of the lymphoscintigraphy trace. Control values were computed during the non-oxygen protocol. The pre-O_2 values were the angular values of the trace before oxygen breathing (baseline values). Increased absorption speed was noted after 30 minutes of 100% oxygen breathing.

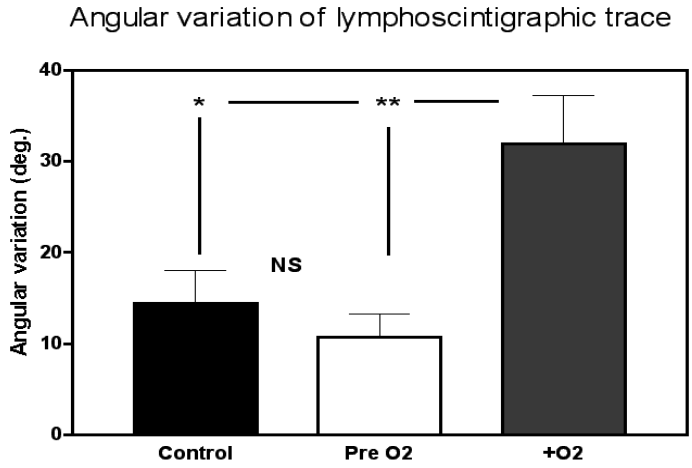

Figure 2. Statistical comparison between the control absorption speed (angular variation) in the axillary region; the pre-oxygen situation and after 30 minutes of normobaric oxygen breathing.*=p>0,05; **=p>0,01

CONCLUSIONS

Breathing normobaric oxygen enhanced protein removal by the lymphatic system in all subjects (**Figure 3**). Our findings support an interest in giving oxygen for at least thirty minutes during on-site first aid in diving-related accidents, as this may be beneficial in increasing elimination of even protein-coated bubbles by the lymphatic bed. Other clinical interest may be in the treatment of lymphoedema, perhaps by administering normobaric 100% oxygen during manual drainage. In order to better understand our observations, new investigations, using different sizes of proteins, are planned to further clarify this phenomenon and explore possible macrophage activity influence.

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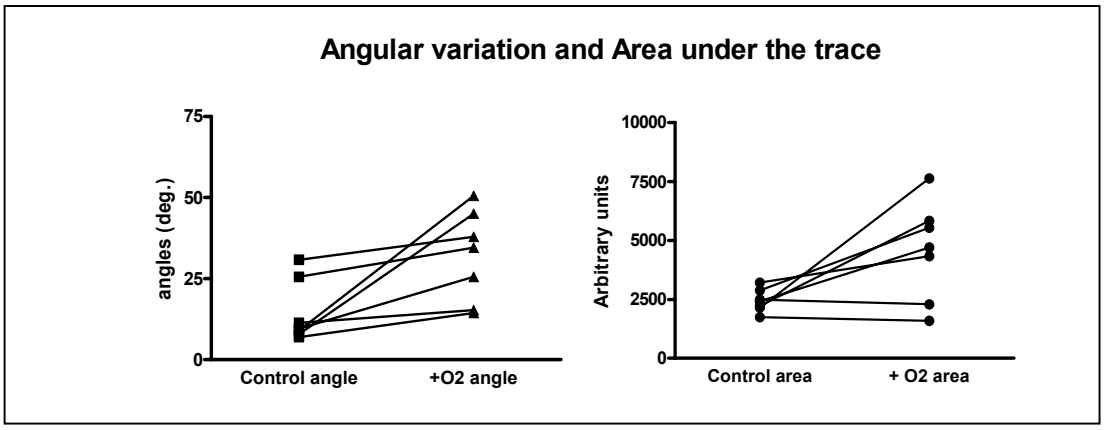

Figure 3. Experimental data comparing (left side) angular variation of the lymphoscintigraphic trace after oxygen breathing, giving us an idea of the increased speed of resorption of marked albumin and the area under the curve, which expresses the amount of total marked proteins detected in the axillary area.

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