ENDOTHELITUM AND BUBBLE INJURY: 
THE ROLE OF ENDOTHELITUM IN DECOMPRESSION ILLNESS
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Introduction
The recent public debate in Norway, initiated by the claim by the so called "Pioneer Divers" that they have been permanently injured as a result of their diving activity, has underscored the need for a fresh look at our diving procedures and the possible health effects of these procedures.

The Norwegian Parliament established a commission to look into the claims made by these divers and concluded that decompression sickness may have been one of the factors responsible for their problems. The divers have been investigated at Haukeland Hospital in Bergen, Norway, showing that about 90% of them have had decompression sickness, and about half of the cases were never reported (preliminary internal report). This is in line with a study we performed 10 years ago, where about 10% of the professional divers and 25% of the sports divers had had symptoms of decompression sickness that never were reported (9). Data from both Norway and from other countries show that even if the incidence of decompression sickness is quite low, about 0.01 to 0.05 % (23), the prevalence is considerably higher. In Norway about 10 divers pro year are treated for decompression sickness, the majority of them with symptoms from the central nervous system. About 30% of them have sequelae from these accidents. Clinical symptoms have traditionally been used for evaluating decompression procedures. This is based on the assumption that if symptoms from muscles and joints (Type 1 symptoms) can be avoided, then these procedures can be considered safe. However, this may not necessary be true. Ten years ago, an international conference on the long term effects of diving was held in Norway, they concluded the following:

"There is evidence that changes in bone, the central nervous system and the lung can be demonstrated in some divers who have not experienced a diving accident or other established environmental hazards. The changes are in most cases minor and do not influence the diver's quality of life. However, the changes are of a nature that may influence the diver's future health ". (26)

Furthermore, a significant number of recreational divers with grade 1 symptoms also have symptoms from the central nervous system (42). As overt clinical symptoms are quite rare in diving, the evaluation of new procedures is increasingly based upon bubbles observed in the pulmonary artery. There is little information about the real incidence of long term effects of diving, nor is there any agreement about the possible mechanisms for such effects. It is generally acknowledged that the injuries to the organism related to diving are caused by gag bubbles. Gas taken up during the dive must be eliminated during and after return to surface. As the partial pressure of gag in the organism will be higher than the environmental pressure, gag may come out of solution, forming bubbles. To prevent this, decompression procedures have been developed. Modern procedures have significantly reduced the incidence of clinical signs of decompression sickness. However, it is generally acknowledged that gag bubbles are formed in the vasculature on most decompressions, as only very low levels of supersaturation are needed for that (16). We observed bubbles in the carotid artery in the majority of divers participating during excursion from saturation on an experimental dive (10). These bubbles have been called "silent bubbles" when no clinical symptoms are present (3). While it is possible that bubbles in the tissue may play a role in DCS (17), vascular bubbles are probably...
the cause of serious symptoms from the lungs and the central nervous system (3; 24; 30). For instance, a study by Palmer (34) demonstrated that the bubbles seen in the spinal cord in severe experimental decompression sickness were all intravascular. As we know that gas bubbles can be observed in the circulation in a majority of divers and thus divers are regularly exposed to such bubbles, it is of importance to determine the effect of the bubbles, how the amount of bubbles can be reduced and how possible harmful effects can be prevented.

**Mechanism of injury**

From our own experimental studies and from studying the literature, our main hypothesis is that the main mechanism for injury to the central nervous system is the effect of bubbles on the vascular endothelium. We have shown in several studies that bubbles will damage or reduce the function of the endothelium in a dose-dependent manner (31) (32), this has also been seen by other authors (36). If gas bubbles reach the brain circulation, they will lead to injury of the Blood-Brain-Barrier within minutes (7; 12).

We have recently performed a pilot study in experienced Navy divers, where we showed that a dive at 18 metres with little bubble formation led to a reduction in endothelial function of the arterial side. Even more surprising was the fact that these divers had an indication of a reduced endothelial function even before they performed the dive (Brubakk et al. in manuscript, Abstract this meeting).

Activation of endothelium will lead to production of so-called Endothelial Micro Particles (EMP) (27). Such activation has been observed in a number of cardiovascular diseases and after using a heart-lung-machine (43). It is therefore not unlikely that gas bubbles can lead to such activation. Studies have shown that circulating activated micro particles can reduce endothelial function (6). This reduction in endothelial function is caused by a reduction in Nitric Oxide (NO) production (18). The activated endothelial cells will have an increased expression of so-called adhesion molecules, VCAM, ICAM and E-Selection. It has been shown that activation of C5a lead to an increased expression of such adhesion molecules and that this will happens after about 4 bouts (2). This is in good agreement with our finding that the reduction in endothelial function could be observed between one and six hours after exposure to gas bubbles (32). We have previously shown that gas bubbles lead to an increase in C5a and that this increase is dose-dependent (4). In a newly finished pilot study an increase in VCAM and ICAM was observed in the blood of divers five minutes after surfacing, this effect lasted twenty-four hours (G. Laden, personal Communication, UHMS 2004). Numerous studies have shown that endothelial dysfunction is independently related to future cardiovascular events (e.g., myocardial infarction, stroke, transient ischemic attack). (22).

Heat Shock Proteins (HSP) are formed in the body when the organism is exposed to a number of stressors like hyper-or hypoxia, heat, cold, exercise and some heavy metals or drugs. HSP has an important function in controlling the folding and structure of proteins and protects the organism from injury (40). The induction of HSP for protection is described below. However, in some cases, expression of HSP may contribute to injury. Divers may be exposed to a number of stressors that could possibly influence the effect of bubbles. It has long been known that infections increase the risk for developing atherosclerosis (37). Saturation divers are exposed to considerable stress (hypermia, hard work, exposure to infections a.s.o.), which could lead to an increase in HSP over longer periods of time. Of particular interest is the exposure to bacteria, as infections still are a significant problem in saturation diving operations (1). Certain bacteria produce HSP which is strongly antigenic and that can trigger a significant immuno response (5). One study demonstrated a considerably stronger
response to endothelial damage in animals with such a response (19). If the bacterial flora in the chamber can produce such an immunological response, this would indicate that saturation diving may have a higher risk of endothelial damage by bubbles than other types of diving.

**Prevention of injury**

In a number of studies in rats, we have shown that the amount of vascular bubbles following a dive can be significantly reduced by performing heavy physical exercise 20 hours before the dive. This effect has disappeared after 48 hours, while exercise closer to the dive has no effect. Exercise with increase in blood flow and increase in shear stress will increase the endothelial production of Nitric Oxide (NO). NO is the most important vasodilator and also reduce the "stickiness" of the endothelial surface (II; 28). We have been able to show that bubble production will be increased by NO blockade and that the effect of exercise may be simulated by adding NO (45-47). The effect of exercise has also been shown in man. In a group of divers performing a dive to 18 meter we had a significant reduction in bubble formation if heavy exercise was performed 24 hours before the dive (15). The above findings were quite surprising and have significantly changed our opinion on how bubbles are formed and how their formation may be controlled.

Traditionally the reduction of bubble formation to prevent decompression sickness (DCS) has been achieved changing ascent rates. Even if the procedures used today have a low incidence of DCS, we have demonstrated experimentally (8) as well as theoretically (Gutvik and Brubakk, UHMS 2004) that there still is considerable room for improvement. We do, however, believe that the above observations indicate a novel and more efficient way of reducing bubble formation.

De novo formation of bubbles requires supersaturations exceeding those seen in the vascular system on bubble formation by a factor of 50-100. It is therefore assumed that bubbles grow from so-called bubble nuclei, these nuclei are about 1 μm and are gas-filled (48). These nuclei are not stable in blood, but on a hydrophobic surface such bubbles will be stable more or less indefinitely (29). Hydrophobic areas exist on the endothelial surface in the form of Caveola, where the production of NO also is localized (38). A reduction of surface tension on such a surface will increase the number of stable nuclei (Vann R, Personal communication). We have previously shown that there is a relationship between surface tension of serum and bubble production and demonstrated that a small reduction in surface tension significantly will increase bubble production (25). We believe that variations in surface tension and/or NO production can explain the large intra- and inter personal variations in bubble formation observed. This could also explain why an increase in body fat lead to an increase in the risk for DCS, as it is also shown that an increase in Low density Lipoproteins will decrease NO production (14). We also believe that this possibly could explain why repeated exposure will reduce the risk for DCS (3).

As mentioned above, we have shown that blocking NO production promote bubble production, in the same study we demonstrated that heavy exercise 20 hours before the dive prevented this (46). This study was performed in rats legs than 280 g. When heavier animals were used (> 300g) this effect could no longer be seen (Wisloff et al 2004 In manuscript). Acute heavy exercise increases blood lipids by approximately 30% immediately after exercise is finished (44). A decrease in surface tension will significantly increase adhesiveness to the vessel wall ((41). However, over the next hours, blood lipids are gradually reduced, this effect has disappeared after few days (20). This effect is more pronounced in the trained than the untrained and is also dependent of intensity of exercise. Twenty-four hours after exercise...
HDL is increased (35). This could be a mechanism to explain why exercise 24 hours before a dive protects lean but not fat animals that are NO blocked. In the lean animals, with lower lipid levels, exercise will reduce blood lipids, allowing endothelial adhesiveness to be reduced sufficiently to allow washing out of bubbles, while the effect is not strong enough in the fat animals with blocked NO. This mechanism could also explain why heavy exercise shortly before decompression could increase bubble formation, more bubble nuclei adhere, so there are more nuclei to row from. A recently finished study from our laboratory demonstrated that exercise performed 75 minutes before decompression had no effect on bubble formation (Berge V et al in manuscript 2004) and that a short acting NO donor (Nitroglycerin) significantly reduced bubble formation if given at the start of a saturation decompression (Mollerlekkken et al in manuscript 2004). Both these findings support the hypothesis that bubble nuclei and their adherence to the vessel wall is critical for, bubble formation in the blood.

Injury by bubbles may be preventable through other mechanisms. As described above, Heat Shock Proteins (HSP) are formed in the body when the organism is exposed to a number of stressors. The protective effect is strongest hours to a day after the stress episode (40). HSP90 is involved in the production of NO (33).

We have shown in rats that increasing body temperature to 42°C 24 hours before the dive, reduces mortality by 50% and that this exposure increased HSP70 but not HS90 and eNOS (Medby, Bye et al 2004 in manuscript). Exercise will also have an effect on HSP expression. A study by Siu et al (39) showed that exercise increased SP70 2100ù in 48 hours after the last exercise bout. This study also showed that exercise reduced apoptosis. Other studies have shown that HSP70 increased immediately after exercise, with a new 24 and 48 hours later (21). The expression of HSP70 following exercise is reduced in older individuals (13). Preliminary data from a pilot study performed at our laboratory show that HSP70 expression in the aorta is correlated to bubble formation. In this study, the dive procedure used gave massive bubble production, it remains to be seen if more moderate bubble production will have the same effect. Our data is supported by data showing that HSP70 was found increased in animals showing signs of decompression sickness (Hang et al UHMS 2004).

Based on the above, we suggest that damage and/or reduction in endothelial function is a central mechanism in the development of serious decompression injury and possibly also in long term effects of diving.

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**Reference List**