Review article
The role of intra-vascular bubbles and the vascular endothelium in decompression sickness
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Key words
Decompression, bubbles, Doppler, endothelium, stress, exercise, review article

Abstract

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Although decompression procedures have been improved over the years, decompression still remains a significant problem in diving. While there is universal agreement that the basic problem of decompression is gas coming out of solution, forming bubbles when pressure is reduced, the exact mechanism of decompression injury is not known. Furthermore, the wide variety of clinical symptoms and the significant difference in individual susceptibility makes identification of the mechanisms involved difficult. Using ultrasound, vascular gas bubbles have been detected in most decompressions, and these bubbles can act on the endothelial lining of blood vessels resulting in impaired endothelial function. Normal endothelial function is a major indicator of cardiovascular health and thus a reduction in vascular bubble formation and hence the risk of endothelial injury is an important goal in decompression. Even if vascular gas bubbles may not be the only adverse effect of decompression, vascular gas bubbles and their adverse effects on the endothelium may be a useful model for decompression injury. This review claims that endothelial dysfunction may be a possible main mechanism for neurological decompression injuries and describes some of the effects of vascular gas bubbles on the endothelium. Furthermore, as the formation of vascular gas bubbles can be significantly influenced by physical exercise and the use of nitric oxide, a novel approach to reducing the risk of decompression injury is suggested.

Defining adverse effects of decompression

Following return to atmospheric pressure after a dive or an exposure to altitude, clinical symptoms and signs can occur. These vary from mild to very severe including death, and have been given a variety of names: decompression sickness (DCS), decompression illness (DCI), 'niggles', aeroembolism, nitrogen disease, diver's palsy, and compressed air illness to name a few. The most commonplace name, the 'bends', is attributed to the posture adapted by fashionable ladies at the turn of the 20th century, the 'Grecian bend'. This posture, bending forward at the waist would give some comfort to a diver in acute pain. As described by Ferris,

"the term is used to denote the syndrome of pain and disability, localized in the locomotor system ... The pain is usually localized in the joints and may radiate up and down the extremities involved ... The subjective pain is usually described as being a deep aching pain, difficult to localize, which – once it begins – usually progresses in intensity with periods of waxing and waning ... When severe it is associated by functional impairment of the involved extremity, with a feeling of numbness and weakness of the part and with faintness."1

Or as was described by Behnke,

"The major symptoms and signs of decompression sickness are pain (bends), asphyxia (choke) and paralysis. Minor effects are rash and fatigue. The parts of the body chiefly involved are the extremities (bends), cardiorespiratory system (choke) and the spinal cord.”2

Even today, there is probably little to add to Behnke’s 1951 description, with the possible exception that the brain may be more frequently involved and that extreme fatigue may be a more serious sign than previously thought.3

Traditionally, the symptoms following decompression (dysbarism) have been categorised according to their anatomical location and severity:

• Type I (mild): muscle and joints, skin, lymphatics, malaise / fatigue
• Type II (serious): spinal, cerebral, vestibular, cardiopulmonary (‘choke’)
• Arterial gas embolism
• Barotrauma.

This classification implies that the different categories are well defined disease entities and that there is reasonable agreement between doctors about the classification. However, studies have demonstrated that there is considerable uncertainty between experts about classification.3 For instance, many cases of cerebral DCS cannot be distinguished from arterial gas embolism or vestibular barotrauma. Furthermore, several studies have shown that joint symptoms alone are uncommon; they are usually accompanied by central nervous symptoms.4,5 According to Diver Alert Network (DAN) data, 40% of symptoms are neurological, 13% are...
vestibular and 22% pain.\textsuperscript{6,7} Extreme fatigue can be classified as a harmless sign or a sign of subclinical pulmonary embolism. Therefore, the term ‘decompression illness’ was suggested to include both decompression sickness and arterial gas embolism.\textsuperscript{8} It was further suggested that the disease should not be classified as Type I and Type II, but instead described according to clinical symptoms and their development. Using this classification scheme, a high degree of concordance between different doctors was reached.\textsuperscript{9}

However, the classification debate loses some of its importance when we realize that all clinical signs of DCS are treated similarly by using oxygen and pressure. Nevertheless, in discussing the more general problems related to the effects of decompression, several other definitions may be used:

- Acute clinical symptoms requiring treatment in individuals who have been exposed to a reduction in environmental pressure
- Acute clinical symptoms in individuals who have been exposed to a reduction in environmental pressure
- Organic and/or functional decrements in individuals who have been exposed to a reduction in environmental pressure
- Vascular gas bubbles without clinical symptoms in individuals exposed to a reduction in environmental pressure.

The first definition is the one traditionally used and is incidentally the one used to evaluate the effectiveness of decompression procedures. This is probably quite accurate if serious symptoms occur. However, decompression illness requiring treatment is a rare disease. In commercial diving, the incidence of treated DCI is probably below 1%.\textsuperscript{10} In recreational divers, the incidence appears to be much lower – about 0.01−0.05%.\textsuperscript{11} However, these general numbers hide the fact that, even in commercial operations, DCS shows considerable individual differences/variability (see below).

Even if it is uncommon, a large proportion of divers have been treated for DCI. In a survey of divers in an off-shore diving company in 1985, 38% of the divers with 1−9 years’ experience and 62% of those with 10–24 years of experience had been treated.\textsuperscript{12} A survey of a large population of Norwegian divers showed that 3% of the recreational divers and 28% of the experienced professional divers had been treated for DCI.\textsuperscript{13} For many years, there has been anecdotal evidence that clinical symptoms of DCS are considerably under-reported. In the Norwegian survey, 19% of the sports divers, 50% of the professional air divers and 63% of the saturation divers reported that they had had symptoms that had not been treated with recompensation; a majority of these symptoms being neurological.\textsuperscript{14} Interestingly, there was a statistical relationship between this and later minor CNS symptoms.

Newer data from DAN have shown that, in recreational divers with DCS, pain is only present in about half of the cases, that injuries of the spinal cord and symptoms from the lungs are quite common and that 17% had experienced extreme fatigue.\textsuperscript{7} Fatigue has been described as a sign of subclinical pulmonary embolism, further supporting the theory that vascular gas bubbles may be an important factor in neurological DCS. If, however, the symptoms are less marked, considerable under-reporting is likely, and the second of the four definitions above perhaps provides a more accurate description.

The third definition includes both acute and chronic changes related to decompression. These may be related to acute clinical symptoms or develop sub-clinically. A recent consensus conference determined that such changes, even in individuals with few or no reported symptoms, have been found in the bones, central nervous system and the lungs of divers.\textsuperscript{14}

The last definition is similar to the so-called ‘silent bubbles’ described by Behnke.\textsuperscript{2} The term silent refers to the fact that these bubbles do not lead to acute clinical symptoms. Most will probably not regard this as DCS. However, the fact that such bubbles are present during most decompressions is similar to the situation in many infectious diseases with detectable pathologic flora and few or no symptoms. The question still remains whether these bubbles have an effect on the organism. 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.

Describing the possible signs and symptoms following decompression does not provide a full understanding of DCS, the presentation of which is protean in nature and severity, and the prognosis varies markedly between patients. A doctor with considerable experience in treating DCS commented that “the signs and symptoms of DCS are more varied than the symptoms of syphilis and diabetes together”.\textsuperscript{6}

The majority of cases of DCS can be classified as a disorder, “a disruption of normal physical or mental functions”.\textsuperscript{15} Probably only neurological DCS fits the dictionary definition of a disease: “a condition of an organ, part, structure or system of the body in which there is incorrect function resulting from the effect of heredity, infection, diet or environment. A disease is a serious, active, prolonged and deep-rooted condition”.\textsuperscript{15}

We have proposed the term ‘adverse effects of decompression’ (AED) as a useful indicator of decompression stress and decompression risk.\textsuperscript{16} This is supported by Thalman who suggested that "minor symptoms like fatigue and transient niggles must be considered as they probably indicate a higher level of decompression stress as completely asymptomatic tables".\textsuperscript{17}
Decompression stress

Stress is a concept that comes from physics, describing the effect of forces against a resistance. However, in medicine and biology, biological stress, a concept developed by Hans Selye, is defined as a general pathophysiological response, where similar symptoms and signs develop in response to a variety of agents and conditions. This phenomenon is termed the ‘general adaptation syndrome’.10 The term has also been used for a long time in psychology to describe the effects on the body that indicate a strong negative psychological or physical pressure or tension exceeding the mental or behavioural resources of the individual.

Decompression acts as a stressor, and decompression stress is the effect on the organism of the physical and physiological factors accompanying decompression. Even without any acute signs and symptoms, vascular gas bubbles can be an indicator of the magnitude of stress. In view of the fact that the majority of dives lead only to minimal symptoms despite the formation of gas bubbles, a major aim in developing safer procedures would be to provide an indication of the risk for injury in a particular dive.

Vascular gas bubbles as a marker of decompression stress

It is generally acknowledged that the injuries to the organism related to decompression in diving are caused by gas bubbles, and that the ‘bends’ is the reaction of the body to bubble formation. The amount, duration and location of the gas phase will influence the risk of acute symptoms and the degree of injury. Following this, it is also reasonable to assume that a reduction in the gas phase will reduce the risk of injury, both acutely and in the longer term. The evolution of a gas phase within the body is outside the scope of reference for this paper and readers are referred to a useful summary.6

Gas bubbles are formed in the vasculature on most decompressions, as only very low levels of supersaturation in the body appear to be needed for bubble formation.19 The evaluation of new decompression procedures is increasingly based on bubble detection in the pulmonary artery using ultrasonic techniques.20 While it is possible that bubbles in the tissue may also play a role in neurological DCS, vascular bubbles are probably the main cause of serious symptoms from the lungs and the central nervous system.22–24 Since gas bubbles may be observed by Doppler or through ultrasonic imaging in the circulation in a majority of divers, and since divers are regularly exposed to such bubbles, it is important to determine their effect, how bubble formation can be reduced and how possible harmful effects can be prevented.

At present, there is only one practical way of evaluating bubble formation, namely by monitoring bubbles in the venous system.20 As all blood enters the lungs through the right side of the heart, it is also reasonable to assume that bubbles in the pulmonary artery might be a good indicator of the total amount of free gas in the body. Whilst the sensitivity and specificity of pulmonary artery Doppler-detected bubbles is somewhat limited in predicting clinical DCS, there is general agreement that the risk of DCS increases with increasing numbers of bubbles.25 For air dives, DCS is always accompanied by vascular bubbles, if all monitoring sites are considered.26 There is even stronger support for the observation that the lack of detectable pulmonary artery bubbles is associated with a low risk of DCS.20 If no bubbles are observed following air dives, any clinical symptoms or signs seen are probably not caused by DCS.27 However, it must be borne in mind that bubble detection is performed intermittently so that bubbles may be missed. Even if the detection of vascular bubbles has the advantage of being an objective indicator, better tools are needed as the same decompression stress may produce quite different amounts of separated gas in different individuals.

In aviators with localized joint pain from DCS, gas could be seen in peri-articular and peri-vascular tissue spaces, and there was a correlation between the occurrence of gas and pain. Ferris and Engels demonstrated in the 1940s that strain and muscular activity were correlated with joint pain in altitude DCS.1 Local compression could reduce or remove the pain in many cases, and pain could be eliminated by occluding arterial inflow to the limb. This suggests that a diver complaining of joint pain has most likely been exposed to two types of decompression stress, namely tissue gas in and around the joint and intravascular gas in the pulmonary circulation.

Decompression risk

In order to develop safer procedures for all divers, it is important to evaluate whether one diver can have a higher risk of DCS compared to another diver. In the literature, several risk factors such as obesity, age and physical activity have been claimed to influence decompression risk.6 It has also been claimed that differences in work load, temperature and blood flow may have a significant effect upon decompression outcome.28 In a major study of tunnellers in the United Kingdom by Colvin, those who had been treated for DCS were compared to workers who had no symptoms.29 Four per cent of the work force contributed to 50% of the treatments for DCS, with no differences in work activities between the groups. Similar findings were reported in a small study from the 1950s, whilst Walder noted similar findings in the 1940s but with a slightly higher incidence (18%) of DCS.30,31 Considerable differences in DCS incidence were noted between different companies in the 2003 report, indicating that operational factors may also play an important role.29 No relationships between the occurrence of DCS and pre-clinical findings were observed; the only significant factors identified being absolute pressure and the duration of exposure. This is similar to the findings of Shields et al in North Sea divers, where the depth and
duration of the dive (expressed as $p^2T$, $p$ in bar and $T$ in minutes bottom time) were related to the incidence of DCS regardless of which decompression tables were used.\textsuperscript{32}

In two separate studies, we performed the same dive (18 metres’ sea water (msw) for 80 minutes) in two groups of similarly aged, well-trained military divers who underwent the same training and activities.\textsuperscript{33,34} There was a significant difference in vascular bubble formation in the two studies, the amount of vascular bubbles in the two groups differing by a factor of approximately twenty. At present, we have no explanation for these findings. According to Lanphier et al, long, shallow or short, deep dives both have a high incidence of DCS pulmonary symptoms (‘chokes’).\textsuperscript{35} The main conclusion from these studies is that, in any particular group of divers, there is a small percentage (approx 5–20\%) that has a significantly higher risk for being injured than the rest of the group. These data also demonstrate that traditional pre-clinical testing of the divers will not necessarily identify those who are most susceptible.

The vascular endothelium and bubbles

The vascular endothelium plays a vital role in homeostasis and is recognized as an organ with important autocrine and paracrine functions. The endothelial cells produce a large number of both vasoconstriction and vasodilating substances, which act on the underlying vascular smooth muscle. Probably the most important endothelial-derived relaxing factor is nitric oxide (NO). NO is produced by the endothelial isoform of nitric oxide synthase (eNOS). In addition to relaxing vascular smooth muscle, NO counteracts the formation of atherosclerosis through inhibition of leukocyte adhesion and invasion, smooth muscle proliferation, platelet aggregation and inflammation.\textsuperscript{36} Abnormalities in one or more of the pathways that ultimately regulate the availability of NO may lead to endothelial dysfunction. Endothelial dysfunction, as defined by impaired endothelial-dependent vasodilatation, has been identified as an independent risk factor and a strong prognostic marker of long-term cardiovascular morbidity and mortality in latent and manifest cardiovascular disease.\textsuperscript{37,38}

Several studies confirm that bubbles will damage or reduce endothelial function in a dose-dependent manner.\textsuperscript{39,40} In the cerebral circulation, they lead to injury of the blood-brain-barrier within minutes.\textsuperscript{41,42} We hypothesise that

\textit{“the main mechanism for dysfunction or injury to the central nervous system after decompression is the effect of bubbles on the vascular endothelium.”}\textsuperscript{43}

In one of the studies mentioned above in navy divers, where a dive to 18 msw led to little bubble formation, a reduction in arterial endothelial function was observed and these divers had reduced endothelial function even before they performed the dive.\textsuperscript{44} This may indicate that diving has a long-term effect on endothelial function, but other lifestyle effects may also be involved.

Activation of the endothelium will lead to production of so-called endothelial micro particles (EMP).\textsuperscript{44} Such activation has been observed in a number of cardiovascular diseases and after using a heart-lung-machine, and it is not unlikely that gas bubbles may lead to such activation.\textsuperscript{45} Madden and Laden showed that bubbles formed during decompression may interact with the endothelium resulting in a loss of integrity which results in an increased shedding of EMP into the circulation.\textsuperscript{46}

Studies have shown that circulating activated micro-particles can reduce endothelial function, and it has been suggested that EMP may be used as a marker of endothelial stress.\textsuperscript{47,48} The reduction in endothelial function is probably caused by a reduction in NO production.\textsuperscript{49} Activated endothelial cells have an increased expression of adhesion molecules (VCAM, ICAM and E-selectin), and activation of C5a leads to an increased expression of such adhesion molecules after about four hours.\textsuperscript{49} This is in agreement with our findings that the reduction in endothelial function could be observed between one and six hours after exposure to gas bubbles, and that gas bubbles lead to an increase in C5a in a dose-dependent manner.\textsuperscript{40,51} In a recent study, increases in vascular cell adhesion molecule (VCAM) and induced cell adhesion molecule (ICAM) were observed in the blood of divers five minutes after surfacing, persisting for 24 hours.\textsuperscript{46}

Heat shock proteins (HSP) are formed in the body when the organism is exposed to stressors such as hyper- or hypoxia, heat, cold, exercise and some heavy metals or drugs. HSP have important functions in controlling the folding and structure of proteins and protecting the organism from injury.\textsuperscript{52} However, in some cases, expression of HSP may contribute to injury. Saturation divers are exposed to considerable stress (e.g., hypoxia, hard physical work and exposure to infections), which could potentially lead to an increase in HSP over longer periods of time. Of particular interest is the exposure to bacteria, as infections are still a serious problem in saturation diving operations.\textsuperscript{53} Certain bacteria, e.g., \textit{Pseudomonas aerogenosa}, which is common in saturation diving, produce HSP that is strongly antigenic and may trigger a significant immune response.\textsuperscript{54} If the bacterial flora in the diving habitats can produce such an immunological response, this would indicate that saturation diving may carry a higher risk of endothelial damage by bubbles than other types of diving. The above also raises the interesting question whether hyperoxia and the stress of the dive prior to decompression play an important role in determining the outcome of decompression.\textsuperscript{55}

Prevention of injury

Traditionally the reduction of bubble formation to prevent DCS has been achieved by changing stop times during decompression. Even if the procedures used today have a low incidence of DCS, we have demonstrated theoretically and experimentally that there is still considerable room for improvement in decompression procedures by reducing
the amount of vascular gas bubbles formed. These observations suggest a novel and more efficient way of reducing the formation of intravascular gas bubbles and hence reducing the decompression stress.

In a number of studies in rats, we have shown that the amount of vascular bubbles following a dive and the incidence of DCS can be significantly reduced by performing severe physical exercise 20 to 24 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 shear stress will increase the production of NO, which also affects the properties of the endothelial surface. We have been able to show that bubble production is increased by blocking NO and that the bubble-preventing effect of exercise can be simulated by exogenous NO. The same effect of exercise in reducing bubble formation with exercise 20 hours pre-dive has also been shown in a group of divers performing a dive to 18 msw. The above findings were quite surprising and have significantly changed our opinion on how bubbles are formed and how their formation may be controlled.

It is assumed that bubbles grow from so-called gas-filled bubble nuclei which are about 1µ in diameter, since de novo formation of bubbles requires high supersaturation pressures that do not occur in diving. These nuclei are not stable in blood, but on a hydrophobic surface such bubbles will remain stable more or less indefinitely. Hydrophobic areas exist on the endothelial surface in the form of caveola, where the production of NO is also localized. A reduction of surface tension on such a surface will increase the number of stable nuclei. We have previously shown in a pig model that there is a relationship between surface tension of serum and bubble production and that a small reduction in surface tension will increase bubble production significantly. Finally, there is a significant increase in caveola and NO after exercise. The effect of this could be that exercise, by increasing NO production, will lead to microbubble detachment from the endothelium, thus allowing them to be transported to the lungs by the blood and actually reduce their number available for future growth during decompression. We believe that variations in surface tension and/or NO production may be a factor that explains the large intra- and inter-personal variations in bubble formation observed in divers.

It has also been shown that an increase in low-density lipoproteins will decrease NO production. Even if obesity does not seem to be related to DCS risk in Colvin’s study, food preferences could have an effect. This might explain why repeated exposure will reduce the risk for DCS. An interesting fact about this adaptation is that it is very specific – if the depth of the dive is changed, the adaptation is lost. This raises the interesting possibility that epigenetic mechanisms might be involved.

As mentioned above, blocking NO production promotes bubble production, and heavy exercise 20 hours before the dive prevented this. This study was performed in rats weighing less than 280 g. When heavier animals were used (>300 g) this effect could no longer be seen. Acute heavy exercise increases blood lipids by approximately 30% immediately after exercise is finished, then, over the next hours, blood lipids are gradually reduced and this effect has disappeared after a few days. This effect is more pronounced in the trained than the untrained rat and is also dependent on the intensity of exercise. Twenty-four hours after exercise HDL is increased. 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 hydrophobicity to be reduced sufficiently to allow washing out of bubbles, while the effect is not strong enough in the heavier animals with blocked NO production. This mechanism could also explain why heavy exercise shortly before decompression could increase bubble formation. If more bubble nuclei adhere, there are more nuclei available for bubble growth.

Injury by bubbles may be preventable through other mechanisms. As described above, HSP are formed in the body when the organism is exposed to a number of stressors. The protective effect is strongest from a few hours to a day after the stress episode. HSP90 is involved in the production of NO. 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 HSP90 and eNOS. Exercise will also have an effect on HSP expression: moderate exercise increased HSP70 by 2,100% 48 hours after the last exercise bout. These studies also showed that exercise reduced apoptosis. HSP70 was also found increased in animals showing signs of DCS.

The incidence of DCS has been reduced over the last 40 years, but the relative number of incidents of DCS involving the CNS has increased. While supersaturation has been a major focus in nearly all research within this field, future research should perhaps focus more on biochemical pathways to uncover the secrets of the bubbles, both in their generation and their pathophysiological effect.

Conclusions

In this review we have concentrated on vascular bubble formation, its detection and effects. We suggest that damage and/or reduction in endothelial function due to the passage of gas bubbles is a central mechanism for the development of serious decompression injury and possibly also for the long-term effects of diving. We further suggest that these effects may be both influenced and prevented. Decompression stress, as defined above, can be used to describe the risk of dysfunction and injury after decompression, but we are well aware that this is only a part of the decompression problem.
The majority of divers do not show any acute clinical signs of DCS. Although the risk of clinical symptoms increases with increasing depth and duration of exposure, only a small proportion of divers develop clinical signs in spite of significant bubble formation. At present, there are no reliable ways of identifying, prior to the dive, those individuals who account for the majority of DCS cases. As diving is performed to greater depths and for longer periods, the search for these identifying factors should be given high priority. One possible approach could be based on the observation that vascular gas bubble formation appears to be significantly influenced by prior physical exercise, and that this mechanism is related to NO production.

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