# Review article

## The aging diver: endothelial biochemistry and its potential implications for cardiovascular health

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### Abstract

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Divers are exposed to circulatory stress that directly affects the endothelial lining of blood vessels, and even asymptomatic dives are associated with inflammatory responses, microparticle release and endothelial dysfunction. As humans age, there is a relative increase in the risk of cardiovascular disease, attributed in part to declining endothelial function. Whether extensive diving in the older diver increases the risk of disease as a result of accumulated circulatory stress, or provides protection through processes of acclimatization remains an open question. We provide a brief review of current knowledge about the separate effects of diving and aging on the vascular endothelium in humans and rodents, and discuss the available data on their combined effects. The aim is to elucidate possible outcomes of the interplay between exogenous and endogenous stress factors for endothelial function and to question potential implications for cardiovascular health in the aging diver.

### Key words

### Introduction

Diving with compressed gas is associated with circulatory stress that affects the cardiovascular system, characterized by altered redox status, activation of inflammatory signalling, microparticle release and a transient dysfunction of the vascular endothelium.[1–5] Any resulting health effects will conceivably depend on individual traits of the diver, including age.[6] There is evidence that the recreational diving population may be aging,[7,8] whilst occupational divers may continue their careers as long as they pass their annual medical examination.

The cardiovascular system undergoes complex phenotypical and functional changes with aging. Age-dependent decline of endothelial function comes as a consequence of increased biochemical stress and results in a gradual progression towards a pro-inflammatory and atherosclerotic phenotype.[9] Whether this is further aggravated by transient stress from diving remains an open question. The contrary may also be the case, with regular diving triggering protective acclimatization.

In this article, we provide a summary of current knowledge about the biochemical effects of diving and aging, separately and combined, on the vascular endothelium, and discuss them in light of cardiovascular health data. The aim is to elucidate possible outcomes of the interplay between exogenous and endogenous stress on vascular endothelial function using data from both human and rodent studies and to discuss the potential implications for cardiovascular health in aging divers.

### Biochemistry of the vascular endothelium

The vascular endothelium is a key regulator of vascular homeostasis.[10] Its function is mediated via the production and release of vasoactive molecules, first described in 1980 and termed acetylcholine-induced endothelium-derived relaxing factors (EDRF).[11] EDRFs regulate the diameter, structure and tone of the vasculature, thereby balancing oxygen supply with the metabolic demands of neighbouring tissues. It was later determined that the essential EDRF was the free radical nitric oxide (NO).[12,13] NO is derived from the amino acid L-arginine by nitric oxide synthase (NOS)-driven catalysis. In the endothelium, NO production is catalyzed by endothelial nitric oxide synthase, eNOS.[14,15] Shear stress generated by flowing blood activates eNOS in healthy vessels and the resulting NO has EDRF function.[16]

Under normal physiological conditions, NO is the sole catalytic product of eNOS. However, when intracellular oxidative stress from exogenous or endogenous sources increases, a switch in eNOS activity may occur, resulting in production of the reactive oxygen species superoxide ($O_2^-$). Enzymatic conversion of $O_2^-$ by superoxide dismutase produces hydrogen peroxide ($H_2O_2$). $H_2O_2$ is highly diffusible and can cause injury to endothelial cells. Also, the interaction of $O_2^-$ with NO generates the reactive nitrogen species peroxynitrite ($ONOO^-$), which inactivates the essential eNOS cofactor tetrahydrobiopterin (BH4). The net result is a loss of eNOS activity followed by endothelial activation and ultimately by endothelial dysfunction. The failure to uphold vascular homeostasis is accompanied by elevated levels of circulating cytokines, intravascular platelet aggregation and inflammation in the surrounding

tissue.[17] Endothelial dysfunction occurs in a number of cardiovascular diseases, e.g., atherosclerosis and coronary artery disease, the risks and progression of which are linked to the severity of endothelial dysfunction.[18]

**Diving impairs endothelial function**

The dynamics of inert gas uptake and elimination during diving add to the physical stress from the hyperbaric environment such that, even in healthy divers, circulatory stress in the form of transient endothelial dysfunction, inflammatory responses and microparticle release are observed following routine dives.[1–4,19] Although no direct correlation between the symptoms of decompression sickness (DCS) and bubbles has been found, DCS risk increases with increasing bubble loads.[20]

It has been hypothesized that a transient loss of vascular homeostasis in response to altered redox status is the underlying cause of DCS, with bubbles acting as an exacerbating factor.[21] Suggested pathophysiological mechanisms linking bubbles to DCS development include direct physical injury to the vascular endothelium, as reported in the pulmonary aorta of pigs after simulated air dives,[22] and biochemical processes, as indicated by complement activation in human serum infused with air bubbles.[23]

**Aging is associated with declining endothelial function**

The gradual decline in vascular endothelial function towards a pro-inflammatory and atherosclerotic phenotype starts in childhood, with clinical symptoms typically appearing in middle age.[12,24,25] This deterioration with age, even in individuals who are not at particular risk for cardiovascular disease, predisposes both sexes to ischaemic heart disease,[26] though men experience earlier and more severe atherosclerosis than women. Middle-aged men have been shown to have a five-fold higher risk than women of dying from cardiovascular disease.[27] Both clinically evident and occult cardiovascular disease increase with age,[28] and this may be a factor to consider for elderly participants in physically demanding activities such as diving.

**Comparative observations from rodents**

The use of animal models in diving research allows for control of environmental and biological factors that might otherwise confound the outcome and for a more liberal use of protocols that lie outside of the boundaries for safe human diving, in order to provoke pathological responses. Studies of diving-related pathology in rodents often use non-invasive measures that are assumed to correlate to human symptomatology. DCS is typically inferred either from post-dive behavioural changes,[29] or from measurements of decompression-induced bubble loads in the circulation.[30,31]

The endothelial dysfunction seen in healthy humans after air dives also appears to occur in both rodents and rabbits.[32,33] The endothelial responses are likely triggered by oxidative stress, as indicated by observed redox-dependent gene expression changes in rat aorta after simulated dives.[32] Also of interest is the potential acclimatization to diving. Rats dived daily on low-stress air profiles have reduced mortality and fewer signs of neuronal impairment after a single provocative dive than their naïve controls.[34] Taken together, these observations support the role of exogenous oxidative stress in the cardiovascular pathophysiology of diving, and the prospect of injury prevention through control of oxidative stress levels.

Rats are also used in comparative studies of cardiovascular aging. Some hallmarks of vascular aging are similar in humans and rats, such as remodelling of the arterial wall, with thickening of the intima-media, vascular stiffening and endothelial dysfunction.[28] Atherosclerosis is normally not seen in rats,[35] but some strains exist in which atherosclerosis develops in response to high cholesterol diets, or as a consequence of targeted genetic modifications (as in the apoE knockout).[36] A severe reduction of flow-induced relaxation in response to increased shear stress has been demonstrated in isolated coronary arterioles of approximately 80-week-old rats (corresponding to a human age of 65 to 70 years).[17] This appears to be related to perturbations in NO metabolism in the vessel wall, that indicate that the aged cells lose their ability to increase NO synthesis even in the presence of abundant substrate. The aging rats also have higher generation of $O_2^-$ in their coronary vessels at basal conditions compared with young controls, consistent with the biochemical changes of the vascular endothelium in aging humans.

**The aging diver: at risk or protected?**

Thus, the aging diver's circulatory system is simultaneously exposed to both transient exogenous stresses from the hyperbaric environment and age-related functional decline of factors that maintain vascular homeostasis. The central question is whether the net effect for the individual diver is detrimental or beneficial for cardiovascular health.

Younger divers produce fewer bubbles than older divers.[6] A decrease of $\dot{V}O_{2max}$ and increased adiposity with increasing age has been postulated to be the main reason for these changes.[6] More recently a positive relationship between age and post-dive bubbles has been reported, as well as the level of post-dive bubbling being both significantly higher in males than in females and associated with weight and body fat mass.[38] In another study, bubble loads in divers were significantly associated with increasing age and decreasing estimated $\dot{V}O_{2max}$, but not with percentage of body fat.[39] However, the correlation between bubble grade and probability of DCS is not close, and the limited epidemiological data available are also conflicting.

The risk of DCS among insured members of the Divers Alert Network (DAN) during the period 2000–2007 peaked at age 35–45 years with 27 cases per 10,000 member-years, then falling to just 16 cases per 10,000 member-years by age 60–69.[7] Reasons for this may be less physiological than behavioural; for example, if divers dive more cautiously as they age. Between 1992 and 2003, the mean age of DAN members increased by one year every four years, whereas the mean age of diving fatalities over the same period increased by two years every four years.[40] The percentage of cardiac-related factors among these diving fatalities increased from less than 5% before age 35 years to 30% from age 50 years onward.[40] Whereas the mean age of diving fatalities in 1992 was about two years older than for DAN members as a whole, by 2003 the mean age of DAN members had risen from 37 to 41 years and the mean age of diving fatalities to 46 years suggesting a greater fatality rate amongst older divers.[40]

Reports of cardiovascular-related death among divers indicate that cardiovascular fatalities peak in the 50- to 60-year age range. Among 947 fatalities in recreational divers, 26% were related to cardiac incidents associated with a history of cardiac disease or age greater than 40.[41] Being male and over 50 is recognized as the principle predictor of non-congenital cardiac incidents in divers. The association with male sex is also seen in younger individuals; the relative risk of cardiovascular mortality in male divers over 30 is six times greater than that of female divers of the same age.[8]

While physical activity in itself provides cardio-protection, diving may offer additional benefit through acclimatization to oxidative stress. Indications of acclimatization to hyperbaric exposure were first seen in caisson workers, where the incidence of DCS was reported to fall markedly over a period of two to three weeks of compressed air work; the effect being lost after off-work breaks of two to 10 days.[42]

The idea was further strengthened in a retrospective study of occupational divers performing repetitive, daily air dives, in whom DCS was treated most frequently early in the period.[43] Biological indications of acclimatization to diving were later reported in a cohort of military diving trainees.[44] There were no incidences of DCS or other medical problems, but there were significant changes in several biomarker levels, leading to the conclusion that extensive diving activates defensive acclimatization towards inflammatory insults.[44]

On an even more basic level, signs consistent with acclimatization to diving have also been observed in the gene expression patterns of peripheral leukocytes from experienced, male divers.[2] Stable changes in the activity of genes involved in apoptosis, inflammation and innate immunity persisted for two weeks after their last dive, consistent with defence against the augmented oxidative stress to which they had been repeatedly exposed during their diving careers.

**Limitations to our understanding**

Aging divers may include unfit individuals or those with chronic or silent cardiovascular disease, who are additionally negatively affected by the circulatory stress associated with diving. Certainly the DAN data supports this possibility, but it is not known if DAN membership holders are representative of the wider diving population or how they might differ from other diving groups such as commercial divers. Likewise, we cannot say whether divers are a self-selected group to which people with pre-existing medical conditions are less likely to belong. Finally, it is not yet known if the life expectancy of lifelong divers is different from that of a comparable non-diving population.

**Conclusion**

Diving and aging independently affect the vascular endothelium and their combined effects need to be better understood. As the function of the vascular endothelium deteriorates with age, the resulting outcome for divers' health depends on a complex interaction between harmful and beneficial factors. Diving may further aggravate an already vulnerable situation, consistent with the elevated risk for male divers of dying in the water from a cardiovascular event. Alternatively diving may provide protection through processes of acclimatization, as suggested by the lower relative risk of decompression sickness in older, experienced divers and the lasting changes in blood biochemistry after extensive diving. Since endothelial biochemistry is comparable between man and rodent, such animal studies may aid in the elucidation of the combined effects of diving and aging on the cardiovascular health of aging divers.

**References**

1  Brubakk AO, Duplancic D, Valic Z, Palada I, Obad A, Bakovic D, et al. A single air dive reduces arterial endothelial function in man. *J Physiol*. 2005;566:901-6.
2  Eftedal I, Ljubkovic M, Flatberg A, Jørgensen A, Brubakk A, Dujic Z. Acute and potentially persistent effects of scuba diving on the blood transcriptome of experienced divers. *Physiol Genomics*. 2013;45:965-72.
3  Thom SR, Milovanova TN, Bogush M, Bhopale VM, Yang M, Bushmann K, et al. Microparticle production, neutrophil activation and intravascular bubbles following open-water SCUBA diving. *J Appl Physiol*. 2012:1268-78.
4  Sureda A, Batle JM, Capo X, Martorell M, Cordova A, Tur JA, et al. Scuba diving induces nitric oxide synthesis and the expression of inflammatory and regulatory genes of the immune response in neutrophils. *Physiol Genomics*. 2014;46:647-54.
5  Theunissen S, Guerrero F, Sponsiello N, Cialoni D, Pieri M, Germonpré P, et al. Nitric oxide-related endothelial changes in breath-hold and scuba divers. *Undersea Hyperb Med*. 2013;40:135-44.
6  Carturan D, Boussuges A, Vanuxem P, Bar-Hen A, Burnet H, Gardette B. Ascent rate, age, maximal oxygen uptake,

adiposity, and circulating venous bubbles after diving. *J Appl Physiol*. 2002;93:1349-56.

7    Denoble PJ, Ranapurwala SI, Vaithiyanathan P, Clarke RE, Vann RD. Per-capita claims rates for decompression sickness among insured Divers Alert Network members. *Undersea Hyperb Med*. 2012;39:709-15.

8    Denoble PJ, Pollock NW, Vaithiyanathan P, Caruso JL, Dovenbarger JA, Vann RD. Scuba injury death rate among insured DAN members. *Diving Hyperb Med*. 2008;38:182-8.

9    Csiszar A, Ungvari Z, Koller A, Edwards JG, Kaley G. Proinflammatory phenotype of coronary arteries promotes endothelial apoptosis in aging. *Physiol Genomics*. 2004;17:21-30.

10   Vita JA, Keaney JF, Jr. Endothelial function: a barometer for cardiovascular risk? *Circulation*. 2002;106:640-2.

11   Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*. 1980;288:373-6.

12   Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*. 1987;327:524-6.

13   Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci USA*. 1987;84:9265-9.

14   Pohl U, Holtz J, Busse R, Bassenge E. Crucial role of endothelium in the vasodilator response to increased flow in vivo. *Hypertension*. 1986;8:37-44.

15   Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*. 1988;333:664-6.

16   Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet*. 1989;2:997-1000.

17   Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*. 1992;340:1111-5.

18   Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Jr., Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation*. 2000;101:948-54.

19   Glavas D, Markotic A, Valic Z, Kovacic N, Palada I, Martinic R, et al. Expression of endothelial selectin ligands on human leukocytes following dive. *Exp Biol Med*. 2008;233:1181-8.

20   Sawatsky DK. *The relationship between intravascular Doppler-detected gas bubbles and decompression sickness after bounce diving in humans* (thesis). Toronto: York University; 1991.

21   Madden LA, Laden G. Gas bubbles may not be the underlying cause of decompression illness – The at-depth endothelial dysfunction hypothesis. *Med Hypotheses*. 2009;72:389-92.

22   Nossum V, Koteng S, Brubakk AO. Endothelial damage by bubbles in the pulmonary artery of the pig. *Undersea Hyperb Med*. 1999;26:1-8.

23   Hjelde A, Bergh K, Brubakk AO, Iversen OJ. Complement activation in divers after repeated air/heliox dives and its possible relevance to DCS. *J Appl Physiol*. 1995;78:1140-4.

24   Jarvisalo MJ, Harmoinen A, Hakanen M, Paakkunainen U, Viikari J, Hartiala J, et al. Elevated serum C-reactive protein levels and early arterial changes in healthy children. *Arterioscler Thromb Vasc Biol*. 2002;22:1323-8.

25   Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. *Circulation*. 2007;115:1285-95.

26   Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Deanfield JE. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol*. 1994;24:471-6.

27   Ng MK, Quinn CM, McCrohon JA, Nakhla S, Jessup W, Handelsman DJ, et al. Androgens up-regulate atherosclerosis-related genes in macrophages from males but not females: molecular insights into gender differences in atherosclerosis. *J Am Coll Cardiol*. 2003;42:1306-13.

28   Lakatta EG. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part III: cellular and molecular clues to heart and arterial aging. *Circulation*. 2003;107:490-7.

29   Buzzacott P, Mazur A, Wang Q, Lambrechts K, Theron M, Mansourati J, et al. A new measure of decompression sickness in the rat. *Biomed Res Int*. 2014;2014:123581. http://dx.doi.org/10.1155/2014/123581

30   Wisloff U, Richardson RS, Brubakk AO. Exercise and nitric oxide prevent bubble formation: a novel approach to the prevention of decompression sickness? *J Physiol*. 2004;555:825-9.

31   Jorgensen A, Foster PP, Eftedal I, Wisloff U, Paulsen G, Havnes MB, et al. Exercise-induced myofibrillar disruption with sarcolemmal integrity prior to simulated diving has no effect on vascular bubble formation in rats. *Eur J Appl Physiol*. 2012:1189-98.

32   Eftedal I, Jorgensen A, Rosbjorgen R, Flatberg A, Brubakk AO. Early genetic responses in rat vascular tissue after simulated diving. *Physiol Genomics*. 2012;44:1202-7.

33   Nossum V, Hjelde A, Brubakk AO. Small amounts of venous gas embolism cause delayed impairment of endothelial function and increase polymorphonuclear neutrophil infiltration. *Eur J Appl Physiol*. 2002;86:209-14.

34   Montcalm-Smith EA, McCarron RM, Porter WR, Lillo RS, Thomas JT, Auker CR. Acclimation to decompression sickness in rats. *J Appl Physiol*. 2010;108:596-603.

35   Suckow MA, Weisbroth SH, Franklin CL. *The laboratory rat*. American College of Laboratory Animal Medicine series. 2nd ed. Amsterdam, Boston: Elsevier; 2006.

36   Ekuni D, Yoneda T, Endo Y, Kasuyama K, Irie K, Mizutani S, et al. Occlusal disharmony accelerates the initiation of atherosclerosis in apoE knockout rats. *Lipids Health Dis*. 2014;13:144.

37   Csiszar A, Ungvari Z, Edwards JG, Kaminski P, Wolin MS, Koller A, et al. Aging-induced phenotypic changes and oxidative stress impair coronary arteriolar function. *Circulation Res*. 2002;90:1159-66.

38   Boussuges A, Retali G, Bodere-Melin M, Gardette B, Carturan D. Gender differences in circulating bubble production after SCUBA diving. *Clin Physiol Funct Imaging*. 2009;29:400-5.

39   Schellart NAM, Vellinga TPvR, van Dijk FJ, Sterk W. Doppler bubble grades after diving and relevance of body fat. *Aviat Space Environ Med*. 2012;83:951-7.

40   Denoble PJ, Marroni A, Vann RD. Annual fatality rates and associated risk factors for recreational scuba diving. In: Vann RD, Lang MA, editors. *Proceedings of the Recreational Diving Fatalities Workshop, April 8-10, 2010*; Durham, NC: Divers Alert Network; 2010. p. 73-85.

41   Denoble PJ, Caruso JL, Dear G de L, Pieper CF, Vann RD. Common causes of open-circuit recreational diving fatalities. *Undersea Hyperb Med*. 2008;35:393-406.

42   Walder DN. Adaptation to decompression sickness in caisson

work. *Biometeorology*. 1967;2(Pt 1):350-9.

43  Doolette DJ. Health outcome following multi-day occupational air diving. *Undersea Hyperb Med*. 2003;30:127-34.

44  Ersson A, Walles M, Ohlsson K, Ekholm A. Chronic hyperbaric exposure activates proinflammatory mediators in humans. *J Appl Physiol*. 2002;92:2375-80.

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