Exercise and nitric oxide prevent bubble formation: a novel approach to the prevention of decompression sickness?

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Nitrogen dissolves in the blood during dives, but comes out of solution if divers return to normal pressure too rapidly. Nitrogen bubbles cause a range of effects from skin rashes to seizures, coma and death. It is believed that these bubbles form from bubble precursors (gas nuclei). Recently we have shown that a single bout of exercise 20 h, but not 48 h, before a simulated dive prevents bubble formation and protects rats from severe decompression sickness (DCS) and death. Furthermore, we demonstrated that administration of N^{ω}-nitro-l-arginine methyl ester, a non-selective inhibitor of NO synthase (NOS), turns a dive from safe to unsafe in sedentary but not exercised rats. Therefore based upon previous data an attractive hypothesis is that it may be possible to use either exercise or NO-releasing agents before a dive to inhibit bubble formation and thus protect against DCS. Consequently, the aims of the present study were to determine whether protection against bubble formation in 'diving' rats was provided by (1) chronic and acute administration of a NO-releasing agent and (2) exercise less than 20 h prior to the dive. NO given for 5 days and then 20 h prior to a dive to 700 kPa lasting 45 min breathing air significantly reduced bubble formation and prevented death. The same effect was seen if NO was given only 30 min before the dive. Exercise 20 h before a dive surpressed bubble formation and prevented death, with no effect at any other time (48, 10, 5 and 0.5 h prior to the dive). Pre-dive activities have not been considered to influence the growth of bubbles and thus the risk of serious DCS. The present novel findings of a protective effect against bubble formation and death by appropriately timed exercise and an NO-releasing agent may form the basis of a new approach to preventing serious decompression sickness.

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Millions of people worldwide partake in recreational and professional diving. Decompression sickness (DCS) following diving or return from elevated pressure is believed to be initiated by the formation of gas bubbles in tissue and blood. Nitrogen dissolves in the blood during dives, but comes out of solution if divers return to normal pressure too rapidly. Nitrogen bubbles cause a range of effects from skin rashes to seizures, coma and death (Francis & Gorman, 1993). The predominant theory is that bubbles grow from preformed nuclei composed of small (approximately 1 μm) stable gas bubbles (Yount & Strauss, 1982).

Recently we have shown that a single bout of exercise 20 h, but not 48 h, before a simulated dive prevents bubble formation and protects rats from severe DCS and death (Wisløff & Brubakk, 2001). Furthermore, we demonstrated that administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME), a non-selective inhibitor of NO synthase (NOS), turns a dive from safe to unsafe in sedentary but not exercised rats (Wisløff *et al.* 2003). Therefore, both acute exercise and NOS affect bubble formation, but they may not be linked. We speculate that both exercise and NOS hinder bubble formation via alteration in vascular endothelial properties since pre-existing gas nuclei are probably attached to the endothelium, where they grow into bubbles that are dislodged into the blood stream (Harvey *et al.* 1944; Harvey, 1951).

Therefore based upon previous data (Wisløff & Brubakk, 2001; Wisløff *et al.* 2003), an attractive hypothesis

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Table 1. Group assignment and number of rats in each protocol

Group	Protocol	Number of rats
I	Exercise or sedentary, 48 h prior to diving	12
II	Exercise or sedentary, 20 h prior to diving	12
III	Exercise or sedentary, 10 h prior to diving	12
IV	Exercise or sedentary, 5 h prior to diving	12
V	Exercise or sedentary, 30 min prior to diving	12
VI	NO-releasing agent or water, 5 days, last time 20 h prior to diving	12
VII	NO-releasing agent or water, once, 30 min prior to diving	12

Total number of rats is presented. There were six exercised and six control rats from groups I–V. Six rats from both groups VI and VII received water instead of a NO donor.

is that it may be possible to use either exercise or NO-releasing agents before a dive to inhibit bubble formation and thus protect against DCS.

However, the correct timing of this type of intervention is not clear, as the benefit of exercise is pronounced at 20 but not 48 h post exercise (Wisløff & Brubakk, 2001; Wisløff *et al.* 2003). As we believe exercise may deplete (wash away) the bubble precursors, it should take 10–100 h to regenerate a depleted nuclei population (Yount & Strauss, 1982). Thus, exercise closer than 20 h prior to the dive should also protect against bubble formation. Consequently, the aims of the present study were to determine whether protection against bubble formation in diving rats was provided by (1) chronic and acute administration of a NO-releasing agent and (2) exercise less than 20 h prior to the dive.

Methods

Study population and NO administration

A total of 84 adult female 310 ± 7 g Sprague-Dawley rats (Møllegaards, Denmark) were maintained six in each cage with light controlled on a 12 h dark–12 h light cycle. Temperature was 21 ± 2°C and humidity 50 ± 4%. Animals were fed a pellet rodent diet *ad libitum* and had free access to water. The rats were assigned into seven groups, as described in Table 1. Rats in groups I–V were used in experiments to determine the duration of the exercise-induced benefit against bubble formation and death. Exercise was performed 48 h, 20 h, 10 h, 5 h, and 30 min prior to the simulated dive in groups I–V, respectively. In groups VI–VII we determined the effect of administration of a NO-releasing agent (isosorbid mononitrate, Roche, Switzerland, 65 mg kg^{-1}) for 5 days (the last time 20 h before the dive) and immediately (30 min) prior to the dive on bubble formation and survival. The isosorbid mononitrate was dissolved in water and administrated to the rats by gastric intubation. Control rats received water by gastric intubation.

Maximal oxygen uptake ($\dot{V}_{O_2max}$) and exercise protocol

Nine days before group assignment, oxygen uptake and respiratory exchange ratio were measured during treadmill running as previously described in detail (Wisløff & Brubakk, 2001). In brief, interval running (1.5 h total duration) alternating between 8 min at 85–90% of $\dot{V}_{O_2max}$ and 2 min at 50–60%. After the exercise session, each rat was rewarded with 0.5 g chocolate (Crispo, Nidar Bergene, Norway). Sedentary rats were given the same reward.

Dive protocol and bubble analysis

Pairs of rats (experimental and control) were compressed at a rate of 200 kPa min^{-1} to a pressure of 700 kPa, maintained for 45 min breathing air and decompressed to the surface (100 kPa) at a rate of 50 kPa min^{-1}. Immediately after surfacing the animals were anaesthetized with Midazolam (Dormicum 'Roche')–fentanyl–fluanison (Hypnorm) (0.1 ml (100 g)$^{-1}$ s.c.), and the right ventricle was insonated using a GE Vingmed Vivid 5 ultrasonic scanner, with a 10 MHz transducer as previously described in detail (Wisløff & Brubakk, 2001; Wisløff *et al.* 2003). Images were graded according to a previously described method (Eftedal & Brubakk, 1997) with the observer unaware of the group allocation of the rat. A pilot study showed that rats surviving 60 min were apparently unaffected by the protocol and lived normally thereafter; thus survival times up to 60 min were recorded were. Surviving rats were killed by decapitation.

The experimental procedures conformed to the *European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes*, and the protocol was approved by the Norwegian Council for Animal Research.

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Table 2. Effects of a NO-donor on bubble formation and survival

	NO donor, 5 days (Group VI)		NO donor, acute (Group VII)	
	Ismo	Water	Ismo	Water
Body weight (g)	310 ± 7	309 ± 10	308 ± 6	307 ± 6
Scan grade	0 (0–5)§	5 (3–5)	0 (0–5)§	5.0 (3–5)
Survival (min)	60 (15–60)§	27 (2–39)	60 (23–60)§	19 (8–60)

§$P < 0.001$, significantly different from its respective control group. Body weights are presented as mean ± S.D.; the rest of the data are given as median (range). Decompression time was 12 min for all groups. Ismo, isosorbid mononitrate.

Table 3. Effects of exercise on bubble formation and survival

Group	Allocation	Body weight (g)	Scan grade	Survival (min)
I	Exercise (48 h prior to diving)	315 ± 6	4 (3–5)	35 (26–60)§
	Sedentary	310 ± 9	5 (—)	12 (4–20)
II	Exercise (20 h prior to diving)	316 ± 9	0.5 (0–2)§*	> 60 (—)§*
	Sedentary	313 ± 8	5.0 (4–5)	13 (2–47)
III	Exercise (10 h prior to diving)	314 ± 9	5 (—)	11 (3–15)
	Sedentary	318 ± 12	5 (—)	14 (2–31)
IV	Exercise (5 h prior to diving)	309 ± 8	4 (2–5)	20 (10–60)
	Sedentary	312 ± 6	5 (3–5)	23 (17–60)
V	Exercise (30 min prior to diving)	315 ± 8	5 (—)	11 (3–23)
	Sedentary	317 ± 9	5 (—)	8 (2–17)

§$P < 0.001$, significantly different from its respective control group. *$P < 0.001$, significantly different from all other goups. Note that rats registered as dead after 60 min were actually still alive at that point, but were killed after 60 min. Body weights are presented as mean ± S.D.; the rest of the data are given as median (range). Decompression time was 12 min for all groups.

Statistics

Data are expressed as mean ± S.D., or as median and range. Non-parametric tests were employed due to the limited number of rats in each group. A Mann-Whitney *U* test was used to evaluate differences in bubble formation, whereas the Gehan generalized Wilcoxon test was used to evaluate differences in survival time between groups. $P < 0.05$ was considered as statistically significant. Group size and statistical power were estimated using nQuery Advisor software (version 3.0, Statistical Solutions Ltd, Cork, Ireland). Based on cautious estimates from a previous study (Wisløff & Brubakk, 2001) six rats in each group would permit us to detect a 15% difference between groups in bubble grade and survival time ($P = 0.01$, power = 0.80).

Results

Isosorbid mononitrate administrated for 5 days and then 20 h prior to the dive significantly reduced bubble formation compared to rats administrated water, and most rats survived for 60 min. The same effect was seen if the NO was given only 30 min before the dive (Table 2).

Exercise 20 h before a dive to 700 kPa lasting 45 min breathing air 'suppressed' bubble formation and most rats survived for 60 min. There was no effect of exercise at any other time point (Table 3).

Discussion

The working hypothesis of this and previous studies (Wisløff & Brubakk, 2001; Wisløff *et al.* 2003) was that bubble precursors (nuclei) adhering to the endothelium are available to grow into bubbles with decompression. In a previous study we showed that basal synthesis of NO is necessary to avoid bubble formation in rats that normally do not produce bubbles (Wisløff *et al.* 2003). Aside from effects on vascular tone, NO has physiological properties that may be antiatherogenic, including inhibition of smooth muscle cell proliferation, platelet aggregation and adhesion, and leucocyte activation and adhesion (Bath, 1993). Previously we have suggested that exercise-induced protection against bubble formation is mediated via the NO pathway; changing the properties of the vascular endothelium reduces the possibility of bubble precursors becoming attached to the vessel wall. This was supported by the observation that administration of a NO donor before a dive protects against bubble formation and death (Table 2). It is reasonable to assume that hard exercise may lead to increased formation of

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bubble precursors or may increase their size (Dervay *et al.* 2002). Our hypothesis is, however, that these bubbles will not adhere to the endothelium if NO is present and thus will not be available for growth when supersaturation is present. Exercise, which is known to acutely increase NO production (Roberts *et al.* 1999) close to the dive, did not protect against bubble formation, whilst administration of a NO donor immediately before the dive did offer protection. Furthermore, in a previous study, we found that NO block increased bubble formation in sedentary but not in exercised rats (Wisløff *et al.* 2003). This indicates that the exercise effect may be mediated by factors other than nitric oxide. One can speculate whether increased blood flow during exercise may simply 'wash away' nuclei. However, this is unlikely, as exercise closer to the dive than 20 h offered no protection. Several studies have shown that passive or active movement during decompression acutely increases bubble formation (Harvey *et al.* 1944; McDonough & Hemmingsen, 1985*a*,*b*). A recent study showed that the lifetime of bubbles formed by exercise is in the order of minutes to a few hours (Dervay *et al.* 2002). Thus, it might be that exercise close to the dive offsets the positive effect of exercise-induced increased NO, and may explain the time-dependent effects of exercise seen in this study (Table 3). Physical activity may trigger a molecular species that is expressed in the endothelium about 20 h later, resulting in the exercise-induced protection against bubble formation. These novel data reveal that NO may be involved in this process (Table 2) and it is therefore reasonable to speculate that exercise 20 h prior to the dive activates the gene transcription of eNOS/iNOS leading to increased NO generation.

To better elucidate the role of NO in the exercise-induced protection against bubble formation, further studies should include rats that normally produce a lot of nitrogen bubbles and (1) determine the effect of exercise 20 h prior to a dive in L-NAME-treated rats, and (2) determine the effect of exercise close to the dive in rats receiving a NO donor prior to the exercise bout.

The present data clearly show that the mechanism of exercise-induced protection requires a time lag of 10–20 h to be fully activated, while a time lag of 20–48 h negates the protective effect. It is known that short-term high-intensity exercise (about 90% of $\dot{V}_{O_2max}$) can induce hypervolaemia (Gillen *et al.* 1991; Richardson *et al.* 1996), and the responses of aldosterone, angiotensin–renin and atrial natriuretic peptide (among others) all have significant consequences that last 24–48 h (Richardson *et al.* 1996). An increase in plasma volume could increase the functionally active capillary bed and the rate of plasma exchange through the muscle bed, which might increase the rate of nitrogen elimination. Interestingly, it is known that volume expansion decreases the severity of DCS (Merton *et al.* 1983). Thus, the well-known volume expansion developed a day after intense exercise may induce the observed exercise-related protection against bubble formation.

Conclusion

Efforts to prevent of DCS have traditionally focused upon the reduction of nitrogen supersaturation in the tissues. It is, however, well documented that the presence of nuclei is probably needed for bubbles to form at the level of supersaturation encountered in human diving (Vann, 1989). The idea that removal of nuclei may prevent DCS is not new. Vann *et al.* (1980) showed that exposure to significantly higher pressures before a dive, which presumably crushed the nuclei, significantly reduced the incidence of DCS. However until now, no practical way of removing nuclei has been suggested.

Pre-dive activities have not been considered to influence the growth of bubbles and thus the risk of serious DCS. The present novel findings of appropriately timed exercise and the use of a NO-releasing agent may form the basis for a new approach to prevention of serious decompression sickness.

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