Bubble Formation and Endothelial Function Before and After 3 Months of Dive Training

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Introduction: It has been suggested that repeated compression-decompression cycles reduce diver susceptibility to decompression sickness (DCS). This study examined whether intensive scuba dive training would reduce bubble formation and modulate endothelial function as shown by skin circulation. ***Methods:*** There were 22 military divers who were studied before and after a 90-d program of physical training and open-sea air diving (mean 67 dives total). Skin blood flow in the forearm was measured at rest (baseline), during post-occlusive hyperemia (endothelium-dependent vasodilatation), and with local heating to 42°C (maximal vasodilatation). Subjects were also examined by pulsed Doppler for venous bubbles 30, 60, and 90 min after surfacing from a hyperbaric exposure to 400 kPa (30 msw) for 30 min in a dry chamber. ***Results:*** None of the divers experienced DCS during the training period. There was no change in weight, body mass index, maximal oxygen uptake, or endothelial function. Bubble grades by the Kisman Integrated Severity Score were significantly decreased immediately after the diving training period (3.6 ± 9.2 vs. 16.4 ± 14.3) and increased 3 mo after this period (10.3 ± 13.9 vs. 3.6 ± 9.2). ***Discussion:*** The results highlight that repeated scuba dives and regular physical exercise activity reduce bubble formation and probably have a protective effect against DCS risk. Although this phenomenon has been observed for decades, the mechanism remains complex and the results cannot elucidate the effects of physical exercise and NO production. Bubble formation could activate the stress response which could be the basis for diving acclimatization.
Keywords: endothelial function, bubble, diving acclimatization.

DECOMPRESSION sickness (DCS) is caused by inert gas bubble formation in blood vessels and tissues resulting from supersaturation during inadequate decompression. The occurrence of many bubbles is clearly linked to a high risk of DCS, and Doppler ultrasonic detection of circulating venous gas emboli after diving is considered a useful index for safe decompression (18,19). When venous gas bubbles occur without any acute clinical signs, authors have termed them "silent bubbles" (3). It has been suggested that the formation of the silent bubbles underlies the etiology of diving acclimation, i.e., decreased susceptibility to DCS associated with repeated compression-decompression cycles (1,9). Although this phenomenon has been observed for decades, the mechanism remains unknown.

Some authors, however, have shown that a single bout of strenuous physical exercise before the dive decreased Doppler-detectable gas bubbles after decompression in rats (28) and in man (4,8). This effect could be related to nitric oxide (NO) production since bubble formation is prevented by administration of NO donors in animals (15,30) and humans (8) while increased by L-Name, a non-selective inhibitor of NO synthase (29). This could be due to a link between vascular endothelium and bubble formation (13,30). Although bubbles are frequent after symptom-free dives, they act as foreign material and pose a stress to tissues (16). Ersson (10) reported mild inflammatory activation in humans subjected to a 2-mo period of daily diving. Impaired arterial endothelial function has been reported in humans after a single simulated air dive (6) sufficient to generate significant amounts of silent bubbles. Whether repeated dives could lead to chronic endothelial change and NO production has never been investigated.

The hypothesis of the present study was that increased frequency and intensity of repeated compression-decompression cycles in humans are able to reduce circulating bubble level and to modulate endothelial function, improving NO production. The aim of the present study was to determine whether intensive scuba diving training is capable of reducing bubble formation and investigate whether this program is able to modulate the endothelial function of skin circulation.

METHODS

All experimental procedures were conducted in accordance with the Declaration of Helsinki, and were approved by the Ethics Committee for Human Research of Marseille (France). Each method and pertaining potential risks were explained to the participants in detail and they all gave written informed consent before the experiment.

Study Population and Diving Training Program

There were 22 healthy men, ages 25.3 ± 0.8 yr (mean ± SD), and admitted to the Explosive Ordnance Disposal Divers course, who volunteered for the study. They had all recently passed medical and physical tests and had

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all received medical clearance as military divers. Before this military course, they were recreational divers and their diving experience was 46 ± 3 dives during the last 5 yr. None had experienced DCS in the past.

For this special military diver course, the men first had a 3-mo basic theoretical formation period without diving activities. The intensive diving training period lasted 3 mo and included repeated open-sea diving (67 mean total number of dives over 90 d) and running. Each day in the morning, they took a 60-min jog in field conditions with physical exercise intensity at 50–60% of maximal oxygen uptake, corresponding to aerobic exercise. The diving profiles were limited to 20 msw depth during the first month (24 air dives in 20 d). Thereafter, subjects performed dives during the second month with maximal depths in the range of 20–40 msw (26 dives in 20 d) and during the last month with maximal depths in the range of 40–60 msw (17 dives in 20 d). Each dive was followed by the standard French Navy decompression schedule. Sea temperatures ranged within 12–20°C. Subjects all performed the same dives under identical environmental conditions. At the end of the diving training program, all subjects were admitted to a 3-mo specific theoretical formation module (corresponding to the explosive ordnance disposal module) with no diving activity, but the same physical exercise activity every morning.

Measurements of Maximal Oxygen Uptake

Maximal oxygen uptake ($\dot{V}o_{2max}$) was determined twice in all subjects using an incremental exercise protocol on a cycle ergometer (Monark Ergomedic 818, Varberg, Suède). First time was before the diving training program, and the second session was performed at the end of the 3-mo period. After 3 min at rest, all subjects carried out a 4-min warm-up period at 60 W, and then the load was increased by 30 W every 2 min until volitional exhaustion. During the entire test, subjects breathed through a mouthpiece in order for expired gas to be analyzed using breath-by-breath rapid response paramagnetic O_2 and infrared CO_2 analyzers (Cosmed Quark PFT ergo®, Rome, Italy).

Hyperbaric Exposure Protocol and Bubbles Analysis

Individual susceptibility for bubble formation was examined in each subject by determining bubble grade after an experimental hyperbaric exposure. This was performed 1 wk before the beginning of the diving training period, 1 wk after the end, and 3 mo after the end of this diving period. Subjects at rest were compressed to 400 kPa (30 msw) at a rate of 100 kPa · min^{-1}, breathing air, and remained at pressure for 30 min. Then decompression was started at a rate of 150 kPa · min^{-1} up to 130 kPa (3 msw), where subjects remained for 9 min before they were decompressed to the surface at the same rate (French Navy MN 90 procedure).

Detection of circulating bubbles was performed by an experienced operator using a pulsed Doppler equipped with a 2-MHz probe (Pioneer® Medical Corp., CA) on the precordial area. Monitoring was performed every 30 min for 90 min after surfacing (first measurement at 30 min after dive). During bubble detection, divers were placed in supine position for 3 min at rest, and then two successive lower limbs flexions were performed in order to improve the detection. Circulating bubbles were graded according to the Spencer scale (24). The bubble grades presented are the maximum grades observed at any observation point, usually 60 min after the dive. The Kisman Integrated Severity Score (KISS) was calculated according to the following formula:

$$KISS = [100/4^{\alpha}(t_4 - t_1)] \cdot [(t_2 - t_1)\,(d_2^{\alpha} + d_1^{\alpha}) + (t_3 - t_2)\,(d_3^{\alpha} + d_2^{\alpha}) + (t_4 - t_3)\,(d_4^{\alpha} + d_3^{\alpha})] / 2$$

where t_i = time of observation in minutes after reaching surface, d_i = Doppler score (grades 0 to IV) observed at time t_i, and $\alpha = 3$ (the parameter α takes into account that the bubble grade is not a linear measure of bubble quantity). The KISS was assumed to be a meaningful linearized measure of post-decompression intravascular bubble activity status which may be treated statistically (18).

Assessment of Cutaneous Endothelial-Dependent and Independent Vasodilatation

Cutaneous blood flow was recorded 1 wk before the beginning and after the end of the training diving period using laser Doppler (LD) flowmetry as previously described (12). To avoid acute effects of diving and/or exercise, vascular reactivity was assessed at least 48 h after the last dive or physical exercise. All procedures were performed in a quiet temperature-controlled room at 24.0 ± 0.3°C. Subjects were asked to empty their bladder before measurements and to remain in the supine position. LD measurements started after at least 20 min of rest. The LD probe (Periflux PF 4001-2, Perimed, Uppsala, Sweden) was always placed on the ventral site of the non-dominant forearm 5 cm below the elbow bend in order to avoid site to site variations. Cutaneous blood flow was measured from a small volume of skin (~1 mm^3) using a laser beam at 650 nm wavelength.

Baseline measurements were performed for at least 3-4 min duration. Thereafter, reactive hyperemia (RH) to an ischemic block was used to evaluate endothelium-dependent vasodilation in cutaneous microcirculation. An arm cuff was placed around the upper arm, and connected to an automatic device (Hokanson, Model E20, Bellevue, WA) which allows rapid inflation and deflation. The arm cuff was inflated to 200 mmHg for 3 min and RH was recorded after cuff deflation.

A 30-min recovery period followed until blood flow returned to baseline values. Then, local heating was used as a non-invasive method to induce endothelium-independent vasodilation (11). The measurement site was warmed to 42°C for 5 min in order to reach a stable plateau of blood flow value using a thermostatically controlled heater of 32 mm diameter (PF 450 Perimed, Uppsala, Sweden) which could be fixed on the same cu-

taneous site. The LD probe was placed in the middle of this area.

All traces were recorded continuously using Perisoft V.5.10 (Perimed Software, Craponne, France). Data were digitized and stored on a personal computer for further analysis. Baseline blood flow is the mean value of all readings over a stable 2-min period. Peak flow was defined as the highest recorded blood flow following cuff deflation (RH_{peak}) or local heating (T_{peak}). For data analysis, cutaneous blood flow was indexed as cutaneous vascular conductance (CVC) calculated as LD flux (mV)/mean arterial pressure (mmHg) (CVC = LD/MAP = mV/mmHg) and normalized to the baseline levels.

Statistical Analysis

Results are expressed as mean ± SE. We verified the normality of our data distribution using a Lilliefor test. KISS bubble score results were compared using the Wilcoxon test for paired values. We used *t*-test for the paired values of endothelial function results and measurements of maximal oxygen uptake. A *P*-value < 0.05 was considered statistically significant.

RESULTS

All participants completed the training period without any medical problem. No signs or symptoms typical for mild or severe DCS were detected during the 4-mo training program. Pre-training and post-training values of bodyweight and BMI were not significantly different (respectively, 74.8 kg ± 5.9 vs. 75.3 kg ± 6.3 and 23.2 kg · m^{-2} ± 1.4 vs. 23.3 kg · m^{-2} ± 1.5). Maximal oxygen uptake was not significantly increased after the training period as compared to pre-training values (55.7 ± 6 ml · kg^{-1} · min^{-1} vs. 54.9 ± 6 ml · kg^{-1} · min^{-1}).

For each subject, KISS bubble scores were significantly lower between the end and beginning of the training diving period (**Fig. 1**). This was true both at rest (1.4 ± 4.4 vs. 4.9 ± 6.5, $P < 0.001$) and after two successive lower limbs flexions in order to improve the detection (3.6 ± 9.2 vs. 16.4 ± 14.3, $P < 0.001$). There was a significant increase in KISS bubble score 3 mo after the end of the training diving period compared with the KISS values recorded immediately at the end of this training diving period (**Fig. 2**). This was true both at rest (4.5 ± 8.3 vs. 1.4 ± 4.4, $P < 0.001$) and after two successive lower limb flexions in order to improve the detection (10.3 ± 13.9 vs. 3.6 ± 9.2, $P < 0.001$).

Baseline CVC values for cutaneous perfusion obtained after the training period were not significantly different (0.096 ± 0.015 vs. 0.084 ± 0.009). The pre- and post-diving periods of normalized RH CVC peak values were, respectively, 1182.3 ± 181.9 and 1152.9 ± 183.3% baseline CVC. No significant differences were detected between the two measurement sessions. Normalized peak CVC values in response to local heating was not significantly different between the two measurement sessions (2094.9 ± 484.6 and 2899.0 ± 847.4% baseline CVC, respectively, for the pre- and post-diving periods).

DISCUSSION

Individual susceptibility for bubble formation was examined in each subject by determining bubble grade after an experimental hyperbaric exposure in a dry chamber. It has been well documented that bubble formation following an in-water dive is considerably higher than following a dry dive. In our protocol, we wanted to eliminate the artifacts of open seawater temperature differences, mental stressors, and physical exercise during diving. We proposed the performance of a hyperbaric exposure at rest in a dry chamber for bubble formation analysis. The main results show a significant decrease in bubble formation immediately after the training diving period and a significant circulating bubble level increase 3 mo after the end of the training diving period.

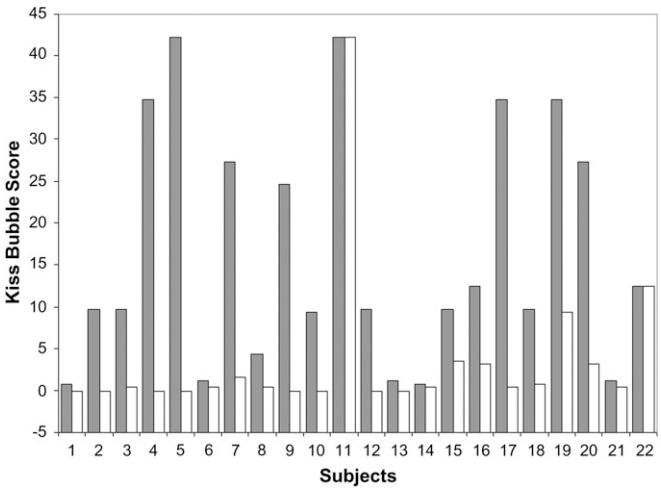

Fig. 1. KISS bubble score in 22 subjects after the same simulated dive in a dry hyperbaric chamber. Gray columns are 1 wk before and white columns are 1 wk after the experimental period.

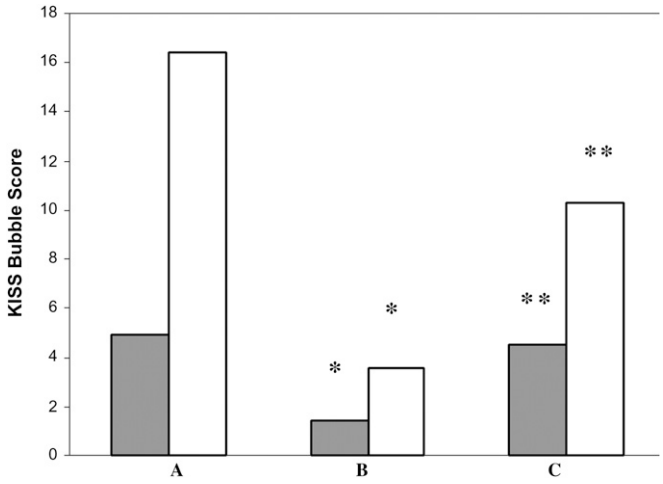

Fig. 2. KISS bubble score after the same simulated dive in a dry hyperbaric chamber, with divers supine for 3 min at rest (gray columns) and with two successive lower limb flexions performed (white columns): A) 1 wk before the experimental period; B) 1 wk after the experimental period; and C) 3 mo after the experimental period. * Significant difference ($P < 0.001$) between the beginning and the end of the experimental period; ** Significant difference ($P < 0.001$) between the end and 3 mo after the end of the experimental period.

DCS risk is related to a high circulating bubble level in the blood vessels (18). Repeated scuba dives and physical exercise reduced bubble formation and probably have a protective effect against DCS risk. Diving acclimatization refers to a phenomenon that occurs when individuals undergoing repeated compression-decompression cycles are able to reduce their susceptibility to DCS. It was described in both human (14) and animal studies (1,25). Although this phenomenon has been observed for decades, the mechanism remains unknown.

In our study, there was no change in maximal oxygen uptake between the beginning and the end of the diving training period as could be expected in well-trained individuals. A protective effect of physical exercise training against the incidence of neurological DCS has been reported in animal models (5,22,28). Behnke (2) reported that aerobically trained runners appeared to be at lower risk for venous gas emboli than sedentary subjects. Finally, Carturan (7) reported that bubble formation after a single air scuba dive correlated significantly with both maximal oxygen uptake ($\dot{V}o_{2max}$) and age. Moreover, Wisloff reported that the decrease in bubble formation in rats previously physically trained was the same as that after a single bout. They concluded that aerobic capacity per se did not influence bubble formation after a single air dive (28). In our well-trained individual study, any inference about the effect of physical exercise and maximal oxygen uptake related to bubble formation cannot be made.

The main mechanism of exercise-induced suppression of bubble formation could be related to NO production (13,29,30). We have investigated endothelial function of skin circulation. The reactive hyperemia test is the most common non-invasive technique used to assess endothelium-dependant relaxation of both conduit and resistance arteries (21). We previously demonstrated that RH response in the cutaneous circulation depends mainly on NO formation (11). In our study, post-occlusive hyperemia shows no change in the endothelial function of skin microcirculation. In our study, any inference about the effect of NO function related to bubble formation cannot be made. The results confirm that the protective effect on bubble formation could result from other biochemical mechanisms and not just from endothelial vascular improvement and NO production.

Authors have previously studied the effects of intravascular bubbles on endothelial-dependent vasodilatation. In the rabbit, endothelial damage has been reported after severe experimental DCS (27). In animal models, intravascular bubbles are known to induce endothelial damage and activation in a dose-dependent manner and lead to decreased endothelial-dependant vasorelaxation of rings in the pulmonary artery (20). Recently, decreased endothelium-dependent vasodilatation of the brachial artery after one dive, sufficient to produce few vascular bubbles but no clinical signs of DCS, was reported in humans (6). Our results suggest that endothelial-dependent vasodilatation is not modified by repeated dives and safe decompression. A hypothesis would be that acute and chronic effects of safe decompression on the endothelium could be different. Alternatively, the effects of decompression on the vascular endothelium depend on the type of vessels considered. In this regard, endothelium-dependent mechanisms regulating the vascular tone vary as a function of vessel size (17,23). It is thus conceivable that decompression may affect the vasculature in different ways according to the type and/or localization of the vessel considered.

There are probably other biochemical mechanisms to explain diving acclimation, including the depletion of complement proteins, thus preventing a massive activation of the complement system (26). Others studies proposed the accumulation of protective factors like heat shock protein (HSP) (14). It is well documented that endurance exercise is a stressor that increases HSP expression (31). It has been demonstrated that heat shock pretreatment before diving enhanced the expression of HSP70 and protected rats from air embolism-induced lung injury (14). Moreover, several investigators have focused on the interaction of endothelial NO synthase with HSP90, emphasizing a possible close link between HSP and the endogenous NO pathway (13). According to our results, it is conceivable that exercise training induced HSP production affects bubble formation after decompression by a different mechanism than NO pathways. The real bioprotective mechanism of HSP70 against DCS has not yet been described and requires further research.

The results of this study highlight that repeated scuba dives and regular physical exercise activity reduce bubble formation and probably have a protective effect against DCS risk. Although this phenomenon has been observed for decades, the mechanism remains complex. In our well-trained individual study, we cannot draw any conclusions about the effects of physical exercise and NO production as there are probably other biochemical mechanisms. For example, bubble formation could activate the stress response, which could be the basis for diving acclimatization. The mechanism underlying this adaptation remains to be elucidated.

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