

Endothelial function may be enhanced in the cutaneous microcirculation after a single air dive

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Key words

Scuba diving; Circulation; Skin; Endothelium; Doppler; Iontophoresis

Abstract

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Introduction: The effects of scuba diving on the vessel wall have been studied mainly at the level of large conduit arteries. Data regarding the microcirculation are scarce and indicate that these two vascular beds are affected differently by diving.

Methods: We assessed the changes in cutaneous microcirculation before an air scuba dive, then 30 min and 24 h after surfacing. Endothelium-dependent and independent vasomotion were successively elicited by iontophoretic administration of acetylcholine and sodium nitroprusside respectively, and cutaneous blood flux was monitored by laser Doppler flowmetry.

Results: The response to sodium nitroprusside was significantly lower 30 min after surfacing than before diving (50 (SEM 6)% of the pre-dive values, $P = 0.0003$) and returned to normal values 24 h post-dive (102 (29)% of the pre-dive values, $P = 0.113$). When compared to pre-dive values, acetylcholine elicited a hyperaemia which was not statistically different 30 min after surfacing (123 (17)% of the pre-dive values, $P = 0.230$), but significantly increased 24 h post-dive (148 (10)% of the pre-dive values, $P = 0.005$).

Conclusion: Microvascular smooth muscle function is transiently impaired after diving. On the contrary, microvascular endothelial function is enhanced for up to 24 h after diving. This further suggests that the microcirculation reacts differently than large conduit arteries to scuba diving. The impact of modifications occurring in the microvascular bed on the physiological effects of diving merits further study.

Introduction

Impaired vasomotion after self-contained underwater breathing apparatus (scuba) diving was first reported in human large conduit arteries when decreased flow mediated dilation (FMD) of the brachial artery was observed after a single simulated air dive at 280 kPa.¹ Post-dive decreased FMD was further confirmed following a single open sea diving with various breathing mixtures including air,^{2–5} nitrox⁶ or trimix.⁷ Although less investigated, the vasodilation induced by direct stimulation of the vascular smooth muscle (VSM) with nitric oxide (NO) donors is also decreased after diving.^{3,5} Altogether, these data indicate that scuba diving impairs both the endothelium and the VSM in large conduit arteries. Additionally, impairment of FMD is maximal 30 min after surfacing and recovers progressively.⁴ Indeed, FMD was still significantly reduced 48 h after the dive and needed three days for returning to pre-dive values.⁴

The microcirculation accounts for about 99% of blood vessels in adults. It is the smallest part of the vascular system and includes vessels with a diameter of less than 150 µm,

i.e., arterioles, capillaries and venules. The microcirculation ensures the exchange of molecules between blood and tissues, as well as the regulation of blood pressure and the control of tissue fluid and oedema.⁸ All of these actions are known to be influenced by the constraints induced during diving.^{9,10} We and others have reported previously decreased microvascular reactivity after a single scuba dive.^{3,5,11–13} However, we also reported that post-dive, macro- but not microvascular impairment was not present when bubble formation was prevented,⁵ suggesting that scuba diving acts differently on these two vascular beds. In the present study, we assessed microvascular endothelium-dependent and independent reactivity and report data suggesting that microvascular endothelial function is enhanced 24 h after an air dive.

Methods

All experimental procedures were conducted in accordance with the Declaration of Helsinki and were approved by the Ethics Committee of the Haute Ecole Paul Henri Spaak, Brussels, Belgium (acceptance number: ISEK – 2009 – 12 –

14 – vs1). The study was conducted during training exercises performed by a local search and rescue dive unit. Dives were performed during September 2012 at Lake Lago D'Orta in Pettenasco, Italy, at an altitude of 294 m.

STUDY POPULATION

Six male divers (age 47 (SEM 4) years; body mass index 27 (0.8) kg.m⁻²) volunteered for this study. The subjects were all experienced divers and had a valid medical certificate for diving at the time of the study. None had experienced decompression sickness (DCS) in the past. All but one were non-smokers. Prior to the experimental protocol, subjects abstained from any physical activity and diving for 72 h. The study participants were not taking medication except one subject who was receiving antihypertensive treatment. Subjects were not asked to fast; however, tea, coffee, alcohol and smoking were prohibited for 6 h prior to the test. Potential risks were explained to all subjects in detail and they gave their written informed consent before the experiment.

DIVE PROTOCOL

Each diver undertook an air dive in field conditions (water temperature 4°C). They were divided into three teams of two divers each. To account for any possible differences due to diving conditions or circadian variations which could influence microvascular reactivity, all dives were performed on the same day always in the morning. The diving site was located near the field laboratory, and divers were transported to the site by a power boat during a 10 min ride. The dive profile was planned as part of a training exercise for local rescue divers. Dive time was 20 min at 30 metres' fresh water (mfw) depth. Then, the ascent rate was 15 m.min⁻¹ with a 150 s pause at 12 mfw according to GAP DivePlanner (2003–2010), version 3.0.425.6, model RGBM, conservatism + 2 (recreational). Subjects used masks and fins and were dressed in dry suits with hood, boots, and gloves. Depth and dive time were monitored by each diver's personal dive computer.

LASER DOPPLER FLOWMETRY

To assess cutaneous microvascular endothelial function we performed iontophoresis with pharmacological agents coupled with laser Doppler flowmetry (LDF) as described previously.³ Cutaneous blood flux (CBF) was recorded in a stable temperature room (22 (1)°C) before the subjects started to equip themselves for the dive and 30 min after surfacing. Each subject was therefore used as his own control. For each diving team, the two subjects were examined in parallel, the entire LDF measurement lasting 15 to 20 min. Before any measurement, subjects were asked to empty their bladder and to remain in the supine position until the end of the measurement. LDF measurements started after at least 15 min of rest. A multifibre laser probe (PF

450-PI, Perimed, Järfälla, Sweden) specially designed to make possible simultaneous current application and CBF recording was placed at the ventral side of the forearm, 5 cm below the elbow bend to avoid site to site variation.¹⁴ CBF was measured from a small volume of skin (1 mm³) using a laser beam at 780 nm wavelength which provides good skin penetration independently of skin color and oxygen saturation.¹⁵ The probe was connected to a LD flowmeter (Periflux PF 5001, Perimed, Järfälla, Sweden).

IONTOPHORETIC STIMULATION

Iontophoresis is a method for non-invasive transdermal drug delivery based on the principle that a charged drug in solution will migrate across the skin under the influence of a direct low-intensity electric current.¹⁵ It makes possible local delivery of small amounts of pharmacological agents, thus avoiding potential systemic effects while delivering drugs in the area of CBF measurement. The laser Doppler probe used had a chamber where we positioned a 0.6 cm² sponge filled with 100 µl of the drug solution. The iontophoretic sponge was connected to a battery powered current supply (Perilont PF 382; Perimed, Järfälla, Sweden), allowing for the delivery of regulated-intensity currents for programmable durations. It allowed the measurement of CBF in the middle of the stimulated region through a hole at the center of the sponge.

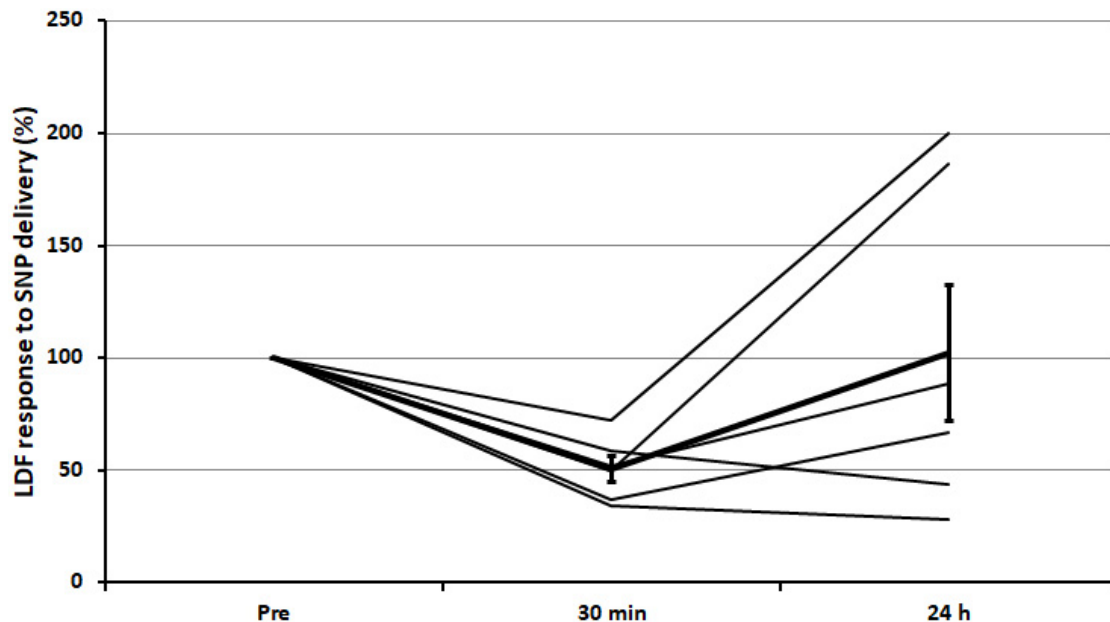
Endothelium-dependent vasodilation was first induced with a 1% acetylcholine (ACh) chloride solution administered with an anodal current (35 s, 0.10 mA). After CBF had returned to baseline values, a cathodal current (35 s, 0.10 mA) was used to deliver a 1% sodium nitroprusside (SNP) solution in order to assess endothelium-independent vasoreactivity. A stable baseline blood flow was measured for 2 min before the current was applied. Measurement of the peak increase in CBF was performed at the peak of the maximal plateau reached after each stimulation to represent the increase in vasodilation. Responses to ACh and SNP were presented as percentages of basal perfusion values measured before the iontophoretic stimulation. All traces were visualised on a personal computer using Perisoft V.5.10 (Perimed Software) and stored for later analysis.

STATISTICAL ANALYSIS

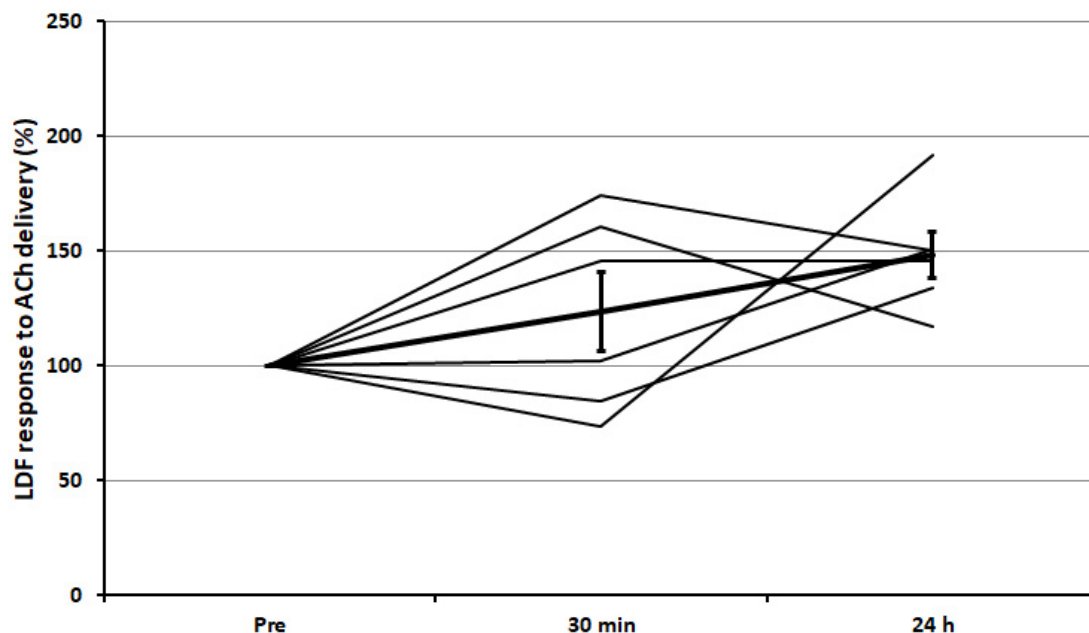
Statistical analyses were performed with the Statistica 10 software programme (Tulsa, Oklahoma, USA). For all data, the Shapiro-Wilk test was first used to test normality. As normality was confirmed for all data, they were presented as mean (SD and SEM). A paired *t*-test was used to compare values obtained for each diver before and after the protocol. Statistical significance was set *a priori* at *P* < 0.05. However, in order to take into multiple comparisons (three for each substance), a Bonferroni correction was applied and, therefore, *P* < 0.016 was considered statistically significant for the comparison of the time of measurement on each substance.

Figure 1

Response of cutaneous blood flow to sodium nitroprusside (SNP) iontophoresis at 30 min and 24 h after an air dive. The increase in cutaneous blood flow is expressed as a percentage of blood flow variation before the dive. Thin lines are individual values; the thick line represents the mean (SEM) value; $P < 0.016$ between before and after diving protocol at 30 min; $n = 6$

**Figure 2**

Response of cutaneous blood flow to acetylcholine (ACh) iontophoresis at 30 min and 24 h after an air dive. The increase in cutaneous blood flow is expressed as a percentage of blood flow variation before the dive. Thin lines are individual values; the thick line represents the mean (SEM) value; $P < 0.016$ between before and after diving protocol at 24 h; $n = 6$



Results

The cutaneous microvascular response to SNP at 30 min post dive was significantly decreased compared to pre-dive measurements (50 (SEM 6)% of the pre-dive values,

$P = 0.0003$), as shown in Figure 1. At the same time, endothelium-dependent hyperaemia elicited by ACh averaged 123 (17)% of the pre-dive values and was not significantly changed ($P = 0.230$; Figure 2). However, 24 hours after this first dive, response to SNP was no longer

different (102 (29)% of the pre-dive values, $P = 0.113$) while ACh-induced hyperaemia was significantly higher than pre-dive values (148 (10)% of the pre-dive values, $P = 0.005$).

Discussion

The main result of the present study is that the impairment of microvascular smooth muscle post-dive lasts less than 24 h and that the microvascular endothelium-dependent response to ACh is increased 24 h post-dive.

The effects of scuba diving on the vascular wall have previously been assessed mainly at the level of the brachial artery. Globally, data from these studies demonstrate that scuba diving decreases both the FMD and the vasodilation induced by NO donors, indicating that the endothelium and the vascular smooth muscle are both impaired.^{1,3,5} The present study assessed vascular function at the level of the cutaneous microcirculation. Only the response of vascular smooth muscle to NO was impaired 30 min after surfacing whereas the response to endothelial stimulation by ACh remained unchanged. This observation agrees with previous studies. Indeed, although they all reported a decreased response to NO donors,^{3,5,13} the response to ACh remained unchanged in two studies^{5,13} while two others reported decreased response to endothelium stimulation by ACh³ or shear stress.¹¹ In the present study, microvascular smooth muscle function was no longer impaired 24 h after the dive, which is shorter than the three days needed for complete recovery of the FMD.⁴

Laser Doppler is based on the reflection of a beam of laser light. The light undergoes changes in wavelength when it hits moving blood cells. The magnitude and frequency distribution of these changes in wavelength are related to the number and velocity of blood cells.¹⁶ LD does not directly measure cutaneous blood flow, but provides an index of skin perfusion, quantified as the product of average red blood cell velocity and their concentration, often referred to as flux. The arbitrary units correspond to the voltage of the analog signal of the LD flowmeter, with the zero-value corresponding to the blood flow value during arterial occlusion. However, it is considered accurate at detecting and quantifying rapid changes in CBF in response to a given stimulus.¹⁴ Additionally, although plasma volume changes have been reported after scuba diving¹⁷ we have shown that this has no effect on the LDF signal, at least in this range.¹³

Besides its action through endothelium, a C-fibre (axon reflex)–mediated mechanism has also been reported during iontophoresis of ACh.¹⁷ However, this mechanism occurs only when the iontophoretic current is equal to or higher than 5.10^{-2} mC·mm⁻². Given the surface of the drug-delivery electrodes (113 mm²), we used a charge density equal to 3.10^{-2} mC·mm⁻² only. A recent study further confirmed that the change in cutaneous microvascular blood flow in response of iontophoresis of 2% ACh in deionised water with 0.1 mA during 30 s was due to ACh only.¹⁹ Thus, it is

unlikely that an axon reflex occurred in our study and the increase in CBF is therefore a response to ACh stimulation. In these conditions, the changes in the cutaneous response post-dive reflect the modifications of microvascular reactivity to ACh.

SNP is known to react with tissue sulfhydryl groups under physiologic conditions to produce NO and thereby directly stimulate VSM relaxation, whereas the mechanisms by which iontophoretic administration of ACh increases CBF in humans are still under debate. Prostanoids, endothelium derived hyperpolarising factors and NO have all been reported to be involved.^{15,20,21} Nevertheless, it was confirmed that ACh and SNP increase skin blood flow through endothelium-dependent and endothelium-independent mechanisms, respectively.^{21,22} Therefore, the increased response to ACh with unchanged response to SNP we observed 24 h post-dive is typical of enhanced endothelium-dependent vasodilation with normal endothelium-independent vasodilation. Moreover, the transient decrease of the response to SNP in the present study clearly indicates that VSM reactivity is impaired 30 min after the dive and recovers within 24 h. As a consequence, the unchanged response to ACh 30 min after surfacing despite a decreased response to SNP suggests that the impairment of vascular smooth muscle function is compensated by enhanced endothelial function. Taken together, our data suggests that air scuba diving might enhance microvascular endothelial function and that this effect may last up to 24 h.

Many mechanisms can explain these changes and hypotheses based on our results are speculative. Because post-dive impairment of FMD results in part from the diving-induced increase of ROS production⁴ and also in part from decompression-induced bubbles,^{5,23} it is tempting to hypothesize that the same mechanisms mediate the effect of diving on the cutaneous microcirculation. However, ROS were shown to decrease the response to ACh iontophoresis without altering that to SNP in the human cutaneous microcirculation,^{24,25} whereas reducing the amount of circulating bubbles has no effect on the post-dive changes of cutaneous microcirculation.⁵ Although we did not measure skin temperature, the immersion must have induced a decrease in the forearm skin temperature, especially in cold water and despite the use of dry suits. Given its role in thermoregulation, this should have resulted in decreased CBF. However, although it was previously shown that a moderate decrease of skin temperature results in progressive decrease of both ACh- and SNP-induced vasodilation,²⁶ in our study only the response to SNP (but not ACh) was decreased at 30 min post-dive. This suggests that the changes in microvascular reactivity we observed are unlikely to result from modifications of the skin temperature. Similarly, we previously reported that neither hyperbaric hyperoxia nor immersion alter the cutaneous responses to iontophoretic administration of both ACh and SNP.¹³ It was also shown that the increased activity of the sympathetic autonomous nervous system, previously reported after diving,²⁷ has no

effect on the amplitude of the forearm skin response to iontophoretic administration of ACh or SNP.²⁸ Finally, an increase of plasma NO was reported during scuba diving.²⁹ Although, the reason and origin of this increased NO production are still unknown, it could explain the decrease in vascular smooth muscle response to the exogenous NO donor SNP through desensitisation of the soluble guanylyl cyclase.³⁰

Enhanced microvascular endothelial function after diving contrasts with the previously reported impaired endothelial-dependent relaxation of conduit arteries as assessed by FMD. This further confirms that the macro and microcirculation can be affected differently by scuba diving. This is consistent with previous reports showing that the brachial artery and the cutaneous microcirculation are affected differently by various conditions such as decompression-induced circulating bubbles^{5,23} and physical exercise training.³¹ The explanations for these territorial differences are still unclear. Differences in vessel calibre-dependent blood rheology and/or signalling pathways may be involved. Indeed, shear stress is lower in the microcirculation than in conductance arteries.³² Along with that, shear stress increases endothelial-dependent vasodilation through an increased NO-dependent mechanism only in the brachial artery³³ and through endothelial NO-dependent and independent pathways in the cutaneous microcirculation.³⁴

LIMITATIONS

The low number of subjects included in the study is certainly its main limitation. Studies including more divers are needed before definitive conclusions can be made. However, differences between the times of measurements were statistically significant. Moreover, at 30 min post-dive, our results agreed with several previous studies from our group and others. As already stated in the discussion, we did not measure skin temperature at the site of measurement, nor did we regulate the temperature of the room where measurements were made. However, although an effect of temperature cannot be ruled out in our study, the room temperature did not vary throughout the experiment, and the previously reported effects of changes in skin temperature are not confluent with the changes observed in our study. In our study, we presented relative amplitudes of the increase of CBF, which gives information about the contribution of each component to the total variability observed. It was reported that wavelet analysis from haemodynamic signals makes possible the determination of different influences on skin blood flow.²¹ This represents a challenge in future research in this field.

Conclusions

Microvascular endothelial function may be enhanced up to 24 h after a SCUBA dive. This contrasts with changes which occur at the level of large conduit arteries and indicate that these two vascular beds may react differently to diving.

However, the reasons for this difference are still unknown. Additionally, whether the changes observed at the level of microcirculation could influence the divers' response to diving and/or decompression needs to be better understood. The impact of modifications occurring in microvascular bed on the physiological effects of diving is worth further studies.

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