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EUBS European Underwater and Baromedical Society

Official Newsletter

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EDITOR **Dr. med. Peter HJ Mueller**
P.O. Box 1225
D-76753 Bellheim/Germany
pete@ejuhm.de

CHAIRMAN of the REVIEW BOARD: Prof. Alf O. Brubakk, NTNU, Trondheim, Norway

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EUBS EXECUTIVE COMMITTEE

EDITORIAL

Dear All,

As previously announced, due to economical reasons this issue combines the March and June editions in time to give you updated membership information and on the upcoming Annual Scientific Meeting in Egypt, which I urge you to read carefully. As a pitfall this issue lacks the information on the nominations for Executive Committee members. This should come separately by mail.

Due to the planned merger of this Journal with the journal of SPUMS we have started to direct publications to the Diving and Hyperbaric Medicine Journal. The editor, Prof. Mike Davis, will attend the EUBS meeting in Egypt and speak to the members of the ExCom and during the General Assembly. We all look foreward to this meeting and hope to meet many of you there.

Peter

LETTERS TO THE EDITOR

ARE PROFESSIONAL DEEP-DIVERS USED AS GUGUINEAPIGS?

Deep diving up to 250m depth has been developped during the seventies and eighties, while oil companies were competing in installation of oil rigs and drilling holes. During the last years this activity has slowed down and the number of saturation divers in the North Sea is actually very small. However, behind the walls a lot of industrial activities and development projects are launched, which means that in the next 10 years the deep diving activity will be increasing again. In the meantime health survey studies of professional divers have been published, amongst which the ELTHI study published in this paper a few months ago. We still do not understand precisely, what somatic and psychologic changes happen while regularly diving with gas mixtures in greater depth. However, as long as we look for the evidence based scientific side, the only thing which is proved is a certain number of dysbaric bone necrosis, which do not necessarely relate with acute DCI manifestations. In some countries controversal reports have been produced, mostly without scientific evidence (unpublished data, uncontrolled, etc) which pretend that more important alterations are found in all the deep divers in a long term, as for instance brain damage. After an expert meeting in Bergen last year, which finished with some consensus agreements, the DMAC experts found it necessary to produce a statement, which represents the actual state of knowledge on an evidence based basis. This statement is not primarely published for medical doctors, but should be spread in the professional diving scene as well. The Diving Medical Advisory Committee DMAC is an expert committee invited by IMCA (International Marine Contractors Association). The EDTC medical subcommittee (European Diving Technology Committee, representing 17 European Countries) is represented in the DMAC and thus I recommend you to check the DMAC website for the **Commercial Diving and Health Statement** (**http://www.dmac-diving.org/guidance/DMAC-Statement-200610.pdf**) summarising the current state of knowledge on the health risks associated with working in the commercial diving industry.

Jürg Wendling
Chairman Medical Subcommittee
Europen Diving Technology Committee (EDTC)
mail@wendling.ch

UNDER-ICE RESEARCH: THE INTERNATIONAL POLAR DIVING WORKSHOP

Since the first ice dives four decades ago in wetsuits without buoyancy compensators and double-hose regulators without submersible pressure gauges, novel ice diving techniques have expanded the working envelope based on scientific need. During this International Polar Year (March 2007-2009), an increased level of attention will be focused on the Arctic and Antarctic and the **International Polar Diving Workshop, Ny Ålesund/Svalbard, 15-22 March 2007** constitutes a contribution from the international polar scientific diving community. Polar Diving Workshop procedures from 1992 (Lang, M.A. & J.R. Stewart, eds.) were re-evaluated through a combined international, interdisciplinary expertise of participating polar diving scientists, manufacturers of dry suits and dive computers, physiologists and decompression experts, and diving safety officers. The proceedings of this workshop will be available by end of July 2007.

Michael A. Lang
Smithsonian Institution
Washington, D.C.
Langm@si.ed

IMPORTANT INFORMATION FOR EUBS MEMBERS

Dear Colleagues,

"It is reminder time again for Membership Fees". The membership fees for all Members of the Society are due again on the 30th June 2007. I look forward to receiving this years fee, by using the form below, or by using the renewal form, which can be obtained from the EUBS website.
Payments can be made by Visa/MasterCard/EuroCard, Sterling Cheque or by Euro cheque, you can also now pay via Paypal on the EUBS website. Please remember to still send me your application forms when paying with PayPal, as PayPal only gives me your payment details and name, and I need to have your address etc to send your member ship card and journals.

Many thanks.
Ms P Wooding
EUBS Treasurer/Membership Secretary
coppernob@freewire.co.uk

EUBS MEMBERSHIP FORM

NAME: _____

ADDRESS: _____

_____

_____

WORK TEL NO: _____ **FAX NO:** _____

HOME TEL NO: _____ **HOME FAX NO:** _____

E-MAIL ADDRESS: _____

Membership fee as per 2006/2007 is due 30th June 2007:

Member – Euro 55 (£37)

Undergraduate Member – Euro 27.50 (£18.50)

Corporate Member – Euro 300 (£202)

Please tick which payment method you are using.

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Please return your form and payment to:
Ms P Wooding
EUBS Membership Secretary
35 Westmede, Chigwell,
Essex, IG7 5LR
United Kingdom
or:
Fax to +44 208 500 1778. (Between 09.00 hrs and 21.00 hrs – UK time)

2nd Announcement EUBS Annual Scientific Meeting 2007

September 8th – 15th 2007

Sharm El-Sheikh, Egypt

The 33rd Annual Meeting of the European Underwater & Baromedical Society (EUBS) will begin on the 8th September with a Welcome Reception in the Hyatt Hotel and it will end at the 15th September 2007. The Conference will not only include invited lecturers, presentations and posters, you will also have the chance to discover the magic of diving in the Sinai. After-hours sessions on culture, music and marine life will be planned to introduce Sinai to our guests.

MAIN TOPICS:

Diving Medicine

- ENT diving related problems: Otitic barotraumas, Diving and HBOT: related middle and inner ear barotrauma. Inner ear decompression sickness. Seasickness. Differential diagnosis of inner ear decompression/round window rupture/inner ear barotraumas
- Dehydration and its relation to decompression
    - The management and treatment of neurological DCI (spinal cord decompression, embolism)
    - Decompression in diving safari (repetitive dives and the use of diving computers)
    - Technical diving and the problems of O2 toxicity and decompression profiles, DCI resulting from Tec Diving, treatment considerations
- Advances in diving research and prevention of dysbaric disorders
- Reverse profile as a contributing factor to DCI (cold versus warm water)
    - Occupational Hazard: u/w videographers and divemaster syndromes
    - Pulmonary oedema & drowning
    - Altitude and diving/flying after diving
    - Criteria and time to return to diving after diving accidents

Diving Physiology

- Acute Oxygen Toxicity
- Oxygen: are we overdosing?
- Underwater Physics & Physiology
- Recent advances in diving equipment & technology
- Diving eligibility/drugs & diving
- Deep Stops: Safer ascents?
- PFO, dynamic changes, latest research
- Thermo-regulation in divers
- Extremes of age and diving

Breath Hold (Apnea) Diving

- Pathophysiology, recent advances, accidents and research

Hyperbaric Oxygen Therapy, Physiology

- Fundamentals of HBOT

Hyperbaric Oxygen Therapy, Clinical & Technological Advances

- Management of critical patients in hyperbaric medicine
- Indications of HBOT: adjunctive/basic/experimental
- Evidence based research in new fields for HBOT
- Advances in medical equipment for critical/intensive care in hyperbaric environments
- Otolaryngological indications for HBOT: Radionecrosis in the head and Neck areas, Necrotizing Otitis Externa, Rhinocerebral Mucomycosis, Sudden Deafness

VENUE:

Official Hotel is HYATT REGENCY Sharm el-Sheikh. Luxury perched above the rich corals of Near Garden reef, with lashings of marble, large rooms, great sea views, a pool and good restaurants. This is a perfect place for a conference on diving medicine. Wireless LAN in hotel available.

HYATT REGENCY Hotel

Sharm el-Sheikh, South Sinai, Egypt

Phone: +20 69 3601234, Fax: +20 69 3603615

SOCIAL EVENTS:

Welcome Reception

The Welcome Reception will take place at HYATT REGENCY Hotel, at 18:30 on 8th September, 2007. Attendance to this event is included in the registration fee. Delegates are kindly requested to indicate in the registration form whether they plan to attend it or not.

Dinners
Dinners will be planned to reflect the nature of the area and give you a taste of Egyptian as well as international cuisine. **Gala Dinner** is planned in the desert and will be prepared by local Bedouins.
For all dinners the dress code will be "desert" casual. Attendance to the announced dinners is included in the fee, but registration is required.

DIVING
Coming to the Sinai, the divers among the conference attendees will have the possibility to explore the beautiful dive sites around Sharm el-Sheikh during, before and after the conference.
We will get special deals from several reputable diving centres for the conference. Half-, full day and night dives can be arranged during the EUBS week, plus special offers for Dive Safaris.

TRIPS AND EXCURSIONS
Visit beautiful Ras **Mohamed National Park** and Marine Reserve either by land or boat. Take the chance to learn about **diving** with an introductory dive or just watch the colorful sealife from above while **snorkeling**.
If you are looking for another challenge, **Camel riding** may be of interest as well as Go-Karts or Quad biking. Get up early in the morning and spend **a day in the desert**: The first highlight will be the **St. Catherine's Monastery**, one of few surviving churches of early Christianity and destination for pilgrims since the 4th century. Combine visiting the monastery with a climb of **Mt. Sinai** which is at a height of 2285m or a stop in **Wadi Feiran**.
Other Destinations include Coloured Canyon, Wadi Kid and Village by car, Cairo by air or car, Gharqana Village with the most northern Mangroves. **After Dinner:** Old Town Tour , Naama Bay, Hard Rock Café and PACHA Disco, Little Buddha, Ghibli Raceway, Cleopark, Down Town Shopping.
Excursions and sightseeing trips will be organized by **EASTMAR TRAVEL**. Please see their website for more information (**www.eastmartravel.com**). Other activities (Horse Riding, Mangrove tours, Spa Wellness packages, Quad Trip in the desert…) can also be arranged. Reservations will be done upon arrival and throughout your stay. Limited "Babysitting Service" upon inquiry and request.

SATELLITE MEETINGS
- Baromedical Association for nurses, operators and technicians (EBAss)
- European Committee for Hyperbaric Medicine (ECHM)
- International Group on High Pressure Biology (IHPBG)
- DAN-Europe & DAN-Egypt Symposium

CALL FOR ABSTRACTS
Abstracts can be submitted for both oral and poster presentation exclusively electronically via this website.
Abstract submission deadline (prolonged): 15th June, 2007
Abstracts must be submitted only electronically to **info@eubs2007.org**, **Re: Scientific Committee**. The instructions must be followed strictly. Notification of acceptance of abstracts will be given by the 15th July, 2007. The **presenting author** must be **registered before 25th July, 2007**, and the **complete paper** should be electronically **sent by 1st August, 2007**. If not, the paper could be withdrawn from the final programme.
It is important to understand that, instead of a Book of Proceedings, we will publish a CD comprising all oral and poster presentations. All abstracts will also be published in the EJUHM. We clearly encourage authors to submit the full text of their accepted papers.
Full papers selected by the Scientific Committee will be peer reviewed and published in the subsequent issues of EJUHM.
Abstract Preparation
Abstracts must be written in English and may **not exceed 300 words**, exclusive of heading. Abstracts should contain a sentence stating the study's **OBJECTIVE**, a brief statement of **METHODS**, a summary of the **RESULTS** obtained and a statement of the **CONCLUSIONS**. Please note that it is not satisfactory to say 'the results will be discussed'. Use a short and specific title. Capitalize initial letters of trade names. Other abbreviations should be spelled out in full at first mention, followed by the abbreviation in parantheses. **International measure units must be used at any section of abstracts, papers, and posters (Pa, meter, kg)**. Equivalence of Pressure units in Absolute Atmospheres (ata) will be always indicated between brackets, as well as other units optionally preferred by the author (feet, pounds, inches).

REGISTRATION

Registration Fees **Fees are in Euros (€)**	On or before 1st June, 2007 (a)	On or before 1st Sept., 2007 (b)	After 1st Sept., 2007
Members	**400 €**	**450 €**	**475 €**
Non-members	**450 €**	**500 €**	**525 €**
Nurses & medical students	**200 €**	**225 €**	**250 €**
Accompanying person	**250 €**	**250 €**	**250 €**

Registration fee includes
- Scientific sessions and exhibition and poster areas
- Welcome Reception on Saturday, 8th September, 2007
- Dinners on Sunday 9th, Monday 10th, Tuesday 11th September, 2007
- Gala Dinner on Thursday 13th September, 2007
- Package including the final program and proceedings book
- Transfers from listed hotels to conference in the morning and to dinners
- Certificate of attendance

Presenting Authors of Submitted Abstracts
Presenting authors are encouraged to register for the Conference. If the paper presented is rejected and the author chooses not to attend, he may request to cancel his registration and will receive a full refund. The cancellation should be received before the 1st of July, 2007.

Official Letter of Invitation
Available on request for certain countries where it is needed. Please send an e-mail to (**info@eubs2007.org**) including the full name and the participant's full mailing address and fax number. Please note, that no financial benefits are granted.

Travel Grants for Students
In the general meeting of the European Underwater & Baromedic Society (EUBS), held in Bled in 1997, there was general agreement that the Society would allocate up to 2500 Euro (approx.) each year to encourage participation of young students in our Society. The money would be offered to students as travel grants for the Society's annual meetings. Please read details on the EUBS website (**http://www.eubs.org**). Please check the button 'Downloads' in the main menue for more detailed information.

TRANSPORTATION
Sharm el-Sheikh International Airport has direct connections with all the major cities in Europe and to Cairo and Hurghada, many airlines offer reasonable charter rates. The airport is 10 km north of Naama Bay and the HYATT hotel. Airport-hotel transfer with EASTMAR TRAVEL is included. For those who do not find direct charters to Sharm el-Sheikh, Cairo and Hurghada are alternative entry ports to Egypt. There are daily connection flights from Cairo to Sharm, tourist busses (Superjet) and a speedboat ferry from Hurghada. If there is a group of people, 5 or more, that needs transportation from Cairo, EASTMAR TRAVEL will be happy to arrange this.

IMPORTANT NOTICE: We strongly recommend to book flights as early as possible !

Local Transport
The Conference venue, the HYATT Hotel, is easily reached from any part of the city by taxi or mini bus. Sharm has public transport tariff boards displayed in various locations around town, as taxis do not use meters. Useful information will be compiled and handed out to you upon arrival.

For more detailed information and Online registration see: www.eubs2007.org

ANNOUNCEMENTS

DAN/ATMO Chamber safety/care and inspection of viewports course and seminar
August 20th and 22nd 2007
Britannia International Hotel, Canary Wharf, London UK

The aim of the course is to provide users of hyperbaric and diving chambers with a basic knowledge of risk assessment and specific hazards associated with the use of such chambers. It will also include a module on the inspection and installation of acrylic plastic view ports as per PVHO(2). After completion of the module the participant will be qualified to inspect and sign off acrylic plastic view ports. Mr Francois Burman (DAN SA) and Mr Robert Sheffield (International ATMO Tx) will run the course, with other guest speakers also in attendance. The course is aimed at Hyperbaric safety directors, supervisors, operators, technicians, nurses, doctors and anyone who has an interest in Chamber safety.
The cost for the two days will be GBP 250.00, this will include a simple lunch on both days as well as tea and coffee throughout. All proceeds will go towards the DAN Recompression Chamber Assistance Program (RCAP).

Contact information: leegriff@aol.com **or** steve@smckenna.freeserve.co.uk **or** guy@daneurope.org

Review Articles

ENDOTHELIAL MICROPARTICLES IN VASCULAR DISEASE AND AS A POTENTIAL MARKER OF DECOMPRESSION ILLNESS

Leigh A. Madden[1] and Gerard Laden[2]

[1] Postgraduate Medical Institute, The University of Hull, Cottingham Road, Hull, HU6 7RX, UK

[2] The North of England Hyperbaric Unit, Hull and East Riding Hospital, Anlaby, HU10 7AZ, UK

Madden LA & Laden G: Endothelial microparticles in vascular disease and as a potential ,arker of decompression illness. Europ J Underwater Hyperbaric Med 2007, 8(1&2): 6-10. Micro-gas emboli are known to be present within the venous circulation following routine hyperbaric exposure. Emboli can be identified/quantified using Doppler and 2D ultrasound; thus functioning as an index of decompression stress. In relation to decompression illness this technique has low sensitivity and specificity. A biological marker of decompression stress would prove a useful tool. Such a marker could be used to gauge the efficacy of prophylaxis. Endothelial cells are known to shed micro-particles during activation and apoptosis. Since microparticles in general express the antigens of the cells from which they were derived, the origin of them can be determined and their phenotype can lead to an insight as to the state of the parental tissue. Microparticles have been studied in many vascular diseases, reviewed here and we hypothesise that micro-gas-emboli have a capacity to damage the endothelium and thus cause a change in the circulating MP population.

Decompression illness, endothelial damage, gas bubbles, microparticles

Decompression illness

When human's breathing air sojourn to high or low-pressure environments they are exposed to the risk of acute decompression illness DCI (the bends). The population at risk is varied and growing, e.g. sport scuba divers, submarine escapees and space explorers. The number of sports diving related cases of DCI has steadily risen in both North America and the UK over the last 10 years. Advancement in techniques for both prophylaxis and treatment of DCI are warranted.

Decompression illness is the result of gas phase forming in tissue and blood, following pressure changes i.e. return to atmospheric pressure (surfacing) or a drop in pressure as in astronaut extravehicular activity. Modern technology has allowed the detection of venous gas following route (believed) safe dive profiles. Contrary to the thinking 100 years ago, venous gas does not routinely lead to overt DCI. Venous bubbles often referred to as "silent" appear to be filtered by the lungs and fails to reach the arterial circulation where their presence is significantly more problematic.

Historically decompression procedures (tables) have been validated following mathematics calculation, animal modelling and human trials. More recently, detection of venous gas, accepted as an index of decompression stress has been used to help validate decompression procedures, as has probabilistic modelling.

Vascular gas can be identified using Doppler and 2D imaging, however these techniques have limitations. For example they tend to uses short time weighted sampling (typically 5 minute) over increasingly extended periods post exposure; they poorly quantify vascular gas. Their sensitivity and specificity in relation to DCI is poorly defined. There are numerous reports of divers with no or low bubble scores becoming ill and asymptomatic diver with high bubble scores.

With the now wide acknowledgement that DCI often attacks the central nervous system, human trials with DCI as one possible end point have become ethically and practically problematic. Finally the postulate that venous gas bubbles are "silent" or biologically inactive regardless of their quantity is naïve. Accordingly a method of biologically describing a sub-clincial dose response to the effect of a decompression procedure would be helpful in all aspects of decompression modelling. A potential biological marker relating to the stress of a dive is the endothelial microparticle. Gas bubbles released into the circulation will interact with the surface of the vascular endothelium and may give rise to a measurable response, linked to the state of the endothelium.

Endothelial function

Vascular endothelium plays a role in the mechanisms of haemostasis, being involved with the vessel itself, platelet interaction and the functions of the plasma. Endothelial function also is implicitly involved in inflammation and repair of damaged tissue. Disruption of the endothelium, either physically or characteristically due to a diseased state such as post-ischemic reperfusion, inflammation, hypertension and others results in a potentially pro-thrombotic endothelial function. In this state the endothelium expresses von Willebrand factor [vWF], P-selectin [CD62P], ICAM-1 [CD54], VCAM-1 [CD106], IL-8 and promotes the activation and adhesion of platelets, via PECAM-1 [CD31]. Thrombin formation is also observed, along with expression of tissue factor and fibrin deposition. Vascular permeability increases and the production of cytokines, chemokines, growth factors and expression of cellular adhesion molecules is upregulated. These responses are usually part of endothelial function rather than dysfunction and are a pre-requisite for tissue repair and wound healing subsequent to the disruption. Endothelial dysfunction usually results from various disease states and is characterised by a reduction in dilatory capacity and decreased NO capacity, as a result of increased oxygen radical production, which reacts with

NO to form peroxynitrate. NO and prostacycline (PGI_2) are vasodilators, involved in maintaining an anti-thrombotic state by preventing the formation of platelet aggregates. NO controls endothelium-dependent vasodilation, leucocyte adhesion, platelet aggregation, expression of adhesion molecules, synthesis of endothelin and inhibition of vascular growth and inflammation (1). NO is produced in endothelium by nitric oxide synthase (eNOS) and is inactivated by oxygen radicals. Production of NO is dependent upon the presence of cofactors (such as tetrahydrobiopterin) and the availability of the substrate L-arginine. Oxygen radicals can be produced by eNOS under conditions of tetrahydrobiopterin or L-arginine deficiency and elevated concentrations of LDL-cholesterol. NADH/NADPH oxidase also produces oxygen radicals when stimulated by TNFa, and is located in the arterial wall, where extracellular superoxide dismutase acts to remove such radicals.

Endothelial dysfunction can be measured and has is implicated in arteriosclerosis (2), in which oxygen radical formation is enhanced.

Oxidative stress results in endothelium mediated vessel dilation and a subsequent increase in cell turnover and death. Endothelial dysfunction is normally reversible (3).

Cytokine activation of endothelial cells results in increased ability to bind circulating leucocytes, by as much as 400% (4). This increase is due to new or increased expession of adhesion molecules E-selectin [CD62E], ICAM-1 and VCAM-1. Under normal physiological conditions endothelial cells bind leucocyes only briefly, but once activated low affinity interactions are formed, which are then disrupted by shear forces, to be reformed once again, causing a rolling of the leucocyte on the cell surface.

This in turn, with the involvement of chemokines, causes firm attachment of leucocytes to the endothelium, where they crawl to the endothelial cellular junctions and extravase into the tissue space causing inflammation. Adhesion molecule expression follows a defined path; E-Selectin expression occurs early in the process of inflammation, around 2-4h after activation and VCAM-1 expression later (12-24h). The pattern of expression can be modified by various chemokines, such as INFγ and IL-4 (5, 6). E-Selectin, ICAM-1 and VCAM-1 possess DNA sequences that bind transcription factors NfκB and activator protein-1 and these are essential for the TNFα mediated activation of endothelial cells (7), showing that these transcription factors are able to modulate the expression of the adhesion molecule expression.

Endothelial microparticles and markers of endothelial perturbation

Established markers of endothelial cell [EC] damage/activation are traditionally soluble, and are measured from circulating blood. Such markers include ICAM-1 and VCAM-1 amongst many others. However, measurement of these markers may well include membrane-bound forms, as they can be removed by filtration (8). This has led to a wide variation in measurements determined by ELISA techniques. These membrane-bound markers are constituents of microparticles [MP]. Endothelium-derived MP [EMP] and the markers they express are indicative of the state of the endothelium, i.e. activated or apoptotic. They were first described as being released from cultured human umbilical vein endothelial cells [HUVECS] upon stimulation with complement (9), and have been studied, as an *in vitro* model for release of MP during activation or apoptosis (10). MP released from HUVECS are phenotypically distinct and have been proposed to be a useful marker for endothelial injury (11) and are presumed to be procoagulant due to the expression of anionic phospholipids.

Microparticles in humans

MP are released by unstimulated endothelium in healthy subjects, and so a basal concentration exists in the circulation. This suggests that endothelial vesiculation occurs under normal physiological conditions (12). They have been postulated to maintain a balance between cell activation, proliferation and death and be involved in the maintenance of homeostasis (13). Plasma membrane vesiculation is part of remodelling and there is evidence that MP can illicit a response in remote cells via their expressed antigens (13). An increase above the basal levels of MP may lead to pathologic disorders, however basal levels are not detrimental. As MP numbers vary according to the method used no comparable intra-study are available, although research to date has been compared to levels found in healthy controls under the same detection conditions.

Microparticles in disease

MP in the blood circulation have been described in many disease states as either increased in their numbers or being of altered composition, reviewed by Horstmann *et al*. (8). They were first identified from platelets by Wolf (14), and have been shown to be released by many different cells in response to activation or cell death, recently reviewed by Nieuwland and colleagues in relation to their role in cardiovascular disease (15). Release of MP from activated cells is time and calcium dependent (16), whereas those released from cells undergoing apoptosis are formed by membrane blebbing, and are positive for annexin V binding. In both cases MP carry the proteins specific to the parent cell from which they were derived, thus allowing identification of relative MP populations. This is particularly useful when the parent cell may have become activated and express proteins specific to the activated state.

Increased numbers of circulating MP have been studied in acute coronary syndromes (17), multiple sclerosis (18) arteriosclerosis (15), diabetes (19, 20), hypertension (21, 22), pre-eclamplsia (23, 24) and sepsis (25) amongst others. MP have been shown to be either elevated or of an altered composition in patients with cardiovascular disease that show impaired endothelial function (15). MP released from endothelial cells may act as marker for vessel wall injury (10).

Microparticles in thrombocytopenic purpura

EMP, released from perturbed endothelium were elevated in thrombotic thrombocytopenic purpura [TTP] (11), a disease where platelet activation is established. Plasma from TTP patients was found to induce a 3-fold increase in ICAM-1 and a 13-fold increase in VCAM-1 expression on *in vitro* culture of renal microvascular endothelial cells [MVECS]. EMP were elevated in patients with TTP, but not when the disease was in remission and were therefore were stated to have the potential to be a useful marker of endothelial injury. CD62E and CD54 expression on EMP from TTP patients was found to be increased significantly (26) and of CD62E positive EMP, 55% displayed expression on vWF. The authors concluded that the EMP were released from activated endothelium in TTP patients. EMP counts returned to normal upon remission. EMP were analysed from cultured brain and MVECS. CD31 and CD42b were used to identify MP of endothelial origin. EMP were found to be pro-coagulant when the cells were stimulated with TNFα (activation) or mitomycin C (apoptosis) (11).

Research into EMP markers by the same group (27) showed that they possess different proteins, which were determined by whether the MP were formed by activation or apoptosis pathways in the endothelial cell of origin. The expression of the inducible markers CD54, CD62E and CD106 were found to be increased in MP from activated cells, compared with those from apoptotic cells and control samples. Annexin V binding to MP was found to be increased in both activation and apoptosis.

Microparticles in coronary disease

EMP were found able to bind platelets *in vitro*, forming aggregates [EMP-P] with a potential involvement in thrombus formation (28). MP were isolated from HUVECS by ultracentrifugation and incubated with isolated platelets before being labelled with CD105 and CD41a. Flow cytometry confirmed aggregates expressing both the endothelial (CD105) and platelet (CD41a) markers had been formed. Similar aggregates could be isolated from healthy subjects and almost all of those which were CD105 positive were also found to express CD31 and two markers of endothelial activation (MCP-1 and CD 62E). Patients with stable coronary disease were found to have a significantly higher concentration of EMP-P (16.7 per γL whole blood) than healthy controls (7.1 per γL). A significant decrease in EMP-P concentration was observed during acute myocardial infarction, which was hypothesised to be due to involvement of these aggregates in thrombus formation in the infarct-related vessel. Levels of circulating EMP-P returned to pre-event concentration at 48h. A previous study observed an increase in EMP within blood of subjects with acute myocardial infarction (17) however, the MP were higher measured days after onset and were compared to healthy controls.

It has also been demonstrated that MP isolated from patients with myocardial infarction have the potential to cause further endothelial dysfunction (29). Rat aortic rings were incubated with MP isolated by ultracentrifugation from patients with myocardial infarction. It was concluded that these MP caused a high degree of endothelial dysfunction in healthy vessels by affecting the NO transduction pathway. The MP significantly decreased relaxations in response to acetylcholine in the aortic rings, and this observation was eliminated upon endothelium removal or the addition of a NO synthase inhibitor. The actual MP, if indeed there was a particular type responsible, were not analysed as to their cellular origin.

Microparticles in multiple sclerosis

The presence of CD31 on endothelium is a prerequisite for extravasion of leucocytes (30) and was found to be increased in the serum of MS patients where brain gadolinium-enhancing lesions were present (31). Circulating EMP were analysed for CD31 and CD51 expression in MS patients and were found to be elevated in disease exacerbation but not when the disease was in remission. The amounts of EMP were found to be 2.45, 0.58 and 0.86 $x10^6$ per mL in MS exacerbation, remission and normal controls, respectively. The median value for all collected EMP was surpassed by 93% of patients with MS in exacerbation and 90% were below the median when in remission, suggesting strong evidence for a role of endothelial damage in the disease process.

Microparticles in sickle cell anaemia

Circulating EC have been analysed in sickle cell anaemia, disease in which the vascular endothelium has a role in pathogenesis (32). A correlation was established between acute painful episodes and circulating EC. Also, the circulating EC were found to express CD54, CD106, CD62E+P, suggesting the endothelium is in an activated state in the illness. MP in sickle cell disease subjects were found to be elevated in crisis and steady state conditions, when compared to normal controls (33), although MP were defined as less than 1γm in size and able to bind annexin V. MP were isolated by ultracentrifugation however, this method was previously shown to vastly underestimate the true number of platelet-derived MP within the plasma (34). These MP were released by endothelial, monocytes, erythrocytes and platelets and a proportion were found to be tissue factor positive, but 13/21 patients had no detectable EMP expressing TF. The majority of TF expression was found to be on monocyte-derived MP (20/21). The expression of TF on MP in sickle disease may be of importance in thrombosis, potentially casusing an activation of clotting pathways and the production of thrombin (33). The authors concluded that the presence of monocyte- and endothelial-MP were a marker of parent cell activation in the disease however, such markers of activation were not measured.

Microparticles in paroxysmal nocturnal haemoglobinuria

A further study focused upon EMP in paroxysmal nocturnal haemoglobinuria [PNH] and sickle cell disease [SCD] in comparison to healthy controls (35). Thrombosis is the major cause of morbidity and mortality in PNH, and is always associated with EC activation and damage. PNH is clinically manifested by haemolysis, which releases free haemoglobin, toxic to EC. The

number of endoglin [CD105] positive EMP was elevated in PNH (0.4 x 10^9/L plasma) and SCD (0.57 x 10^9/L) when compared to controls (0.18 x 10^9/L). A subpopulation of CD105+CD54+ EMP were also elevated in PNH (0.24 x 10^9/L) and SCD (0.25 x 10^9/L) than controls (0.11 x 10^9/L). This was thought to show that the vascular EC show an inflammatory phenotype. No correlation between EMP and thrombotic events in the disease states was evaluated however, since thrombosis is a major source of mortality in PNH a link was intimated and the EMP phenotype was said to be a marker of severity of vascular disease and of diagnostic use.

Microparticles in Meningococccal sepsis

Meningococccal sepsis is caused primarily by the release of endotoxin by bacteria. Plasma from patients (n=7) with this disease were analysed for MP and compared to healthy controls (n=5) (36). Elevated levels of various MP, with procoagulant properties (TF positive) were found, although CD62E+ defined EMP were elevated but not significantly so. EMP concentration was 61 x 10^6 per L plasma in patients, compared with 18 x 10^6 in controls. All cell derived MP showed large variations in the 36h testing period. Interestingly, TF activity was identified in a non-surviving patient, and was found to occur on monocyte-derived MP, as identified by CD14/TF dual staining. Also, thrombin generation was seen to occur when MP from this patient was incubated with normal plasma, and furthermore this generation was delayed significantly delayed by pre-incubation with antibodies against TF or factor VII.

Microparticles in diabetes

In type I diabetes EMP levels were significantly raised (26 x 10^9/L plasma), when compared with control subjects, and patients with type II disease did not show an increase (37). Healthy controls were found to have 14 +/- 16 x 10^9 EMP per L plasma. Platelet-derived MP were also elevated in type I disease, and annexin V positive MP were elevated in type II disease. Leucocyte-derived MP were found to be elevated in both type I (38 x 10^9/L) and type II (37 x 10^9/L), when compared to controls (14 x 10^9/L). The results were thought to suggest that EMP were a marker of endothelial damage associated with diabetic nephropathy in type I diabetic patients. The authors speculated that MP in diabetes which possess a procoagulant function exacerbates cell activation and contributes further to the disease progress and therefore may be a target for therapeutic intervention (37).

EMP, as with other soluble cytokine receptors may act to neutralise ligands destined for their parent cells, effectively removing or at least decreasing their potential effect. Therefore EMP may have a protective function, preventing further EC activation.

In summary EMP may provide a valuable insight into the state of vascular endothelium in many disease states, some of which have been highlighted here.

EMP and decompression

We recently conducted a randomised crossover trial of divers (N=24) subject to 2.8atm for 78min bottom time and decompressed using USN standard air tables. Endothelial MP were identified from blood samples taken pre-, during and up to 24h post-hyperbaric exposure, using flow cytometry, after labelling with the relevant fluorescent-tagged antibodies PECAM-1, CD34, CD42b, CD51, CD54, CD62E+P and VCAM-1.

Having established a coefficient of variation for mean fluorescence values of CD markers of approximately 50% (N=46 samples) no analysis between groups was thought meaningful. Wilcoxon's signed-rank test was used for within group analysis, with pre-dive values used as a base-line control. In the dive group significant increases were observed in some markers 5 min following decompression [CD54 (P=0.030; N=20), CD106 (P=0.013; N=23)]. After 12h decompression most markers studied had significantly increased [CD34 (P=0.041; N=15), CD51 (P=0.007; N=20), CD54 (P=0.005; N=21), and CD106 (P=0.001; N=24)] however, no significant change was observed in CD62E+P expression.

The significant increase in endothelial specific markers within the circulating MP population is indicative of endothelial damage. There is evidence of possible EC activation following hyperbaric exposure, as indicated by the increase in CD106. Further investigations are necessary to correlate changes in MP population with diving stress.

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Corresponding author:
Leigh A. Madden
Postgraduate Medical Institute
The University of Hull
Cottingham Road
Hull
HU6 7RX
United Kingdom
l.a.madden@hull.ac.uk

Zetterstrøm Award Winner 2005

RECOMPRESSION WITH OXYGEN TO 160 kPa ELIMINATES VASCULAR BUBBLES, BUT DOES NOT PREVENT ENDOTHELIAL DAMAGE

Andreas Møllerløkken[1], Vibeke Nossum[2], Wenche Hovin[1], Mikael Gennser[3], Alf O. Brubakk[1]

[1]) Baromedical and Environmental Physiology Group, Dept. Circulation and Medical Imaging, NTNU, Trondheim, Norway
[2]) Thelma AS, Trondheim, Norway
[3]) Department of Defence Medicine, Swedish Defence Research Agency, Centre for Environmental Physiology, Karolinska Institutet, Stockholm, Sweden

Møllerløkken A et al.: Recompression with Oxygen to 160 kPa eliminates vascular bubbles, but does not prevent endothelial damage. Europ J Underwater Hyperbaric Med 2007, 8(1&2): 11-16. Treatment of decompression sickness is a significant problem especially when time from the insult to the initiation of treatment may be delayed by several hours. As an emergency procedure, in-water treatment with oxygen has been recommended. The present study was initiated to determine the effect of recompression to 160 kPa breathing 100% oxygen for 60 min, 60 min after a strenuous dive to 600 kPa for 30 min breathing air. A total of 17 pigs were divided into an experimental and a control group. The pigs underwent a dive to 600 kPa for 30 min breathing air and were decompressed at a rate of 200 kPa/min. 60 min after surfacing, the animals in the experimental group were recompressed to 160 kPa breathing 100% O_2 for 60 min, while the control group remained at the surface breathing air. Following recompression, the bubbles in the pulmonary artery were rapidly reduced and no bubbles reappeared after ending the treatment. Vessels (pulmonary artery, carotid artery) from both groups showed a reduced relaxation response to acethylcholine. The relaxation response to bradykinin was on the other hand close to what is expected as normal. Recompression to 160 kPa 60 min after surfacing removes the vascular gas bubbles, but does not prevent endothelial damage.

Oxygen recompression, endothelial damage, gas bubbles

INTRODUCTION

Treatment of decompression sickness (DCS) still remains a significant problem, especially in situations where several hours will elapse before the divers can be brought to a recompression facility. A large number of those treated have significant sequelae, and most divers treated for DCS today have signs and symptoms from the central nervous system (CNS) (1, 2).

The purpose of all decompression procedures is to prevent injury to the diver, and it is generally agreed that these injuries are caused by the formation of gas bubbles in the body. Vascular gas bubbles are formed in nearly all decompressions (3), and the risk of developing DCS increases with the number of gas bubbles. Based on previous work in our laboratory we have formed the hypothesis that it is gas bubble formation in the vascular system which is the main initiator of serious DCS (4). Large numbers of vascular gas bubbles have been shown to cause mechanical damage to the endothelium (5). The damage from smaller numbers of gas bubbles may not necessarily be caused by mechanical effects on the endothelium but could be due to interaction between the bubble surface and components of the blood. However, large numbers of bubbles have been shown to tear endothelial cells from their basal layer causing the endothelial nuclei to protrude into the lumen (5). This leads to increased permeability for proteins in the endothelial layer. If the endothelial lining becomes disrupted or damaged by gas emboli, endothelium dependent vasodilatation could be depressed. Smaller numbers of gas bubbles have been shown to activate the complement system and subsequently an inflammatory response (6). A previous study showed that complement activation led to reduced endothelial function without any visible damage to the endothelial layer (6).

Our laboratory has previously performed an experimental series where the effect of recompression to 200 kPa on air or treatment with 100 kPa oxygen on CNS injury following an air dive was studied (7). Only one animal in the air group had any changes in the CNS, none of the animals had any endothelial functional deficit. Another study found that recompression to 200 kPa eliminated the vascular gas bubbles significantly faster than breathing oxygen at 100 kPa, and no additional effect of adding oxygen or increasing the total pressure to 400 kPa could be seen (4). In these studies, the time to treatment was determined using maximum bubble formation and the treatment duration was set by determining when the bubbles disappeared. It has been reported that time to treatment is not an important factor in determining outcome of treatment (8, 2), although others have shown the importance of early treatment (9, 10). It appears that given long delays (6 h or more), further delay no longer affects the outcome significantly (2, 10). Thus, to achieve the best possible outcome the diver should be treated promptly, and longer delay than a few hours should not be allowed. These days much recreational diving takes place in remote locations where it may take many hours and even days to transport an injured diver to a recompression chamber. It is in view of this development that there has

been renewed interest regarding in-water recompression treatments. Presently the recommended procedure for in-water recompression treatment is 190 kPa pressure (9 msw) with oxygen or air breathing (11). However, if the same treatment effect could be obtained at a lower pressure this would reduce the risk of oxygen seizures. The present study was initiated to determine if treatment at 160 kPa using oxygen would be effective.

METHODS

All 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.

Study population

A total of 17 pigs (*Sus scrofa domestica*), both female and male, were used in this study. The pigs were 12 weeks old and weighing 23.5 ± 5.6 kg. All animals arrived at the centre for experimental animals at St Olavs Hospital in Trondheim, and had one week of acclimatisation before start of the study. The pigs were randomly divided into two groups: an experimental group (n= 9) and a control group (n=8).

Surgical procedures

Before the experiment, the pigs were fasted for 16 h with free access to water. On the day of the experiment, they received premedication with 10 ml Stresnil (Azaperon, Janssen-Cilag Pharma, Vienna) and 2 ml Stesolid (Diazepam 5 mg · kg^{-1}, Dumex-Alpharma AS, Copenhagen). After 20 min, atropine sulfate (Atropin, 1 mg iv; Nycomed Pharma) was given via an ear vein. Anaesthesia was induced by thiopental sodium (5 mg · kg^{-1} Pentothal Natrium, Abbott Scandinavia) and ketamine (20 mg · kg^{-1} Ketalar; Pfizer). The anaesthesia was maintained by a continuous iv infusion of ketamine in 0.9% NaCl (30 mg · kg^{-1} · h^{-1}) together with bolus doses of α-chloralose in 0.9% NaCl (10-15 mg · kg^{-1} injected iv; 0.25% solution). A tracheotomy was performed to allow the pigs to breathe spontaneously through an endotracheal tube (Tracheal Tube, 7.00 mm ID, Portex). Throughout the experiments, the pigs were in a supine position. The depth of anaesthesia was maintained at an even level, as judged by clinical observation of the experimental animal, its breathing frequency, blood pressure, blood gas measurements and amount of CO_2 in the expired gas. Blood-samples were taken at regular intervals throughout the entire experiment for blood-gas analysis (both arterial and venous), and were analysed on an ABL 700 blood gas analyzer (Radiometer, Copenhagen).

Core body temperature was measured continuously throughout the experiments by a rectal thermometer, and was adjusted through regulation of the chamber temperature. The body temperature was kept between 38.0 °C and 39.0 °C.

After the observation period, the endotracheal tube was removed and the pigs were allowed to wake up and followed for up to one week. The pigs received a daily injection of 3 ml Penovet vet (Penovet vet, 300 mg/ml, Boehringer Ingelheim, Denmark) and were observed by a veterinary once a day.

Pressure profile and breathing gas

All animals were compressed to 600 kPa for 30 min while breathing air. Both compression and decompression was at a rate of 200 kPa/min. After surfacing, the control group was observed for 3 h while continuing breathing air. The experimental group was observed at the surface for 60 min before they were recompressed to 160 kPa for 60 min while breathing 100% O_2. After 60 min, the pigs were again decompressed to the surface and followed for an additional 60 min (Fig 1).

Bubble detection

A transesophageal echcardiograpic probe was introduced through the mouth and was placed in a position where a good image of the right ventricle and the pulmonary artery could be seen as described by (12). The transducer was connected to an ultrasonic scanner (CFM 750; Vingmed Sound, Horten, Norway). From the images, the amount of bubbles in the right ventricular outflow tract is given as number of bubbles per square centimetre (bubbles · cm^{-2}) as described by (13).

Endothelial function

A modified tissue-bath technique was used as described previously (5). The pulmonary artery from the right lung and the right carotid artery were carefully dissected and stored in oxygenated (5% CO_2; 95 % O_2) sodium-Krebs buffer solution for a maximum of 24 h. The vessels were cut into cylindrical segments with lengths ranging from 1.0-1.5 mm. Each cylindrical segment, four from each vessel, was mounted on two parallel L-shaped metal prongs and immersed in temperature-controlled (37.0 ^{o}C) tissue baths containing a sodium-Krebs buffer with the following composition: 119mM NaCl, 10 mM $NaHCO_3$, 1.2 mM $MgCl_2$, 4.6 mM KCl, 1 mM NaH_2PO_4, 1.5 mM $CaCl_2$, and 11 mM glucose. Air comprising 5% CO_2 in O_2 was bubbled continuously through the sodium-Krebs buffer to keep it at pH 7.4. The contractile capacity of each vessel segment was examined by exposure to a potassium-rich (60mM) Krebs buffer solution. The vessels were pre-contracted with cumulative doses of noradrenaline and the relaxation response was tested with cumulative doses of acetylcholine (ACh) (0.8 x 10^{-9}-0.8 x 10^{-4} M) and bradykinin (BK) (10^{-11}- 10^{-6} M). The maximum relaxation response (I_{max}) was defined as the maximal dilatory response regardless of the concentration induced by an agonist, and is expressed as a percentage of the pre-contraction induced by a pre-contracting agent. The performance of the vascular smooth muscle cells was evaluated with cumulative doses of sodium nitroprusside (SNP) (10^{-9}-10^{-5} M). Dose-response curves for all agonists were calculated.

Statistical analysis

The data were analyzed using SPSS 13.0. Since the number of animals in each group is small, the tension data were subject to analysis using the Mann-Whitney test for non-parametric data between the groups. A student t-test was used to compare the number of bubbles during the observation period. $P< 0.05$ was accepted as significant.

RESULTS

Bubbles appeared in the pulmonary artery in all animals immediately after decompression from the first dive. The number of bubbles seen in the two groups was not significantly different. Following recompression, the bubbles in the pulmonary artery were rapidly reduced and no bubbles reappeared after ending the treatment (Fig 2).

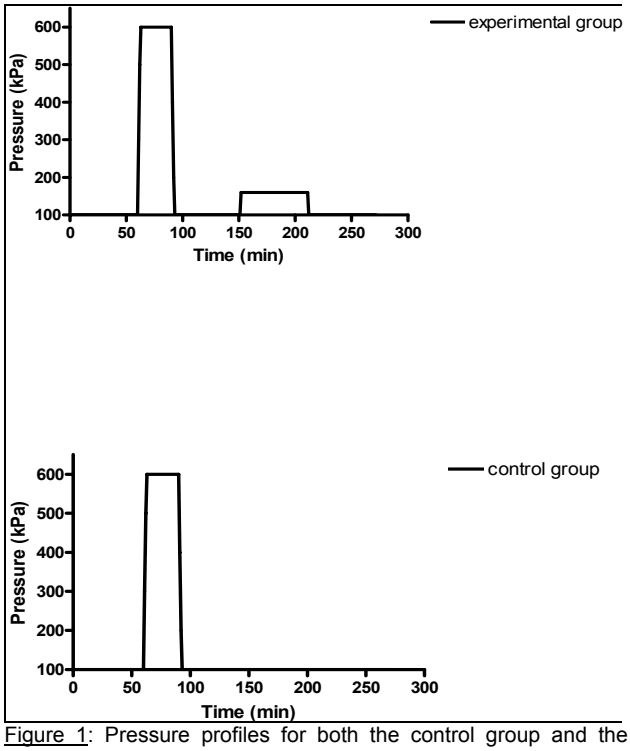

Figure 1: Pressure profiles for both the control group and the experimental group.

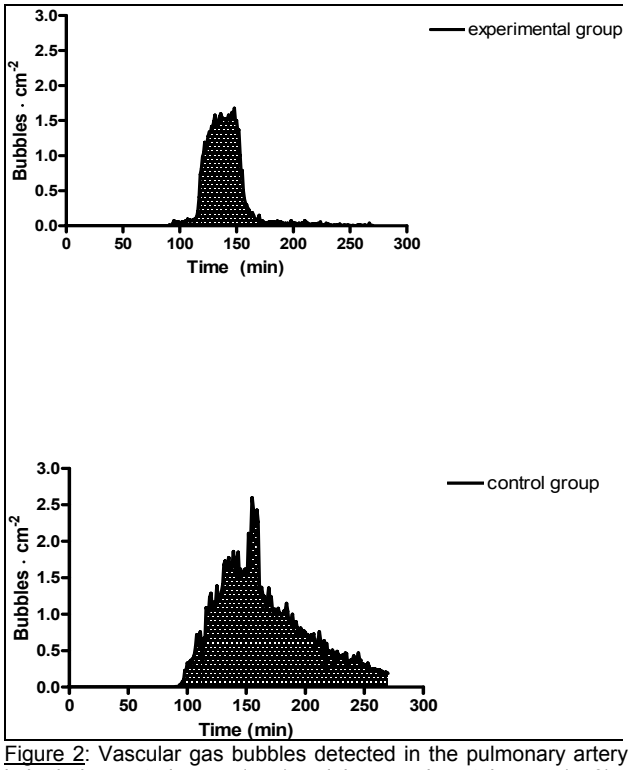

Figure 2: Vascular gas bubbles detected in the pulmonary artery in both the control group (n=5) and the experimental group (n=9).

Three out of eight animals in the control group died within 15 min after decompression and were hence excluded from further comparisons. Of the remaining five animals in the control group, three of them were sacrificed due to signs of neurological DCS within the first post-dive period (<12h). One of the nine animals in the experimental group died in the same period.

Both the experimental group and the control group showed a reduced relaxation response to ACh compared to earlier studies (6, 14). The experimental group also showed a lower I_{max} response (10.7 ± 12.1) in the pulmonary artery to ACh compared to the non-treated control group (Table 1) (p = 0.04). The relaxation response in the treatment group was also lower in the carotid artery, but this difference was not significant. From the dose-response curves, it appears that the dose-related relaxation response to ACh in the pulmonary artery was lower at $0.8x10^{-7}$M and through all higher concentrations for the experimental group compared to the control group (Fig 3).

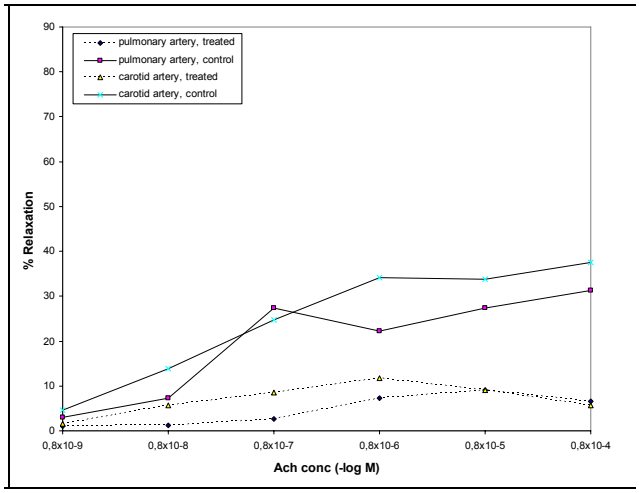

Figure 3: Dose-response curves for the response to acetylcholine in the experimental group (n=6) and the control group (n=5), in both the pulmonary artery and the carotid artery. The response in the pulmonary artery was significantly lower in the experimental group compared to the control group, * *P<0.05*. There was no significant difference between the responses in the carotid artery between the groups.

The relaxation response to BK was on the other hand close to what was expected (15, 16) in both the pulmonary artery and the carotid artery (Fig 4).

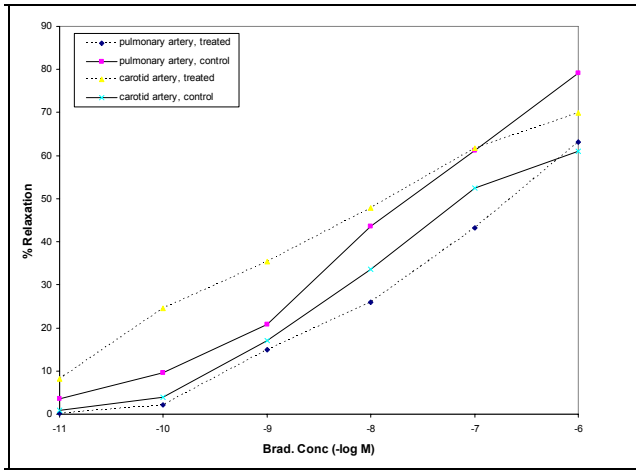

Figure 4: Dose-response curves for the response to bradykinin in the experimental group (n=6) and the control group (n=5), in both the pulmonary artery and the carotid artery.

Application of the endothelial-independent agonist SNP resulted in no significant differences in I_{max} between the two groups in either carotid or pulmonary arteries (Fig 5).

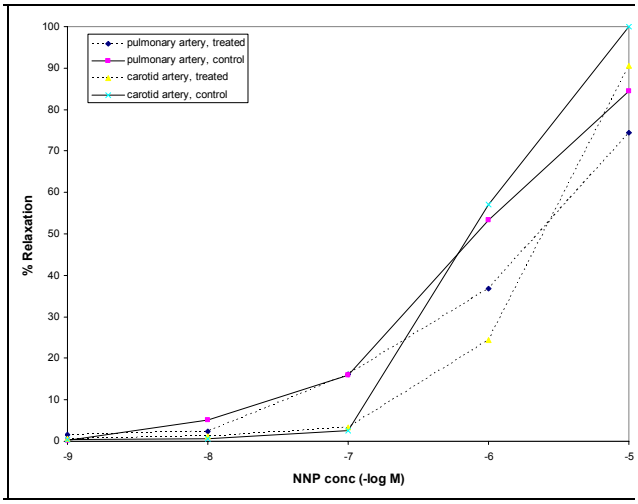

Figure 5: Dose-response curves for the response to sodium nitruprusside (SNP) in the experimental group (n=6) and the control group (n=5), in both the pulmonary artery and the carotid artery.

Table 1: Comparison of values for the experimental group and the control group. Maximum %-relaxation values (I_{max}) for acetylcholine (Ach), bradykinin (BK) and sodium nitroprussid (SNP). Values are presented as the mean (SD) in each group.

Group allocation	I_{max} Pulmonary artery			I_{max} Carotid artery		
	Ach	BK	SNP	Ach	BK	SNP
Control (n=5)	44.0 (37.8)	79.2 (12.0)	84.4 (7.8)	39.5 (24.6)	60.9 (29.9)	100.0 (15.7)
Experimental (n=6)	10.7 (12.1)*	63.1 (26.5)	74.5 (27.2)	12.7 (10.9)	70.0 (22.6)	90.7 (46.8)

* *P = 0.04*

Discussion

The main finding in this study was that recompression to 160 kPa breathing 100% O_2 for 60 min, 60 min after a strenuous dive to 600 kPa with 30 min bottom time did rapidly remove the vascular bubbles in the pulmonary artery. The treatment did not, however, prevent a reduction in the endothelial response to ACh.

The efficiency of the recompression is seen in figure 2. Within 6 min, there was a 50% reduction in the number of bubbles. After the treatment was ended and the animals were decompressed to the surface, there was no reappearance of vascular gas bubbles. For the control group, the time to 50 % reduction was 43 min. There were still detectable vascular gas bubbles in the control group after 3 h of observation. Our laboratory has previously reported the effect of 100 kPa O_2 and recompression to 200 kPa with air on organ injury following decompression in the pig (7). The results from this study indicated that rapid treatment using oxygen at the surface or air at 200 kPa could prevent injury. Our laboratory has also reported that a recompression to 200 kPa eliminates the gas bubbles significantly faster than breathing oxygen at 100 kPa (4). In these previous studies, the treatment was initiated when the number of vascular gas bubbles were at peak values, and from these studies it became evident that by recompressing to 200 kPa breathing air, the mean treatment time at 76 min was not sufficient to eliminate the excess nitrogen from the dive. The present study shows that recompressing after 60 min on the surface to 160 kPa with O_2 for an additional 60 min adequately removed the excess nitrogen from this specific dive.

Endothelial function of the pulmonary artery was determined as previously described (5). The same method was applied to the carotid artery. Due to technical problems we only have endothelial measurements from six of the nine animals in the experimental group, but the I_{max}-response to ACh in the pulmonary artery was significantly impaired in the experimental group compared with the control group. The response to ACh was 10-12 % for the experimental group, in both pulmonary and carotid artery. A normal relaxation response, based on earlier studies, is expected at least between 50-60% (5, 14). The low response to ACh was also present when investigating the carotid artery in the experimental group, but the difference did not reach statistical significance. The control group also showed a low relaxation response to ACh both in the pulmonary artery and in the carotid artery, but the response was considerabley better when compared with the experimental group (Table 1). In contrast, the response to BK can be considered to be normal (15, 16) in both the pulmonary artery and the carotid artery in both groups. Also the endothelium independent response to SNP seems unaffected by the dive, the vascular bubbles and the treatment with recompression and oxygen. This confirms that the change in vasoactive response is only related to endothelial function and not to function in the vascular smooth muscle layer.

The finding of impaired endothelial response to ACh but not to BK indicates that the endothelial response to ACh is affected by different mechanisms. In fact, from table 1 it is temping to speculate that the treatment used in the present study, did not improve the outcome from the dive, but actually lead to more damage to the endothelium. Since the damage occurred despite removal of the vascular gas bubbles, it indicates that the response to ACh seems to be affected by breathing 100% O_2. Whether we would have found the same response if we had chosen to use 100% O_2 at 100 kPa in the treatment regime, and not at 160 kPa, is speculative. A previous study showed that the use of 100 kPa O_2 after a dive to 500 kPa for 40 min breathing air, with decompression at 200 kPa/min did not affect the endothelial function which was found to be normal (7). In the same study, a group of animals was also recompressed to 200 kPa breathing air, which had no effect on the endothelial response. Hence, in the present study, the impaired endothelial function to ACh seems to be through the use of 100% O_2 at elevated pressure. The present study does not allow us to point out the exact mechanism for the observed effect, but regardless of mechanism it seems that treating the animals with 100% O_2 for 60 min at a pressure of 160 kPa 60 min after a strenuous dive weakens the endothelial relaxing response to ACh, but not to BK.

The endothelium controls vascular tone by secreting relaxing and contracting factors (EDRFs). The

endothelium can regulate the release of EDRFs in response to humoral stimulation by vasoactive substances such as ACh and BK (17). ACh is known to induce dilation in vascular smooth muscle via the M_2 and M_3 muscarinic receptors located on endothelial cells, and has become a standard method for determining endothelial function (18, 19, 20). BK is known to cause vasodilatation through the B_2 receptor and subsequent effects on NO, prostacyclin and EDHF production (21). Studies have shown that there is a possible beneficial vascular effect of angiotensin converting enzyme (ACE) inhibitors related to increased availability of BK (17). Recent studies indicate that BK stimulates the release of tissue plasminogen activator (tPA) from the human vasculature in a dose-dependent manner (22), and animal studies suggest that BK is 1000-fold more potent than agonists such as histamine, norepinephrine, vasopressin and ACh in stimulating the acute release of tPA from the vasculature (23). It is tempting to speculate that the response to BK seen in the present study is caused by an acute release of tPA. Also, since ACh and BK act via different receptors, it seems likely that in our experiment, the vascular gas bubbles and the oxygen have affected the M_2 and M_3 receptors, but not the B_2 receptor.

The in-water treatment tables recommended by Edmonds indicate that the recompression depth should be 190 kPa (24). However, at this pressure there is still some risk of inducing oxygen convulsions, and hence we chose a shallower treatment depth of 160 kPa (25). By using oxygen, the gradient for nitrogen elimination is increased. Further, extra nitrogen loads are avoided, and the depths required for the exposure time are decreased (26).

After the initial dive, three of the animals in the control group died within 10 min after surfacing. Since those deaths occurred before the scheduled treatment these animals were excluded from the analysis. For the remaining animals there was no significant difference in survival time between the two groups in this study, but there was a trend towards better survival in the experimental group compared with the control group.

The important finding from this study was that by waiting for 60 min before starting the recompression treatment, the rapid removal of bubbles was not able to prevent endothelial damage. Brubakk *et al.* (7) showed that by initiating treatment at peak bubble numbers, no reduction in endothelial function was found, whether the treatment consisted of 100% oxygen breathing at surface or recompression to 200 kPa on air. It would thus appear that it is not the treatment depth but the time to treatment that is of importance here. Hence, by waiting for 60 min before start of treatment, the function has already been impaired to a point where recompressing to 160 kPa with O_2 for 60 min had little effect. Similar changes were also observed in the carotid artery. There is an increased risk of developing serious DCS when a large number of bubbles can be detected in the vascular system (3). Previous findings showed a relationship between gas bubbles and mechanical endothelial damage, and that the injuries were acute (5, 27). The mechanical injuries were also related to the number of gas bubbles. Infusion of a controlled number of gas bubbles did not lead to any mechanical damage, but a dysfunction related to the vasoactive response in the endothelium was found (6) which indicated a biochemical or an immunological response to the bubbles that developed with time.

We observed impaired endothelial response to ACh on the venous side of the circulation. The endothelial response on the arterial side did not reach statistical significance, but showed a low response as well (Table 1). In human studies, a reduction in arterial endothelial function following an air dive with few venous bubbles has been found (4). It can not be excluded that there has been shunting of gas bubbles from the venous to arterial side subsequent to this dive. There is a relationship between neurological DCS and an open foramen ovale (PFO) (28), and gas bubbles may pass through intrapulmonary arteriovenous shunts (29). This would support the hypothesis that vascular gas bubbles are the main problem in serious DCS. Arterial dysfunction might also be a result of an immunological responses which have transmitted from the venous side to the arterial side of the circulation, activated endothelial cells may reduce endothelial function downstream from the injury.

In-water treatment with oxygen has been recommended as an emergency treatment for DCS, and the present study has demonstrated that recompressing to 160 kPa for 60 min breathing O_2 is effective in removing gas bubbles even if treatment is started 60 min after surfacing. However, in the present study the treatment did not protect from endothelial damage.

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Author contributions:

Andreas Møllerløkken, Cand Scient, Physiology, PhD student. Department of Circulation and Medical Imaging, Faculty of Medicine, Norwegian University of Science and Technology, Medisinsk Teknisk Forskningssenter, N-7489 Trondheim, Norway. Has designed, performed and been the main contributor to the manuscript.

Vibeke Nossum, PhD. Thelma AS, Pb 6170, Sluppen, 7435 Trondheim, Norway. vin@thelma.no. Had the main responsibility for the endothelial measurements and has contributed to the summarizing of the present paper.

Wenche Hovin, M.Sc. Biotechnology. Department of Circulation and Medical Imaging, Faculty of Medicine, Norwegian University of Science and Technology, Medisinsk Teknisk Forskningssenter, N-7489 Trondheim, Norway. Wenche.hovin@ntnu.no. Has participated both in performing the experiments and given valuable keypoints to the manuscript.

Mikael Gennser, Dr Med. Department of Defence Medicine, Swedish Defence Research Agency, Centre for Environmental Physiology, Karolinska Institutet, Stockholm, Sweden. mikaelge@foi.se. Has given important clues during the planning of the study, and throughout the process of writing this paper.

Alf O Brubakk, Dr. Med. Department of Circulation and Medical Imaging, Faculty of Medicine, Norwegian University of Science and Technology, Medisinsk Teknisk Forskningssenter, N-7489 Trondheim, Norway. alf.o.brubakk@ntnu.no. The navigator throughout the entire study. Has given important intellectual contributions both in the stage of designing the study, and throughout the entire process of writing this manuscript.

Corresponding author:

Andreas Møllerløkken
Department of Circulation and Medical Imaging
Faculty of Medicine
Norwegian University of Science and Technology
Medisinsk Teknisk Forskningssenter
N-7489 Trondheim, Norway
Phone: +47 73 59 89 07
Fax: +47 73 59 86 13
Email: andreas.mollerlokken@ntnu.no

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