Thesis

de

Cerebro urinatorio

ab

Andrea Rosenio, Physico, natione Sueco,

via et ratione explorata et conscripta,

Facultati anaesthesiae adhibendae

et

patientibus valde laborantibus curandis,

apud institutum scientiis clinicis provehendis,

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Summarium latinum

Homines apti sunt vitae terrestri. Is, qui apparatu urinatorio[1] praeditus urinatur, se alienis periculosisque rebus exponit. Cum urinator deorsum in aquam se propulerit, aqua circumjecta eum pressura intensiore premet, quo profundius in aquam descenderit. Pressura aucta reddit in aere spirato et oxygenium et nitrogenium noxia systemati nervoso. Nitrogenium efficit ut urinator aliquid simile ebrietati sive narcosi patiatur cum animo confuso et ignavo, necnon moribus mire novis. Illa animi stupefactio gravior fit quo profundius descenderit urinator. At urinatore ad aquae superficiem reverso stupefactio omnino tollitur. Pressura aucta, oxygenium excitat urinatori rigorem et distentionem nervorum, quod quidem urinatori, dum urinatur, morti esse potest. Urinatori praeter 150 metra descendenti systema nervosum ob auctam pressuram excitare potest tremulos, paroxysmos, veternum et lethargum, et diminuere vim cogitandi.

Aucta illa pressura gasis nitrogenii in eo aere, quem urinator, aqua submersus, inspirat, efficit ut nitrogenium in telam cellulosam accipiatur. At, dum urinator sursum ad aquae superficiem se confert et sic pressura minuitur, tela cellulosa nitrogenium acceptum exsolvit. Nitrogenium, quod a tela cellulosa solutum est, in sanguine dissolvitur et ex corpore evanescit per pulmones. Si autem urinanti pressura citius diminuetur quam nitrogenium dissolutum ex corpore evanuerit, copia dissoluti nitrogenii esse potest ita magna ut bullae crescant in et sanguine et tela cellulosa. Periculum morbi urinatorii maior est cum magna copia bullarum nitrogenii in sanguine exstiterit. Hic quidem morbus urinatorius et cerebro et systemati nervoso nocere potest.

Inquisitiones doctae docent eos homines, qui per temporis longum spatium artem urinatoriam exercuerunt, pati memoriam peiorem et difficultates animum intendendi. Rogandum est igitur si urinatio cerebro noceat.

Tria proteina, quae et ‘tau’ et glutinosum fibratum acidiosum proteinum (GFAp) et neurofilamentum leve (NfL) vocantur, cerebro laeso in sanguinem manant.[a] At et proteinum ‘tau’ et proteinum ‘GFAp’ possunt dissolvi in sanguinem, si cellulae nervorum moventur vel quodam modo affligantur — etiam si nullae cellulae noceantur.

Inquisitio haec doctoralis duas res excutit: primum, partim an pressura aucta commoveat systema nervosum ita ut proteinum ‘tau,’ proteinum ‘GFAp,’ et proteinum ‘NfL’ dissolvantur in sanguine, partim an respiratio oxygenii statim post urinationem diminuat copiam bullarum nitrogenii in sanguine et sic reddat minus periculum morbi urinatorii. Haec thesis doctoralis constat ex tribus partibus.

Prima pars:

Decem mercenarii urinatores, quatuor diebus urinabantur in mari inter altitudem 52—90 metrorum. Copia proteini tau aucta erat 98.8 centessimis post quartum diem urinationis. At illa mutatio proteini ‘tau’ cum copia bullarum nitrogenii in sanguine non cohaesit.

[a] Sequentia nomina a interprete ficta Latine sunt: tau, glutinosum fibratum acidiosum proteinum (GFAp), Neurofilamentum leve (NfL), anglice sonant tau (tau), glial fibrillary acid protein (GFAp) and Neurofilament light (NfL).

Secunda pars:
Quatuor decim nautae (mares et feminae) submarini in cella pressoria (cella pressurae temperandae) inclusi sunt per 36 horas pressura adhibita ita ut submersi essent ad altitudinem 30 metrorum. Deinde pressura gradatim et lente diminuebatur per 70 horarum spatium. Nulla mutatio percepta est in proteinis 'tau,' 'GFAp,' et 'NfL.'

Tertia pars:

Quadraginta octo mercenarii urinatores, inclusi in cella pressura, aquae plena, passi sunt eam pressuram, quae ad 42 metrorum altitudinem invenitur, per 10 minutarum spatium. Hoc facto, per semi-horam una pars urinatorum respirabant oxygenium, altera pars eorum aerem respirabant. Post biduum, eidem urinatores eandem urinationem in eadem cella facint per idem tempus una re mutata. Post hanc secundam urinationem, prima illa pars urinatorum aerem respirabant, altera pars oxygenium. Copia proteini 'tau' aucta est 31.5 centessimis post urinationem. Copia bullarum nitrogenii in sanguine minor fuit apud hos qui oxygenio usi sunt quam apud illos qui aere usi sunt. At illa mutatio proteini 'tau' cum copia bullarum nitrogenii in sanguine non cohaesit.

His rebus factis viaque et ratione consideratis, patet post urinationem factam urinatoribus respirationem oxygenii praestans remedium esse contra nitrogenii bullas in sanguine. Hoc remedium, igitur, diminuit periculum morbi urinatorii. Ad haec, proteinum 'tau' post urinationem auctum est. Quae mutatio videtur pendere ab nervis, nescio quo modo, motis. Opus est aliis futuris inquisitionibus ut intelligamus quo modo proteinum 'tau' mutetur post urinationem.

Concerning the use of the latin word 'urinator' for 'diver', see reference 1.

Diving and the brain

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Diving and the brain

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English text with summaries in Latin and Swedish

Abstract

Introduction
There are reports that long-term diving is associated with cognitive impairments. This raises the question if diving itself is harmful to the brain in the absence of decompression sickness or hypoxia. Protein tau (tau), glial fibrillary acid protein (GFAp) and neurofilament light (NfL) are biomarkers whose concentrations in blood increase after traumatic brain injuries, cerebral hypoxia, and stroke, though both tau and GFAp are alleged also to change in response to cellular stress without overt damage. Inert gas bubbles are common in the blood after diving and the amount of bubbles present correlates to the risk of developing decompression sickness.

The present dissertation investigates if exposure to increased ambient pressure causes tau, GFAp, or NfL concentrations in blood to increase, and if breathing oxygen after diving decreases the amount of nitrogen bubbles in blood. It includes three studies, which resulted in four papers.

Methods
Ten professional divers dived in the open sea over four days in the first study. Maximum dive depths ranged from 52–90 metres of seawater. Concentrations of tau, GFAp and NfL, and the amount of nitrogen bubbles in the blood was measured using Doppler ultrasound (Paper I). In the second study, 14 submariners were pressurised in a dry hyperbaric chamber to an equivalent of 30 metres of seawater and remained at that pressure for 36 hours. Thereafter, pressure was slowly decreased over 70 hours. Concentrations of tau, GFAp and NfL were measured before, during and after exposure (Paper II). In the third study, 48 professional divers were pressurised twice, 48 hours apart, to an equivalent of 42 metres of sea water for 10 minutes in a water-filled hyperbaric chamber. After one dive, oxygen was breathed for 30 minutes, with air breathed after the other. Concentrations of tau, GFAp and NfL (Paper III), and the amount of nitrogen bubbles in blood (Paper IV) after diving were analysed.

Results

Protein tau increased by 98.8% after four days of deep open water diving (Paper I) and by 31.5% after exposure to a pressure equivalent of 42 metres of seawater (Paper III). GFAp and NfL did not increase, and there were no associations between the amount of gas bubbles in blood and changes in protein tau (Paper I and III). Tau, GFAp or NfL concentrations did not change in response to 36 hours of exposure to a pressure equivalent of 30 metres of seawater, followed by slow decompression (Paper II). The amount of nitrogen gas bubbles in blood were significantly lower among subjects that had breathed oxygen after being pressurised in a water-

filled hyperbaric chamber to an equivalent of 42 metres of depth compared to those that breathed air (Paper IV).

Conclusions

Protein tau increases after diving, presumably due to neuronal stress. Unchanged NfL and GFAp concentrations suggest that neither frank neuronal injury nor astrocytic injury are involved. Oxygen breathing after diving effectively reduces the amount of nitrogen gas bubbles in blood, which decreases the risk of decompression sickness.

Key words
biomarkers, brain, central nervous system, decompression sickness, dive research, diving, neuronal damage, saturation diving, tau protein, venous gas embolism

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Sammanfattning på svenska

Människan är anpassad för ett liv på land. Den som dyker utsätter sig för olika risker. När dykaren simmar nedåt kommer det omgivande vattnet att utöva ett allt högre tryck mot kroppen ju djupare hen kommer. Förhöjt tryck gör både kvävgas och syre i andningsluften skadliga för nervsystemet. Kvävgasen orsakar så kallad djupberusning med förvirring, avvikande beteende och slöhet. Besvären blir alltmer påtagliga ju djupare dykaren tar sig ned men de försvinner helt och hållet när hen simmar tillbaka till ytan. Höga syrgastryck kan ge krampanfall, vilka är livshotande eftersom de sker under vatten. Vid dykning djupare än 150 meter påverkas nervsystemet av omgivningstrycket i sig självt med skakningar och ryckningar, slöhet och försämrad tankeförmåga som följd.

Det förhöjda kvävgastrycket i inandningsluften vid dykning gör att det sker ett upptag av kvävgas i kroppens vävnader. Både dykets djup och dess tidslängd avgör hur mycket kvävgas som tas upp. Mest kvävgas ansamlas i kroppen vid djupa och långvariga dyk. När dykaren simmar tillbaka upp mot vattenytan kommer den upplagrade kvävgasen att lösa sig i blodet och föras till lungorna där den lämnar kroppen. Om omgivningstrycket minskar alltför snabbt hinner inte kvävgasen lämna lungorna lika snabbt som den kommer ut i blodet. Kvävgasen kan då bilda bubblor i blod och vävnader. Det är vanligt att det finns kvävgasbubblor i blodet efter dykning utan att det ger upphov till obehag men risken för dykarsjuka ökar om det bildas en stor mängd kvävgasbubblor i kroppen. Dykarsjuka kan skada hjärnan och nervsystemet.

Forskningen är inte entydig men det finns vetenskapliga studier som visar att vissa av hjärnans funktioner, exempelvis reaktionsförmågan och närminnet, är försämrade hos personer som under längre tid ägnat sig åt dykning. Frågan är om dykning är skadligt för hjärnan.

Tau (tau), gliafibrillärt surt protein (glial fibrillary acid protein, GFAp) och neurofilament light (NfL) är tre proteiner som läcker ut i blodet vid skador på hjärnan. Men både tau och GFAp kan även utsöndras vid påverkan av hjärnans celler utan att det förekommer en direkt cellskada.

Föreliggande avhandling undersöker två saker; dels om ett ökat omgivningstryck påverkar nervsystemet på ett sådant sätt att tau, GFAp och NfL frisätts i blodet, dels om syrgasandning direkt efter dykning minskar mängden kvävgasbubblor i blodet och därmed minskar risken för dykarsjuka. Projektet består av tre studier, som har lett fram till fyra publikationer.

I den första studien dök 10 yrkesdykare under fyra dagar i havet till som mest 52–90 meters djup. Protein tau hade ökat med 98,8% efter fyra dagars dykning men det förelåg inget samband mellan mängden kvävgasbubblor i blodet och förändringen i tau. Mängden GFAp och NfL i blodet var inte förändrade.

I den andra studien trycksattes 14 ubåtsmän och -kvinnor i en tryckkammare till motsvarande 30 meters vattendjup och förblev där under 36 timmar. Sedan sänktes trycket långsamt under 70 timmar. Koncentrationerna av tau, GFAp och NfL steg inte.

I den tredje studien trycksattes 48 yrkesdykare två gånger vardera, med 48 timmars mellanrum, till 42 meters djup under 10 minuter i en vattenfylld tryckkammare. Efter ett av dyken andades

de syrgas, efter ett andades de luft under 30 minuter. Mängden kvävgasbubblor i blod var lägre efter syrgasandning än efter luftandning. Protein tau steg i medeltal med 31,5% efter dykning. Det fanns inget samband mellan förändringen i tau och mängden kvävgasbubblor i blodet. Mängden GFAp och NfL ökade inte.

Resultaten visar att syrgasandning efter dykning är ett effektivt sätt att minska bubbelbildning i blodet efter dykning vilket minskar risken för dykarsjuka. Protein tau ökade efter dykning, sannolikt på grund av nervpåverkan. Oförändrade nivåer av GFAp och NfL talar emot att det uppstått bestående skada på nerver eller påverkan på omkringliggande celler i hjärnan.

Table of contents

Abbreviations

AD	Alzheimer's disease
AG	anaesthetic gases
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATA	atmosphere absolute
BBB	blood-brain-barrier
CAGE	cerebral arterial gas embolus
CAW	compressed air worker
CK	creatinine kinase
CNS	central nervous system
CO	cardiac output
CO_2	carbon dioxide
CSF	cerebrospinal fluid
DCI	decompression illness
DCS	decompression sickness
DNC	SwAF diving and naval medicine centre
DU	doppler ultrasound
EEG	electroencephalogram
ELISA	enzyme-linked immunosorbent assay
EOD	explosive ordnance disposal
F_iO_2	fraction inspired O_2
GABA	gamma-aminobutyric acid
GFAp	glial fibrillary acidic protein
Hb	haemoglobin
Hct	haematocrit
HBO	hyperbaric oxygen
HIS	high intensity spots
HIIT	high intensity interval training
HMS	his majesty's ship
HPNS	high pressure neurological syndrome
i.e.	id est, latin for 'that is'
IGN	inert gas narcosis
ISF	interstitial fluid
kDa	kilodalton
KISS	Kisman integrated severity score
KM	Kisman-Masurel
KM_{max}	maximum Kisman Masurel bubble grade
kPa	kilopascal (1000 Pascal)
mmHg	millimetres of mercury
MPa	megapascal (1000 kPa)
MRI	magnetic resonance imaging
msw	metres of seawater
NfL	neurofilament light
NMDA	N-methyl-D-aspartate
NNT	number needed to treat
NSE	neuron-specific enolase
PFO	patent foramen ovale
Pa	pascal
$PaCO_2$	arterial carbon dioxide pressure
P_ACO_2	alveolar carbon dioxide pressure
P_AH_2O	saturated vapour pressure of water in the alveoli
P_{Amb}	ambient pressure
P_AO_2	alveolar oxygen pressure
per se	latin for 'by itself' or 'in itself'
PNS	peripheral nervous system
pO_2	oxygen partial pressure
RLS	right to left shunt
RNS	reactive nitrogen species
ROS	reactive oxygen species
S100B	S100 calcium binding protein beta
SCG	Swedish coast guard
SD	standard deviation
SI	Système International d'Unités
Simoa	single molecule array
SOD	superoxide dismutase
SP	Swedish police
SPECT	single photon emission computed tomography
SwAF	Swedish armed forces
tau	protein tau
TEE	transoesophageal echocardiography
UBO	unidentified bright objects
UCH-L1	ubiquitin carboxy-terminal hydrolase L-1
UPTD	unit pulmonary toxic dose
VGE	venous gas emboli

Introduction

Most living beings exist in environments to which they have adapted specifically. However, humans are unique in that we have conquered almost all of the varied environments on earth, whether hospitable to us or not. Despite our limitations as a species, humans have dived since the beginning of recorded history, and most likely before. With time, breath-hold techniques have been replaced by diving bells and breathing apparatuses, and it is now possible for humans to explore the underwater world fully. Despite this, diving still necessitates that humans enter an environment to which we are not physiologically adapted, and therefore it is not without risks.

Each year, a number of people around the world die or are left disabled after diving incidents. It is well known that diving may cause decompression sickness (DCS), which can afflict the nervous system. Other risks associated with diving are drowning, hypothermia and barotrauma to ears, lungs, and sinuses. Diving protocols are designed to mitigate risks and professional diving is governed by safety regulations. Despite all precautions, there are reports that long-standing diving is associated with decline in cognitive function among its practitioners, even in the absence of known injurious causes like DCS. Results from published studies are conflicting but could it be that increased ambient pressure, which is unavoidable when diving, is in itself harmful to the brain? And if so, by what mechanism? Or could it be that repeated episodes of mild, asymptomatic, and thus unnoticed DCS cause brain damage that gradually builds up and eventually becomes symptomatic?

If diving is harmful to the brain, blood levels of proteins used as markers of neuronal injury could be expected to increase afterwards. The present dissertation investigates the effects of diving on the nervous system. It includes three studies that resulted in four papers. Two studies investigate changes in blood concentrations of proteins specific to the brain after diving, and a third investigates the same changes after a 106-hour stay in a hyperbaric chamber. One of the studies also assessed if oxygen breathing after diving changes the amount of inert gas bubbles in venous blood and reduces the risk of DCS.

This dissertation begins with a review of physical principles relevant to diving, followed by a description of how inert gases behave in the body when there is a change in ambient pressure. Thereafter, potential mechanisms by which diving may affect the nervous system are presented. The formation of inert gas bubbles after diving and the effects of DCS are described, also explaining how exposure to increased partial pressures of gases, and increased ambient pressure itself may impair the nervous system during exposure. Studies reporting on cognitive dysfunction after diving, on cerebral lesions among divers, and dive studies measuring biomarkers of neurological injury are summarised. Finally, the individual studies in the dissertation are presented and their results discussed.

Part I

Pressures and gases

"We live submerged at the bottom of an ocean of the element air, which by unquestioned experiments is known to have weight."

Evangelista Torricelli, 1644[2]

Composition of the atmosphere

The earth is surrounded by a cloud of gas which constitutes our atmosphere. The main contents of air are oxygen (21%) and nitrogen (78%)[b]. A small part (1%) is made up of noble gases, carbon dioxide (CO_2) and hydrogen.[3]

Gas	Chemical symbol	Fraction of total volume (%)
Nitrogen	N_2	78.08
Oxygen	O_2	20.95
Argon	Ar	0.93
Carbon dioxide	CO_2	0.03[c]
Neon	Ne	0.0018
Helium	He	0.0005
Krypton	Kr	0.0001
Hydrogen	H_2	0.00005
Xenon	Xe	0.0000087

Table 1 *Contents of the atmosphere*[3]

Pressure

Pressure is defined as the force applied to an area, and can be described by the formula:

$P = F/A$

where P is pressure, F force and A area.[4]

Many units are in use to describe pressure, but the SI[d] unit for pressure is Pascal (Pa), where one Pa equals the force of one Newton per square meter (N/m^2).[5] One kPa is 1000 Pa and one MPa is 1000 kPa.

[b] As seen in Table 1, oxygen and nitrogen fractions in air are not exactly 21% and 78%, but these figures are used as approximations throughout the text.

[c] This reference used dates from 2014. The fraction of CO_2 has increased since then and is now about 0.04% (IPCC 2021)

[d] The international system of units (Système International d'Unités), abbreviated SI.

Atmospheric pressure

Atmospheric pressure is generated by collisions of gas molecules and is dependent on the mass of air, i.e.[e] the height of the air column, above the ground.[5] Thus, atmospheric pressure varies depending on location, for example at sea level or on top of a mountain, and it will change slightly with local factors such as wind velocity and density changes due to temperature. Atmospheric pressure, is, by convention held to be 101.3 kPa at the level of the sea, which is the equivalent of one atmosphere absolute (ATA),[5] but to simplify calculations, pressure at sea level is often approximated to 100 kPa.[6]

Pressure at depth

Water has a much higher density than air. When we dive, ambient pressure will increase as the water above exerts a pressure equal to about 100.5 kPa per 10 metres of sea water (msw) depth.[5] The change in pressure per 10 meters submerged, again to simplify calculations, can be approximated to 100 kPa.[6,7] Relative change of pressure per msw is greatest close to the water surface and steadily diminishes with depth. Diving from the surface to a depth of 10 msw doubles ambient pressure to approximately 200 kPa, while a further 10 metres deeper, will increases pressure by only 50%, to approximately 300 kPa. A further descent to 30 msw will increase pressure by about 33% to 400 kPa, and so forth.

Unit of pressure	Values of equal pressures
kPa	101.3
mmHg	760
Atmosphere (ATA)	1
Bar	1.013
Feet sea water (fsw)	33.07
Metres sea water (msw)	10.08

Table 2 *Units of equal pressure.[5] It should be noted that the relationship between ATA and depths of seawater are affected by temperature and salinity and that the values for fsw and msw in the table are approximates.*

Immersion in water

When a diver is immersed in water, gravitational effects are lost as pressure acts from all sides on the body,[5] as according to Pascal's principle.[f] One consequence of this is that blood contained in the venous system of the legs when standing on the ground will be redistributed and thoracic blood volume increased. Cardiac output (CO) can increase by as much as 35%,[8] and lung compliance will be reduced. The increased ambient pressure is transmitted to the tissues, where it counteracts capillary filtration of fluids and causes fluid to remain in the vascular system. Raised atrial filling pressures and increased CO leads to increased diuresis and possibly dehydration,[8] which has been reported after diving.[9] Cerebral perfusion increases when diving, primarily due to the increased CO, but as gas density

[e] Abbreviation of the latin words *id est* meaning 'that is'.

[f] Pascal's principle states that "a pressure exerted anywhere in a confined incompressible fluid is transmitted equally in all directions throughout the fluid such that the pressure ratio remains the same" (Lippmann 2016).

increases with depth and the surrounding water impedes breathing, the amount of CO_2 could build up in blood[5] and cause cerebral vasodilation with a further increase in cerebral perfusion.[10]

Behaviour of gases

Boyle's law

The relationship between the pressure and volume of a gas, given that the temperature is constant, is described by Boyle's law,[g] which could be written:

$$PV = k$$

where P is pressure, V volume and k a constant.[4]

In practical diving this means that when pressure increases with depth, all gas-filled parts of the body will be compressed, and their volumes will diminish if no new gas is supplied. The need to equalise pressure in the ear when diving illustrates this: extra air needs to be blown into the middle ear to counteract squeeze. At the end of the dive upon moving to the surface, gases will expand, and if they are not exhaled they can cause barotrauma to lungs, ears, and sinuses.

Dalton's law

According to Dalton's law,[h] in a mixture of gases the partial pressure of a particular gas is proportional to its fraction of the total gas volume.[4] As oxygen and nitrogen constitute 21% and 78% of air respectively, their partial gas pressures are 21% and 78% of the total air gas pressure which, if at sea level and ambient pressure (~100 kPa) gives an oxygen partial pressure of 21 kPa, and 78 kPa for nitrogen.

When diving, the tensions of inhaled gases will change with ambient pressure. Hence, partial pressures of oxygen and nitrogen in inhaled air will increase as a diver descends, and decrease when the diver eventually returns to the surface again. At a depth of 40 msw, the ambient pressure is ~500 kPa, so the partial pressure of oxygen in inhaled air will be 500 x 0.21 = 105 kPa, while that of nitrogen will be 390 kPa.

[g] Boyle's law states: "*if the temperature remains constant, the volume of a given mass of gas is inversely proportional to the absolute pressure.*" (Lippmann 2016)

[h] Dalton's law states: "*the total pressure exerted by a mixture of gases is equal to the sum of the partial pressures that would be exerted by each of the gases if it alone occupied the total volume.*" (Lippmann 2016)

Depth	Ambient pressure	Partial pressure of oxygen	Partial pressure of nitrogen
Sea level	100 kPa	21 kPa	78 kPa
10msw	200 kPa	42 kPa	156 kPa
20msw	300 kPa	63 kPa	234 kPa
30msw	400 kPa	84 kPa	312 kPa
40msw	500 kPa	105 kPa	390 kPa
50msw	600 kPa	126 kPa	468 kPa

Table 3 *Partial pressures of oxygen and nitrogen in inhaled dry air at 10 msw increments up to a depth of 50 msw. Both pressure at sea level and changes in pressure per 10 msw are approximated to be 100 kPa. Oxygen and nitrogen fractions are approximated to be 21% and 78% respectively.*

The general gas equation

For an ideal gas the following relationship exists, according to the general gas equation:

$$PV = nRT$$

where P is pressure, V volume, n the amount of gas in moles, R the universal gas constant, and T temperature in Kelvin.[4]

The general gas equation has limitations because it does not take molecular interactions into account and becomes inaccurate in states of high pressure or low temperature, and it does not apply to gases with strong intermolecular forces.[11] Still, the general gas equation describes the principal relationship for a gas between pressure, volume, temperature and amount of substance. The formula can be rewritten as:

$$P = nRT/V$$

from which it can be concluded that the pressure exerted by a gas is proportional to its amount.

Henry's law

The amount of dissolved gas in a volume of fluid is proportional to the partial pressure of the gas in equilibrium with that fluid.[12] This phenomenon is stated in Henry´s law.[i] Partial pressures in gas and fluid are the same but it is important to appreciate that the tension an amount of gas dissolved in a fluid exerts is also related to its solubility in that particular fluid.[5,13] Based on solubility, the pressure exerted by a gas in a fluid or tissue could be expressed as:

$$P = C/S$$

where P is the gas pressure, C the concentration of dissolved gas and S is the solubility coefficient.[13]

Hence, when the partial pressure of nitrogen is identical in blood and in adipose tissue, the molar concentrations of nitrogen will be different, due to the fact that nitrogen is around five times more soluble in fat than in blood.[4] The ratio of concentrations of a substance between two compartments at equilibrium could be expressed as a partition coefficient. For example, nitrogen's partition coefficient for olive oil/blood is 5.05/1.0, while for helium, a substance much less soluble in fat, it is 1.9/1.0.[4]

Diffusion of gases

Movement of molecules of gas in the body is driven by differences in their partial pressures, also called gradients of pressure.[13,14] Diffusion is a process whereby gas molecules move from a point of higher pressure towards lower pressure. A large difference in pressure between two points increase rate of diffusion, whereas the rate is slower for more massive molecules.[j] The rate of diffusion also depends on the surface area available for the process and decreases with diffusion distance[k].[13] The movement of gas will continue until equilibrium[l] between two compartments ensues.[3] Physiological equilibrium of gaseous pressures thus depends not only on the amount of a particular gas in each compartment but also on its solubility in the two compartments concerned, as it is gas pressures that equilibrate, not the number of gas molecules.[5] When a tissue has reached equilibrium for a gas and no more movements of gas molecules take place, it is said to be saturated at that pressure.[3,14,15] If the concentration, hence pressure, of dissolved gas in adjacent compartments changes, then movement of gas will again take place in the direction of the lower concentration, until a new equilibrium is reached.

Inert and metabolic gases

Gases such as nitrogen, helium and argon do not take part in physiological processes in the body and are therefore named 'inert' gases.[3,16] Their partial pressures in the body are in equilibrium with the ambient air. Oxygen and CO_2 are metabolic gases[17] and are consumed and produced, respectively, in relation to metabolic activity in the body; their partial pressures in blood and tissues can differ substantially from their proportions in air.

[i] Henry's law states: "*At a constant temperature, the amount of a gas that will dissolve in a liquid is proportional to the partial pressure of the gas over the liquid.*" (Lippmann 2016)

[j] Graham's law states that "*the rate of diffusion of a gas is inversely proportional to the square root of its molecular weight*". (Brubakk 2003)

[k] Fick's law of diffusion could be expressed "*The transport of gas by diffusion through the tissue is proportional to the area over which diffusion occurs and the partial pressure gradient, and inversely proportional to the diffusion distance*." (Brubakk 2003)

[l] In physiology, equilibrium is a condition of steady state, a situation where all opposing processes occur at equal rates, so that no overall change happens.

Alveolar gas contents

Uptake and elimination of gases takes place in the lung. If the partial pressure of a gas is higher in the alveoli than in blood, that gas will diffuse into the blood until pressure equilibrium ensues, and vice versa.[13] The breathing gas that the diver inhales will be saturated with water vapour in the airways, then mix with CO_2 from the blood in the alveoli.[13] Thus, inhaled oxygen and nitrogen are diluted and their partial pressures in the alveoli are lower than in dry air, as the sum of all gaseous partial pressures should equal total ambient pressure. At a given temperature, the saturated vapour pressure of water is that at which liquid and vaporised water are in equilibrium. The equilibrium depends solely on temperature and is not affected by changes in ambient pressure.[18] At 37 degrees centigrade, the saturated vapour pressure of water is almost 6.3 kPa. Partial pressure of CO_2 in blood is regulated through rate and depth of breathing and it is normally kept at about 4.5–6.0 kPa in arterial blood.[19] Increased ambient pressure does not affect production of CO_2. However, diving is associated with a risk of CO_2-retention,[5] potentially caused by factors such as altered regulation of breathing due to hyperoxia, increased work of breathing related to either equipment or gas flow characteristics, or due to increased density of gases at depth.[5,18]

Alveolar content of oxygen can be estimated according to the formula:[20]

$P_AO_2 = F_iO_2(P_{Amb} - P_AH_2O) - PaCO_2/0.8$

P_AO_2	alveolar oxygen pressure
F_iO_2	fraction inspired O_2
P_{Amb}	ambient pressure
P_AH_2O	saturated vapour pressure of water in the alveoli
$PaCO_2$	arterial carbon dioxide pressure
0.8	an approximation of the respiratory quotient[m]

When breathing air, the alveolar content of nitrogen will, according to Dalton's law, constitute about 78/79 of $P_{Amb} - (P_AH_2O + P_ACO_2 + P_AO_2)$.

P_ACO_2	alveolar carbon dioxide pressure

Because their partial pressures do not change with ambient pressure, the proportional effect of water vapour and CO_2 on total gas pressure in the alveoli will diminish as ambient pressure increases. Mixing inhaled air with CO_2 and water vapour in the alveoli will diminish oxygen partial pressure by almost 38% at the surface but only by about 4% at a depth of 80 msw.

[m] The respiratory quotient states the relationship between produced (exhaled) CO_2 and consumed (inhaled) O_2 (CO_2-production/O_2-consumtion) during metabolism. Different amounts of oxygen are consumed in relation to released CO_2 depending on which nutrient is metabolised. Lipid metabolism has a respiratory quotient of 0.7, while it is about 0.8 for proteins. Carbohydrate metabolism has a respiratory quotient of 1.0. The respiratory quotient is often held to be 0.8 as a general approximation. (Camporesi 2003)

Gas	Atmospheric pressure (kPa)	Alveolar pressure (kPa)		
		At surface	At 40 msw	At 80 msw
Oxygen	21	13.1 (21)	97.1 (105)	181.1 (189)
Nitrogen	78	74.3 (78)	386.3 (390)	698.3 (702)
Water	0	6.3 6.3%	6.3 1.3%	6.3 0.7%
Carbon dioxide	0.04	5.3 5.3%	5.3 1.1%	5.3 0.6%

Table 4 *Differences in gas content in dry air and in the alveoli at surface, 40 and 80 meters of depth. This is a simplified, theoretical, depiction of gaseous pressures. The proportions of oxygen and nitrogen in air are approximated to be 21% and 78%, respectively. Partial pressures of dry inhaled oxygen and nitrogen in dry air, not yet mixed with water vapour and CO_2, are shown in brackets. Alveolar partial pressure of CO_2 and water vapor are presumed to be 5.3 and 6.3 kPa, respectively. Their proportions of total gas pressure at different depths are shown as percentages. Pressure at surface is presumed to be 100 kPa and to increase with 100 kPa per 10 msw of depth.*

In this dissertation, partial pressures of breathing gases are stated as if they were dry and not diluted by CO_2, i.e., as inhaled and before being mixed with water vapour in the airways and CO_2 in the alveoli.

Inert gas kinetics

Nitrogen uptake

Where air is used as breathing gas during diving, the alveolar partial pressures of nitrogen will increase with depth, and nitrogen will be taken up in the blood.[14] An equilibrium with arterial blood is reached swiftly[21] and the dissolved nitrogen transported throughout the body. Tissues will also take up nitrogen, but at a slower rate than in the blood. The rate of uptake depends mainly on the pressure gradient between blood and tissue, blood flow to the tissue, and also the specific nitrogen blood-tissue partition coefficient.[14] Tissues that are well perfused such as muscles, will be delivered more nitrogen during a set period of time than tissues with less perfusion, for example adipose tissues. Ease of diffusion between compartments will also affect rate of uptake.[3] Tissues where the solubility for nitrogen is low will reach an equilibrium with the blood faster than tissues with a higher solubility for nitrogen,[21] for example fat, because the latter can accommodate more nitrogen molecules at the same pressure.[5] Total uptake of nitrogen depends on diving depth and time spent under water, up until saturation develops and no more nitrogen can be taken into the tissues regardless of how long time the divers spends at depth.[14,15] When the diver ascends to the surface the process will be reversed.[3]

Compartments

One way to portray inert gas movements in the body in relation to changes in ambient pressure is to use a compartment model, as in pharmacokinetics. A compartment does not represent a specific organ or part of the body, but a group of tissues or parts of tissues where uptake and release of a particular

inert gas happens in a similar way.[3,14,22,23] Different models use various numbers of compartments for each inert gas, with some using up to 16.[24]

Each theoretical compartment is designated a unique half time for a specific inert gas, which reflects the rate of inert gas change after a shift in ambient pressure.[3,12,25,26] Compartments that represent well-perfused aqueous tissue with low solubility for inert gases are designated as having short half times, for example five minutes, because they equilibrate fast with blood partial pressures;[21] in some models, compartments that need a longer period for uptake and release of gases, for example low-perfusion adipose tissue, could have half times up to several hundreds of minutes long.[24]

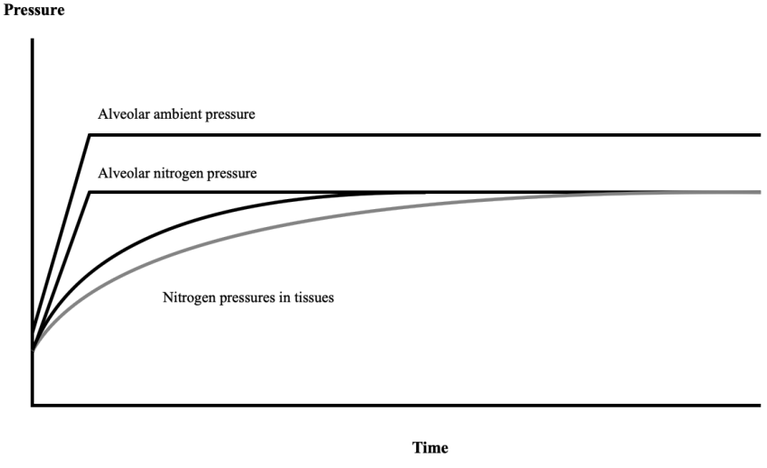

Figure 1 *Uptake of nitrogen in tissue. Theoretical depiction with two compartments*

While compartments with short half times often are saturated after an ordinary dive, slower compartments will still be taking up inert gas when the dive ends. Consequently, gas tensions in different compartments will vary, until the point at which they are all saturated and have the same partial gas pressures, for example during a saturation dive.[15,21]

Nitrogen release

As the diver ascends and ambient pressure decreases, a state of supersaturation will occur in tissues when inert gas pressure exceeds the surrounding gas tensions.[3,12,14,22] The inert gas will be released from the supersaturated tissues to the blood, and finally the alveoli, at rates determined by the same factors as uptake of gas.

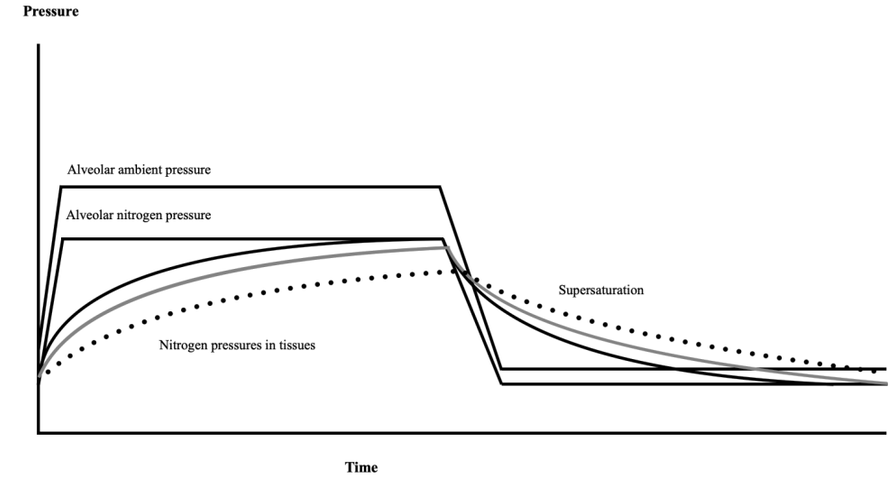

Figure 2 *Uptake and release of nitrogen in tissues with different saturation half-times. Theoretical depiction with three compartments.*

Supersaturation will first come about in compartments that were fully equilibrated with alveolar inert gas pressure before ascent, and if the diver continues towards the surface, slower compartments will also develop a supersaturated state, as ambient pressure eventually drops below their inert gas tissue tensions.[3] If the diver stops at a new depth, all compartments will equilibrate at the new pressure, and either take up or release inert gas at rates determined by their respective half times.

Bubbles

"The formation of gas bubbles in the living body during or shortly after decompression evidently depends on the fact that the partial pressure of the gas or gases dissolved in the blood and tissues is in excess of the external pressure."

Boycott, Damant and Haldane, 1908[25]

Behaviour of bubbles

Bubble formation

When the sum of all gases present in a tissue exceeds the ambient pressure, there is a risk that gas will come out of solution and form bubbles in either fluids or tissues.[22,27] The process of bubble formation, technically named nucleation, is not completely understood. Nucleation *in vitro*[n] in a homogenous solution like water can only happen if supersaturation is greater than approximately 10.0 MPa (~1000 msw,[23,28]), which is an immense pressure difference, not least from a physiological point of view. This huge overpressure is needed to overcome the strong surface tension of evolving microscopic gas bubbles. In contrast, when micron-sized particles, microscopic voids, or impurities are present in a fluid, bubbles can form while exposed to far lower supersaturation through a process called heterogenous nucleation;[21,23,28,29] venous gas bubbles have been detected in human blood after decompression from hyperbaric exposures as low as 138 kPa.[30] Current theory holds that in states of supersaturation, inert gas bubbles form and grow around pre-existing micronuclei, which are minuscule voids, smaller than a few micrometres.[14,28,31-33] The definitive sources of micronuclei are not known, but microscopic gas bubbles can form in blood through cavitation,[29,34] when turbulent flow generate areas of negative pressure[o], or through tribonucleation,[14,23,31] a process where movement of structures such as joints or heart valves generate small spots with negative pressure within tissue fluid or blood, and hence localised supersaturation may lead to the formation of microscopic voids: micronuclei.

Bubble growth

The fate of an inert gas bubble formed around a micronucleus is determined by its surface tension, gas tension within the bubble, and the magnitude of the surrounding pressure.[14,23,28,34] When forces acting on a bubble are in equilibrium, its size does not change. Decreased ambient pressure will cause an inert gas bubble to grow and increased pressure will compress it.[34] Surface tension will cause microscopic gas bubbles to implode but surfactants can counteract this, and bubbles formed in vascular crevices can assume irregular shapes that negate surface tension. The inward force caused by surface tension is inversely proportional to bubble radius: it reduces when a bubble grows and therefore, the larger the bubble is the easier it can expand. If the inert gas pressure outside a bubble is lower than within, then the bubble will shrink and vice versa. When growing bubbles absorb inert gas from surrounding tissues, they act as inert gas reservoirs and slow down inert gas elimination from the body.[22,35]

[n] latin for 'in glass' – used for a process taking place outside its biological context

[o] The phenomenon is illustrated by bubbles formed around a rotating propeller, where bubbles are formed under water, without contact with air.

Bubble theory and bubble reality

Traditional decompression theory states that there is a level of supersaturation, or critical overpressure, that can be tolerated in blood or a tissue before inert gas bubbles are formed. It is sometimes called the maximum value or M-value,[3,23] and is expressed as a quotient, e.g. 2:1 means that when tissue inert gas tension becomes twice the ambient pressure, bubbles will form. M-values are higher for faster compartments and also grow with increasing dive depths. It was originally believed that no inert gas bubbles would form if critical overpressure or M-values were not exceeded but that changed when doppler ultrasound (DU) was introduced to diving research. Doppler ultrasound investigations have shown that inert gas bubbles are also common after uneventful dives that are well within the limits of classical decompression model predictions, which forecast that all inert gas should remain in solution. It seems that inert gas can form bubbles whenever supersaturation to any degree exists in the blood or tissues,[3,23,27,34,36,37] and it may well be that micronuclei exist in the body at all times,[31,34] even when not diving. With this in mind, modern decompression models often now not only compute the theoretical amount of dissolved gas in different compartments in order to avoid supersaturation, but also presume that microscopic inert gas bubbles exist and aim to avoid their growth.[34]

Venous gas emboli

Inert gas bubbles that form in blood during decompression are believed to originate at the distal end of the capillary bed, or in venules where supersaturation may arise because intravascular pressure is low and nitrogen pressures are elevated when nitrogen diffuses out of tissues.[14,26] The bubbles are therefore often referred to as venous gas emboli (VGE).[14,38,39] Unless supersaturation is massive, it is unlikely that gas bubbles will form in the arterial system because inert gas pressures are swiftly and effectively equilibrated when blood passes through the lung, meaning that blood will no longer contain supersaturated gas when it leaves the lung circulation.[14,26] If the local tissue concentration of inert gas becomes sufficiently high, it is believed that bubbles can form *in situ,*[p] [14] and this process is called *autochthonous*[q] bubble formation.[26]

The fate of bubbles

Venous gas emboli will be transported via the venous blood to the lungs.[14,26] After passing through the right heart, they will typically be trapped in the pulmonary capillary network.[27,39] VGE that are lodged in the network will, as all VGE in the body, eventually shrink and disappear as their inert gas content equilibrates with the blood. Shunting to the systemic arterial circulation can happen, especially if VGE are copious enough to obstruct the pulmonary circulation and cause pulmonary filling pressures to increase, either through a *patent foramen ovale*[r] (PFO) or some other cardiac defect, or through local shunts in the pulmonary circulation.[14,26,33,37,39]

[p] Latin for 'on site' or 'in position', meaning that something happens in the original place

[q] Autochthonous is derived from Greek and in natural science used to denote something that is formed in its present position.

[r] The foetus does not breathe but depends on the placenta for oxygenation of the blood. Most of the oxygenated blood coming from the placenta flows directly from the right to the left atrium of the heart through an opening called *foramen ovale* and then to the left ventricle and exits via the aorta. This is an effective arrangement in the unborn because blood must not flow through the still unused lungs. After birth, the *foramen ovale* closes and the pulmonary and systemic circulations are separated, but in about 25–30% of adults a gap remains open (Mitchell 2016). Such a persistent gap between the atria is called a *patent foramen ovale* or a PFO. The filling pressures are higher on the left side of the heart and usually there is no or minimal flow through a PFO. But if the pressures on the right side of the heart are increased, for example when coughing, during physical straining or if large numbers of VGE get lodged in the pulmonary circulation, blood could flow through the PFO from the right to the left side, a process referred to as 'embolisation'.

Wet and dry dives

Whether a dive is made in water or in a dry hyperbaric environment can affect the evolution of VGE. A study on 14 divers found that the bubble load that result after a dive in water was larger than following a simulated dive in a dry hyperbaric chamber, even when the pressure was identical.[40] Possible reasons for this difference in VGE production are differences in physical activity, in tissue perfusion due to temperature, or immersion effects.

Measuring venous gas emboli

Doppler ultrasound can be used to estimate the amount of VGE in blood.[41] Bubbles scatter ultrasound more effectively than red blood cells, creating a typical sound that is discernible to a trained operator.[38] As per a recent consensus statement[42], ideally DU monitoring should be performed over the precordium to capture the entire systemic venous return. However, monitoring over peripheral veins such as the subclavian vein, can be used in addition to increase sensitivity.[42,43] The Kisman Masurel (KM) grading system can be used to evaluate DU signals[41,42] and is based on three types of ordinal data: frequency (bubbles per cardiac cycle); percentage (percentage of cardiac cycles with bubbles) for resting measurements, or duration (number of cardiac cycles with bubbles) for exercise measurements; and amplitude of bubble sounds (compared to blood flow/cardiac sounds) (Table 5).

Code	**Frequency (f), bubbles/cardiac period**	
0	0	
1	1–2	
2	several, 3–8	
3	rolling drumbeat, 9–40	
4	continuous sound	
Code	**Rest % (p)**	**Movement duration (d)**
0	0	0
1	1–10	1–2
2	10–50	3–5
3	50–99	6–10
4	100	> 10
Code	**Amplitude (A)**	
0	No bubbles discernable	
1	Barely perceptible, $A_b << A_c$	
2	Moderate amplitude, $A_b < A_c$	
3	Loud, $A_b \approx A_c$	
4	Maximal, $A_b > A_c$	

Table 5 *The Kisman Masurel coding system. A_c is amplitude of blood flow/cardiac sounds and A_b is the amplitude of bubbles.[42] From Møllerløkken A, Blogg S L, Dolette D J et al. (2016) Consensus guidelines for the use of ultrasound in diving research. Reproduced with permission from Diving and Hyperbaric Medicine.*

These parameters are scored and the resulting three-digit code then converted to a grade, as shown in the Table 6. There are twelve KM grades, ranging from 0 to IV.

fpA fdA	Bubble grade	fpA fdA	Bubble grade	fpA fdA	Bubble grade	fpA fdA	Bubble grade
111	I-	211	I-	311	I	411	II-
112	I	212	I	312	II-	412	II
113	I	213	I+	313	II	413	II+
114	I	214	II-	314	II	414	III-
121	I+	221	II-	321	II	421	III-
122	II	222	II	322	II+	422	III
123	II	223	II+	323	III-	423	III
124	II	224	II+	324	III	424	III+
131	II	231	II	331	III-	431	III
132	II	232	III-	332	III	432	III+
133	III-	233	III	333	III	433	IV-
134	III-	234	III	334	III+	434	IV
141	II	241	III-	341	III	441	III+
142	III-	242	III	342	III+	442	IV
143	III	243	III	343	III+	443	IV
144	III	244	III+	344	IV-	444	IV

Table 6 *Conversion of Kisman Masurel codes to Kisman Masurel bubble grades.[42] From Møllerløkken A, Blogg S L, Dolette D J et al. (2016) Consensus guidelines for the use of ultrasound in diving research. Reproduced with permission from Diving and Hyperbaric Medicine.*

Bubbles can be measured both when subjects rest passively and after a short bout of physical movement, for example, squatting when standing, or forcefully flexing the legs 2–3 times while lying down; these impactful movements are intended to release VGE from the vascular walls, and help the DU operator to determine if there are any bubbles present should a resting load not be apparent.[42]

DU has shortcomings:

- There is a risk that only a section of the blood flow is scanned, which gives a limited depiction of VGE content in the circulation.[41]
- It is operator-dependent and there is a risk of interobserver variability.[12] If the same DU operator makes all measurements, the potential errors will likely be systematic instead of random.
- Only VGE can be detected. Static, inert gas bubbles in tissues will not be noted.
- There is a risk that small inert gas bubbles are not detected above the background noise from the red blood cells.[41]

Doppler ultrasound recordings should begin ideally within 15 minutes after a dive has ended and should be repeated at least once every 20 minutes for at least 120 minutes.[42]

When multiple VGE recordings are made according to the KM grading system, they can be integrated according to the Kisman Integrated Severity Score (KISS),[41,44] which gives a value for the amount of VGE to evolve over a certain time. KISS is calculated using the following formula:

$$KISS = \frac{100}{4^{\alpha}\left(t_n - t_1\right)} \sum_{i=1}^{n} \frac{(t_{i+1} - t_i)\left(d_{i+1}^{\alpha} + d_i^{\alpha}\right)}{2}$$

where α = 3, d is KM grade, t time in minutes since end of decompression and n number of measurements.

VGE measurements made using the KM grading scale generate ordinal data,[38] and any statistical techniques used in their analyses should be non-parametric.[42] Transformation of results using KISS will produce 'linearized' numerical values;[41] even so, they are based on ranked data and parametric statistical techniques should be avoided.[42]

Decompression sickness

Decompression sickness is a condition that is unique to diving and activities where changes of ambient pressure take place, such as compressed air work in tunnels, caissons, and high-altitude aviation.[27] Decompression sickness is a complex disease, probably better likened to a medical syndrome, that can affect many different organs and manifest with various symptoms including itchy skin, skin rash, fatigue, joint pain, sensory loss, paresis, dyspnoea, coma, and death.[26,27,45] Common to all manifestations is an aetiology that involves the formation of inert gas bubbles in blood or tissues.[22,26,27] Older descriptions of DCS used the terms type 1 and type 2, with the former involving the musculoskeletal system and the latter indicating the presence of neurological symptoms.[27] Modern consensus discussions have adopted the use of a descriptive classification system for DCS.[45] It can thus be described as 'musculoskeletal', 'cutaneous', 'neurological' (spinal, cerebral or peripheral), 'vestibulocochlear', 'lymphatic' or 'cardiopulmonary' depending on how it manifests. Description of symptomatology should preferably be complemented with an indication on how the symptoms evolve, the recommended terminology being 'static', 'remitting', 'progressive' and 'relapsing'.

Neurological decompression sickness

Neurological DCS often affects the spinal cord,[26] with para- or tetraplegia, bladder atony and sensory losses as typical symptoms. The proposed pathophysiology of spinal DCS involves venous stasis and direct mechanical damage, both caused by inert gas bubbles.[22,26] The spinal cord contains an abundance of myelin and has a relatively low blood flow, two factors that are alleged to favour formation of inert gas bubbles.[26] In contrast to the spinal cord, the brain is richly perfused, which makes it probable that cerebral VGE will shrink and disappear before they cause manifest damage, but arterialised VGE could potentially cause ischemic lesions in the brain.[14,22,45]

Risk of decompression sickness

When established protocols are adhered to, DCS is uncommon in modern diving. Its incidence has been estimated to be 0.01–0.095% per dive, depending on the population concerned,[27,33,46] and incidence is allegedly up to five times higher among divers with a PFO.[33,46] In saturation diving, the

incidence has been stated to be 0.2%.[47] Analyses involving large populations of divers have reported correlations between the amount of VGE in blood and DCS rate.[12,41,48] DCS is more common when VGE loads are high, while an absence of detectable VGE is associated with a low risk of DCS, but there is a considerable interindividual variability in VGE load among subjects after similar dive exposures.[49] Quantification of VGE after diving seems to have low specificity for DCS in an individual diver[43,48,50,51] and inert gas bubbles may exist in the body even if no VGE are found using DU. Subjects with low VGE loads after diving can develop DCS. Still, for want of any other method, measurement of VGE is used to assess decompression stress[s] after diving.[38,41,52] Doppler ultrasound may be used to evaluate decompression regimens and dive protocols, where lack of or scarce amounts of VGE are regarded as signs of safety,[43,52] but the use of VGE as a surrogate measure for DCS has been questioned.[53]

Silent bubbles

The presence of VGE without clinical signs of DCS are sometimes referred to as 'silent bubbles'.[23,41,54] It has been speculated that they could exert harmful, albeit subtle effects in the body that are sometimes called subclinical DCS, which could eventually build up to form long-term neurological impairments or cause inflammatory activation.[52] Silent bubbles have also been implicated in fatigue after diving, which is a common experience among divers.[3]

The endothelium and inflammation

According to classical teaching, DCS is caused by VGE in the blood and tissues and great efforts have been made to minimize the risk of bubble formation after diving or other hyperbaric exposures.[14,26,27] However, the notion of DCS as 'bubble disease' may be too simplistic. There is a growing quantum of data implying that DCS is a systemic disease involving the endothelium, and that inflammatory processes might play a role in the pathogenesis and manifestations of symptoms.[52,55] Endothelial dysfunction has been alleged in subjects without DCS after diving to a depth of 18 msw for 60 minutes,[56] after being compressed to 280 kPa for 80 minutes,[57] and after repeatedly diving to between 55–80 msw.[58] However, in another study, repeated diving over three days to 18 msw for 47 minutes showed significant endothelial impairment only when inspired oxygen fractions were increased.[59] It is possible that VGE, also in the absence of DCS, affect the endothelium either mechanically or by acting as a foreign material triggering the immune system,[60] but its association with vascular dysfunction is unclear.[57,61] Endothelial dysfunction has been hypothesized to be the main cause of DCS,[62] but this notion has not won widespread acceptance. Despite increasing evidence that DCS is a complex phenomenon, the formation of inert gas bubbles is still held to be *sine qua non*[t] for DCS.[22,26,27] Additionally, the presence of VGE could be fully integrated into an endothelial pathophysiological theory.[52]

Cerebral arterial gas embolism

A decrease in ambient pressure at the end of a dive could cause pulmonary barotrauma with secondary embolisation of gas into the arterial cerebral circulation, a phenomenon called cerebral arterial gas embolism (CAGE).[63] Neurological symptoms like coma, hemiplegia, seizures, and blindness, which appear within minutes of surfacing are common.

[s] Decompression stress could be described as the amount of inert gas dissolved in the body directly after a dive, sometimes also referred to as inert gas load. It is determined by diving time, diving depth, breathing gas used and rate and character of decompression. High decompression stress is associated with increased VGE load (Germonpre 2017).

[t] Latin, literally meaning 'without which not', used for an essential condition or a thing that is absolutely necessary. (Hornby 1989).

Decompression illness

DCS and CAGE are two separate disease entities, but they are both part of the concept of decompression illness (DCI).[64] DCI is sometimes used interchangeably with DCS, which is incorrect. When the works of other authors are referred to in this dissertation, the designations DCS and DCI are as used in the original texts.

Ways to reduce venous gas emboli load

Conservative diving

Different strategies can be employed to reduce the amount of VGE, and thus the risk of DCS after diving. One way is to change the profile of a dive, making it more conservative by decreasing the depth, slowing the ascent rate at the end of dive and shortening the dive duration in comparison to the mandate of the table or official protocol; this will reduce the inert gas load caused by a dive,[29,65,66] although conservative profiles can still result in DCS.[67] Decompression stops at a few meters of depth will allow for release of inert gas, and thus decrease potential supersaturation before the final ascent.[65]

Physical activity before diving

Fit divers are considered to have a lower risk of developing DCS.[68] Several studies have shown that exercise before diving may reduce the amount of VGE detectable after diving,[69-72] though the mechanism for this effect has not been clarified; however, two studies did not find any beneficial effect of physical training on VGE load before compression in either a hyperbaric chamber or diving in water.[73,74] Thus, the effect of exercise prior to diving in terms of benefit is still equivocal.

Physical activity during decompression

Physical activity during decompression was associated with lower VGE loads in a study investigating 39 males after a dive to 45 msw,[44] and in another study where 10 subjects dived to 30 msw,[75] probably because increased peripheral blood flow eases inert gas removal from the tissues.

Vibration treatment before diving

At least two studies have shown that a 30-minute session with whole-body vibration one hour before diving to 30 or 33 msw for 20–30 minutes is associated with fewer post-dive VGE in comparison to controls.[76,77] It has been hypothesised that vibration mechanically reduces the amounts of micronuclei in the body and thereby reduces VGE formation.

Heat exposure before diving

It has been reported that heat exposure via a 30 minutes sauna session reduces VGE load after diving.[36,68,78] The mechanism of action is not known but increased levels of heat shock proteins, changed levels of nitric oxide and dehydration have all been implicated as possible mediators.[67]

Rehydration

If dehydration leads to a reduced cardiac output and reduced tissue perfusion, it could hamper release of tissue nitrogen at the end of dive and increase the risk of DCS, because more nitrogen will remain in the tissues. A study following eight professional divers found that active rehydration with 1300 mL of fluid during the hour preceding a dive (30 msw for 30 min) reduced post-dive VGE load.[79]

Effects of breathing different gases

"You know as well as I do, Professor, that man can live underwater, provided he can carry with him a sufficient supply of breathable air."

Captain Nemo in Twenty thousand leagues under the sea by Jules Verne, 1870[80]

Breathing oxygen

The oxygen window

During metabolism, oxygen is consumed and replaced by CO_2. The respiratory quotient (RQ)[u] states the relationship between evolved CO_2 and consumed O_2 during metabolism. When an average mixed diet is eaten the RQ is ~0.8,[18] which indicates that not all oxygen molecules are replaced by CO_2. The solubility of CO_2 in blood is about 20 times greater than that of oxygen.[4,13] Due to these two factors, primarily the high solubility of CO_2, the total gas pressure is lower on the venous side compared to the arteries; this reduces the risk of supersaturation in blood, and hence, the risk of bubble formation is lowered. [3,14,21] This phenomenon is referred to as the 'oxygen window'.

Increased inert gas elimination

Breathing of either normobaric[v] or hyperbaric oxygen[w] (HBO) will reduce the nitrogen content in the lung. The resulting difference in nitrogen pressure between alveoli and blood causes nitrogen to leave the blood and be exhaled.[81] The subsequent reduction in nitrogen partial pressure in arterial blood will in turn cause nitrogen to leave the tissues, and diffuse into the blood, in a continuous process. The outflow of nitrogen from the body can be significant if oxygen is breathed long enough.[14] It should be noted that this process of 'denitrogenation'[81] is driven by differences in nitrogen partial pressures and happens whenever oxygen is breathed, whether diving has taken place or not.

If oxygen is breathed when there is an excess of nitrogen in the body, which is usual after diving, the resulting inert gas pressure gradient will lead to faster transport of nitrogen out of the tissues.[14,21] Reduced nitrogen pressure in tissues and fluids will decrease the risk that nitrogen bubbles are formed and existing inert gas bubbles will lose their content and shrink faster. Oxygen can thus be used after diving to increase the speed of elimination of nitrogen from the body and decrease the risk of developing DCS.[54] It has also been hypothesized that oxygen breathing can reduce the number of micronuclei in the body, which theoretically can decrease the risk of DCS.[81]

Oxygen toxicity

Oxygen is necessary for human life. The consequences of hypoxia with anaerobic metabolism, lactate accumulation, acidosis, brain damage, seizures and death are well-known.[82-84] But hyperoxia can also be dangerous. Partial pressures of oxygen higher than approximately 140–160 kPa are toxic to the nervous system and could manifest as tonic-clonic seizures.[85-87] There is variation in susceptibility to oxygen toxicity between individuals, but also from day to day for a single person. The higher oxygen partial pressure (pO_2) is above 160 kPa and the longer the time of exposure, the greater is the risk that seizures will develop. Increased partial pressure of CO_2, exercise, and immersion in water all increase

[u] See footnote m on page 26.

[v] Normobaric oxygen is oxygen at a pressure of 100 kPa.

[w] Hyperbaric oxygen is oxygen at a pressure higher than 100kPa.

the effects of oxygen toxicity and hence the risk of seizures. Convulsions may well be the first manifestation of oxygen toxicity, but other less severe preceding symptoms include facial twitching, nausea, tinnitus, dizziness, incoordination, tunnel vision and dysphoria. Oxygen induced seizures are generally short and self-terminating but for a diver under water they could be fatal and thus, prevention is important. Therefore, professional diving is regulated both in terms of time of exposure and partial pressure of oxygen allowed. Hyperoxia is reported to decrease cerebral blood flow.[88,89]

Oxygen becomes harmful to the lung when its partial pressure exceeds 50kPa.[86,90] A correlation between pO_2 and time of exposure exists, alongside a measurable decrease in lung function. Initial symptoms of pulmonary oxygen toxicity are substernal discomfort and tracheal irritation, later followed by chest tightness and coughing. Dyspnoea can develop. The detrimental effect of oxygen on lung function can be quantified with spirometry as the change in vital capacity.

Oxygen dose is measured as units of pulmonary toxic dose (UPTD),[87] where one UPTD equals the breathing of 100% oxygen for one minute at a pressure of 101 kPa; 615 UPTDs has been used as limit of maximum safe exposure. However, the clinical usefulness of UPTD in diving medicine has been questioned due to variability in repetitive measurements of vital capacity that impedes its use, due to its poor predictive performance after short oxygen exposures and when in-water dives are compared to dry hyperbaric oxygen exposures.[90]

Oxidative stress

Reactive oxygen species (ROS), also called oxygen free radicals, are unstable oxygen derivatives produced during normal cellular respiration.[91] Typical representatives of the ROS group are superoxide (O_2^-), hydroxyl radical (HO) and hydrogen peroxide (H_2O_2).[91,92] They are produced mainly in the mitochondria and are present in low and stable levels in cells. Reactive oxygen species have important physiological functions, including cell signalling, regulation of vascular tone, inflammatory reactions, and defence against bacteria.[91,93,94] However, they can also cause protein oxidation, nucleic acid damage and lipid peroxidation, which are all harmful to the cell.[91,95] Superoxide dismutase (SOD), catalase, vitamins E and C, and glutathione peroxidase are examples of antioxidants that neutralise ROS and uphold what is referred to as the 'redox balance'.[92,95-97] Oxidative stress is a term used to describe a state of imbalance between ROS and antioxidants, where ROS activity surpasses systems responsible for their removal.[98] The brain could arguably be seen as vulnerable to oxidative stress on account of its high rate of oxygen consumption.[97] Detrimental effects of ROS activity have been hypothesised to play a role in the pathophysiology of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis.[96,97]

Exogenous factors could increase the amount of ROS. During normal metabolism about 1–2% of the oxygen molecules are converted to ROS[99] and this fraction is thought to increase in states of normobaric hyperoxia.[95,98-100] However, in one study, no biochemical signs of lipid peroxidation were found when subjects breathed 60% oxygen at sea level for 30 minutes.[101] Studies report that SCUBA

diving[x], saturation diving[y] and treatment with HBO, all conditions where not only oxygen partial pressures but also ambient pressures are increased, may result in oxidative stress and increased levels of ROS.[93,95,102-105] But when subjects were pressurised up to 203 kPa in a hyperbaric chamber while breathing oxygen, no clear evidence of a dose dependent oxidative stress response was found.[106] High oxygen partial pressure in tissues could lead to increased levels of reactive nitrogen species (RNS);[93,98] typically, NO reacts with superoxide (O_2^-) resulting in the formation of peroxynitrite (ONOO-), which could damage neuronal cells.[107] It has been proposed that reactive oxygen species (ROS) may be involved in the pathophysiology of oxygen induced seizures.[106] Breath-hold diving is also alleged to induce oxidative stress.[108]

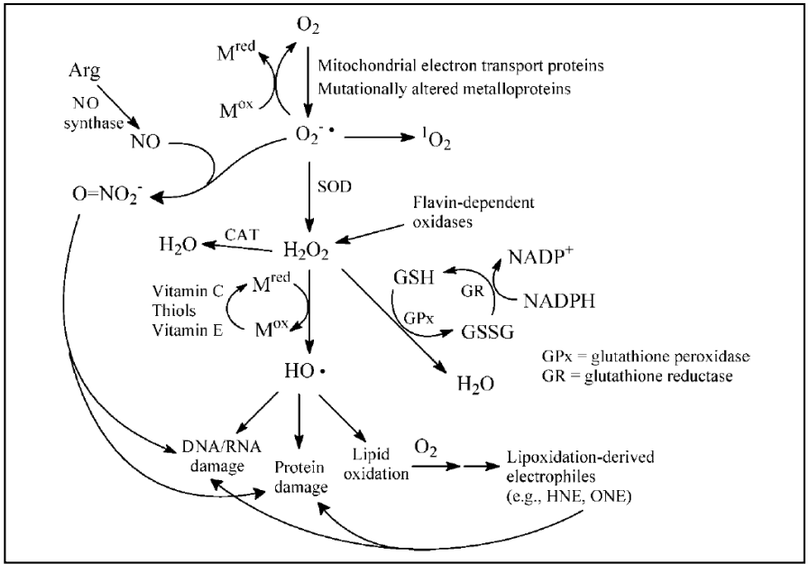

Figure 3 *Schematic depiction of formation and fate of ROS.[97] From Sayre LM, Perry G, Smith MA (2008) Oxidative stress and neurotoxicity. Chem Res Toxicol 21(1):172-88. doi:10.1021/tx700210j. Reproduced with permission from ACS Publications. Further permissions related to the material excerpted should be directed to ACS Publications.*

Oxygen breathing before diving

The effect of oxygen breathing before diving was evaluated in a study on 21 subjects who performed four sets of two dives, breathing either air or normobaric oxygen for 30 minutes before a particular dive.[54] The dive depths were 30 msw over a duration of 30 minutes, and the interval between dives in the same set was two hours. The following four combinations of breathing regimens before diving

[x] SCUBA is an acronym for 'self-contained underwater breathing apparatus'. The diver carries one or more cylinders with compressed gas with her, and diving time is defined by the amount of gas available in the cylinders. SCUBA breathing circuits are commonly open, which means that exhaled gas is not recycled but leaves the system, diving to depths of about 30–40msw. More complex systems, where some or all exhaled gas is recycled after carbon dioxide has been removed and oxygen replenished, are typically used for deeper diving.

[y] Extended and deep dives result in long decompression obligations. To use working time more effectively and decrease DCS risk deep divers could be contained under pressure for prolonged periods of time, days to weeks. Typically, they live in a pressurised chamber on the dive site and are transported in a separate tank or bell to actual diving depth, which correspond to the pressure inside the accommodation chamber. The inert gas pressure in the divers' tissues during this period is in equilibrium with the increased ambient pressure and referred to as 'saturated'. The divers are only decompressed to surface at the end of a working period. (Bennet 2016)

were compared: air–air, oxygen–oxygen, air–oxygen, oxygen–air. After each dive, DU was used to measure the VGE load, with results from the set where both dives were preceded by air breathing used as baseline; each subject served as their own control. Oxygen breathing before diving lead to a significant decrease in VGE load. The most effective VGE reduction was seen after the second of two dives with oxygen breathing before both. Interestingly, the effect of a reduction in VGE load after oxygen breathing before the first dive seemed to remain in effect when the second dive was preceded by air breathing.

Another study investigating different oxygen breathing regimens involved six recreational divers, who each made four dives to 30 msw with 20-minute bottom times.[81] The dives were separated by two weeks. Before the first dive, all subjects breathed air for 20 minutes while the second dive was preceded by normobaric oxygen breathing for 20 minutes. In the third and fourth dives, no surface air or oxygen was administered, instead subjects breathed oxygen for 20 minutes at depths of 6 msw and 12 msw respectively, before descending to 30 msw. Normobaric oxygen significantly reduced VGE load after diving, with oxygen breathing at depth being even more effective, perhaps due to the increased pressure reducing micronuclei.

A study on six healthy sports divers investigated the effect of oxygen breathing, or whole-body vibration, or both, during a 30-minute session one hour before diving. Oxygen breathing was associated with fewer VGE after diving compared to controls but whole-body vibration was more effective than oxygen in reducing VGE.[77]

Oxygen breathing during diving

One way to mitigate DCS risk is to increase oxygen content in the breathing gas used. Commercially available mixtures of 'nitrox' breathing gas containing lower amounts of nitrogen than air, for example EAN 32 (32% oxygen and 68% nitrogen), can be used together with dive profiles intended for air dives to reduce the amount of nitrogen taken up during a dive and add a margin of safety.[109] At shallow depths is it possible to breathe pure oxygen while diving, which eliminates uptake of nitrogen altogether. However, breathing increased fractions of oxygen during a dive places limits on dive depth, due to the risk of oxygen induced seizures, especially with prolonged exposure.[86,87] The study on the six recreational divers related above[81] showed that breathing pure oxygen for 20 minutes at depths of six and 12 msw effectively reduced VGE load after a dive to 30 msw for 20 minutes.

Oxygen breathing after diving

A study on 19 professional divers investigated the effect of administering oxygen for 30 minutes, after diving to a depth of 30 msw for 30 minutes, on VGE load.[110] Each diver performed three dives, separated by three days. A safety stop was made at the end of each dive for nine minutes at three msw. After the first dive no oxygen was breathed, while after the second and third dives, at 10 minutes after surfacing oxygen was breathed at either sea level or at a depth of six msw. It was noted that post-dive oxygen breathing reduced the amount of VGE and was most effective at a depth of six msw.

In a study on 48 professional divers, it was shown that breathing normobaric oxygen in the 30 minute period immediately after a dive to 42 msw for 10 minutes, with a safety stop for three minutes at five msw, significantly reduced the amount of VGE.[111] Each diver performed two dives, 48 hours apart,

breathing air after one dive and oxygen after the other, thus serving as their own controls. The effect of delaying the start of oxygen breathing until 15 minutes after surfacing was assessed. The reduction in VGE load after diving was judged to be more pronounced when oxygen breathing was started immediately after diving.

Breathing gases other than oxygen

Anaesthetic gases

Many gases have the potential to affect our nervous system when inhaled. The most well-known are probably anaesthetic gases (AG) that are widely used to render patients unconscious but also free of pain during operations. Modern anaesthetic gases are metabolised to some extent but are otherwise taken up, distributed and eliminated in a way similar to inert gases and their effects are correlated to their partial pressures; they are dosed accordingly.[112] Originally, it was believed that the effects of AG were caused by changes in the neuronal cell membrane and their potencies were thought to be correlated to their lipid solubility,[113] but modern hypotheses on the AG mechanisms of action describe their direct actions on receptor proteins affecting synaptic transmission and membrane ion channels.[113,114] It has been proposed that anaesthetic drugs induce unconsciousness by modifying synaptic transmission in the CNS, with inhibitory activity increased and excitatory neurons suppressed. Gamma-aminobutyric acid (GABA) and N-methyl-D-aspartate (NMDA) receptors are proposed to be important targets for modern anaesthetics.[113,114]

Inert gases

Nitrogen

When the partial pressure of nitrogen increases, it affects the nervous system.[16,115,116] As this sensation is similar to the effects of AG, the phenomenon often is referred to as inert gas narcosis (IGN). When breathing air, symptoms of IGN usually become detectable at ambient pressures of about 400 kPa, when the partial pressure of nitrogen is about 300 kPa, and they gradually become more evident with increasing depth. There is a variation in individual susceptibility to IGN but at pressures of 700–800 kPa, most subjects breathing air will be clearly affected. Early symptoms of IGN are mild cognitive impairment and euphoria, followed by more severe cognitive and neuromuscular dysfunction. At an ambient pressure of about 800–1000 kPa pronounced intellectual impairment, confusion and stupefaction are described among subjects breathing air. Hallucinations and unconsciousness are associated with yet higher pressures. It has been speculated that IGN may be caused in part by changed GABA-receptor function secondary to increased nitrogen pressure.[117,118] Elevated concentrations of CO_2 can amplify the effects of IGN.[115,116] The effects of IGN appear early and are not dose dependent, i.e., IGN does not get worse over time if ambient pressure remains the same, and its symptoms recede swiftly when pressure is reduced, but it has been speculated that there might be a residual effect after diving.[117] Stress and anxiety could make IGN symptoms more debilitating, although prior experience of IGN and active mental strategies can alleviate impairments, at least subjectively. As cognitive functions such as reasoning, memory, concentration, and attention are affected early in its onset, IGN is a risk factor for dive accidents.

Helium

Helium has no known effects on the CNS at pressures less than 4 MPa. Above that pressure, experimental data suggest that helium could have narcotic potential, but this hypothesis has been disputed.[116] To reduce the narcotic effects of nitrogen at depth, helium is used either in combination

with pure oxygen in a mixture called 'heliox', which is devoid of nitrogen, or in a mixture of oxygen, helium and nitrogen that is referred to as 'trimix'.[119,120] Helium molecules are smaller than nitrogen molecules and will therefore diffuse faster between bodily compartments. Helium is also less soluble than nitrogen, which means that a smaller amount of gas will be taken up by the body at equilibrium. Hence, compared to nitrogen, there will be less inert gas in the tissues with the potential to form bubbles upon decompression. Helium has a lower density than nitrogen, which makes it easier to breathe when ambient pressure is increased but its thermal conductivity is higher than that of nitrogen.[6]

Other inert gases

Inert gases like neon, argon and krypton have been shown to have similar effects as nitrogen, but at different pressures.[16,115,116,119] Xenon has narcotic effects at sea level and is therefore unsuitable as a breathing gas for diving. Argon and krypton are regarded as roughly twice and ten times as narcotic as nitrogen, respectively, which also makes them unsuitable as diving gases.[16] Neon is much less narcotic than nitrogen but expensive; in addition, it has a higher density than helium.[119] Hydrogen is very light and has been used in deep diving as it is easy to breath even at great depths. It has central nervous system (CNS) effects at pressures above approximately 2.5 MPa, which are described as psychedelic more than narcotic.[116] One major problem with hydrogen is that it is explosive in mixtures containing more than 4% oxygen, which limits its use to depths exceeding 35 msw.

Carbon dioxide toxicity

The narcotic potential of CO_2 is about 20 times that of nitrogen, but adaptation to abnormally high levels is possible.[121] Even small increases in pCO_2 from 'normal' levels may impair judgement, and it has been speculated that hypercarbia could be involved in fatal diving accidents. A modest increase in CO_2, at partial pressures of about 6–10 kPa, will cause tachycardia, hypertension, flushing, anxiety, subjective dyspnoea, loss of coordination, confusion and, eventually, lethargy. When pCO_2 exceeds 10 kPa, severe mental impairment and eventually unconsciousness will ensue. Death from respiratory depression or seizures will follow if pCO_2 continues to increase.

Adverse effects of pressure on the nervous system

"Man under pressure is a potent source of invaluable information about human physiology and thus he is a very attractive person."

Børge Minsaas, 1983[122]

Increased ambient pressure can itself be detrimental to the nervous system, independent of breathing gas used. 'High-pressure neurological syndrome' or 'high-pressure nervous syndrome' (HPNS) describes a set of physiological reactions in humans who are exposed to increased ambient pressure, which are noticeable at depths exceeding ~150msw.[123-126] Typical initial symptoms are opsoclonus[z], slow tremor, nausea, and vertigo. With increasing pressure, tremor amplitude increases and myoclonic jerks appear. Problems with coordination, mood changes, somnolence, and loss of consciousness are described. Ultimately, continued compression can lead to convulsions and death. The clinical signs are accompanied by electroencephalogram (EEG) changes. A difference in susceptibility exists between individuals but the symptoms are, for the most part, related to rate of compression and pressure attained. A reduced rate of compression can mitigate and delay symptoms of HPNS and periods of constant pressure can allow for adaptation. Humans may be compressed to considerable depths of hundreds of msw, but it takes several days to reach such depths safely. A small fraction of either nitrogen or hydrogen added to a breathing mixture of oxygen and helium could alleviate or delay HPNS symptoms,[123,125] while anaesthetic and sedative substances do have a suppressive effect on HPNS.[126] Drugs such as flunitrazepam and ketamine have been reported to ease its manifestations. The pathophysiology of HPNS is not completely known. As it is a multifaceted syndrome there may be more than one causative mechanism, but its effects are at least partly independent of elevated gas pressures.[123,125] It is generally considered that the effects of HPNS subside and disappear as pressure is diminished.

[z] Opsoclonus refers to involuntary, irregular, conjugated both vertical and horizontal eye movements (Anderson 1988).

Long-term effects of diving on the nervous system

"I hereby have to declare that two workers, having passed seven hours straight in compressed air, have experienced rather sharp pain of the joints half an hour after leaving the mines. The first one complained of an extremely sharp pain of the left arm, the second experienced a similar pain of the knees and the left shoulder. A few rubs with alcohol soon took the pain away in both individuals and they could nonetheless continue their work the following days."

Jacques Triger, 1845[127]

Is diving harmful to the nervous system?

That neurological DCS can cause disability among stricken divers is well-known,[27,128-130] but the question remains whether uneventful diving, without DCS or hypoxia, could confer damage on the nervous system with long-lasting effects. Neurological impairments related to increased ambient pressure have been reported after deep saturation diving and there have been concerns that HPNS symptoms seen in deep diving on some occasions might persist and become long-lasting or even permanent.[124,131] However, many of the studies reporting negative effects of deep diving on the CNS were published at least 30 years ago,[131-133] and reflect diving practices that are long since modified.[124] Several,[117,134-142] but not all,[143-148] later studies on non-saturation diving report that diving may be associated with cognitive[å] impairments. However, almost all published studies are retrospective or cross-sectional, which makes it impossible to determine causality and potential confounding factors could make interpretation of results difficult.

One possibility is that cognitive long-term effects of diving do exist and can be attributed to the accumulated effect of subclinical, unrecognised, neurological DCS. This notion is supported by studies that find associations either with dive experience, i.e. probably not diving time, but rather the number of decompressions, or the presence of PFO and cognitive or radiological CNS abnormalities among divers.

Another possibility is that there are one or more mechanisms that have not yet been defined, which are mediated through increased ambient pressure, increased partial gas pressures, or some other pathophysiological process, by which diving activities could result in nervous system impairments. This hypothesis does not necessarily preclude the notion that subclinical neurological DCS could harm the nervous system, indeed they could be seen as complementary and not mutually exclusive.

A third possibility is that there is no specific effect of diving on the brain. Results yielded from dive studies are conflicting, and no unequivocal evidence exists to attest that exposure to an increased ambient pressure *per se*[ä] causes permanent damage to the CNS.[107,129,149,150]

There has been some debate as to whether professional offshore divers, who also participate in saturation diving, may have been damaged by their work. A Norwegian governmental report[151]

[å] Cognitive functions could be described as mental processes concerned with learning and memory, language, visuospatial (footnote bb on page 49), executive (footnote cc on page 49) and psychomotor functions. (Knopman 2014)

[ä] Latin, meaning 'by itself' or 'in itself'.

concluded that divers employed between 1965–1990 'often or quite often' had problems with joint pains (83%), memory (55%) or *"psychological issues"* (42%). Among former divers, 40% underwent some sort of medical treatment, which was higher than expected for Norwegian males of the that age, and many had disability pensions. In another study, despite reports of lower health-related quality of life among former North Sea divers especially in those who had suffered one or more DCS incident,[152] it was found that their mortality was not higher than age-matched controls'.[153,154]

In a British governmental report on the long term effects of diving, questionnaires were sent to 2,958 divers and 2,708 non-diving offshore workers.[155] The range of dive types made by the participants included Scuba diving, surface oxygen decompression diving[ö], mixed gas bounce diving,[aa] and saturation diving. Response rates were low, at 56% and 51% respectively, and after exclusions only 1,540 divers and 1,035 non-divers were finally assessed. Health-related quality of life outcome measures were similar in all groups and divers received less medical treatment than controls, but 18% of divers complained of problems with 'forgetfulness or loss of concentration', compared with only 6% of non-divers. Divers were also more likely to report problems with joint pain or muscle stiffness and hearing impairments, which were judged to be caused by work related factors such as welding. Subjective symptoms of 'forgetfulness or loss of concentration' were associated with length of diving career. A subgroup of divers with 'forgetfulness or loss of concentration' was assessed with magnetic resonance imaging (MRI) and neuropsychological testing. The low response rates raise questions about whether the studied sample adequately represented the whole population of divers. Nonetheless, when the report was summarised, it was stated that the investigators "*did not identify any long term health effects associated with professional diving amounting to a clinical abnormality"*[155] Results from the report were also published as separate journal articles.[138,156]

The effects of diving on the nervous system have been discussed further at several scientific meetings. International consensus meetings on the long-term effects of diving were held in 1983, 1993, and 2005. At the last meeting, the following concluding statement was agreed upon: "*There is evidence that changes in lung function, CNS, bone and cochleo-vestibular system can be demonstrated in some occupational divers. The magnitude of these changes is highly variable and has the potential to influence divers' quality of life. The knowledge about the precise mechanisms is still limited and calls for further research. The knowledge calls for preventive measures, including health surveillance."*[157]

In two major textbooks on diving medicine, it is concluded that no clear evidence exists that diving incurs lasting neurologic or neuropsychological disturbances,[129,158] and in a review on the neurological effects of diving, it was stated that "*…the results from epidemiological and clinical studies regarding long term neurological effects from deep diving are conflicting and still not conclusive.*"[150]

One important question exists with regards to what constitutes a relevant neurological or neuropsychological impairment. It has been proposed that a long-term effect of diving should be defined as a finding or symptom that is: "*Outside the range of normal in an appropriately matched population, causally related to diving, persisting beyond the acute and rehabilitation phase of a diving accident, having no explanatory non-diving pathological features"* and that it also should produce "*…a demonstrable reduction in the performance or quality of life of the diver*."[130]

[ö] Surface decompression refers to a diving regimen where all or a part of the decompression needed at the end of a dive is carried out in a hyperbaric chamber and not in the water. (Hamilton 2003)

[aa] A bounce dive is a dive to an essentially constant depth until ascent, which may involve decompression stops. It could also be used to describe dives with a very short time spent at the bottom. (Hamilton 2003)

Neuropsychology

Several studies have used neuropsychological testing to determine the effects, if any, of diving on the CNS. Typically, cognitive functions such as memory, language, attention, reaction time, visuospatial abilities,[bb] and executive functions[cc] have been assessed. Study results are not only conflicting but at the same time difficult to compare or contrast, as the neuropsychometric test types varies between studies, and the clinical significance of one or more deviated psychometric test result is seldom obvious. It is beyond the scope of this text to discuss methodological concerns relevant to neuropsychometric testing in dive research but it should be noted that many factors other than dive exposure itself, such as motivation, anxiety, testing conditions, intellectual ability, non-reported episodes with DCS and age may affect results.[159-161] When results from neuropsychological tests are interpreted, their validity, reliability and sensitivity must be considered.[160-163] Almost all neuropsychological studies on diving are retrospective, which makes it impossible to assess causality.

Longitudinal prospective studies with appropriate control groups are difficult to conduct but are needed in the future to obtain reliable neuropsychological data on the effects of diving on the CNS. Standardised use of neuropsychological tests in diving studies would facilitate research progression and make meaningful comparisons and compilations possible.

Changes in cognition after deep diving involving saturation exposure have been studied. When 25 professional saturation divers underwent neuropsychological examinations before and after dives to depths between 198–335 msw, over 26–31 days, no neuropsychological changes were found that persisted beyond 10 days.[164] Most divers performed only one, and no more than two dives.

In a study on commercial off-shore divers, 82 subjects were neuropsychologically tested before and after 3–3.5 years of saturation diving.[131] Sixty-four of the subjects dived to depths of 300–500 msw during 18–34 days and were tested also after each deep dive. A difference in test results larger than 10% between two sampling points was defined as a mild to moderate change in neuropsychologic function and was found in about 20% of the subjects. Reduction in 'spatial memory' was the main cognitive dysfunction found but tremors and 'autonomic dysfunctions' were reported as well. Negative correlations existed between number of days in saturation or number of years performing saturation diving, and results on memory and 'visuomotor' tests. The authors speculated that neuropsychological changes could, among others, be caused by hydrostatic pressure, silent bubbles, reduced cerebral blood flow or predisposed sensitivity to nitrogen or HPNS. The study has been criticised for using poor statistical methods.[129]

[bb] *"Visuospatial function is the ability to specify the parts and overall configuration of a percept, appreciate its position in space, integrate a coherent spatial framework, and perform mental operations on spatial concepts"* (Salimi 2018)

[cc] Executive functions are *"mental functions related to planning, evaluation, judgement, and management of other mental abilities"* (Hogan 2019).

A retrospective cohort of 156 divers, of whom 133 had participated in saturation dives and 40 had dived to depths exceeding 180 msw, were compared to 100 non-diving controls.[133] The findings showed that the divers had more neurological symptoms, mainly paraesthesias, tremors, and lower extremity motor and sensory disturbances, than controls. Almost a quarter of the divers (21%) reported problems with concentration and with both short and long-term memory. Divers drank more alcohol than controls, 51% of them reported having experienced DCS, 33% had experienced neurological symptoms during decompression, and 14% had lost consciousness at some time during diving, which all could be considered as confounding factors. The divers' symptoms were significantly correlated to diving exposure, DCS and age, but a shortcoming of the study was that no examinations had been made before diving. When the 40 (≥180 msw) saturation divers were tested further and compared to the same 100 controls at 1–7 years after their last deep dive, it was found that they had more neurological symptoms in comparison, mainly regarding concentration difficulties, paraesthesias and sensory disturbances.[132] The mean time in saturation was 378 days. Exposure to deep diving and age were correlated to neurological symptoms. Divers drank more alcohol and had lower education than controls.

In another study, 96 professional deep divers, of whom 21% reported at least one episode of neurological DCS and had been referred to specialist care due to health problems, underwent neuropsychological examinations with the results compared to a sample of 60 controls matched for age and education.[165] The professional divers had significantly lower scores on tests of attention, concentration, memory, 'processing speed' and 'mental flexibility'. Results may have been influenced by the fact many of the tests were interrelated and no statistical corrections were made for multiple testing. It should be remembered that the cohort studied was a selected subgroup of all deep divers.

In a British governmental report on the long term effects of diving referred to earlier in this text,[155] some of the divers who had reported complaints of 'forgetfulness or loss of concentration' of 'moderately' or 'extremely' severity were recruited to further investigations where they underwent neuropsychological testing. Their results were compared to those of divers with the same general background who had denied or reported only slight problems with 'forgetfulness or loss of concentration', as well as to the results of non-diving off-shore workers.[138] There were 94, 89 and 92 subjects in the three groups, respectively. Divers with 'forgetfulness or loss of concentration' had an overall decrease in neuropsychological test performance compared to the other two groups, particularly regarding memory functions other than working memory. Executive functions were similar for the groups. Deficits were described as mild and reduced memory function was associated with mixed gas bounce diving and surface oxygen decompression diving.

A study on the effects of non-saturation diving compared 20 experienced construction divers with 18 years of diving experience on average, to 32 trainees at a professional diving school, and 20 not fully age-matched non-diving construction workers.[147] One of the construction divers had participated in saturation diving for a short period. Fourteen (70%) of the experienced divers reported one or more episodes of DCI, one of whom had experienced neurological manifestations. In the final analyses, test results for the diver with neurological DCI were excluded. For the remaining subjects, all diving had been made with surface supplied air or nitrox to a maximum depth of about 50 msw. Among the trainees, none had performed more than 100 dives. There were no significant differences in self-reported neuropsychiatric symptoms between the groups, but the experienced divers had longer reaction times compared to control groups, while their other neuropsychometric test results were all normal. The experienced divers were recruited from two major construction companies. The author

discussed the possibility of selection bias, as divers who left the company might have had more impairments compared to those who had chosen to stay in the profession.

A total of 50 subjects were included in a longitudinal study on the effects of diving; however, only 37 of them were followed up during the whole study period of 12 years.[148] Total number of dives during the period were not associated with any adverse neuropsychological effects, but divers who reported incidents with DCI performed worse in a memory test and had more self-reported neuropsychiatric symptoms.

Professional abalone divers in Australia and Tasmania have been neuropsychologically examined in different studies, with conflicting results. In one study, neuropsychological test results for 48 professional abalone divers who dived regularly to between 6–30 msw with surface supply compressed air, sometimes spending hours at depth, and 47 local fishermen were compared.[146] Divers performed slightly worse than the fishermen on memory testing only, and the divers test results all, including those examining memory, fell within normal reference limits. In summary, no cognitive defects were found among the divers "*in spite of evidence of their exposure to decompression stress*". Yet, in another study that compared neuropsychological performance among 33 abalone divers, of whom 11 had stopped diving, to 33 matched non-diving controls, divers performed worse than controls when neuropsychologically tested, and they also had more tremors.[139] The divers' reaction times were shorter but their error rates higher than those of controls. Unfortunately, it was not clearly reported why 11 subjects had discontinued diving. In a third study, 80 abalone divers underwent neuropsychological testing and were interviewed about lifestyle and diving practices.[140] Multiple linear regression was used in analyses of the results. The authors concluded that there was a relationship between unsafe diving practice and problems in 'visual function', 'psychomotor abilities' and short-term memory. In short, these studies on abalone divers gave no clear evidence that shallow water diving in itself caused brain damage.[149]

In Chile, 104 artisanal divers and 58 non-diving fishermen were interviewed and their executive functions assessed.[145] The divers had performed a median of 150 dives with surface-supplied air during the preceding year, of which 83% went to a depth ≤ 30 msw and 99% of the dives were ≤ 50 msw. Three quarters of the divers were judged to have experienced DCS, and it was alleged that 20 divers reported "*having had cerebral air gas embolism"*. No differences in executive functions were found between the divers and the non-divers as groups, but a dose-related relationship between frequency and severity of DCI and a decrease in executive functions among divers was reported.

In a study where 43 professional divers with at least 15 years of active service were compared to 68 non-diving matched controls in a study using neuropsychological testing, divers had better results on two subtests, 'finger tapping test' and 'digit memory span test', but both groups had values within the normal reference ranges.[144]

When 17 'experienced' military divers, with a history of 500–1200 diving hours, and eight 'very experienced' military divers, with a history of >2.800 diving hours, were retrospectively compared to 12 and 11 non-diving controls, respectively, the 'very experienced' divers had longer reaction times compared to control subjects.[134] All dives were less than ≤ 60 msw and performed while breathing compressed air. The study groups were small, but the results suggested that extensive diving was associated with decreased cognitive performance. A strength of the study was that a training phase was

given before the actual tests took place, to control for a potential training effect on results from the neuropsychological tests.

Another retrospective study compared 16 divers who had made ~1700 dives during on average 13 years, 16 divers with ~3500 dives during about 20 years, and 18 healthy controls without diving experience.[137] None of the divers reported any occurrence of DCI. All participants were psychometrically tested. The most experienced divers had worse 'visuo-constructional' and 'visual long-term memory' test results; the authors hypothesized that asymptomatic VGE could cause cognitive impairments among divers.

In yet another retrospective cohort study, 44 recreational SCUBA divers with average experience of about 660 dives each and no history of DCS were compared to 24 boxers with at least five years of experience and no history of major head trauma, and 37 healthy physically active non-diving ordinary controls.[136] Mean ages were similar for all three groups. Most dives (84%) were ≤ 40 msw. Reaction times were faster for divers while their short-term memory was worse than for ordinary controls. No difference in memory function was seen between divers and boxers.

Hypoxemia could harm the nervous system if severe, prolonged, or recurrent. Here too, results from studies are conflicting. One study compared 12 experienced breath hold divers[dd], with a mean best static apnoea time of 371 seconds and mean experience of apnoea training of 105 months, and 12 novice breath hold divers with a mean best static apnoea time of 243 seconds and 8.75 months of apnoea training experience, to 12 healthy subjects with no breath hold experience.[166] Age and education were similar among the groups. The results suggested that the experienced breath hold divers had short-term memory impairments. Contrary to this, a study on 21 breath-hold competitors with a mean static apnoea time of 294 seconds and mean experience of apnoea training of about 76 months, found no abnormal neuropsychometric results among its participants.[167]

Radiology

If diving damages the brain, divers could be expected to have abnormal findings on MRI of the brain. One methodological problem is that abnormal MRI findings, often described as a 'high intensity spots' (HIS) or 'unidentified bright objects' (UBO), are present among subjectively healthy non-diving persons, and their prevalence increases with age.[168,169] Results from controlled radiological studies on divers are conflicting, with some reporting a higher incidence of MRI abnormalities among divers compared to controls,[170-172] while some report the opposite,[132,143] and some do not find any differences between the groups.[141,173-177]

All published studies are retrospective and recruitment bias or confounding may have influenced results, with the cause of detected MRI findings remaining uncertain. If a lesion or abnormality is found on MRI, it could represent effects of a known or asymptomatic DCS, effect of ageing, trauma or another disease affecting the brain, such as hypertonia or atherosclerosis.

[dd] Breath-hold diving is also called free diving. The diver remains under water for as long as one breath lasts, which could be a considerable amount of time for a trained and motivated individual. Breath-hold diving is the oldest diving technique known. In its simplest form no equipment is needed, but often face masks, fins and thermal protection suits are used. It is also a sport where it is possible to compete in different disciplines, for example 'static' and 'dynamic' apnoea are two. In the first, subjects do not move, while in the second they swim horizontally with or without fins, depending on discipline, while holding their breaths.

As for neuropsychological research on the effects of diving, prospective and appropriately controlled studies would be needed to obtain reliable radiological data on the effects of diving on the CNS.

In one study, 105 professional divers, including some with experience of saturation diving, were examined with MRI and the results compared to scans of 49 non-diving controls. No statistically significant difference in the number of subjects with HIS was seen between the groups.[176] When a subgroup of 37 subjects who had made deep saturation dives were analysed, the number of subjects with HIS was significantly less (19%) among divers compared to non-diving controls (43%).[132]

When 59 divers, five having experience of saturation diving, together with 48 matched non-diving controls were examined with brain MRI, no statistically significant difference in number of lesions were found between the groups.[177] Seven divers had had non-neurological DCS. Eighteen percent of non-divers had more than three hyperintense white matter spots compared to only 12% of the divers.

Some of the subjects described in the British governmental report on the long term effects of diving[155] were also examined with MRI. Of 95 divers with 'forgetfulness or loss of concentration', 86 (91%) had white matter abnormalities while the same abnormalities were found among 80 out of 97 examined divers (83%) without complaints of forgetfulness. Among non-diving offshore workers 73 out of 88 subjects (83%) had white matter abnormalities. Periventricular hyperintensities were found to be associated with 'forgetfulness or loss of concentration' but it was also stated in the report that the radiological abnormalities found did not "*amount to pathological change known to be typical of a disease state.*"

Seventy commercial divers with at least one year of experience and 47 healthy age-matched non-diving controls were examined with brain MRI.[174] HIS was found among 34% of the divers and 42% of the controls, but the difference was not statistically significant. In control subjects, presence of multiple lesions was correlated to smoking, alcohol, head trauma and cerebrovascular risk factors.

In contrast, more brain MRI lesions were found in 113 male professional divers without a history of DCS than 65 non-diving controls.[172] In the divers, 23% had lesions in comparison to 11% of controls.

In a study on shallow compressed air diving, 30 experienced divers, who on average had performed just less than 1000 dives to a depth of 30 msw, were compared to 30 non-diving controls matched for age and sex.[175] MRI spots of high intensity were seen in 33% of the divers and 30% of the controls.

When 52 recreational divers and 50 matched non-diving controls were compared, MRI revealed 86 lesions among divers and 14 among controls, but the majority of lesions (79%) were found in 14 of the 52 divers; no differences compared to controls were found in the remaining divers.[171] However, this study has been criticised for risk of recruitment bias,[129] and the presence of PFO, that may have influenced the results, was not assessed.

Only one structural brain abnormality was found when 17 elite breath hold divers and 50 age-matched controls were examined with MRI.[173] When nine of the breath hold divers were examined again one year later, no new brain lesions were found.

Neuropsychology and radiology

The association between MRI brain abnormalities, neuropsychometric results, and diving history among 20 experienced compressed air divers with no history of neurological DCI was assessed, and compared to results for 20 non-diving controls matched for age, alcohol, and smoking habits.[141] Even though no diver reported a history of neurological DCI, eight had had skin or joint pains, and four divers had performed deep saturation dives to depths between 210–600 msw. MRI detected abnormalities in 60% of divers and 45% of controls, but the difference was not statistically significant. Neuropsychological test results regarding 'mental flexibility', 'visual tracking' and 'recall of nonverbal material' were significantly worse for divers compared to controls. Of note, no correlations were found between psychometric test results and MRI abnormalities, but the latter were for divers correlated with number of hours diving to 40–60 msw while breathing air.

Brain MRI results from 19 compressed air workers (CAW) were compared to 11 workers with no exposure to compressed air.[170] Significantly more lesions were found among CAW but most of them were found in a subgroup of seven CAW; the other CAWs did not have more lesions than the controls. Neuropsychometric testing showed no significant differences between the groups. Prevalence of PFO, which might have caused the uneven distribution of lesions, was not assessed.

In another study, 24 navy divers and 24 non-diving navy employees of matching age and with matching smoking habits used as controls were examined with brain MRI and clinical neurologic examination.[143] They were also evaluated using neuropsychometric tests. The divers had a mean diving experience of ~1400 hours, and 84% of all dives were made to depths of less than 20 msw. The clinical neurologic examination was unremarkable for all participants. Divers had longer reaction time on attention tests, and there were differences between the groups in other psychometric subtests, but as all results were *"subclinical and within the range of applied tests"*. The authors concluded that they had found no evidence of decreased neuropsychological performance due to long-term diving. On MRI, HIS were detected in 25% of the divers and 42% of the controls.

In a study on long term effects of recreational diving, 215 divers were examined with functional brain imaging using single-photon emission computerised tomography (SPECT) and neuropsychological testing was performed.[135] The authors concluded that frequent diving, more than 100 times per year, to depths greater than 40 msw in cold water may have a negative effect on the CNS and should not be considered a recreational activity.

Another study investigated the long-term effects of professional diving.[142] Two groups, including 52 professional scuba divers with at least 2000 dives each, and 52 age-matched non-diving controls, were investigated using MRI. Among divers, "*modest*" white matter alterations were detected in the anterior part of cerebrum and decreases, again "*modest*", in attention and memory functions were described.

Radiology and PFO

The difference in MRI study results could, at least theoretically, be explained by uneven rates of PFO or other shunt,[178-180] or differences in shunt sizes,[178,180] between groups of subjects. Several studies have assessed the proportion of divers with PFO and related it to MRI findings.

In one study, 87 sport divers with a minimum experience of 160 dives were examined with transcranial doppler sonography; in 25 (29%) of the divers right-to-left shunting was demonstrated, with 13 (18%) deemed to have a PFO of high haemodynamic relevance.[178] Eleven (13%) of the divers had one or more brain lesion. Seven (11%) of those who did not have a shunt had one lesion each, while four (16%) divers with shunts contributed 34 lesions between them: one subject with a small PFO had one lesion, and three subjects with large PFOs had multiple lesions. Multiple lesions were only found among divers with large PFOs, which suggested that embolisation might have taken place.

To retrospectively assess the risk of DCS in the presence of PFO, 52 recreational divers who had made at least 200 dives using compressed air, and 52 healthy non-divers, were examined with MRI and transesophageal echocardiography (TEE). Divers also filled out a questionnaire about health status, diving habits and prior DCS episodes.[179] Of divers and controls, 13 (25%) and nine (17%) had a PFO, respectively. Forty-one MRI lesions were reported in 19 divers (37%), with seven lesions in six controls (12%). Neurological DCS was reported in 4/13 (31%) of divers with a PFO and 4/39 (10%) of divers without PFO, while 4/13 (31%) of divers with, and 2/39 (5%) without PFO were alleged to have experienced episodes with "*air embolism"*. About twice as many ischemic lesions were seen in divers with PFO compared to them without. Using a logistic regression model, it was concluded that the risk for DCI was 4.5 times greater for divers with a PFO compared to divers without.

Contrary to these findings, in a retrospective uncontrolled study of 50 healthy divers, 36% had a PFO but no correlation could be established between presence of PFO and number of HIS.[181]

In another study, MRI revealed HIS in 44% of 32 asymptomatic professional divers and in 22% of 32 non-diving age-matched controls.[180] Divers were assessed with transcranial doppler for the presence of a right-to-left shunt (RLS), which was found in 15 out of 32 (47%) divers. There were no differences in the rates of HIS between divers with or without RLS but divers with larger RLS had a higher prevalence of hyperintense spots than divers with a small RLS or no RLS (75% vs 25%).

Neuropsychology, radiology and PFO

In a study on a group of 200 volunteer recreational divers with at least five years of diving experience, at least 200 performed dives, and no DCS, 50 divers were randomly selected and 42 were examined with MRI, TEE, and neuropsychometric testing.[117] A significant PFO was detected in 38%, and UBO were found in 12% of the divers, but no correlation was found between PFOs and UBO. Neuropsychometric test results from two historic control groups were used for comparison, one with 161 non-divers and one with subjects exposed to neurotoxic solvents. When neuropsychometric test results were analysed, it was concluded that divers had worse short-term memory and worse 'visual-spatial performance' compared to the 161 non-diving controls but neuropsychometric test results were not correlated to either PFOs or UBOs.

A review published in 2008 concluded that cerebral MRI findings among divers had "*…so far not been linked with a reduction in neuropsychological performance,…*"[182] and a recent report on health surveillance in deep diving concluded that "*…the relationship between development of MRI changes on one side and the presentation and progression of symptoms and recognised illness on the other hand remains to be established.*"[124]

Biomarkers of neuronal injury

On the use of biomarkers in dive research

Patients with residual neurological DCS symptoms have been likened to patients with mild brain trauma, as they may exhibit similar symptoms.[129] Several biomarkers with an established use in research on brain trauma and neurodegenerative diseases have been studied also in the context of diving.[183-190] It is plausible that biomarkers of neuronal injury could be increased in DCS with clinical signs of neurological dysfunction, or after episodes of marked cerebral hypoxia during diving. But in studies that report increased concentrations of neuronal biomarkers after uneventful diving without known hypoxic insults or neuronal DCS, interpretation becomes more precarious. If ordinary diving affects the brain, it could be expected to bring about a measurable change in neuronal biomarkers, but an important question asks: if these markers always herald neuronal damage, might it be subclinical and potentially reversible, or do they also increase as a result of neuronal stress?

Biomarkers of neuronal origin could be measured in cerebrospinal fluid (CSF) or blood.[191] The latter is a more convenient mode of sampling for the subject. The concentration in blood is lower than in CSF,[191] but an exact quotient cannot be stated, and may be different for each biomarker. It is not known how biomarkers in CSF reach the blood;[192] potentially, they could leave the CNS with CSF through the arachnoid granulations into venous sinuses inside the skull, or they could filter through an intact or defective blood-brain-barrier (BBB) into the venous system.[192] There is no validated blood marker for BBB damage or dysfunction, though beta-trace protein (prostaglandin D synthase) and beta-2-transferrin, both synthesised in the CNS, have been used to detect CNS origin of fluid.[193] Biomarkers could also be transported within the glymphatic system out of the CNS.[194,195] The glymphatic system is, in many aspects, thought to have an analogous function to the lymphatic system in the rest of the body. It forms a drainage system of waste products out of the CNS, though the role of the glymphatic system for neuropeptide clearance has been questioned.[196] Dehydration could cause increased concentrations of proteins in blood, including neuronal biomarkers.

Tau protein

Tau protein (tau) is an intracellular protein that is abundant in unmyelinated axons.[96,191] It can also be found to a far lesser extent in astrocytes and oligodendrocytes, and outside the nervous system, in the liver, kidneys, and testes.[191] Six isoforms of tau exist (352–441 amino acids, 45–65kDa) in the CNS,[197] while a seventh isoform with an additional exon (110kDa) called 'big tau', is found mainly in the peripheral nervous system (PNS)[96,198] and in neurons that extend into the PNS, like spinal motor neurons or the optic nerve.

Tau is important for cytoskeleton strength[96,198] but is also involved in such diverse activities as cellular morphogenesis and division, and intracellular transport.[96,199,200] Phosphorylation of tau is a normal process, which regulates its affinity to intracellular microtubulei.[201,202] However, it can become pathophysiological, for example in Alzheimer's disease (AD) and other neurodegenerative diseases,

where tau is a component of neurofibrillary tangles, a pathological feature observed in the CNS of these diseases.[191,197,201] Neurofibrillary tangles can also be found in patients with chronic traumatic encephalopathy.[191,192,203] It has been suggested that oxidative stress causes increased tau phosphorylation.[96] Changes in tau concentrations are thought to be specific to neuronal processes but at least one study reports an increase in tau after high intensity interval training (HIIT) without known neuronal involvement.[204]

Tau can be passively released into the extracellular space after manifest neuronal cell injury or death but it can also be actively released into the interstitial fluid (ISF) secondary to both physiological and pathophysiological stimuli,[195,199,200,205] with increased concentrations in the CNS within hours of a stimuli.[205] It has been suggested that excitatory neuronal activity can increase extracellular tau levels[205] and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor may be responsible for regulation of tau release from intact neurons.[199] One proposed route of elimination of tau from the ISF into the blood is across the BBB,[192] another is via the glymphatic system.[195] Little is known about tau elimination from the blood. It may be enzymatically degraded[195] and excreted with urine. Elimination half-time for tau is probably less than 24 hours in human blood,[195,206] but elimination of tau from the CNS seems to be slower, with reported half-times in humans ranging between 11 to 23 days.[200,205] Diurnal variation could potentially confound measurements of biomarkers of neuronal injury, but studies on patients with Alzheimer's disease and older healthy volunteers,[207] neurosurgical patients,[208] and patients with suspected normal pressure hydrocephalus or pseudotumor cerebri[209] found no indices of a circadian pattern for tau when repeatedly sampled in CSF.

Studies on patients with cardiac arrest[206,210,211] and traumatic brain injuries[212-214] have showed that tau has potential as marker of cell death and neurological damage, but it also seems to be useful as marker of cerebral contusion.[215-217] Tau levels may also increase in the absence of overt brain damage.[183,185,218,219]

Absolute protein tau values obtained when a particular batch of samples is analysed depends on tau concentration in the calibrator solution. As yet, there are no standardised tau calibrators,[195] so each used has a different tau concentration; thus, absolute values yielded for a certain tau sample may vary between batches. Therefore, it is prudent to compare relative tau change when results from different studies are compared, as absolute values may be misleading.

In a study on boxers,[215] mean tau in CSF was 58 pg/mL among 30 subjects 1–6 days after a bout, compared to 49 pg/mL following ≥ 14 days without boxing, and 45 pg/mL among matched controls. In 28 ice hockey players with concussion, a median blood tau value of 10 pg/mL at one hour after concussion was found in comparison to 4.5 pg/mL preseason.[216] Tau levels decreased during the first 12 hours after concussion but remained significantly elevated for at least a further 132 hours. In another study, 87 ice hockey players had a median tau value of 2.5 pg/mL one-hour post-concussion, compared to a median of 2.1 pg/mL among 74 hockey players sampled at preseason.[217] However, in contrast, one study found lower blood concentrations of tau in blood, 15.1 pg/mL, taken from 16 subjects sampled within seven days of concussion compared to healthy controls, who had a concentration of 22.2 pg/mL.[220] Additionally, a study on 11 subjects reported that HIIT was associated with increased tau levels in blood, with median tau values in blood of 12.5 pg/mL before and 21.4 pg/mL immediately after the first training session.[204] However, a two-week period of HIIT three times a week seemed to blunt the tau release.

At 48 hours after cardiac arrest, 308 patients with poor neurological outcome had a median tau concentration of 49.5 pg/mL in blood.[210]

Increased blood concentrations of tau were reported after uneventful anaesthesia in combination with orthopaedic surgery in 30 patients, with a 257% increase at six hours postoperatively.[218] After that, tau levels decreased and no neurological symptoms were reported. Even larger relative changes in tau were observed in 25 patients undergoing cardiac surgery.[219] Median tau had increased from 3.2 pg/mL before surgery to 21.8 pg/mL after it ended. Tau levels decreased swiftly postoperatively, with median tau being only ~2x its baseline value after 24 hours; when sampled seven days postoperatively, median tau no longer differed from its baseline value. Patients were neither neurologically nor neuropsychologically assessed. In contrast, the same study reported that tau levels were unchanged after otolaryngeal surgery in 26 patients, and among 16 patients with myocardial infarction.[219]

In a study on 16 divers participating in a breath-hold competition, tau increased to 196% of baseline levels within one hour after protracted apnoea, and the level of tau correlated to apnoeic time.[221] Confusingly, tau concentrations then steadily decreased when sampled further after hypoxic events at 21 and 37 hours after the first breath hold, but according to the authors, distribution of data was wide and baseline tau values were, for unknown reasons, significantly higher among competitors compared to five control subjects.

When the concentration of tau in CSF among seven patients treated for DCS was analysed and the results compared to seven age-matched controls, no changes in tau were found, though it must be noted that only one of the patients had DCS with neurological manifestations.[186]

Three studies have assessed the effect of hyperbaric exposure on tau levels in blood. In one, 14 submariners were saturated at 401 kPa for 36 hours and then decompressed slowly over 70 hours.[184] Tau was sampled before, during, and directly after hyperbaric exposure had ended and at about 25–26 hours thereafter. Oxygen partial pressure did not exceed 50 kPa and nitrogen pressure during the 36 hours at depth was approximately 350 kPa. No changes in tau concentrations were noted at any point, neither among the submariners nor the 12 subjects in the control group. Dehydration was controlled for.

However, in an uncontrolled study where 10 professional divers performed one or two daily open-water dives increasing in depth up to 52–90 msw over the course of four days, mean tau increase was 98.8%.[183] Protein tau concentration was 0.50 pg/mL after four days of deep diving. At depths exceeding 40 msw, divers breathed a mixture of oxygen, helium, and nitrogen ('trimix'). The oxygen partial pressure in the breathing gas was 130 kPa at depth and could increase to 160 kPa during decompression. Divers breathed oxygen for 10 minutes after each deep dive. Nitrogen partial pressures in the breathing gases were approximately 176–193 kPa at depths of 82–90 msw. Venous gas emboli loads were not correlated to increases in tau. Possible dehydration was not controlled for, but tau was the only measured biomarker protein that increased, which made dehydration less plausible.

In another study, when 32 professional divers performed two identical dives separated by 48 hours to 42 msw while breathing air, tau levels were increased by 29.1% and 33.9% with absolute levels of 2.18 pg/mL and 2.23 pg/mL at 120 minutes after the first and second dive, respectively.[185] The increases observed were statistically significant at 120 minutes after each dive and at 30–45 minutes after the second dive, when tau had increased to 2.11 pg/mL. The oxygen partial pressure at depth was 109 kPa while nitrogen partial pressure was 406 kPa. Divers breathed normobaric oxygen for 30 minutes after one of the two dives, but analyses showed that it did not influence tau levels. Protein tau was the only biomarker that increased, so significant dehydration seemed unlikely. A shortcoming of the study was that it lacked a control group.

Neurofilament light

Neurofilament light (NfL) is one of five intermediate filaments[ee] (68 kDa) in the cytoplasm of neurons in both CNS and PNS.[222,223] They are mainly found in the cytoskeleton of myelinated axons, but also in cell bodies, dendrites, and synapses. The main functions of neurofilaments are related to structure of the cell and its components as well as to cell signalling.

NfL concentrations increase as a result of axonal damage and elevated levels are always pathological.[224] NfL, as well as neurofilament medium and heavy, exists also in PNS. Therefore, even though NfL increase is specific for neuronal injury or damage, it is not specific for CNS.[222,225] The kinetics for NfL are slower than for tau, with an expected peak no earlier than 10–12 days after an insult,[217,226] but significant increases have been seen within 48 hours.[217,218]

Serum NfL concentrations are found to increase after boxing,[215] in patients with concussion,[217] traumatic brain injuries,[191,224,226,227] and neurodegenerative diseases such as Alzheimer's disease and multiple sclerosis.[191,224,228] Increased levels of NfL in blood have been reported already at six hours after uncomplicated anaesthesia with orthopaedic surgery, and when sampling ended at 48 hours postoperatively, NfL levels had yet not decreased.[218] NfL is reported to increase after cardiac, but not otolaryngeal surgery.[219] The increase in NfL concentration in blood after cardiac surgery was seen at 24 hours but was even higher seven days later. In the same study, myocardial infarction was not associated with increased NfL concentrations.[219]

As with tau, NfL has been studied in conjunction with diving and saturation exposure. Neither repeated open-water diving to 82–90 msw using trimix as breathing gas,[183] nor diving to 42 msw in a water-filled hyperbaric chamber elicited any changes in NfL concentrations in blood,[185] though in these two studies, no samples were obtained later than 2–4 days after exposure. In line with these findings were the results from the study where submariners were exposed to 401 kPa for 36 hours and then slowly decompressed over 70 hours.[184] No changes in NfL blood concentrations were seen, neither when sampled during increased ambient pressure nor directly, or one day after, hyperbaric exposure had ended with the last sample taken about 132 hours (5.5 days) after start of the initial compression.

Subjects participating in a breath-hold competition were sampled within one hour after each of three hypoxic events taking place within 16–21 hours after each other.[221] NfL concentrations in blood were not changed at any point when measured up to 38 hours of the first hypoxic event.

Glial fibrillary acidic protein

Glial fibrillary acidic protein (GFAp) is an intermediate filament protein (50 kDa) present almost exclusively in astrocytes and myelin-producing oligodendrocytes but also to a small extent in testes.[191] There are no differences in GFAp values between the sexes, which suggests that the testes are not an important source of GFAp. Astrocytes are part of the blood-brain-barrier (BBB)[194,229] and of the glymphatic system,[194] and GFAp is important not only for cellular structure but also for synaptic transmission and BBB integrity.[191,230,231] Based on results from animal research, it has been claimed that increased astrocytic activity, in response to stressors such as neurotrauma or ischemia, could result in increased concentrations of GFAp.[229,232,233] Astrocytes seem to respond to oxidative stress and astrogliosis is associated with increased levels of GFAp.[234]

[ee] Intermediate filaments are cytoskeletal proteins important for cellular mechanical strength. Intermediate filaments have a diameter of about 10nm, which is intermediate between two other important elements of the cytoskeleton, actin filaments (about 7nm) and microtubuli (about 25nm) (Herrmann 2016).

Increased concentrations of GFAp have been reported after boxing,[215] traumatic brain injury,[191,227] intracerebral haemorrhage,[235] and in neurodegenerative disease.[232] GFAp did not increase among runners who were tested after finishing a marathon.[236] In the aforementioned studies on open-water[183] and tank divers,[185] GFAp did not increase after diving. At one point after diving to 42 msw in a tank, GFAp was inexplicably decreased in one of the analyses. In the study on hyperbaric saturation exposure,[184] at one point, GFAp decreased in the non-exposed control group, a change that could not be explained by diurnal variation.

Calcium binding protein beta

Calcium binding protein beta (S100B) is a protein mainly expressed in astrocytes and Schwann cells but also in adipose and skeletal muscle cells.[236,237] It is therefore not specific to the CNS. S100B has been reported to increase after different forms of cerebral injury such as carbon monoxide poisoning,[238] cardiac arrest,[211,239] and traumatic brain injury.[237,240] Gross increases in plasma S100B could be attributed to either neuronal cell death or an impaired BBB. A study on 18 marathon runners without cerebral concussion reported significant increases in S100B after the race, probably due to musculoskeletal strain, as creatinine kinase (CK) was also increased.[236] When 11 athletes engaged in six sessions of structured HIIT over two weeks, S100B levels increased after the first as well as the last session;[204] CK values were not reported. A study on ice hockey players reported higher S100B values at one hour after concussion compared to pre-season,[216] and in a study on boxers, mean S100B was increased 1–6 days after a bout compared to results found after 14 days without boxing, and to controls.[215] However, no CK values were reported in these two studies. In another study on boxers, hits to the head were associated with an increased S100B, in contrast to hits to the torso, but due to elevated levels of CK the origin of S100B could not be determined indisputably.[241] In yet another study involving trauma to the head, there were no differences in serum levels of S100B between boxers and controls.[242]

In one study on nine highly trained breath hold divers and six healthy controls, S100B increased within 10 minutes after protracted apnoea among divers but not among controls.[243] However, in another study on 16 breath-hold diving competitors, S100B did not change after either static or dynamic apnoea.[221] In addition, S100B did not increase when five divers performed three identical dives each over two days, to a depth of 15 msw for 56 minutes.[187]

A study on 16 divers performing daily open-water no-decompression dives to 18 msw for four days found a significant increase in S100B but a concomitant increase in CK led the authors to conclude that the release of S100B might have been muscular and not cerebral in origin.[188] VGE were recorded using 2-dimensional ultrasound but no association between VGE and S100B was found which strengthened the notion of muscular release.

There was no difference in S100B concentration in blood among 59 divers with neurological DCS compared to 37 asymptomatic divers with comparable diving profiles.[189] Twenty-one patients treated for DCI in a hyperbaric chamber were sampled for S100B and CK, but neither analysis was significantly higher than expected in the healthy population.[190]

Neuron-specific enolase

Neuron-specific enolase (NSE) is a glycolytic enzyme mainly localised in neuronal cell cytoplasm, but it can also be found in neuroendocrine cells, oligodendrocytes, erythrocytes, and blood platelets.[191] Neuron-specific enolase increases in response to manifest neuronal injury such as cardiac arrest hypoxia,[211,239] and traumatic brain injury[191] but when NSE is studied in other contexts, results are

contradictory. No significant changes in NSE have been found among ice hockey players with concussions compared to preseason values,[216,217] although NSE was increased in boxers receiving hits to the head.[241,242] HIIT was associated with increased NSE levels in blood among 11 athletes after the first and the last of six training sessions made over two weeks.[204] In the study mentioned earlier concerning 59 divers with DCS,[189] NSE concentrations were significantly higher for divers with DCS compared to asymptomatic divers but in a the study on 16 divers that performed daily open-water no-decompression dives to a depth of 18 msw,[188] NSE was not increased after diving. Neuron-specific enolase values are increased in the presence of haemolysis, due to high NSE content in red blood cells.[191]

Ubiquitin C-terminal hydrolase-L1

Ubiquitin C-terminal hydrolase (UCH-L1) is a protein found in neuronal cell cytoplasm; though it is not specific to CNS, it can be found in PNS as well as in smooth muscle, neuroendocrine and endothelial cells.[191] There are reports that serum UCH-L1 increase in patients with traumatic brain injury.

Amyloid beta

Amyloid beta peptide is part of insoluble extracellular plaques seen in AD. Amyloid beta peptide was increased 30 days after TBI in a study on 34 patients and 69 controls.[212] Hypoxia has been implicated as a factor promoting plaque production, and Aβ peptide has been measured in 16 subjects taking part in a breath-hold competition. It was increased in blood after static apnoea but not after dynamic apnoea where the subjects swam during the breath-hold period.[221]

Decompression sickness and tau, NfL, GFAp and UCH-L1

An ongoing single-centre study (ClinicalTrials NCT03192956), is investigating changes in tau, NfL, GFAp and UCH-L1 before and after HBO therapy among divers with DCS.

Part II

The dissertation

Papers included

I. **Serum tau concentration after diving – an observational pilot study**
Rosén A, Oscarsson N, Kvarnström A, Gennser M, Sandström G, Blennow K, Seeman-Lodding H, Zetterberg H
Diving and Hyperbaric Medicine 2019;49(2):88–95.
PMID 31177514

II. **Biomarkers of neuronal damage in saturation diving – a controlled observational study**
Rosén A, Gennser M, Oscarsson N, Kvarnström A, Sandström G, Blennow K, Seeman-Lodding H, Zetterberg H
European Journal of Applied Physiology 2020;120(12):2773–2784.
PMID 32975632

III. **Protein tau concentration in blood increases after SCUBA diving: an observational study**
Rosén A, Gennser M, Oscarsson N, Kvarnström A, Sandström G, Seeman-Lodding H, Simrén J, Zetterberg H
European Journal of Applied Physiology 2022;122(4):993–1005.
PMID 35142945

IV. **Venous gas bubble load after immediate or delayed normobaric oxygen breathing post-decompression**
Gennser M, Blogg S L, Rosén A
Manuscript 2022.

Aims of the studies

Paper I

To determine changes in protein tau, GFAp, NfL and UCH-L1 concentrations after diving to depths of up to 90 msw and to explore any associations between these biomarker concentrations and VGE loads after the same dives.

Paper II

To determine whether concentrations in blood of protein tau, GFAp, NfL and UCH-L1 would increase during or after a saturation exposure at 401 kPa.

Paper III

To test the hypothesis that diving to 42 msw for 10 minutes would incur a change in protein tau, GFAp or NfL, and to investigate if there were associations between protein tau, GFAp or NfL concentrations in blood and VGE loads after the same dives.

Paper IV

To determine the effect of breathing normobaric oxygen for 30 minutes immediately after diving to 42 msw for 10 minutes on VGE load, and to assess if this effect changed when oxygen breathing was delayed by 15 minutes after diving ended.

Ethics

All studies were approved by Swedish ethical review authorities. The studies were also registered at ClinicalTrials.gov. Written informed consent was obtained from all participating subjects.

Ethical approvals:

Paper I	EPN Dnr 292-17
Paper II	EPN Dnr 022-17
Paper III	EPN Dnr 352-14 with supplementary approvals T847-15, T1032-18, 202-05525
Paper IV	EPN Dnr 352-14 with supplementary approvals T847-15, T1032-18

Registration at ClinicalTrials.gov:

Paper I	NCT03190252
Paper II	NCT03192930
Paper III	NCT02468752
Paper IV	NCT02468752

Methodology

Paper I

Design
Prospective observational cohort study

Location
Swedish armed forces (SwAF) naval base in Skredsvik, Sweden

Subjects
Ten professional male divers.

Intervention
The subjects performed one or two daily dives over four days. On the first day, two subjects dived to 34 msw and eight subjects dived to 50–52 msw. Dive depths increased over time and on the fourth day the subjects who had initially dived to 34 msw had reached 52 msw. The remaining eight subjects reached depths of 82–90 msw.

Air was breathed during dives up to 40 msw and mixtures of oxygen, helium and nitrogen ('trimix') were used for the deeper dives. Partial pressure of oxygen was 130 kPa during descent and at depth. In the last stage of decompression, an oxygen partial pressure of 160 kPa was accepted. Pure oxygen was breathed during 10 minutes after dives deeper than 60 msw.

Data collection
Venous blood samples were collected before the first and after the last dive. The eight deep divers were monitored for the presence of VGE up to 120 minutes after diving, using precordial DU; measurements were made at rest and after the subjects performed three vigorous knee bends. Results were scored using the KM grading system. KM data recorded during four days of diving were converted to an individual KISS value for each subject. All DU measurements were made by the same operator.

Analyses
Tau, GFAp, NfL, and UCH-L1 concentrations were measured, and statistical significance tested with Wilcoxon-signed rank test. Using Spearman's rank correlation test, maximum KM bubble grade (KM_{max}) values after the last dive were tested for correlation with tau concentration at the same point in time, while KISS values were tested for correlation with tau concentrations after the last dive and for correlations with absolute changes in tau from before to after the dives. For all tests, a probability value of 0.05 or less was considered significant.

Paper II

Design
Prospective, controlled cohort study

Location
His majesty's ship (HMS) Belos, SwAF naval base, Karlskrona, Sweden

Subjects
The intervention group consisted of 14 submariners from the SwAF.
The control group consisted of 12 subjects who either had passed a dive medical examination or were employed as Swedish Navy mariners.

Intervention
The submariners were compressed to 401 kPa in a dry hyperbaric chamber. They remained pressurized for 36 hours and were then slowly decompressed over 70 hours. The total duration of hyperbaric exposure was 106 hours.

Data collection
Venous blood samples were obtained from both groups before start of compression of the intervention group, shortly before start of decompression at 33–34 hours, and when hyperbaric exposure had ended. A fourth blood sample was obtained from subjects in the intervention group after a further 25–26 hours.

Towards the end of decompression, at 98 hours, subjects in the intervention group, then at a pressure of 131 kPa, were monitored for the presence of VGE using precordial DU. Monitoring with DU continued for three hours after the final decompression with 30 minutes intervals. All DU measurements were made by the same operator.

Analyses
The concentrations of tau, GFAp, NfL, and UCH-L1 and albumin concentrations in blood were measured at all sampling points. Haemoglobin (Hb) and haematocrit (Hct) were measured in the intervention group before and directly after hyperbaric exposure.

For changes of tau, GFAp, and NfL within each group, Fisher's non-parametric permutation test for matched pairs was used. For the same comparison between the two groups, Fisher's non-parametric permutation test was used. Hb, Hct, and albumin concentrations obtained during the study were compared to baseline concentration for the relevant group using the Wilcoxon signed-rank test. In comparisons of albumin concentrations between groups, the Mann-Whitney U test was used.

For all tests, a probability value of 0.05 or less was considered significant.

As no VGE were found, no analyses of this parameter were possible.

Paper III

Design
Prospective observational cohort study

Location
SwAF diving and naval medicine centre (DNC), Karlskrona, Sweden

Subjects
Thirty-two professional divers employed at SwAF, the Swedish coast guard (SCG) or the Swedish police (SP).

Intervention
Study subjects performed two identical dives in a water-filled hyperbaric chamber pressurised to an equivalent of 42 msw for 10 minutes. A three minutes safety stop at an equivalent to five msw was included at the end of each dive. The dives were separated by a 48-hour interval. Air was used as breathing gas during dives.

The study was carried out in two sets, each involving 16 divers. In the first set, each diver randomly breathed either air or normobaric oxygen for 30 minutes after the first dive, beginning immediately after surfacing. After the second dive, air or oxygen was again breathed for 30 minutes, but the breathing gas was switched for each subject. In the second set, breathing of normobaric oxygen or air for 30 minutes was deliberately delayed and started at 15 minutes after each dive. Oxygen was randomly breathed after one dive and air after the other, exactly as in the first set. Both the divers and experimental personnel were blinded to which breathing gas was used after a particular dive.

Data collection
Three blood samples were obtained at each dive, before, at 30, or 45 minutes after diving depending on study set, and at 120 minutes after diving.

The presence of VGE was monitored with precordial DU every five minutes during the first 30 minutes and every 15 minutes thereafter for a further 90 minutes post-dive. DU measurements were made at rest and after the subjects had performed three vigorous knee bends. Results were scored using the KM grading system. For each subject, all KM grades collected during 0–30 minutes and 0–120 minutes after diving were converted to KISS: $KISS_{30min}$ and $KISS_{120min}$. All DU measurements were made by the same operator.

Analyses
Tau, GFAp, and NfL concentrations in blood before and after each dive were compared. Samples obtained before the second dive were used not only in comparisons to results after the second dive, but also as a fourth, late sample 48 hours after the first dive. Tau, GFAp and NfL concentrations were also analysed according to breathing gas used, without regard to dive order.

To assess the effect of diving 48 hours prior to the second dive on tau, GFAp, and NfL concentrations, samples obtained before the first and second dive were compared, with subjects breathing oxygen or air after the first dive analysed separately.

Fisher's non-parametric permutation test for paired observations was used for all analyses described above.

To assess the effect of breathing normobaric oxygen on tau, GFAp, and NfL, changes in their concentrations after dives followed by oxygen breathing were compared to matching results obtained after dives with air breathing afterwards, using Fisher's non-parametric permutation test.

Both absolute values for tau, GFAp, and NfL concentrations at 30 or 45 and 120 minutes after diving and absolute changes in GFAp, NfL, and tau concentrations at these points were tested for correlation with KM_{max} after knee bends and for correlation with KISS-values for 0–30 and 0–120 minutes after diving ($KISS_{30min}$ and $KISS_{120min}$), using Spearman's rank correlation test.

For all tests, a probability value of 0.05 or less was considered significant.

Paper IV

Design
Prospective, double-blinded, cross-over trial

Location
SwAF DNC, Karlskrona, Sweden

Subjects
Forty-eight professional divers employed at SwAF, SCG or SP.

Intervention
Study subjects performed two identical dives in a water-filled hyperbaric chamber pressurised to an equivalent of 42 msw for 10 minutes. A three minutes safety stop at an equivalent to 5 msw was included at the end of each dive. The dives were separated by a 48-hour interval. Air was used as breathing gas during dives.

The study was carried out in three sets, each involving 16 divers. In the first set, each diver randomly breathed either air or normobaric oxygen for 30 minutes after the first dive, beginning immediately after surfacing. After the second dive, air or oxygen was again breathed for 30 minutes immediately after surfacing, but the breathing gas was switched for each subject. In the second and third sets, a 30-minute bout of normobaric oxygen or air breathing was deliberately delayed, instead being started at 15 minutes after each dive. Oxygen was randomly breathed after one dive and air after the other, exactly as in the first set. Both divers and experimental personnel were blinded to which breathing gas was used after a particular dive.

Data collection
The presence of VGE was investigated with precordial DU every five minutes during the first 30 minutes and every 15 minutes thereafter for a further 90 minutes. DU measurements were made at rest and after subjects had performed three vigorous knee bends. Results were scored using the KM grading system. For each subject, all KM grades collected during the first 75 minutes after oxygen or air breathing had ended were converted to KISS: $KISS_{30\text{-}105minutes}$ when oxygen or air breathing began immediately, and $KISS_{45\text{-}120minutes}$ when use of breathing gas was delayed for 15 minutes. All DU measurements were made by the same operator.

Analyses
Subjects were used as their own controls in analyses. Therefore, only subjects who had VGE after at least one dive could be included in the comparisons. The proportion of subjects in each group with VGE was compared using Fisher's exact test. In comparisons involving KM_{max} and KISS results, Wilcoxon signed rank test was used for analyses within groups while the Mann-Whitney U test was used in comparisons between groups. For all tests, a probability value of 0.05 or less was considered significant.

Results

Paper I

Tau
Protein tau concentration increased after deep open water diving.

The mean value of tau was 0.322 pg/mL (standard deviation [SD] 0.315 pg/mL) before diving, and 0.500 pg/mL (SD 0.337 pg/mL) after the last dive on the fourth day (p=0.016). Median tau concentration also increased, from 0.200 pg/mL (range 0.100–1.10 pg/mL) before to 0.450 pg/mL (range 0.100– 1.20 pg/mL) after diving. The relative change in mean tau concentration was 98.8%.

No correlations were found between serum tau protein concentrations after diving with either KM_{max} or KISS values.

GFAp and NfL
No significant changes in GFAp or NfL concentrations were seen. Results of UCH-L1 analyses had a high level of imprecision and could therefore not be used.

Paper II

Tau, GFAp and NfL
No significant changes in tau, GFAp or NfL concentrations were found at any point in the intervention group, which had been exposed to an increased ambient pressure.

In the unexposed control group, GFAp was decreased when the second sample was taken at 33–34 hours (p<0.01) shortly before slow decompression of the intervention group started. At this point, there were also significant differences in mean absolute changes of GFAp and NfL between the two groups (p=0.02 for both proteins), though NfL never changed significantly within neither group. GFAp concentration in the control group increased in the third and last sample taken, and no further differences between the groups were seen, neither regarding GFAp nor NfL. Protein tau did not change significantly at any point. Results of UCH-L1 analyses were to imprecise to be used.

Haematocrit, haemoglobin and albumin
Mean Hct had increased from 45.6% to 47.9% (p=0.02) among subjects in the intervention group after hyperbaric exposure but Hb and albumin remained unchanged. In the control group, albumin concentration was decreased from 47.6 g/L to 44.9 g/L (p=0.02) at 33–34 hours but increased to 45.8 g/L (n.s. compared to 47.6 g/L) at 105–108 hours, when the intervention group left the hyperbaric chamber. There were no significant differences in albumin concentrations between the groups at any point.

Venous gas emboli
No VGE were detected at any point.

Paper III

Tau

Protein tau concentration in blood was significantly increased at 120 minutes after both dives.

When the subjects' results were analysed as one group, irrespective of breathing gas used after a dive, tau concentrations in the blood increased similarly after both dives being highest at 120 minutes after diving. The increases were statistically significant at 30–45 minutes after the second dive ($p<0.01$), and at 120 minutes ($p<0.01$ / $p<0.01$) after both dives. The relative increase in tau concentration was 29.1% (SD 44.7%) after the first, and 33.9% (SD 81.7%) after the second dive. Mean tau increase at 120 minutes after all 64 dives was 31.5% (SD 66.4%).

One subject had a deviant tau increase of 428% at 120 minutes after one dive. When this result was excluded, the mean tau increase for all other subjects was 25.2% (SD 43.7%) at 120 minutes after diving. Absolute tau concentrations were 2.18 pg/mL (SD 1.47) and 2.23 pg/mL (SD 1.56) at 120 minutes after the first and second dive, respectively.

Dives with oxygen and air breathing following the dive were also analysed separately, with mean tau concentrations being significantly increased at 30–45 minutes following dives with oxygen breathing ($p=0.03$) and at 120 minutes regardless of breathing gas used after diving ($p<0.01$ / $p<0.01$).

GFAp

Glial fibrillary acidic protein concentrations were significantly decreased at 30–45 minutes ($p=0.04$) but not at 120 minutes after the first dive, if subjects were analysed as one group irrespective of post-dive breathing gas used. No significant changes in GFAp concentrations were found after the second dive. When analysed based on post-dive breathing gas, no significant changes in GFAp were found.

NfL

No significant changes in NfL concentrations were found in any analyses.

Effect of breathing oxygen

Comparing samples taken after diving when breathing air to matching samples with oxygen showed that there was no effect of normobaric oxygen breathing after diving on tau or NfL concentrations. No differences in GFAp concentrations were seen at 30–45 minutes post-dive between subjects breathing oxygen or air, but GFAp concentrations were significantly higher at 120 minutes after diving for subjects breathing air compared to oxygen ($p=0.04$).

Residual effect of the first dive

Samples taken before the first and second dive were compared, separately for subjects breathing air and oxygen after the first dive, but no differences were found. Hence, there were no residual effects after the first dive. After 48 hours, tau increases noted after the first dive had returned to values observed before the dive, and concentrations of tau, GFAp and NfL were similar before each of the two dives.

Venous gas emboli

Neither tau, GFAp or NfL concentrations nor their changes were at any point correlated with VGE_{max} after flexing the legs or KISS values.

Paper IV

There was a significant decrease in VGE load when oxygen was breathed after diving. Results are shown in Table 1 in Paper IV.

Immediate oxygen breathing

In three of 16 divers, neither dive resulted in detectable VGE, thus these divers' data could not be used in the analyses. Of subjects who breathed oxygen for 30 minutes immediately after diving 2/13 (15.4%) had VGE after it ended compared to 11/13 (84.6%) of subjects breathing air. The absolute reduction of VGE rate at 30 minutes after diving was 69.2% after immediate oxygen breathing ($p=0.0021$).

Median maximum KM grades for the whole measurement period after diving, 120 minutes, were 0 both at rest and after flexing the legs among subjects breathing oxygen, and were III- and III, respectively, among subjects breathing air. The differences in median maximum KM grades between the groups were significant ($p=0.022$ for rest measurements and $p=0.016$ for measurements after flexing the legs). Median maximum KM grades during 75 minutes after oxygen or air breathing had ended, 30–105 minutes after the dive, were 0 both at rest and after flexing the legs among subjects that had breathed oxygen, and were II and III, respectively, among subjects that had breathed air. The differences in median maximum KM grades between the groups were significant ($p=0.0012$ for rest measurements and $p=0.0034$ for measurements after leg flexions).

KISS were significantly lower after dives followed by oxygen breathing. Results are shown in Table 7.

Delayed oxygen breathing

In eight of 32 divers, neither dive resulted in detectable VGE, thus their data could not be used in the analyses. When oxygen breathing was delayed to 15 minutes after diving, 9/24 (37.5%) had VGE after 45 minutes compared to 17/24 (70.8%) among subjects breathing air, giving an absolute reduction of VGE of 33.3% for delayed oxygen breathing ($p=0.042$).

There were no differences in median maximum KM grades between the studied groups before delayed breathing of either oxygen or air began.

Median maximum KM grades for the whole measurement period after diving, 120 minutes, were II at rest and III after flexing the legs among subjects breathing oxygen, and between III- and III at rest and KM III after flexing the legs among subjects breathing air. The differences between the groups were not statistically significant.

KM grades obtained after the delayed oxygen or air breathing had ended were also compared. During the period 45–120 minutes after diving, median maximum KM grades were 0 at rest and 0 after flexing the legs among subjects that had breathed oxygen, and were 0 and III- at rest and after flexing the legs, respectively, among subjects that had breathed air. Again, the differences between the groups were not statistically significant.

KISS were significantly lower after dives followed by delayed oxygen breathing compared to dives followed by air breathing. Results are shown in Table 7.

Comparison between the effects of immediate and delayed oxygen breathing

Median maximum KM grades and KISS scores were, with one exception, similar for dives where air was breathed afterwards, regardless if it was begun immediately or was delayed 15 minutes. Median maximum KM grades collected during the whole measurement period, 0–120 minutes, were

significantly lower when oxygen breathing was initiated immediately and not delayed for 15 minutes ($p<0.05$ for measurements both at rest and after flexing the legs) but there were no statistically significant differences in median maximum KM grades registered during the 75 minutes following oxygen breathing.

When KISS calculated for the first 75 minutes following immediate or delayed oxygen breathing, $KISS_{30\text{-}105minutes}$ and $KISS_{45\text{-}120minutes}$, respectively, were compared to each other, no statistically significant differences in VGE loads were found between the two regimens. Though, there were statistically significant differences in KISS values between the two groups when the whole measurement periods, 0–120 minutes after diving, were compared, both at rest ($p<0.05$) and after flexing the legs ($p<0.01$).

0–120 minutes		
Immediate oxygen breathing	**rest**	**flex**
Air	5.5 (0–48.7)	19.8 (0–54.7)
	11.8 ±15.3	19.8 ±16.6
Oxygen	0 (0–14.1)	0 (0–21.4)
	1.3 ±3.9	2.2 ±5.9
p-value	0.0041	0.0022
Delayed oxygen breathing	**rest**	**flex**
Air	6.4 (0–49.9)	17.0 (0–54.0)
	11.7 ±13.6	18.3 ±16.9
Oxygen	0.9 (0–26.2)	3.6 (0–35.8)
	5.4 ±8.1	9.4 ±11.6
p-value	0.020	0.026
30–105 minutes / 45–120 minutes		
Immediate oxygen breathing	**rest**	**flex**
Air	5.9 (0–49.9)	13.9 (0–56.1)
	11.4 ±15.3	20.1 ±17.1
Oxygen	0 (0–7)	0 (0–16)
	0.7 ±2.0	1.2 ±4.4
p-value	0.005	0.0034
Delayed oxygen breathing	**rest**	**flex**
Air	3.6 (0–45.7)	12.6 (0–53.0)
	8.6 ±12.7	15.9 ±16.9
Oxygen	0 (0–22.6)	0 (0–30.7)
	2.5 ±5.8	6.1 ±10.0
p-value	0.015	0.016

Table 7 *Comparisons between median (range) and mean (±SD) KISS after air and oxygen breathing, at rest and after flexing the legs. For comparisons between immediate and delayed oxygen breathing regimens, see Table 1 in Paper IV.*

Discussion

Protein tau

In the present dissertation, protein tau concentrations in blood increased after deep open water diving (Paper I), and also after repeated diving in a water-filled hyperbaric chamber (Paper III), though no changes in tau concentrations were detected neither during nor after a saturation exposure in a dry pressure chamber (Paper II). No associations between changes in tau and VGE loads were found (Papers I and III).

Why was tau increased after diving?

Protein tau concentration could have increased in blood after diving due to:

- neuronal damage,
- increased tau release from neuronal cells,
- increased transport or diffusion of tau out of the CNS,
- decreased elimination of tau,
- increased release of tau from the PNS, or
- increased release of tau from outside the nervous system.

Dehydration, which is common after diving, may be a possible confounding factor that increased tau blood concentration. Another potential cause of change in tau that is not related to diving exposure is diurnal variation, if this factor has an effect on tau.

Neuronal damage?

It was most likely not frank neuronal damage that caused an increase in tau after diving. Only deep saturation diving, neurological DCS or CAGE have been convincingly associated with injury to the CNS, and neither of these factors affected participating subjects in Papers I–III. Although absolute tau values cannot be compared reliably between studies, the concentrations of tau observed after diving in Papers I and III were much lower than those reported after traumatic brain injuries and cerebral hypoxia. If neuronal cell damage caused the increase in tau in Papers I and III, it is likely that GFAp and NfL would increase as well, which was not the case, although sampling might have been performed too early to detect changes in NfL.

Studies where MRI results of divers have been compared to those of controls have not been conclusive for brain damage after ordinary diving. Abnormal neuropsychometric test results observed in divers are not necessarily caused by brain damage, and may be due to factors other the diving in itself. There is one published study on tau and DCS, but it reports that only one of seven subjects had neurological DCS, which makes it impossible to draw any reliable conclusions in this regard.

In summary, there is no convincing evidence that diving causes brain damage and thereby a rise in tau.

Increased release of tau from neuronal cells?

Tau is reported to be released from neuronal cells in response to both physiological and pathophysiological stimuli. Diving could potentially affect the nervous system, for example through increased gas pressures, changes in ambient pressure or changes in cerebral perfusion. Increases in tau seen after diving may thus be caused by neuronal stress with release of tau from intact cells.

Increased transport or diffusion of tau out of the central nervous system?
It is not known whether the increase in tau observed after diving is caused by increased BBB permeability for tau, or increased clearance by the glymphatic system, but both mechanisms are possible theoretically. The fact that neither GFAp nor NfL increased in the same way as tau in Papers I and III makes increased glymphatic clearance from the CNS less probable, and unchanged blood levels of GFAp suggest that BBB dysfunction is not responsible.

Decreased elimination of tau?
Decreased elimination of tau could hypothetically result in increased blood concentrations. Published studies regarding the effects of diving on liver[244,245] and kidney[246,247] functions are few and mostly concern saturation dives. Applied pressures range from 0.56–6.7 MPa. As an exception, one study investigated the effects of high altitude diving on liver function.[248] Nevertheless, given the available data, there is no obvious mechanism that would alter tau elimination in blood after the hyperbaric exposures employed in Papers I–III.

Increased release of tau from the peripheral nervous system?

The increase in tau observed after diving could be caused by a release of tau from the PNS. In future studies, measurements of 'big tau' would make it possible to assess the impact of diving on the PNS.

Increased release of tau from outside the nervous system?

Increased release of tau from the kidneys or testes could theoretically cause an increase in blood concentrations of tau. A few studies report on hormonal concentrations and semen quality in relation to hyperbaric exposure,[249-251] but there are no data which show that diving causes a release of tau from kidneys or testes.

Central or peripheral neuronal damage?

Alpha-internexin is a neurofilament found mainly in the CNS,[252] while Peripherin is a neurofilament present mostly in the PNS and in CNS neurons with peripheral projections.[252,253] In future studies, they could potentially be used a markers of neuronal damage specific to the CNS and PNS respectively.

Dehydration?

Diving can result in dehydration. Paper II was designed to control for changes in subject hydration status. The absolute increase in Hct was 2.3% but Hb and Alb were unchanged. Significant dehydration was considered unlikely. No neuronal biomarkers increased in Paper II, but GFAp was decreased at one point. In Papers I and III, dehydration was not controlled for, but the periods of exposure were much shorter. If dehydration occurred after the dives, it would be expected to have caused an increase not only in tau but in GFAp and NfL as well, which was not seen.

Diurnal variation?

The question of diurnal variation of tau as a potential confounding factor was discussed in Paper I. Studies where tau has been sampled repeatedly in CSF do not report any significant diurnal variation of protein tau. In Paper II, all samples, except at baseline, were made at approximately the same time of the day, which made diurnal variation less likely as a confounding factor. In Paper III the dives were short and all samples were taken within two hours of each other, which excluded diurnal variation as a plausible intraindividual confounding factor. However, as the experimental dives were spread out over whole days, it is possible theoretically that subjects sampled in the afternoon had different baseline values than morning subjects, which could have biased the results.

What stimulus caused tau to increase?

Stimuli that could potentially cause the increases in tau observed after diving were:

- increased partial pressure of oxygen,
- increased partial pressure of nitrogen,
- breathing of helium,
- immersion effects on the circulation with an increased cerebral perfusion,
- decompression stress
- increased pressure *per se*.

	Paper I	Paper II During hyperbaric exposure	Paper II After hyperbaric exposure	Paper III
Number of exposed subjects	10	14	14	32
Relative increase in tau	98.8%	0	0	Dive one 29.1% Dive two 33.9% Mean both dives 31.5%
Exposure	620–1000 kPa[1]	401 kPa	401 kPa for 36 hours. Decompression over 70 hours	521kPa
Exposure duration	10–20 minutes[1]	33–34 hours	106 hours	10 minutes
Number of exposures	3–5 dives over 4 days	One (saturation)	One (saturation)	2 dives separated by 48 hours
Nitrogen pressure in breathing gas at depth (approximate)	176–202 kPa[2]	351 kPa[3]	Decreasing from 351 kPa to 78 kPa	406 kPa
Oxygen pressure at depth	130 kPa (160 kPa during final decompression)	50 kPa	Never exceeding 50 kPa	109 kPa
Immersion in water	Yes	No	No	Yes
Helium in breathing gas	Yes	No	No	No

Table 8 *Physical stimuli in Papers I – III.*
1: Eight out of the 10 divers reached a depth of 82–90msw (920–1000 kPa) and remained there for 20 minutes. The remaining two divers reached 52 msw (620 kPa) and remained there for 10 minutes.
2: Oxygen partial pressure was held at 130 kPa at depth. The diluent gas used at depths between 40–65 msw contained 50% helium and 35% nitrogen and at depths deeper than 65 msw it contained 70% helium and 20% nitrogen. ((620 – 130) x 35/85 = 202, (1000 – 130) x 20/90 = 193 and (920 – 130) x 20/90 = 176.
3: Oxygen content was reduced to 12.5% at depth (12.5 x 401 = 50, rounded off) and nitrogen content proportionally increased to approximately 87.5%, which gives a nitrogen partial pressure at depth of about 351 kPa (87.5 x 401 = 351, rounded off).

It should be remembered that observed changes in tau may have been caused by combined effects, synergistic, additive, or otherwise interlinked. It is also possible that change in tau is not linear in relation to exposure, or that there is a stimulus threshold for tau release.

Increased partial pressure of oxygen

Based on observed effects of oxygen on the lung, it is considered that an oxygen partial pressure of less than 50 kPa is harmless to humans. High partial pressures of oxygen are known to be toxic to neurons and could induce seizures when partial pressures exceed 160 kPa, but it is possible that the brain is affected before clinical manifestations become obvious. There are reports that diving causes oxidative stress, but it is not known whether it influences tau levels in blood. Biomarkers indicative of oxidative stress were not analysed in any of the Papers (I–III). In Paper II, oxygen partial pressure did not exceed 50kPa and tau did not change. During diving, tau changed more for subjects exposed to an oxygen partial pressure of 130 kPa during diving, and up to 160 kPa while decompressing (98.8%, Paper I), than for those exposed to 109 kPa (31.5%, Paper III). However, the statistical analyses in Paper III showed that tau concentration in blood was not influenced by normobaric oxygen breathing after diving. The relationship between tau concentration in the blood and exposure to partial pressures of oxygen higher than 100 kPa remains to be investigated, but it seems that oxygen partial pressures of that value or a lower magnitude do not affect the brain.

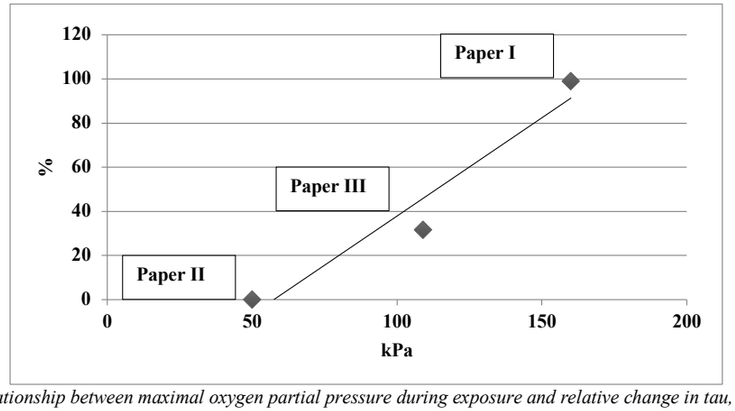

Figure 4 *Relationship between maximal oxygen partial pressure during exposure and relative change in tau, in Papers I–III.*

Increased partial pressure of nitrogen

It could be speculated that increased partial pressures of nitrogen may stimulate neuronal cells and thereby promote release of tau, but that notion is not consistent with results from Papers I–III. Subjects had the longest exposure to increased nitrogen pressures during the saturation study (Paper II), where no change in tau was found at all. The highest nitrogen pressure was experienced during the 42 msw dives (Paper III), followed by the saturation exposure (Paper II), and the lowest nitrogen pressures were present in the deep open water dives (Paper I) where tau increase was the largest. Thus, in this dissertation with its limited sets of observations, tau changes cannot be explained by exposure to increased partial pressures of nitrogen.

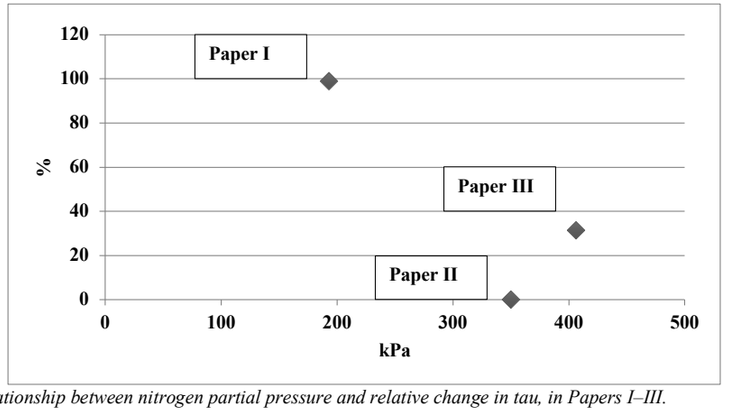

Figure 5 *Relationship between nitrogen partial pressure and relative change in tau, in Papers I–III.*

Breathing of helium

Helium was used as a breathing gas in Paper I only, and it cannot be judged if that impacted the changes found in tau. Helium has no known narcotic properties at the dive depths reached in Paper I.

Immersion effect

Cerebral perfusion increases after immersion, which might affect tau release. In-water diving and dry hyperbaric chamber exposures reportedly give rise to different VGE loads even when ambient pressure is the same, with a greater VGE load seen after diving in water. In the two experiments where tau increased, (Paper I and Paper III) dives were performed in the water, while the study included in Paper II was performed in a dry hyperbaric chamber. Although no correlation between tau and VGE was found, is it possible that this difference in exposure also affected tau changes.

Decompression stress

There were no associations between tau and VGE found in Papers I and III, although increases in tau and VGE were observed, whereas in Paper II neither VGE nor tau were seen to be increased. There is a possibility that the lack of association between tau and KM_{max} are false negative findings due to small sampling sets and a narrow distribution of VGE data, especially in Paper I. In both Papers I and III, VGE recordings were made for up to 120 minutes, on eight subjects in Paper I and 32 subjects in Paper III. Association between VGE and tau was tested after each dive in Paper III, while in Paper I only KM_{max} recorded after the last dive was tested for association with tau sampled after a total of four days diving, though it should be noted that the last dive was the deepest and most stressful.

KISS values are calculated to reflect the integrated VGE load over a certain time, but even so, no associations between KISS and tau values were observed in either Paper I or Paper III. The distribution of KM_{max} recorded after deep open water dives are shown in Figures 3 and 4 in Paper I, and distribution of KM_{max} recorded after flexing the legs in Paper III are shown in Figure 6 below. Most (45%) of all bubble grades recorded in Paper III were KM 0, followed by KM III (25%), KM III- and III+ (both 12.5%) and a few KM II (5%). This means that in cases where VGE were observed, 23% of the bubble grades were KM III+, 46% were KM III and 31% were either or KM III- or KM II.

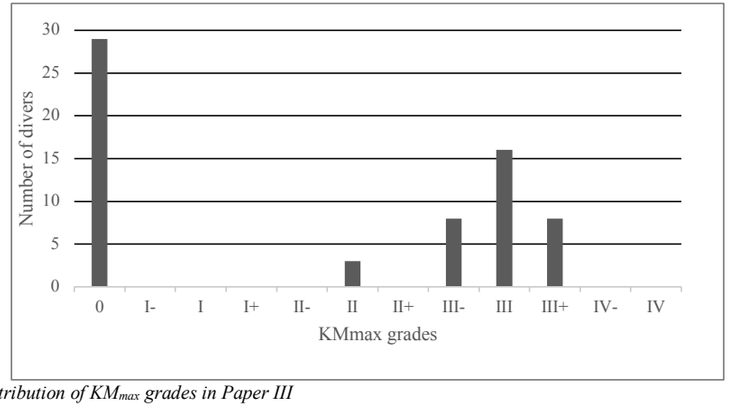

Figure 6 *Distribution of KM_{max} grades in Paper III*

In short, tau changes in Papers I and III were not associated with VGE or KISS values. These data suggest that it is unlikely that tau increases observed after diving were due to decompression stress or subclinical DCS, although it is possible that a larger dataset with a wider range of KM grades would have yielded statistically significant associations between VGE load and change in tau.

Effect of increased pressure

The manifestations of HPNS proves that the CNS can be affected by pressure alone. Overt symptoms of HPNS have only been observed at depths greater than ~1.6MPa, but it is possible that the nervous system is subclinically affected at shallower depths. In Papers I–III, tau increased more as the depth of exposure increased. Although a 36-hour exposure to 401 kPa followed by slow decompression (Paper II) did not result in increased tau levels, 10–20 minutes-long exposures to 521–1000 kPa (Paper I and III) did. Based solely on these three observations, the increases in tau concentrations observed were more likely related to maximum ambient pressure, with time of exposure being of less importance, though such a notion must be regarded as speculative given the small sample sizes.

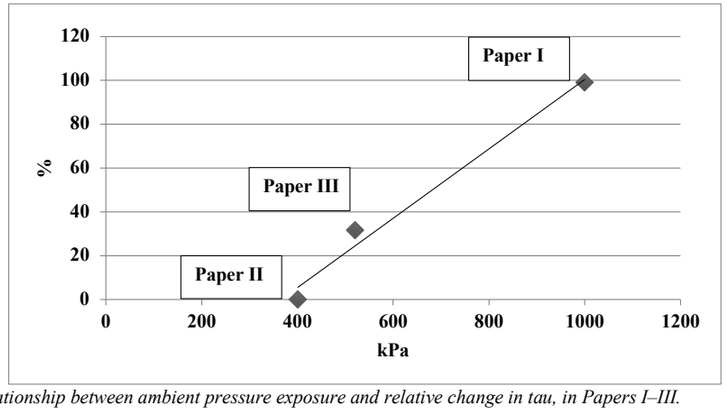

Figure 7 *Relationship between ambient pressure exposure and relative change in tau, in Papers I–III.*

Was there a difference between diving to 42 or 90 metres?

Absolute changes in tau values after deep open-water dives (52–90 msw, 620–1000 kPa, Paper I) and chamber dives (42 msw, 521 kPa, Paper III) could not be compared statistically, because the results came from different batches of samples. When the relative changes in tau (%) observed after these markedly different dive exposures were compared using the Mann-Whitney U test for independent samples, the probability value obtained was 0.075. Hence, in terms of statistics, there was no significant difference in relative change of tau concentration in blood after diving in a hyperbaric chamber to 42 msw (31.5%) compared to open water dives to 52–90 msw (98.8%). However, it should be remembered that the sample size of open water deep divers in Paper I was small, with only eight subjects reaching depths of 82–90msw. For the study in Paper III, four times as many subjects were recruited who performed identical dives in a very controlled environment, thus, the results of Paper III could arguably be seen as more robust. Given the small dataset in Paper I, chance may have influenced the probability value yielded and at what probability level a difference should be acknowledged is a matter of convention. The differences observed between the two exposures remain interesting and relevant, not least for the generation of hypotheses regarding tau and diving.

Do incorrect sampling times lead to incorrect results?

In the diving studies, tau was sampled twice within 30–120 minutes after diving (Paper III) or at about 120–180 minutes after the last dive (Paper I), whereas in the study on saturation diving (Paper II), the first sample after baseline was made at around 33–34 hours after compression while the subjects were still under pressure. When tau was measured in conjunction with anaesthesia and surgery, maximum tau values were reported at the end of cardiac surgery and within six hours of orthopaedic surgery, after which they decreased. In Paper III, tau had increased within two hours after diving. It is possible that there was a transient tau increase after compression in the saturation study (Paper II), but that tau levels then decreased and reached baseline levels before sampling after 33–34 hours. Results from Paper III show that tau, in a normobaric environment, returns to baseline within 48 hours, but the process might be much faster and completed within 33–34 hours, even in the presence of an increased ambient pressure.

GFAp

Results for GFAp have been difficult to interpret because they do not fit into one pattern. GFAp increases were not observed after diving or hyperbaric saturation exposure, which implies that diving would not affect or damage astroglial cells, or the BBB. However, GFAp was seen to be decreased at one point after diving in Paper III, and at one point among non-exposed subjects in the control group in Paper II. These decrements had no obvious cause, such as diurnal variation, or pre-analytical sampling error. GFAp is a well-established biomarker, and it was analysed in a reliable facility. Thus, these spurious decreases in GFAp are conundrums.

NfL

NfL values did not change after diving or hyperbaric saturation exposure, suggesting that frank axonal damage did not occur, but interpretation is hampered by the fact that samples were obtained too early to definitely rule out an increase in NfL. Maximum values of NfL could be expected no earlier than around 10–12 days after an insult. However, significant increases have been reported previously six hours after anaesthesia in conjunction with orthopaedic surgery, and within 24 hours after cardiac

surgery. In this dissertation, the last samples were obtained either two (Paper III), four (Paper I) or five days (Paper II) after hyperbaric exposure.

Oxygen breathing after diving

Oxygen breathing after diving effectively reduced VGE load. These findings are in line with earlier reports and consistent with physiological knowledge of the behaviour of inert gas bubbles. Strengths of the study were that it was blinded and that the subjects served as their own controls.

Paper IV documented and quantified experimentally the positive effect of oxygen breathing, which was shown to be considerable. If oxygen breathing was initiated immediately after surfacing, only 15% of divers had VGE at the end of the administration period compared to 85% of divers breathing air, giving an absolute VGE reduction of 70%. When oxygen breathing was delayed by 15 minutes, the positive effect was diminished, as 38% of subjects had VGE at the end of oxygen administration compared to 71% of subjects breathing air. The absolute VGE reduction was 33% for delayed breathing of oxygen.

Number needed to treat (NNT) is a clinical concept used to describe how effective a certain treatment is when the outcome measure of interest is dichotomous; it is often explained as the number of patients that need to be treated in order to achieve a favourable outcome for one patient.[254-256] However, it is important to appreciate that correct use of NNT requires that the baseline risk of patients, the intervention and comparator, the outcome measure, and the follow-up period are all clearly stated. Number needed to treat is calculated as 1/absolute risk reduction.[254] To illustrate the effect of oxygen breathing on VGE load, NNT for immediate oxygen breathing after diving was calculated to be 1/ (0.85-0.15) = 1.43, which was rounded up to 2. Number needed to treat for delayed oxygen breathing, starting after 15 minutes, was 1/ (0.71-0.38) = 3.03, which was rounded up to 4. Both NNT 2 and NNT 4 could be regarded as low values, which suggests that breathing normobaric oxygen for 30 minutes is an effective method to eliminate VGE in blood after diving to 42 msw for 10 minutes. Though, it may be necessary to prolong the period of oxygen breathing beyond 30 minutes after more extensive dives because tissues that have longer saturation half-times will have taken up more nitrogen, which takes longer time to release.

Both immediate and delayed oxygen breathing significantly reduced KISS values, and no difference was seen between KISS for the 75-minute period following the two different oxygen breathing regimens ($KISS_{30\text{-}105minutes}$ and $KISS_{45\text{-}120minutes}$). Still, KM grades were significantly lower for subjects that breathed oxygen compared to those that breathed air only when oxygen had been administered immediately after diving and the proportion of divers with VGE was less after immediate oxygen breathing than after delayed oxygen breathing. When the whole measurement period was considered, both KM grades and KISS were lower when oxygen breathing was initiated immediately compared to when it was delayed by 15 minutes. These findings indicate that oxygen administration should start as soon as possible after diving to be most effective.

It is possible that the effect of oxygen breathing was diminished once VGE had formed, as growing bubbles absorb surrounding inert gas and thus, decrease inert gas partial pressure in the tissues. In turn, this reduces the inert gas available for diffusion and delays elimination of inert gas out of the body.

Venous gas emboli will shrink and naturally disappear, which means that the risk of overestimating the effect of any intervention used to reduce bubble loads will increase over time. It is possible that the apparent effect of delayed oxygen breathing was augmented because it continued for 15 minutes longer than the immediate oxygen breathing.

It is likely that in ordinary diving environments and situations, the immediate administration of oxygen will be impossible or difficult, whether that be due to lack of an oxygen source or to logistical barriers such as divers being involved in moving their equipment from a boat to shore causing a delay, for example. The results from Paper IV showed that oxygen breathing administered within 15 minutes after surfacing would still reduce VGE load, and thus is worthwhile.

The risk of DCS is positively associated with VGE load after diving. Reducing VGE load could therefore be expected to reduce the risk of DCS. Oxygen breathing after diving is already, to an unknown extent, used by both professional and recreational divers to increase nitrogen elimination, reduce surface interval time, and reduce the risk of DCS.

Exposure to oxygen partial pressures above 50 kPa are considered injurious to the lung, and oxygen partial pressures above 160 kPa could cause seizures. In Paper IV, normobaric oxygen was breathed for only 30 minutes, yielding an UPTD of 30, a dose that could be considered negligible in terms of lung damage; as normobaric oxygen was administered there was no risk of seizures.

Shortcomings

The interpretation of this dissertation is hampered by the fact that Paper I was based on a small sample of 10 subjects, of whom eight only were exposed to pressures between 920–1000 kPa. When tau increased (Papers I and III), the lack of sampling points made it impossible to determine both maximum values of tau and to describe changes in tau after diving in detail. The fact that samples were taken at different points in time in Papers I, II and III precluded comparisons of tau concentrations between the studies. Dehydration was only controlled for in Paper II, not in Papers I, III and IV, and oxidative stress was not assessed in any of the Papers. The lack of control groups in Papers I and III weakens the results. That no NfL samples were collected 10–12 days after diving or saturation exposure is also a shortcoming, because any potential increases might only have been demonstrable at this time point and may have been missed in the present studies.

Conclusions

Protein tau

Protein tau increases in blood after diving, making it a promising marker of dive-related stress that is presumably neuronal in nature. The cause of this increase is unknown, but could be due to increased ambient pressure, exposure to hyperbaric oxygen, or increased cerebral perfusion secondary to immersion in water. The increase in tau concentration is probably not associated with VGE load, or nitrogen partial pressures, and there is no evidence that increases in tau concentrations seen after diving reflect frank neuronal injury.

When studying changes in biomarkers after diving, optimally, blood sampling should be continued for hours after hyperbaric exposure and also include follow-up samples obtained 10–14 days later.

Future blood analyses of tau, GFAp, and NfL in divers should ideally be complemented with analyses of ‘big tau’, markers specific to peripheral nerve injuries, markers specific to central nervous injuries, and markers of oxidative stress.

Oxygen breathing after diving

Oxygen breathing after diving reduced VGE load and hence, the risk of DCS. Most likely, the effect of breathing oxygen is more pronounced if begun immediately after surfacing. Breathing 100 kPa oxygen for 30 minutes did not affect tau concentrations, was without clinical adverse effects, and within what could be considered safe limits regarding both pulmonary and CNS toxicity.

Future perspectives in biomarker research

Future dive research on tau could be carried out in many ways, but two questions stand out as important:

- is there a quantitative relationship between tau and dive exposure, i.e. diving time and dive depth?
- by what mechanism does tau increase in blood?

A large study with dives to various depths and of different durations could determine if there is a quantitative relationship between tau and dive exposures. An appropriate control group with individuals who do not dive but perform a physical activity at a corresponding level of exertion would strengthen validity of the results.

To investigate if exposure to oxygen partial pressures exceeding 100 kPa increases tau levels in blood, subjects can be pressurised in a dry hyperbaric chamber while breathing either oxygen or air with samples taken afterwards. Though, the risk of oxygen induced seizures will restrict possible pressure exposures.

A study on divers with DCS where tau, GFAp, and NfL in blood is analysed is ongoing. When the results are compiled, they will provide information on tau levels in divers with neurological DCS and hopefully it will be possible to relate tau concentrations to clinical symptoms.

If in the future there proves to be a quantitative relationship between protein tau concentrations in blood and dive exposures, and if tau concentrations are highest in patients with neurological DCS, measurements of tau could be used in a manner analogous with the use of VGE measurements today, to assess diving protocols and diving procedures according to risk.

Possible diurnal variation of neuronal biomarkers could be ruled out or confirmed by sampling tau, GFAp and NfL every three hours over 24 hours in a cohort of healthy people.

A controlled experiment, where half of the subjects are immersed in water with heads above the surface for 60 minutes, with blood sampled before and afterwards, then analysed primarily for tau but also GFAp and NfL, could determine if immersion can elicit an increase in these biomarkers. It would also be valuable to obtain a sample at around 10–14 days after exposure, to fully assess the effect on NfL.

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Paper I

Errata Paper I

Missing data section, page 93
The sentence reads:
"*Over the first three days, VGE data were not available for any dives and no VGE data were collected for the pair of divers who were diving no deeper than 52 msw."*

The sentence should read:
"*Over the first three days, VGE data were not available for all dives and no VGE data were collected for the pair of divers who were diving no deeper than 52 msw."*

Serum tau concentration after diving – an observational pilot study

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Key words
Tau protein; Decompression sickness; Venous gas emboli; Diving research; Biomarkers; Central nervous system; Stress

Abstract
(Rosén A, Oscarsson N, Kvarnström , Gennser M, Sandström G, Blennow K, Seeman-Lodding H, Zetterberg H. Serum tau concentration after diving – an observational pilot study. Diving and Hyperbaric Medicine. 2019 June 30;49(2):88–95. doi: 10.28920/dhm49.2.88-95. PMID: ????.)
Introduction: Increased concentrations of tau protein are associated with medical conditions involving the central nervous system, such as Alzheimer's disease, traumatic brain injury and hypoxia. Diving, by way of an elevated ambient pressure, can affect the nervous system, however it is not known whether it causes a rise in tau protein levels in serum. A prospective observational pilot study was performed to investigate changes in tau protein concentrations in serum after diving and also determine their relationship, if any, to the amount of inert gas bubbling in the venous blood.
Methods: Subjects were 10 navy divers performing one or two dives per day, increasing in depth, over four days. Maximum dive depths ranged from 52–90 metres' sea water (msw). Air or trimix (nitrogen/oxygen/helium) was used as the breathing gas and the oxygen partial pressure did not exceed 160 kPa. Blood samples taken before the first and after the last dives were analyzed. Divers were monitored for the presence of VGE at 10 to15 minute intervals for up to 120 minutes using precordial Doppler ultrasound.
Results: Median tau protein before diving was 0.200 pg·mL^{-1} (range 0.100 to 1.10 pg·mL^{-1}) and after diving was 0.450 pg·mL^{-1} (range 0.100 to 1.20 pg·mL^{-1}; $P = 0.016$). Glial fibrillary acidic protein and neurofilament light protein concentrations analyzed in the same assay did not change after diving. No correlation was found between serum tau protein concentration and the amount of inert gas bubbles.
Conclusion: Repeated diving to between 52–90 msw is associated with a statistically significant increase in serum tau protein concentration, which could indicate neuronal stress.

Introduction

Diving is a widespread recreational and professional activity. While diving using air as the breathing gas, the body accumulates nitrogen due to an elevated ambient pressure. The amount of nitrogen or other inert gases taken up in the tissue depends on diving depth and time spent underwater. When the diver ascends towards the surface and decompresses, the ambient pressure falls and nitrogen leaves the tissues. If decompression is too rapid, then there is a risk that nitrogen could come out of solution, forming bubbles in blood and tissues. Intravascular nitrogen bubbles mainly form in the venous system and they are therefore named venous gas emboli (VGE).[1]

The formation of VGE in the body is considered to be a cause of decompression sickness (DCS). VGE passing into the arterial circulation through veno-arterial shunts in either the heart or the lungs could occlude arteries, disrupting both blood supply and normal tissue function. Disparity in bubble location could explain the varied clinical symptoms associated with DCS, which range from itchy skin, fatigue and pain, to neurological lesions, seizures, coma, and death.[2] Even uneventful dives, without clinical signs of DCS, can give rise to VGE; these so-called 'silent bubbles' can be regarded as a normal phenomenon after diving. Analyses of large groups of divers show that DCS is more common when the VGE load is high after diving. Conversely, when no VGE can be detected, the risk of DCS seems low.[3] VGE

load can be quantified by Doppler ultrasound examination of the heart or major vessels using the Kisman-Masurel (KM) grading system. This is an ordinal scale based on categorical data describing amplitude, frequency and duration of VGE.[4]

High partial pressures of both oxygen and nitrogen are known to disturb normal function of the human brain. Oxygen can be harmful to the central nervous system (CNS) at partial pressures exceeding 160 kPa, 66 metres' sea water (msw) when a diver breathes air, with the toxic effect increasing with partial pressure and length of exposure. Signs of oxygen toxicity include sensory and behavioural changes, dizziness, and seizures.[5] The narcotic effect of nitrogen becomes increasingly apparent at depths exceeding 30 msw when a diver breathes air, but individual susceptibility varies. Nitrogen narcosis manifests as impaired cognitive and neuromuscular performance.[6] In order to regulate the partial pressures of oxygen and nitrogen and their effects at greater depths, gas mixtures containing nitrogen, oxygen and helium are used and commonly referred to as 'trimix'.

Exposure to high ambient pressure, equivalent to diving depths of more than 150 msw, can cause neuromuscular dysfunction, a condition termed the high-pressure neurological syndrome (HPNS). Nausea, dizziness and tremors are common symptoms. With increasing depth, myoclonic episodes appear. Factors such as individual susceptibility, compression rate and breathing gas mixture affect the clinical manifestations. The causal mechanism of HPNS is partly unknown though it has been shown to be independent of elevated gas pressure.[7]

Tau protein (tau) is a microtubular protein abundant in neuronal axons, predominantly in thin unmyelinated axons of the cortex. It can also, to a lesser extent, be detected in the liver, kidneys and testes.[8] Increased tau levels are found in blood serum in conjunction with dementia, traumatic brain injury (TBI),[8–10] cerebral concussion, boxing,[11,12] and hypoxic brain injury, where it correlates with outcome.[13,14] Tau levels in blood serum rise early, within 24 hours, after cerebral damage. A delayed secondary peak appears a few days after an hypoxic injury.[14] A recent study on patients undergoing surgery and general anesthesia showed a transient rise of serum tau levels.[15] High intensity interval training can also lead to increased serum tau levels in the bloodstream; however, a two-week period of such training is alleged to blunt the tau release during subsequent training sessions.[16] Transient hypoxia during breath-hold diving has been associated with elevated tau levels, but a small pilot study on divers with DCS found no statistically significant elevation of tau concentration in cerebrospinal fluid (CSF).[17,18]

Neurofilament light protein (NfL) is a structural axonal protein which is found mainly in myelinated subcortical axons.[8] Serum NfL levels correlate with outcome in patients with TBI, but their rise is slower than that for tau, reaching a maximum beyond 10 days following the insult.[19] Glial fibrillary acidic protein (GFAp) is expressed almost solely in astrocytes. Elevated blood serum levels of GFAp have been reported within 24 hours after TBI.[8]

The potential influence of diurnal variation on neuronal fluid biomarker results has been a subject of scientific discussion.[20–22] However, a study including patients with Alzheimer´s disease and older healthy volunteers concluded that there was no circadian pattern for tau in CSF.[23] Another study on neurosurgical patients showed no diurnal variation in CSF tau levels.[24] Likewise, there was no significant diurnal variation in CSF tau levels among older patients with idiopathic normal pressure hydrocephalus or pseudotumor cerebri, when studied through sequential CSF sampling.[25] Most likely serum tau levels reflect those in CSF. It is not known whether a hyperbaric exposure alone, without hypoxia, is associated with a rise in serum tau levels.

We hypothesized that diving, by way of the previously discussed consequences of exposure to an elevated ambient pressure, affects the central nervous system and causes a rise in serum tau protein concentration in blood. Our primary objective was to investigate changes in serum tau concentration after diving to depths of up to 90 msw. The secondary objective was to investigate if there was an association between serum tau concentration and VGE load after the same dives.

Methods

The study was prospective and observational. It was conducted in accordance with the Declaration of Helsinki, approved by the regional ethical committee in Gothenburg, Sweden (Dnr 292-17) and registered at ClinicalTrials.gov (NCT03190252).

SUBJECTS

Ten male military divers participating in professional naval dive training on the Swedish west coast from 12–15 June 2017 took part in the study. Subject characteristics are described in Table 1. All subjects gave their written informed consent. A control group containing non-diving military divers was initially planned. However, difficulties in subject recruitment meant that an appropriate control group could not be formed.

DIVING PROTOCOL

The participants performed one or two dives a day over four days, as shown in Figure 1. Dive depths were planned to increase with each subsequent dive. One diver did not dive on the third day. Eight subjects dived to 50–52 msw on the first day and reached 82–90 msw on the fourth day. For the two remaining divers, maximal depth ranged between 34 msw on the first day and 52 msw during the last dive.

Figure 1
Diving protocol. Divers 7–10 performed two 65–66 msw dives on the 13 June; divers 1 and 2 performed two 52 msw dives on the 15 June; diver 10 did not dive on the 14 June

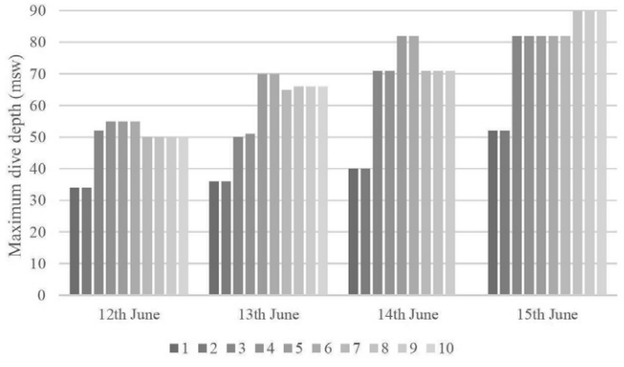


Table 1
Baseline and demographic data; for categorical variables *n* is presented; for continuous variables mean (SD) and median (range) are presented

Male	10
Age (years) Mean (SD) Median (range)	 38.4 (8.2) 39.5 (27.0–52.0)
BMI (kg·m^{-2}) Mean (SD) Median (range)	 25.6 (1.2) 25.4 (24.4–28.1)
Prior DCS	4
Excessive physical activity or diving ≤ 48 hours prior:	
No	6
One dive 15–20 msw	4
Medication:	
No	8
Diclofenac	1
Phenylpropanolamine (only on 1st dive of day)	1

Median time spent at maximum depth during the first three days was 20 min (range 10 to 25 min). On the fourth day, time spent at maximum depth was 10 min for dives to 52 msw and 20 min for dives to 82–90 msw. All subjects used electronically controlled closed circuit rebreathers. Air was used as the diluent gas for dives less than 40 msw and trimix was used for all dives deeper than 40 msw. For dives between 40-65 msw the trimix diluent gas contained 15% oxygen, 50% helium, 35% nitrogen. During dives deeper than 65 msw the diluent contained 10% oxygen, 70% helium and 20% nitrogen. The rebreather equipment maintained a constant oxygen partial pressure of 130 kPa while the divers descended and were at depth. During the final decompression phase, an oxygen partial pressure of 160kPa was allowed. Decompressions were planned according to the VPM-B algorithm with conservatism factor 2.[26] Immediately after dives deeper than 60 msw 100% oxygen was breathed for 10 minutes.

DATA COLLECTION

Venous blood samples were obtained from all participants before the first dive (Sample 1, baseline, 12 June 2017 between 11:30–12:50) and approximately two to three hours after the last dive (Sample 2, 15 June 2017 between 15:35–17:05). Samples were collected in gel tubes (Vacuette no. 454420, Hettish Labinstrument AB, Sweden) and immediately centrifuged for 10 minutes at 2,200 rpm and 20ºC (Sorvall ST 8/8R Centrifuge, Thermo Scientific, Germany). Directly afterwards, aliquots of 500 µL serum were frozen on dry ice and then stored at -78ºC until analyzed. Tau concentration was measured using the Human Neurology 4-Plex A assay (N4PA) on an HD-1 single molecule array (Simoa) instrument according to instructions from the manufacturer (Quanterix, Lexington MA, USA). For quality control (QC) samples, with tau concentrations of 0.70 pg·mL^{-1}, 1.4 pg·mL^{-1} and 24.1 pg·mL^{-1}, coefficients of variation (CVs) were 8.1%, 11.9% and 6.2%, respectively. The N4PA assay is designed to measure four biomarkers, namely tau, GFAp, NfL and ubiquitin carboxy-terminal hydrolase L1 (UCHL-1). Therefore, results for all these four biomarkers were obtained. For QC samples, with NfL concentrations of 101.2 pg·mL^{-1}, 8.0 pg·mL^{-1} and

Table 2
Serum tau protein values before (sample 1) and after (sample 2) diving; the mean (SD) and median (range) are presented for each parameter; for comparison the Wilcoxon signed-rank test was used

Sample 1 (pg·ml^{-1}) *n* = 9 Mean (SD) Median (range)	 0.322 (0.315) 0.200 (0.10–1.10)	
Sample 2 (pg·ml^{-1}) *n* = 9 Mean (SD) Median (range)	 0.500 (0.337) 0.450 (0.10–1.20)	
Delta-tau (pg·ml^{-1}) Mean (SD) Median (range)	 0.211 (0.145) 0.300 (0.0–0.40)	 *P* = 0.016
Delta-tau (%) Mean (SD) Median (range)	 98.8 (96.0) 100 (0.0–30)	 *P* = 0.016

Figure 2
Serum tau protein values before and after diving (*n* = 9). One diver is not included due to a missing sample 1 before diving. For two divers, increase in serum tau protein value (0.2 pg·mL^{-1} – 0.5 pg·mL^{-1}) was identical. They are represented by one line

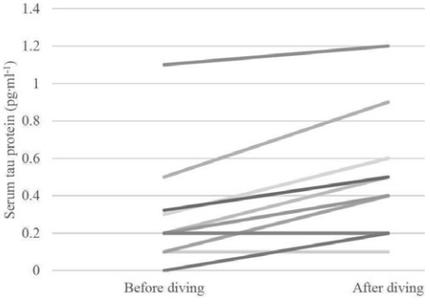


Table 3
Serum GFAp and NfL values before (sample 1) and after (sample 2) diving; for continuous variables mean (SD) and median (range) are presented; for comparison the Wilcoxon signed-rank test was used

	GFAp	*P*-value	NfL	*P*-value
Sample 1 (pg·ml^{-1}) *n* = 9 Mean (SD) Median (range)	 68.644 (24.086) 59.0 (41.0;108.5)		 9.956 (7.663) 7.800 (5.4–30.0)	
Sample 2 (pg·ml^{-1}) *n* = 10 Mean (SD) Median (range)	 65.090 (13.834) 62.9 (45.2–86.6)		 8.960 (5.830) 7.200 (5.7–25.3)	
Delta (pg·ml^{-1}) Mean (SD) Median (range)	 -3.644 (25.834) -0.400 (-39.4–32.6)	 0.678	 -1.056 (1.982) -1.400 (-4.7–1.6)	 0.173
Delta (%) Mean (SD) Median (range)	 3.5 (37.0) 0.0 (-39.9–62.0)	 0.678	 -5.9 (19.7) -15.7 (-26.9–28.1)	 0.173

14.8 pg·mL^{-1}, CVs were 5.0%, 9.5% and 3.5%, respectively and for QC samples, with GFAp concentrations of 75.3 pg·mL^{-1}, 95.6 pg·mL^{-1} and 118.9 pg·mL^{-1}, CVs were 2.2%, 9.4% and 4.9%. The results of UCHL-1 analyses were discarded due to an unacceptably high level of imprecision as CVs were 44.9% and 121.0% for QC samples with UCHL-1 concentrations of 8.4 pg·mL^{-1} and 9.7 pg·mL^{-1} respectively.

Within 20 minutes after surfacing, each diver was monitored for the presence of VGE, at 10 to 15 minute intervals for up to 120 minutes, using precordial Doppler ultrasound (DBM9008; Techno Scientific Inc., Ontario, Canada). VGE load was assessed while the subjects lay in the left lateral decubitus position at rest and measurements were also made following movement (knee bends made whilst still lying down) and graded according to the KM scale.

The Kisman integrated severity score (KISS) algorithm[27] was used to convert KM grade measurements collected during the four-day study period into one mean score for each diver (VGE-KISS).

STATISTICS

Results for tau and its association with VGE, were compiled by an independent statistical company (Statistiska Konsultgruppen, Gothenburg, Sweden) using SAS® v9.3 (Cary, NC, USA). Statistical analysis for GFAp and NfL were performed using IBM SPSS® v24 (IBM, Armonk NY, USA) and Spearman´s correlation tests involving VGE KISS were performed using Microsoft© Office Excel 2018 (Microsoft Corporation, Redmond WA, USA). The study group was small and serum levels of tau, GFAp and NfL before diving were not normally distributed. Therefore, a non-parametric statistical technique was used for statistical inference.

Primary outcome

Serum tau levels before and after diving are presented as both mean (SD) and median (range: min/max) values. Differences in the tau levels between sample 1 and sample 2 (delta-tau) were presented both as an absolute (pg·mL^{-1}) and a relative change (%). Statistical significance was tested using the Wilcoxon signed-rank test.

Secondary outcome

Correlation between the maximum VGE loads measured after the last dive and the sample-2 serum tau concentrations was tested using Spearman´s correlation test and presented as scatter plots.

Following the initial compilation of the results, correlation between the KISS scores and the sample-2 serum tau concentrations, and between KISS and delta tau, was tested using the Spearman correlation test.

GFAp and NfL

GFAp and NfL levels before and after diving were presented as both mean (SD) and median (range: min;max) values. Differences in the GFAp and NfL levels between sample

Figure 3
Serum tau protein after the last dive versus maximal KM grade at rest (*n* = 8); Spearman rank correlation coefficient 0.2, *P*-value: 0.6

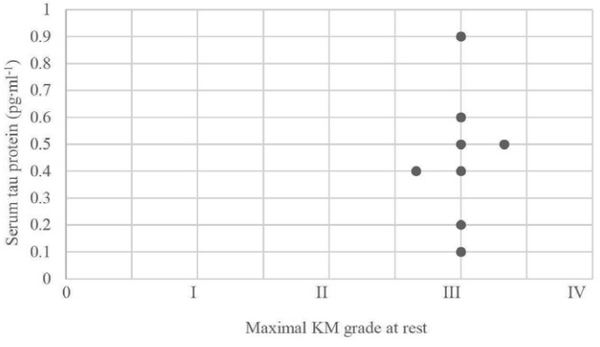
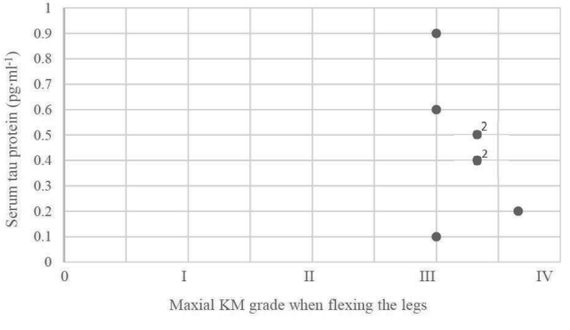


Figure 4
Serum tau protein after the last dive versus maximal KM grade when flexing the legs (*n* = 8); Spearman rank correlation coefficient -0.4, *P*-value: 0.33



1 and sample 2 (delta-values) were presented both as an absolute ($pg \cdot mL^{-1}$) and a relative change (%). Statistical significance was tested using the Wilcoxon signed-rank test.

Missing data

Tau sample 1 was missing for one diver. Over the first three days, VGE data were not available for any dives and no VGE data were collected for the pair of divers who were diving no deeper than 52 msw.

Results

PRIMARY OUTCOME

Among the nine divers with baseline samples, seven had increased serum tau concentrations after four days of diving and none showed a decrease. Both the absolute and the relative changes in median serum tau concentration between sample 1 and sample 2 were statistically significant (Table 2, Figure 2).

SECONDARY OUTCOME

Eight of the 10 divers were monitored for VGE after the last dive of the series; across these subjects the median KM grade was III at rest and III+ following the knee bends. With regard to maximum KM grades, six subjects had grade III, one III- and one III+ measurements at rest. Following knee bends, three were graded KM III, four III+ and one IV-. With the observed narrow distribution of KM grades, no statistical correlation was found between serum tau protein concentration and maximum VGE load after diving (Figure 3 and Figure 4). Similarly, there was no statistically significant correlation between the VGE-KISS scores and sample-2 serum tau concentration ($R^2 = 0.15$, $t = 1.02$) nor between VGE-KISS and delta-tau ($R^2 = 0.002$, $t = 0.12$).

GFAp AND NfL

Neither GFAp nor NfL concentrations changed significantly after diving (Table 3).

Discussion

In this prospective pilot study, diving over a four-day period was associated with a statistically significant rise in serum tau concentration. The median tau value increased 2.5 times. This serum tau change is comparable to changes in plasma tau and CSF tau observed in earlier studies in athletes and after mild concussion injuries.[8,9] Causality between diving and serum tau concentrations is still uncertain, due to the lack of a control group and the small number of observations. Yet, as the divers' tau values after diving were compared to values obtained shortly before the first dive, the results are consistent with causation.

The KM grading system is the gold standard method of assessing VGE load after diving, as confirmed in a recent concensus,[28] but it is subjective and non-linear. Furthermore, all categorization results in a loss of information and reduced precision. A majority of KM grades after diving were III at rest and III or III+ following knee bends. In this study, there was no statistically significant correlation between maximum VGE load and tau levels nor between VGE-KISS scores and tau levels, but the narrow distribution of KM grades and the small set of observations precludes conclusions. A future study involving a larger cohort of divers, with a wider range of KM grades, would make it possible to investigate if there is a correlation between tau and VGE.

Our objective was to investigate changes in serum tau concentration after diving, but the assay used for measurement also provided us with results for GFAp and NfL. The absence of change in NfL concentration was expected, as NfL is a slow biomarker for axonal injury, reaching its maximum no earlier than 10 days following a traumatic injury.[19] GFAp, a protein highly expressed in astrocytes, appears to have similar kinetics in blood as tau.[8] The unchanged GFAp concentrations may thus suggest a limited involvement of astrocytes in response to diving exposure, though the small size of the study makes such a conclusion speculative.

High partial pressures of oxygen could potentially affect the CNS negatively. Oxygen partial pressure in the breathing gas did not exceed 160 kPa during the study. This is considered a safe limit during diving and does not give rise to subjective symptoms. Despite this, even a modest increase in oxygen partial pressure could be a contributing cause of elevated serum tau protein after diving and furthermore, nothing is known about any relevant effects of breathing gases containing helium. Studies investigating HPNS have shown that exposure to increased ambient pressure affects the nervous system through mechanisms unrelated to the partial pressures of breathing gases and VGE. It is possible that the CNS is affected by pressure at depths shallower than those associated with manifestations of HPNS and this could be a cause of elevated tau.

The lack of a control group is a shortcoming of this study. There was a difference between the time of day when samples 1 and 2 were taken. Studies show no diurnal variation in CSF tau levels,[23–25] making it improbable that they should fluctuate in the blood significantly during the day. Nevertheless, a representative control group could have ensured that no confounding factors, such as diurnal variation, were responsible for changes in serum tau. Ideally in future studies, tau should be sampled at the same times of day and the results compared to a representative control group. In the context of hypoxic brain injury, studies have shown that the increase in serum tau levels reach a maximal elevation within 24 hours, though sometimes there is a delayed peak at about 72 hours.[14] The change in serum tau levels after a far milder but prolonged impact, such as repeated diving, are unknown. Additional sampling of venous blood at other points might have yielded even higher serum tau values.

The small size of the study was an important limitation. Mean values were potentially unreliable and misleading. For that reason, both mean and median values were presented and a non-parametric statistical technique was used for inference. Another limitation was that only serum samples were available. Tau concentrations are, for unknown reasons, higher in plasma than in serum, but the ultrasensitive method employed still allows accurate measurement of serum tau concentrations. Meaningful associations of serum tau concentrations and neuronal injury in other conditions have been reported before.[13,29] Therefore, we consider this limitation minor.

No subject reported excessive physical activity within the 48 hours before the study, but it is possible that dives made by four of the participants shortly before the study did influence their results. None of the dives prior to the study were reported to be deeper than 20 msw, which could be considered at most moderately stressful for a trained diver. No strenuous physical activity was performed during the study dives. Therefore, it is unlikely that the results were confounded by either prior diving or physical exertion during the study dives.

The study group consisted exclusively of trained male navy divers. Even though there was a considerable age difference between participants, they all met the physical and medical demands required by the navy and so in this respect the group was homogenous. Four of the 10 subjects had a past history of DCS. This is potentially the result of a professional diving career and not necessarily due to an increased individual susceptibility of the nervous system to hyperbaric exposure.

Conclusion

Despite its limitations, this pilot study showed that repeated diving to depths between 52–90 msw using a trimix breathing gas was associated with a statistically significant rise in tau protein levels in serum. A larger, controlled study is needed both to validate these results and to investigate the relationship between VGE and tau. Further studies on tau and diving should ideally also be carried out on divers with DCS.

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Conflicts of interest

HZ has served at scientific advisory boards for Roche Diagnostics, Wave, Samumed and CogRx and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. KB has served on scientific advisory boards for Roche Diagnostics, Fujirebio Europe, IBL International, Eli Lilly and Alzheon and is a co-founder of Brain Biomarker Solutions in Gothenburg AB. No other authors have reported any conflicts of interest.

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Paper II

Errata Paper II

Materials and Methods section, page 2776
The *sentence* reads:
"At 105 h, when the intervention group reached a pressure equivalent to 3msw (131 kPa), research personnel entered the chamber to monitor the subjects for VGE using precordial Doppler ultrasound (DBM9008, Techno Scientific Inc, Ontario, Canada)."

The sentence should read:
"At 98 h, when the intervention group reached a pressure equivalent to 3msw (131 kPa), research personnel entered the chamber to monitor the subjects for VGE using precordial Doppler ultrasound (DBM9008, Techno Scientific Inc, Ontario, Canada)."

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ORIGINAL ARTICLE

Biomarkers of neuronal damage in saturation diving—a controlled observational study

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Abstract

Purpose A prospective and controlled observational study was performed to determine if the central nervous system injury markers glial fibrillary acidic protein (GFAp), neurofilament light (NfL) and tau concentrations changed in response to a saturation dive.

Methods The intervention group consisted of 14 submariners compressed to 401 kPa in a dry hyperbaric chamber. They remained pressurized for 36 h and were then decompressed over 70 h. A control group of 12 individuals was used. Blood samples were obtained from both groups before, during and after hyperbaric exposure, and from the intervention group after a further 25–26 h.

Results There were no statistically significant changes in the concentrations of GFAp, NfL and tau in the intervention group. During hyperbaric exposure, GFAp decreased in the control group (mean/median − 15.1/ − 8.9 pg·mL^{-1}, $p < 0.01$) and there was a significant difference in absolute change of GFAp and NfL between the groups (17.7 pg·mL^{-1}, $p = 0.02$ and 2.34 pg·mL^{-1}, $p = 0.02$, respectively). Albumin decreased in the control group (mean/median − 2.74 g/L/ − 0.95 g/L, $p = 0.02$), but there was no statistically significant difference in albumin levels between the groups. In the intervention group, haematocrit and mean haemoglobin values were slightly increased after hyperbaric exposure (mean/median 2.3%/1.5%, $p = 0.02$ and 4.9 g/L, $p = 0.06$, respectively).

Conclusion Hyperbaric exposure to 401 kPa for 36 h was not associated with significant increases in GFAp, NfL or tau concentrations. Albumin levels, changes in hydration or diurnal variation were unlikely to have confounded the results. Saturation exposure to 401 kPa seems to be a procedure not harmful to the central nervous system.

Trial registration ClinicalTrials.gov NCT03192930.

Keyword Biomarkers · Central nervous system · Diving · hyperbaric · Neuronal damage · Saturation diving

Abbreviations

BMI	Body mass index
CNS	Central nervous system

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CV	Coefficients of variation
DCS	Decompression sickness
GFAp	Glial fibrillary acidic protein
Hb	Haemoglobin
Hct	Haematocrit
kPa	Kilopascal
msw	Meters of seawater
NfL	Neurofilament light
SwAF	Swedish armed forces
tau	Protein tau
UCH-L1	Ubiquitin carboxy-terminal hydrolase L-1
VGE	Venous gas emboli
QC	Quality control

Introduction

Divers are repeatedly exposed to increased ambient pressures and both caisson and tunnel workers might have to work in a compressed air environment, typically exposed to 1.5–7 times normal atmospheric pressure (Le Péchon 2010). The hyperbaric environment is potentially dangerous to the human body and adverse effects can manifest both during and after exposure.

Divers are often exposed to increased partial pressures of oxygen. By maintaining the oxygen fraction in the breathing gas above what would constitute a normoxic partial pressure, the uptake of inert gas is reduced and the time needed for decompression reduced. However, increased partial pressures of oxygen have toxic effects, mainly affecting the lungs and the central nervous system. The toxic effect of oxygen on the central nervous system (CNS) depends mainly on its partial pressure, time of exposure, and individual susceptibility. Toxicity could be aggravated by exercise, hypothermia, immersion in water or hypercarbia (Edmonds 2016a). Oxygen toxicity becomes increasingly noticeable at partial pressures exceeding 150 kPa, equivalent to a depth of about 60 m of seawater (msw) when breathing air (Hamilton 1989). Symptoms of oxygen toxicity are facial twitching, nausea, dizziness, tinnitus, tunnel vision and generalized seizures (Edmonds 2016b).

Nitrogen affects the nervous system when its partial pressure increases, which typically manifests as changed behaviour, impaired intellectual performance, and deteriorating neuromuscular function. The symptoms usually become apparent as the nitrogen pressure exceeds 300 kPa, equivalent to a depth of about 30msw when breathing air, and gradually worsen with increasing pressure. Factors, such as compression rate and level of carbon dioxide as well as anxiety and stress, accentuate the effects (Bennet 2003).

While diving using air as breathing gas, nitrogen will accumulate in the body due to the increased ambient pressure. Deep, extended or repeated diving could result in considerable uptake of nitrogen. When the diver returns towards the surface and ambient pressure decreases, nitrogen will leave the tissues and enter the blood before finally being exhaled through the lungs. At this stage, supersaturation can lead to formation of inert gas bubbles in both blood and tissues, which is common after diving. Intravascular inert gas bubbles form mainly in the venous system and are referred to as venous gas emboli (VGE) (Francis 2003). The amount of VGE is positively correlated with decompression speed (Germonpré 2017), but there is a considerable individual variability in the amount of VGEs observed in divers after similar decompression regimens (Papadopoulou 2018). Inert gas bubbles are considered to be the cause of decompression sickness (DCS). Analyses of large groups of divers show that DCS is more common when the VGE load is high. (Nishi 2003, Eftedal 2007). Still, divers may exhibit a high VGE load without any signs of DCS and divers without detectable VGE can develop DCS.

Saturation diving is a technique developed to handle the risk of DCS in conjunction with prolonged or deep dives. In contrast to usual diving, where each session begins and ends at the surface, in saturation diving, the divers remain under pressure for long periods of time, usually days to weeks. The inert gas pressures in the divers' tissues are, during this period, in equilibrium with the ambient inert gas pressure and they are, hence, saturated (Powell 2014).

Though neurological impairment is evident during exposure to increased partial pressures of nitrogen and oxygen, and in connection to DCS involving the central nervous system, possible neurological long-term effects of uneventful diving remain to be fully elucidated (Grønning and Aarli 2011). Experienced saturation divers have, when compared to non-diving controls, been found to have more subjectively reported problems with memory and concentration, as well as more neurological symptoms when objectively examined (Todnem 1990,1991), but confounding factors (e.g., DCS) exist and causality between hyperbaric exposure per se and neuropsychological sequelae remains to be unequivocally proven. There are reports of objectively confirmed impaired memory function among both saturation divers with subjective forgetfulness (Taylor 2006) and experienced recreational divers without a history of DCS (Hemelryck 2014). Assessment of cognitive function using neuropsychological tests showed a correlation between frequent recreational diving in cold water and decreased cognitive performance (Slosman 2004) and worse visual–motor performance and short-term memory has been reported among recreational divers (Balestra 2016). In contrast, another study comparing professional divers to matched controls found no difference in neuropsychometric test results (Cordes 2000), and a 12-year longitudinal study on professional non-saturation divers concluded that divers without a history of DCS did not show signs of impaired nervous system function (Bast-Petrersen 2015).

It is unknown to what extent factors, such as increased hydrostatic pressure or higher than normal oxygen and nitrogen partial pressures, influence neurons in the CNS. If there is a cumulative effect on the CNS due to multiple exposures to a hyperbaric environment, it should be possible to measure acute effects after provocative dives, even if those effects are subclinical and do not give rise to any subjective symptoms.

We speculated that a saturation dive would infer neuronal stress and possibly be harmful to the CNS, which eventually could translate into long-term neurological sequelae. To test the hypothesis that a saturation dive might cause injury to the CNS, we selected a number of CNS-specific molecules in blood, Glial fibrillary acidic protein (GFAp), Neurofilament light protein (NfL) and tau protein (tau), the concentrations of which are known to increase in response to different types of brain trauma, including mild traumatic brain injury (Zetterberg 2016), and neuronal stress (Evered 2018, Sato 2018). Our aim was to analyse the concentrations of these proteins in blood among individuals exposed to an increased ambient pressure and compare the results with those obtained in unexposed individuals.

GFAp has a molecular weight of 50 kDa and is expressed mainly in astrocytes in the CNS. Elevated levels of GFAp in blood have been reported after traumatic brain injury (Zetterberg 2016, Gill 2018) and after intracerebral haemorrhage (Foerch 2012). However, a change in GFAp concentration is also claimed to reflect astrocytic plasticity in response to neuronal stress and increased neuronal metabolic and immunologic activity (Wang 2009, Brenner 2014, Femenia 2018).

NfL is a structural axonal protein, with a molecular weight of 68 kDa, found mainly in myelinated subcortical axons. NfL levels in blood are increased in patients with traumatic brain injury (Shahim 2016, Zetterberg 2016), axonal injury due to multiple sclerosis (Khalil 2018), and sports-related concussion (Shahim 2018), but even uneventful anaesthesia has been associated with increased blood levels of NfL (Evered 2018).

Tau is a microtubule-binding protein present in neuronal axons, mainly in the cortex, with a molecular weight of 55–62 kDa, which is thought to be a marker of both axonal damage and neuronal plasticity in response to stress. Tau could be both passively released as a result of cell death but also actively secreted in connection to increased neuronal activity (Sato 2018). Increased levels of tau have been reported in the context of dementia, brain injury (Zetterberg 2016, Mattson 2017), cerebral concussion (Shahim 2014, Zetterberg 2016) and boxing (Neselius 2012, Zetterberg 2006), but also after less evident neuronal trauma as protracted apnea (Gren 2016) and uneventful anaesthesia (Evered 2018). A small pilot study found elevated tau levels in blood after diving (Rosén 2019). However, another small pilot study found no increase in tau levels in cerebrospinal fluid among divers with DCS (Shahim 2015).

We had the possibility to analyse GFAp, NfL, and tau using a 4-plex Single molecule (Simoa) assay, in which also ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) was included. This is a cytoplasmic neuronal protein present in the CNS, the peripheral nervous system, the neuroendocrine system, endothelial and smooth muscle cells. Increased concentrations of UCH-L1 have been reported in patients with traumatic brain injury but the analytical performance of the biomarker in the 4-plex assay is variable (Zetterberg 2016).

Materials and methods

The study was prospective, controlled and observational. It was conducted in accordance with the Declaration of Helsinki, approved by the regional ethical committee in Gothenburg, Sweden (Dnr 022-17), registered at ClinicalTrials.gov (NCT03190252) and carried out at the naval base in Karlskrona, Sweden, as two separate but identical trials, between March 17th and 23rd 2017, and between January 11th and 17th 2018, while the Swedish armed forces (SwAF) performed validation tests of a decompression table to be used during submarine rescue. Written consent was obtained from all subjects before participation in the study.

The intervention group consisted of 14 Swedish Navy submariners participating in the SwAF submarine rescue training. The control group consisted of 12 people who either had passed a dive medical examination or were employed as Swedish Navy mariners. None of the control subjects undertook any diving activities during the study.

Data concerning gender, age, weight, height and current medication were collected from all subjects and baseline venous blood samples taken (sample 1, between 09:20 and 14:00). The intervention groups were compressed in a dry hyperbaric chamber to 401 kPa, equivalent to a depth of 30msw, and remained at that pressure for 36 h. They were then decompressed at a rate of 0.5 m/h for 30 h, thereafter 0.375 m/h for 40 h. After 70 h of decompression, normal atmospheric pressure was reached and the subjects left the hyperbaric chamber, at which point they had then been subject to an increased ambient pressure for 106 h. The oxygen partial pressure inside the chamber was kept at or near 50 kPa at depth, which was accomplished by increasing the amount of nitrogen in the breathing gas. During decompression, the oxygen partial pressure was maintained at 50 kPa until 15msw (251 kPa) was reached. At that pressure, the breathing gas was switched to air and the decompression speed was reduced. During night hours, lights in the hyperbaric chamber were turned off and subjects in the intervention group lay in their beds. Some subjects in the control group were part of the ships rota of duties including

night service. The start of compression of the intervention group was defined as 0 h. Venous blood samples were obtained from all subjects in both groups at 33–34 h (sample 2, between 06:10 and 08:00) and at 104–108 h (sample 3, between 05:30 and 09:40). For practical reasons, some samples in the control group had to be obtained shortly before the hyperbaric exposure ended at 106 h. Blood samples taken from the intervention group at 33–34 h were collected by a research nurse who was pressurized to 401 kPa and after sampling decompressed to atmospheric pressure. Sample 3 was obtained from the intervention group directly after the hyperbaric exposure had ended. About 25–26 h after the hyperbaric exposure ended, a venous blood sample was collected from subjects in the intervention group (sample 4, between 08:00 and 09:10). At 105 h, when the intervention group reached a pressure equivalent to 3msw (131 kPa), research personnel entered the chamber to monitor the subjects for VGE using precordial Doppler ultrasound (DBM9008, Techno Scientific Inc, Ontario, Canada). After the final decompression, repeated precordial Doppler ultrasound examinations were carried out until more than 3 h had elapsed.

All samples were analysed for GFAp, NfL, tau, UCH-L1 and albumin. They were collected in gel tubes (Vacuette no. 454420, Hettish Labinstrument AB, Sweden) and centrifuged for 10 min at 2200 rpm and 20° centigrade (Sorvall ST 8 / 8R Centrifuge, Thermo Scientific, Germany). Directly afterwards, aliquots of 500 µL serum were frozen on dry ice and then stored at − 78° centigrade until analyzed. GFAp, NfL, tau and UCH-L1 concentrations were measured using the Human Neurology 4-Plex A assay on an HD-1 Simoa instrument according to instructions from the manufacturer (Quanterix, Billerica, MA, USA).

For quality control (QC) samples with GFAp concentrations of 113.1 pg·mL^{-1} and 88.8 pg·mL^{-1}, coefficients of variation (CVs) were 4.4% and 4.2%, respectively, for QC samples with NfL concentrations of 13.8 pg·mL^{-1} and 7.5 pg·mL^{-1}, CVs were 4.6% and 5.7% and for quality control (QC) samples with tau concentrations of 1.5 pg·mL^{-1} and 23.9 pg·mL^{-1}, CVs were 9.0% and 6.2%, respectively. The results of UCH-L1 analyses were discarded due to an unacceptably high level of imprecision as CVs were 105.6% and 21.7% for QC samples with UCH-L1 concentrations of 2.8 pg·mL^{-1} and 11.0 pg·mL^{-1}, respectively.

Albumin concentration was measured using an immunoturbidimetric method on Elecsys (Roche Diagnostics, Penzberg, Germany).

In the intervention group, samples 1–3 were taken in doublets and each extra sample was analysed using a hand-held blood analyser (i-STAT® 1, Abbott Point of Care Inc, IL, USA), which determined haematocrit (Hct) conductometrically and provided a calculated haemoglobin (Hb) value based on the Hct value. All samples for Hct and Hb taken during hyperbaric exposure (sample 2) had to be discarded, as potential VGE induced by decompression of the samples interfered with the results of the measured conductivity.

Statistics

Statistical results for GFAp, NfL and tau were compiled using SAS® v9.3 (Cary, NC, USA) in collaboration with an independent statistical company (Statistiska Konsultgruppen, Gothenburg, Sweden). Concentrations of GFAp, NfL and tau were presented as both mean (± standard deviation) and median (min;max) values. Differences between baseline and sample 2–4 were presented as absolute (pg·mL^{-1}) changes. Fisher's non-parametric permutation test was used for analyses of absolute changes within groups as well as for comparison of absolute changes between the two groups.

Statistical analyses for Hb, Hct and albumin were performed using IBM SPSS® v24 (IBM, Armonk, NY, USA). Concentrations of albumin, Hb and Hct were presented as both mean (± standard deviation) and median (min;max) values. Within each group, absolute values of sample 2–4 were compared to baseline values using Wilcoxon signed-rank test. For comparison of albumin values between groups, Mann–Whitney *U* test was used.

In all comparisons, a two-sided *p* value of 0.05 or lower was considered statistically significant.

Missing data

There were no missing data for GFAp, NfL or tau. In the intervention group, data on Hct were missing for one subject at baseline due to sampling error, on albumin for two subjects in sample 2, and for one subject in sample 3. In the control group, data on albumin were missing for two subjects at baseline and for one subject in samples 2 and 3. All missing data on albumin were caused by lack of sample volume.

Results

Demographics

There were significant differences in body mass index (BMI) and age between subjects in the two groups. Subjects in the control group had higher BMI and were older. The proportion of subjects taking medicines was the same for both groups, but type of medication diverged. In the intervention group, one subject used antihistamines, another paracetamol and a third contraceptives and metformin. One subject in the control group had cardiac medication and two subjects used

Table 1 Demographics and baseline variables

Variable	Intervention group ($n=14$)	Control group ($n=12$)	p value
Gender			
Female	1 (7.1%)	0 (0.0%)	
Male	13 (92.9%)	12 (100.0%)	
Age	29.9 (8.5) 27.5 (21.0; 51.0) $n=14$	45.1 (10.1) 47.5 (29.0; 58.0) $n=12$	< 0.01
BMI	24.9 (1.9) 24.7 (21.3; 28.7) $n=14$	26.9 (2.8) 27.3 (23.0; 32.6) $n=12$	0.04
Medication			
No medication	11 (78.6%)	9 (75.0%)	
Acetylsalicylic acid, Enalapril, Simvastatin and Esomeprazole	0 (0.0%)	1 (8.3%)	
Antihistamine	1 (7.1%)	0 (0.0%)	
Paracetamol	1 (7.1%)	0 (0.0%)	
SSRI	0 (0.0%)	2 (16.7%)	
Contraceptives and Metformin	1 (7.1%)	0 (0.0%)	

For categorical variables *n* (%) is presented

For continuous variables Mean (Standard deviation) / Median (Min; Max) / *n* = is presented

For comparison between groups Fisher's Non-Parametric Permutation test was used

antidepressants. Results for demographic data are shown in Table 1.

GFAp

There was no difference between the groups in GFAp concentration at baseline. In the intervention group, there were no statistically significant changes in the concentration of GFAp at any point. GFAp concentration in the control group was significantly reduced in samples collected at the same time as the hyperbaric exposure was ongoing (mean/median, − 15.1/ − 8.9 pg·mL^{-1}, $p<0.01$). As a consequence, the differences in absolute change between the groups were statistically significant at this point (17.7 pg·mL^{-1}, $p=0.02$). GFAp concentration then increased in the control group, and after hyperbaric exposure, there was no difference between the groups. Results for changes in GFAp are shown in Tables 2, 3 and Fig. 1.

NfL

There was no difference between the groups in NfL concentration at baseline. In the intervention group, an increase in NfL concentration was seen during hyperbaric exposure (mean/median 1.01/1.24 pg·mL^{-1}, $p=0.06$), after which NfL concentrations decreased. Concentration of NfL did not change significantly in the control group but the observed mean concentration was decreased. The difference in absolute NfL concentration changes between the groups was statistically significant during hyperbaric exposure of the intervention group (2.34 pg·mL^{-1}, $p=0.02$) but not when decompression had ended (1.62 pg·mL^{-1}, $p=0.07$). There were no significant changes in the intervention group. Results for changes in NfL are shown in Tables 2, 3 and Fig. 2.

Tau

There was no difference between the groups in tau concentration at baseline. Variations in mean and median tau concentrations during the study did not reach statistical significance in either group. Results for changes in tau are shown in Tables 2, 3 and Fig. 3.

Haematocrit and haemoglobin

Directly after hyperbaric exposure, Hct had increased significantly (mean/median 2.3%/1.5%, $p=0.02$) in the intervention group, whereas an increase in mean Hb that did not reach statistical significance was seen (4.9 g/L, $p=0.06$). Hct and Hb were not assessed in the control group. Results for changes in Hct and Hb are shown in Table 4.

Albumin

There was no difference in albumin concentration between the groups at baseline. In the intervention group, there were no significant changes in albumin concentration,

Table 2 Changes in GFAp, NFL and tau – comparisons within groups

		Intervention group ($n=14$)			Control group ($n=12$)		
		Absolute value			Absolute value		
Sample 1 Before exposure	GFAP (pg/mL)	62.1 (33.0) 56.7 (25.7; 150.2)			63.2 (24.8) 61.9 (20.3; 113.4)		
	NfL (pg/mL)	8.29 (4.86) 6.22 (4.2; 19.01)			8.69 (5.02) 7.18 (4.12; 21.13)		
	Tau (pg/mL)	0.25 (0.23) 0.20 (0.01; 0.88)			0.34 (0.62) 0.16 (0.01; 2.28)		
		Absolute value	Absolute change compared to baseline	*p* value within group	Absolute value	Absolute change compared to baseline	*p* value within group
Sample 2 At 33–34 h of hyperbaric exposure	GFAP (pg/mL)	64.7 (30.4) 63.7 (25.4; 133.9)	2.60 (17.50) 4.76 (−33.61; 31.38) (-6.44; 11.12)	0.58	48.1 (23.7) 49.4 (1; 93.2)	−15.1 (18.8) −8.9 (−62.2; 3.8) (−26.1; -6.2)	< 0.01
	NfL (pg/mL)	9.30 (4.69) 7.58 (4.31; 20.47)	1.01 (1.80) 1.24 (−3.57; 4.42) (0.05; 1.88)	0.06	7.36 (3.16) 7.8 (1; 13.07)	−1.33 (3.68) 0.15 (-11.98; 1.16) (−3.60; 0.26)	0.25
	Tau (pg/mL)	0.365 (0.35) 0.26 (0.01; 1.12)	0.11 (0.34) 0.01 (−0.19; 1.11) (−0.03; 0.30)	0.26	0.20 (0.21) 0.16 (0.01; 0.75)	−0.14 (0.66) 0.02 (−2.16; 0.39) (−0.54; 0.11)	0.81
		Absolute value	Absolute change compared to baseline	*p* value within group	Absolute value	Absolute change compared to baseline	*p* value within group
Sample 3 At 104–108 h After hyperbaric exposure	GFAP (pg/mL)	63.0 (25.3) 64.1 (26.9; 121.9)	0.97 (17.81) −5.80 (−28.3; 32.28) (−7.96; 9.75)	0.84	67.7 (38.3) 58.4 (30.8; 174.8)	4.44 (22.76) 1.34 (−21.46; 61.44) (−6.86; 17.98)	0.56
	NfL (pg/mL)	8.79 (4.21) 6.73 (4.38; 18.14)	0.49 (1.46) 0.914 (−2.40; 2.47) (−0.280; 1.18)	0.22	7.57 (3.37) 6.87 (3.52; 16.16)	−1.12 (3.33) −0.17 (−11.44; 0.92) (−3.16; 0.13)	0.22
	Tau (pg/mL)	0.23 (0.22) 0.10 (0.01; 0.79)	−0.02 (0.29) −0.05 (−0.50; 0.78) (−0.16; 0.13)	0.77	0.15 (0.06) 0.16 (0.01; 0.25)	−0.18 (0.62) 0 (−2.12; 0.14) (−0.56; 0.03)	0.41
		Absolute value	Absolute change compared to baseline	*p* value within group			
Sample 4 At 131–132 h After hyperbaric exposure	GFAP (pg/mL)	53.3 (28.0) 57.2 (17; 119.5)	−8.80 (23.76) −7.11 (−66.88; 35.75) (−21.48; 2.66)	0.19			
	NfL (pg/mL)	7.96 (3.66) 6.78 (4.22; 16.97)	−0.33 (1.87) 0.05 (−4.19; 2.7) (−1.31; 0.57)	0.52			
	Tau (pg/mL)	0.34 (0.32) 0.23 (0.01; 1.22)	0.09 (0.35) 0 (−0.26; 1.21) (−0.06; 0.28)	0.50			

Mean (Standard deviation) / Median (Min; Max) / (Bootstrapped (10,000 replicates) 95% Confidence interval for mean) / are presented. For comparison of changes within groups the Fisher's Non-Parametric Permutation test for matched pairs was used

neither during nor after hyperbaric exposure. Albumin concentration had decreased significantly in the control group when sampled concurrently with the hyperbaric exposure (mean/median − 2.74 g/L/ − 0.95 g/L, $p=0.02$). There was, however, no statistically significant difference between the two groups at any point. Results for changes in albumin are shown in Table 4.

Table 3 Changes in GFAp, NfL and tau – comparison between groups

		Intervention group ($n=14$)	Control group ($n=12$)		
Sample 1 Before exposure		Absolute value	Absolute value		*p* value
	GFAP (pg/mL)	62.1 (33.0) 56.7 (25.7; 150.2)	63.2 (24.8) 61.9 (20.3; 113.4)		0.92
	NfL (pg/mL)	8.29 (4.86) 6.22 (4.2; 19.01)	8.69 (5.02) 7.18 (4.12; 21.13)		0.84
	Tau (pg/mL)	0.25 (0.23) 0.20 (0.01; 0.88)	0.34 (0.62) 0.16 (0.01; 2.28)		0.83
	Variable	Absolute change	Absolute change	Absolute difference in mean change between exposed and controls	*p* value
Sample 2 At 33–34 h of hyperbaric exposure	GFAP (pg/mL)	2.60 (17.50) 4.76 (−33.61; 31.38) (−6.44; 11.12)	−15.1 (18.8) −8.9 (−62.2; 3.8) (−26.1; −6.2)	17.7 (4.6; 31.8)	0.02
	NfL (pg/mL)	1.01 (1.80) 1.24 (−3.57; 4.42) (0.05; 1.88)	−1.33 (3.68) 0.15 (−11.98; 1.16) (−3.60; 0.26)	2.34 (0.44; 4.76)	0.02
	Tau (pg/mL)	0.11 (0.34) 0.01 (−0.19; 1.11) (−0.03; 0.30)	−0.14 (0.66) 0.02 (−2.16; 0.39) (−0.54; 0.11)	0.25 (−0.07; 0.72)	0.27
	Variable	Absolute change	Absolute change	Absolute difference in mean change between exposed and controls	*p* value
Sample 3 At 104–108 h After hyperbaric exposure	GFAP (pg/mL)	0.97 (17.81) − 5.80 (−28.3; 32.28) (−7.68; 10.08)	4.44 (22.76) 1.34 (−21.46; 61.44) (-6.55; 18.01)	−3.47 (−19.21; 11.63)	0.68
	NfL (pg/mL)	0.49 (1.46) 0.91 (−2.40; 2.47) (−0.28; 1.18)	−1.12 (3.33) -0.17 (-11.44; 0.92) (−3.14; 0.13)	1.62 (−0.00; 3.91)	0.07
	Tau (pg/mL)	−0.02 (0.29) − 0.05 (−0.50; 0.78) (−0.16; 0.13)	−0.18 (0.62) 0 (−2.12; 0.14) (−0.55; 0.03)	0.16 (−0.13; 0.59)	0.52

Mean (Standard deviation) / Median (Min; Max) / (Bootstrapped (10,000 replicates) 95% Confidence interval for mean) are presented. Calculation of confidence interval for continuous variables (absolute change) is based on bootstrapping of 1000 replicates picking the 2.5 and 97.5 percentiles of the 10,000 mean differences as confidence interval. For difference in change between groups Mean (95% CI) is presented. For comparison between groups the Fisher's Non-Parametric Permutation Test was used

Venous gas emboli

No VGE were detectable using precordial Doppler ultrasound.

Conclusion

In the present study, hyperbaric exposure to 401 kPa during 36 h was not associated with statistically significant increases in GFAp, NfL or tau concentrations in serum. Oxygen partial pressure never exceeded 50 kPa during exposure and the decompression regimen employed was selected to be conservative, without any need for oxygen breathing during the ascent (Gennser 2019). The nitrogen partial pressure reached levels that would be expected to mildly affect the CNS. By keeping known pertinent factors at or below levels where they would be expected to cause harmful effects, study subjects were kept as safe as possible although the environmental conditions were sufficient to induce neurological stress.

Tau, GFAp, and NfL are established as markers of brain injury after trauma and cerebral apoplexia, but they have also been reported to increase in conjunction with far less traumatising neurological stressors, such as protracted apnea, deep diving, and uneventful anaesthesia. Furthermore, both GFAp and tau are claimed to be secreted in response to increased neuronal activity and stress. It is plausible that

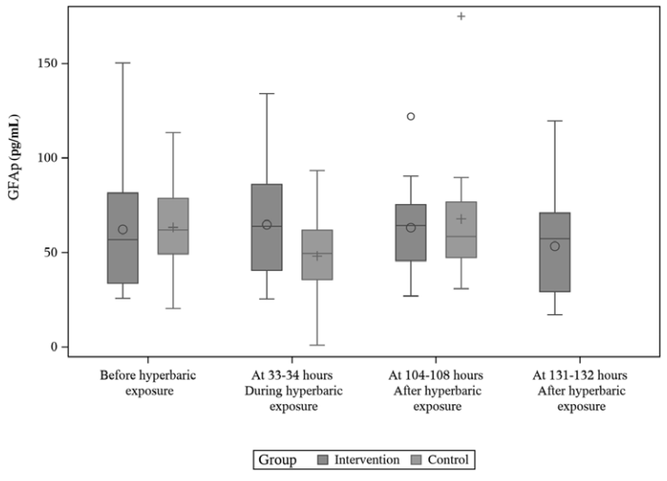

Fig. 1 GFAp levels (pg/mL) before, during and after hyperbaric exposure

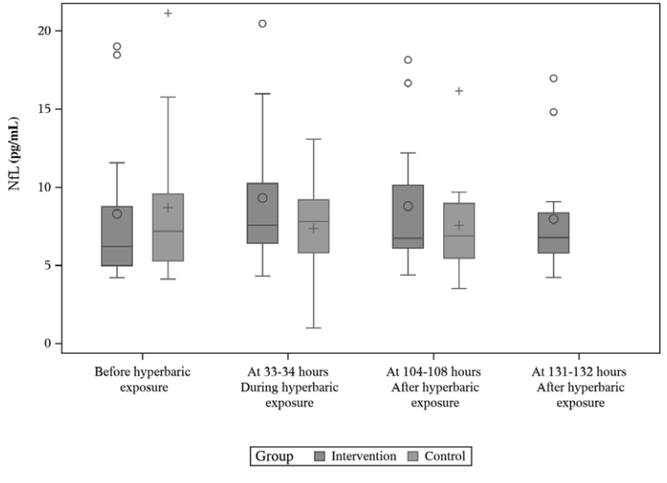

Fig. 2 NfL levels (pg/mL) before, during and after hyperbaric exposure

the CNS is affected, prior to clinical symptoms becoming evident, by increased ambient pressures, increased nitrogen and oxygen partial pressures, or a combination of these and, hence, these potentially neurological harmful exposures could result in elevated levels of tau, GFAp and NfL.

During hyperbaric exposure, an increase in NfL concentration was seen in the intervention group, but it did not reach statistical significance. At the same time, mean, but not median, NfL concentration decreased in the control group, which resulted in a statistically significant difference in NfL absolute change between the groups at that point. Like NfL, GFAp concentration decreased in samples collected from the control group, while the intervention group

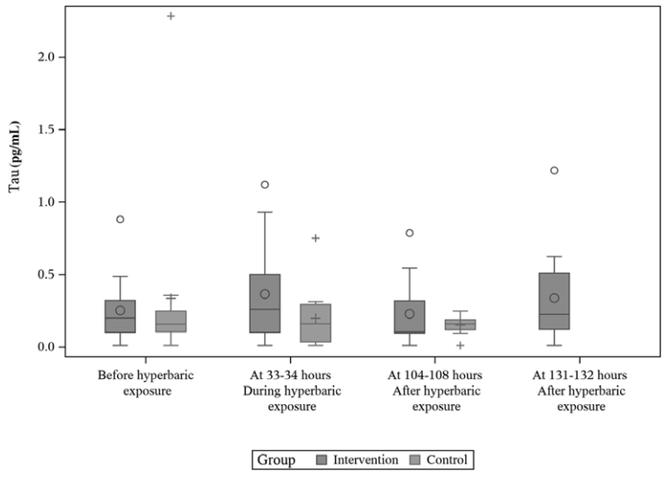

Fig. 3 Tau levels (pg/mL) before, during and after hyperbaric exposure

was pressurized, causing a statistically significant difference in absolute GFAp change between the groups.

A strength of the study was that samples were collected during subject exposure to 401 kPa. It was thereby possible to obtain analyses devoid of VGE, at a point when the subjects were influenced by hydrostatic and gas pressures alone. Decompression of samples collected from the intervention group during hyperbaric exposure might have generated VGE in the test tubes, but the size and structure of GFAp, NfL, and tau molecules make it most unlikely that they could have been degraded or deformed by gas bubbles. There was no neuronal tissue in the venous blood samples which precluded further increase in GFAp, NfL, and tau concentration in the test tubes during decompression.

After completed decompression, no VGE was found in any of the subjects. Although there is a known variability in VGE formation after diving, the lack of VGE in our study most likely could be explained by a conservative decompression regimen. In the absence of observed VGE, changes in GFAp, NfL and tau would likely have been caused by increased ambient pressure alone.

The results from this study contrasted with results from a small pilot study on 10 divers, where tau concentration had increased significantly, but NfL and GFAp concentrations were unchanged after repeated diving during four days to at most between 52 and 90 m (Rosén 2019). However, hyperbaric exposure to 401 kPa followed by slow decompression is a qualitatively different exposure compared to repeated and deep diving and the results could be consistent with each other. In the diving study, subjects used trimix, a breathing gas containing oxygen, helium and nitrogen, and partial pressures of oxygen were kept at 130 kPa, whereas in the present study, the breathing gases were oxygen and nitrogen with the partial pressure of oxygen never exceeding 50 kPa. Factors, such as helium or oxygen partial pressures, might have caused the increased tau value seen in the aforementioned diving study, in which the decompression stress was also greater. Contrary to the present study, in the diving study, Doppler detectable VGE was present after most of the dives. Additionally, immersion in water causes redistribution of blood flow, which could potentially change cerebral perfusion and cerebral gas pressures, and thereby affect the release of GFAp, NfL, and tau into the blood. It is possible that the hyperbaric exposure and decompression regimen employed in this study was not sufficiently challenging to affect the CNS in such a way that serum GFAp, NfL or tau concentrations were increased, given that the oxygen partial pressure was kept below what is considered a toxic level, and the decompression stress appeared mild.

Samples taken during and after hyperbaric exposure were collected at approximately the same time of day, which excludes diurnal variation as a cause. Diurnal variation of Hct and albumin has been reported to be < 3% (Sennels 2011, Andersen 2015). It is also unlikely that the decrease in GFAp and NfL in the control group represented an impaired circadian rhythm, as this phenomenon was seen neither in the intervention group nor later. It seems implausible that the lower GFAp and NfL concentrations could be explained by age or BMI but unknown confounding factors and selection bias may have affected the results. Measurement error is improbable as the internal quality control samples gave the expected values.

Table 4 Changes in Haemoglobin, Haematocrit and Albumin – comparisons within and between groups

Sample	Variable	Intervention group ($n=14$)		Control group ($n=12$)		p value between groups
		Absolute value	p value within group	Absolute value	p value within group	
Sample 1 **Before exposure**	Albumin g/L	46.84 (3.66) 46.25 (41.7; 54.2) (44.72; 48.95) $n=14$		47.62 (2.86) 47.25 (43.90; 51.90) (45.57; 49.67) $n=10$		0.41
	Haemoglobin g/L	158.00 (13.06) 161.50 (136.0; 190.0) (150.46; 165.54) $n=14$				
	Haematocrit (%)	45.61 (2.76) 46.00 (40.0; 49.0) (43.95; 47.28) $n=13$				
Sample 2 **At 33–34 h of hyperbaric exposure**	Albumin g/L	45.78 (3.97) 46.65 (40.0; 53.1) (43.26; 48.31) $n=12$	0.53	44.88 (2.942) 46.30 (39.10; 49.20) (42.91; 46.86) $n=11$	0.02	0.39
Sample 3 **At 104–108 h** **After hyperbaric exposure**	Albumin g/L	46.69 (3.81) 46.60 (40.6; 54.3) (44.39; 48.99) $n=13$	0.81	45.79 (2.59) 46.20 (39.40; 48.50) (44.05; 47.53) $n=11$	0.19	0.42
	Haemoglobin g/L	162.86 (7.62) 161.50 (150.0; 180.0) (158.46; 167.26) $n=14$	0.06			
	Haematocrit (%)	47.86 (2.25) 47.50 (44.0; 53.0) (46.56; 49.16) $n=14$	0.02			
Sample 4 At 131–132 h After hyperbaric exposure	Albumin g/L	46.02 (3.19) 45.20 (42.1; 52.7) (44.18; 47.87) $n=14$	0.12			

For continuous variables Mean (Standard deviation) / Median (Min; Max) / (95% Confidence interval for mean) / $n=$ are presented

Within each group, absolute values for haemoglobin and haematocrit were compared to baseline using Wilcoxon signed-rank test. For comparison of albumin values between groups Mann–Whitney test was used

It is possible that level of hydration did influence the measured concentrations of GFAp, NfL, and tau. Hb, Hct (Matomäki 2018) and albumin (Miller 2019) have been used to assess hydration status. Prolonged exposure to increased ambient pressure may result in decreased Hb levels (Hofsø 2005, Luczynski 2019) that return to prior levels after cessation of exposure, which could influence estimation of hydration status. The study was conducted at a naval base in Sweden. Hospital laboratory facilities were too far away to make centrifugal measurement of Hct possible. The i-STAT blood analyser was chosen to measure Hb and Hct because it was portable. The important fact that i-STAT determined Hct conductometrically was, unfortunately, not recognised until the study was actually carried out. Consequently, all Hct samples taken during hyperbaric exposure had to be discarded, as potential VGE induced by decompression of the samples interfered with the results of the measured conductivity. This error could have been avoided with better planning and is an obvious shortcoming of the study that should be avoided in the future.

Mean Hct and Hb values obtained in the intervention group had increased after hyperbaric exposure but the change was only significant for Hct. Valid albumin measurements from both studied groups were available before, during and after hyperbaric exposure. There were never any significant differences in albumin values between the groups, but the spurious decrease in albumin in the control group at the time when the intervention group was exposed to hyperbaric pressure makes a relative dehydration in the intervention group possible. Measured albumin values are

by many hospital laboratories reported to be 10–15% lower if taken during bed rest. During the present study, subjects in the intervention group were mainly inactive in a hyperbaric chamber while subjects in the control group moved as usual. This may have biased the albumin results giving comparatively lower albumin results in the intervention group, which would point to a larger actual difference in albumin values between the groups than measured. Due to the lack of Hct measurements during hyperbaric exposure assessment of hydration was less precise, but the small increase in Hct after exposure and the unchanged albumin values among exposed individuals make it improbable that significant dehydration confounded the results. Differences between the groups during hyperbaric exposure were predominantly due to the decrease in GFAp and NfL in the control group. If dehydration was present among individuals in the intervention group, a significant increase in GFAp, NfL, and tau would have been expected, which was not the case.

There were a few GFAp, NfL, and tau values, most notably in the intervention group, that differed considerably from the median. It is unclear if they were caused by measurement errors or physiological diversity. These values represented 1–2 individuals in the control group and 1–3 individuals in the intervention group, respectively, depending on protein analysed. As a result, the distribution of results was partly skewed but as both mean and median values were reported and a non-parametric statistical technique used, disproportionate weight to outliers was averted.

The study was carried out during naval training. Hence, the number of eligible study subjects was limited and the hyperbaric regimen predetermined. The small number of subjects was a weakness of the study. There is a possibility that an effect did exist and that a larger study would have yielded statistically significant results.

In conclusion, hyperbaric exposure to 401 kPa for 36 h followed by slow decompression over 70 h was not associated with statistically significant increases in GFAp, NfL or tau concentrations. Diurnal variation, changes in albumin levels or hydration were considered unlikely to have confounded the results but the lack of information about Hct made assessement of hydration status during hyperbaric exposure less precise. A larger study with an appropriate control group and measurement of Hct levels during hyperbaric exposure is needed to validate these results. Future studies on divers both with and without DCS would be desirable to establish the role of GFAp, NfL, and tau in hyperbaric research. Nevertheless, in view of the presented results, saturation exposure to 401 kPa seems to be a procedure not harmful to the CNS.

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Author contributions The project was conceived and designed by AR and MG. AR, AK and NO collected the blood samples. KB and HZ were responsible for blood analyses. AR drafted the manuscript, which NO, AK, MG, GS, KB, HSL and HZ critically revised. All authors approved the final version before submission.

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Compliance with ethical standards

Conflicts of interest HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

Availability of data and material The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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Paper III

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ORIGINAL ARTICLE

Protein tau concentration in blood increases after SCUBA diving: an observational study

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Abstract

Purpose It is speculated that diving might be harmful to the nervous system. The aim of this study was to determine if established markers of neuronal injury were increased in the blood after diving.

Methods Thirty-two divers performed two identical dives, 48 h apart, in a water-filled hyperbaric chamber pressurized to an equivalent of 42 m of sea water for 10 min. After one of the two dives, normobaric oxygen was breathed for 30 min, with air breathed after the other. Blood samples were obtained before and at 30–45 and 120 min after diving. Concentrations of glial fibrillary acidic, neurofilament light, and tau proteins were measured using single molecule array technology. Doppler ultrasound was used to detect venous gas emboli.

Results Tau was significantly increased at 30–45 min after the second dive ($p < 0.0098$) and at 120 min after both dives ($p < 0.0008$/$p < 0.0041$). Comparison of matching samples showed that oxygen breathing after diving did not influence tau results. There was no correlation between tau concentrations and the presence of venous gas emboli. Glial fibrillary acidic protein was decreased 30–45 min after the first dive but at no other point. Neurofilament light concentrations did not change.

Conclusions Tau seems to be a promising marker of dive-related neuronal stress, which is independent of the presence of venous gas emboli. Future studies could validate these results and determine if there is a quantitative relationship between dive exposure and change in tau blood concentration.

Keywords Biomarkers · Brain · Central nervous system · Diving · Diving research · Proteins · Venous gas embolism

Abbreviations

BMI	Body mass index
CNS	Central nervous system
CV	Coefficient of variation
DCS	Decompression sickness
GFAp	Glial fibrillary acidic protein
KISS	Kisman integrated severity score
KM	Kisman Masurel
kPa	Kilopascal

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msw	Meters of seawater
NfL	Neurofilament light
Simoa	Single molecule array
SwAF	Swedish armed forces
tau	Protein tau
UCH-L1	Ubiquitin carboxy-terminal hydrolase L-1
VGE	Venous gas emboli
QC	Quality control

Introduction

It is well-known that diving is not without certain risks. The diver is exposed to increased ambient pressure that causes inert gas accumulation and inhaled gases can, at sufficient depth, exert a noxious effect on the central nervous system (CNS). Oxygen becomes harmful to the CNS when its partial pressure exceeds 160 kPa, equivalent to a depth of about 66 m of seawater (msw) when breathing air. Symptoms of oxygen toxicity range from cognitive and sensory impairment to manifest convulsions (Bitterman 2004). Adverse effects of nitrogen, typically behavioural and intellectual disturbances, become apparent as the partial pressure of nitrogen exceeds 300 kPa (equivalent to a depth of about 28 msw when breathing air) and gradually worsen with increasing pressure (Clarke 2015).

Rapid ascent at the end of a dive may cause barotrauma to the lungs and sinuses. Nitrogen taken up by the body during diving may come out of solution as ambient pressure decreases and form gaseous bubbles in both blood and tissues, which is a common occurrence after diving (Nishi 1981, Eckenhoff 1990). Nitrogen bubbles in the blood can be detected by Doppler ultrasound and are referred to as venous gas emboli (VGE) (Blogg et al. 2014). The amount of VGE in the blood after diving depends on both the amount of accumulated inert gas and decompression stress, but a substantial individual variability exists (Germonpre and Balestra 2017, Papadopoulou et al. 2018). Though VGE is a normal phenomenon after diving, release of dissolved gas as bubbles is considered to be the cause of decompression sickness (DCS) (Eftedal et al. 2007, Blogg et al. 2014). The risk of DCS is correlated to VGE load after diving (Sawatzky 1991, Eftedal et al. 2007). Oxygen breathing employed before (Castagna et al. 2009, Bosco et al. 2010), during (Bosco et al. 2010) and after diving (Blatteau and Pontier 2009) have all been associated with reduced amounts of inert gas bubbles (VGE) in blood, as have active hydration (Gempp et al. 2008), whole body vibration (Germonpre et al. 2010) and sauna-induced heat exposure (Gempp and Blatteau 2010) before diving.

Historically, there have been discussions as to whether repeated, and especially deep diving could be harmful to the nervous system. Several radiological studies involving divers have been published, but the results yielded are hard to interpret, as both the cause and clinical significance of observed CNS lesions often remain uncertain (Knauth et al. 1997, Tetzlaff et al. 1999, Grønning and Aarli 2011, Kohshi et al. 2014, Coco et al. 2019). There have been reports of impaired cognitive performance among both professional and recreational divers without documented DCS, but results from published studies are conflicting. Neuropsychological changes were found in 20% of saturation divers when tested before and after 3.5 years of diving (Vaernes et al. 1989). Saturation divers with self-reported forgetfulness and loss of concentration were found to have mild cognitive deficits when tested objectively and compared to matched controls (Taylor et al. 2006). In a retrospective study, experienced saturation divers did not only have more neurological symptoms but also exhibited more subjective problems with memory and concentration than individuals in a non-diving control group (Todnem et al. 1990, 1991) and experienced recreational divers without a history of DCS have been shown to have a worse short-term memory compared to non-diving control subjects when tested after an average of 12 years diving (Hemelryck et al. 2014). Studies have found inferior neuropsychometric test results among recreational (Balestra et al. 2016), professional (Ergen et al. 2017) and experienced breath-hold divers (Billaut et al. 2018) compared to non-diving controls. Depth and number of dives have been reported to have a negative influence on cognitive performance among recreational divers when tested retrospectively (Slosman et al. 2004). However, contrary to these results, one retrospective study found no difference in neuropsychometric test results when professional divers were compared to matched controls (Cordes et al. 2000), nor was there evidence of neuropsychological impairment among professional, non-saturation divers without a history of DCS when followed-up over 12 years in a longitudinal study (Bast-Pettersen et al. 2015). Post-dive cognitive function studies such as these are hard to interpret, as confounding factors might influence the results, and decreased neuropsychological test performance is not tantamount to neurological impairment.

Increased partial pressure of oxygen (Ferrer et al. 2007, Camporesi Bosco 2014, Bosco et al. 2018) and nitrogen (Bhullar et al. 2016), present during diving, could cause oxidative stress and increased levels of reactive oxygen species (ROS), which could potentially harm cells in the CNS. If diving has a detrimental effect on the brain, it would likely induce changes in the concentration of biochemical markers known to increase in response to CNS trauma or neuronal stress. GFAp is an astrocytic protein involved in several neuronal processes, including synaptic transmission. It has been studied both as a marker of neuronal damage in the context of traumatic brain injury and cerebral haemorrhage when venous concentration increases (Foerch et al. 2012, Zetterberg and Blennow 2016, Gill et al. 2018), and as a marker of degenerative disease (Siracusa et al. 2019). Animal studies suggest that increased immunologic and neuronal activity, as well as neuronal stress could result in changed concentrations of GFAp (Wang and Hatton 2009, Brenner 2014, Femenia et al. 2018). Neurofilament light (NfL) is a neuronal cytoskeletal component found mainly in myelinated subcortical axons. Serum NfL concentrations are increased in patients with cerebral traumas, ranging from sports related concussions (Shahim et al. 2018) to severe traumatic brain injuries (Zetterberg and Blennow 2016, Shahim et al. 2016). Neurodegenerative disorders such as multiple sclerosis and Alzheimer's disease are associated with increased NfL concentrations in blood (Bergman et al. 2016, Kahlil et al. 2018). Uncomplicated general anaesthesia in conjunction with orthopedic surgery has also been associated with increased blood levels of NfL (Evered et al. 2018).

Tau is a microtubular protein present mainly in unmyelinated cortical axons but also, to a lesser extent, in the liver, kidneys and testes. Tau could be both passively released as a result of manifest axonal damage (Zetterberg and Blennow 2016) and actively secreted in connection to increased neuronal activity in response to stress (Sato et al. 2018). Neurodegenerative diseases, sports related concussions, boxing and traumatic brain injuries are all associated with increased tau levels in blood (Zetterberg et al. 2006, Neselius et al. 2012, Zetterberg and Blennow 2016, Mattson 2017, Shahim et al. 2018). As with NfL, increased blood concentrations of tau have been found after uneventful general anaesthesia in conjunction with orthopedic surgery (Evered et al. 2018). Physiological stressors such as protracted apnea among breath-hold divers (Gren et al. 2016) and high intensity interval training (Battista et al. 2018) have both been associated with increased tau levels in blood. A small pilot study found increased serum tau levels in blood after repeated deep, open sea diving (Rosén et al. 2019). In contrast, tau levels were not increased in cerebrospinal fluid in a small study on divers with DCS, though only one of seven patients in the study had CNS symptoms (Shahim et al. 2015). In addition, serum tau did not increase in response to nitrox saturation exposure in a study on submariners (Rosén et al. 2020).

The present study tested the hypothesis that diving to 42 msw would, via neuronal stress, incur a change in GFAp, NfL and tau. It was also investigated whether breathing normobaric oxygen after diving changed the amount of GFAp, NfL and tau in blood.

Materials and methods

The study was conducted at the Swedish Armed Forces (SwAF) naval base in Karlskrona, Sweden, during June 2015 and November 2018, as part of a project where subjects performed identical dives breathing either air or normobaric oxygen after decompression. The study was prospective, observational, registered at ClinicalTrials.gov (NCT02468752) and approved by Swedish ethical review authorities. Participants were recruited among professional divers from the SwAF, the Swedish Coast Guard and the Swedish Police. Written consent was obtained from all subjects before the start of the study.

Altogether, 33 professional divers were recruited. Data concerning age, gender, weight, height, medication and prior DCS were collected from all subjects. A water-filled hyperbaric chamber was used to simulate diving. After entering the chamber, the study subjects immersed themselves in water and rested in a horizontal position close to the bottom of the chamber for the duration of the experiment. All study subjects breathed air using an open circuit system.

The chamber was electronically pressurized to 521 kPa (equivalent of 42 msw) for 10 min by an external operator. Compression speed was 200 kPa/min, and decompression speed 90 kPa/min (equivalent to 9 msw/min), with a safety stop made at 151 kPa (equivalent to 5 msw) for 3 min. After surfacing, either oxygen or air was breathed for 30 min using tight-fitting face masks with demand valves. The breathing gas a particular study subject used after the first dive was randomly chosen by an independent operator and hence unknown to both study subjects and study personnel. In June 2015 (16 subjects, 32 dives), oxygen or air breathing commenced immediately after surfacing, while in November 2018 (16 subjects, 32 dives), mask breathing was deliberately delayed by 15 min. After a predetermined interval of 48 h, all subjects performed an identical second dive, with a switch of the breathing gases afterwards. Study subjects and all study personnel were unaware of the breathing gas used after a particular dive throughout the trial.

Immediately after surfacing, the subjects were monitored for VGE by a blinded operator using precordial Doppler ultrasound (DBM9008, Techno Scientific Inc, Ontario, Canada). Measurements were made every five minutes during the first 30 min and every 15 min thereafter for a further 90 min. Venous gas emboli were measured using the Kisman Masurel (KM) grading system, which is an ordinal scale based on categorical data describing amplitude, frequency and duration of VGE (Kisman 1978a). Maximal KM grades were recorded for every subject after each dive (VGE_{max}). For each subject, the Kisman Integrated Severity Score (KISS) algorithm (Kisman et al. 1978b, Jankowski et al. 2004) was used to convert all KM grades collected

from 0–30 min and 0–120 min after diving into individual integrated scores ($KISS_{30min}$ and $KISS_{120min}$).

Venous blood samples were collected 1–3 h before the first dive (sample 1, baseline), after mask breathing had ended (sample 2, 30–45 min after diving) and 120 min after diving (sample 3). For the second dive, the same blood protocol was performed, with the resulting samples named 4–6. Besides providing a baseline value before the second dive, Sample 4 was also used as the last (48 h after diving) in the series of samples taken after the first dive.

Plasma EDTA tubes (Vacuette nr 454410, Hettish Labinstrument AB, Sweden) were used for blood collection and samples were centrifuged for 15 min at 2500 rpm and 4° centigrade (Sorvall ST 8/8R centrifuge, Thermo Scientific, Germany). Directly after centrifugation, aliquots of 500 µL plasma were frozen at −18° centigrade for 1–4 days, transported on dry ice and then stored at −78° centigrade until analysed.

GFAp, NfL and tau concentrations were measured using the Human Neurology 4-Plex A assay on an HD-1 Single molecule array (Simoa) instrument (Quanterix, Lexington, MA, USA). The 4-Plex assay also included ubiquitin carboxy-terminal hydrolase L-1 (UCH-L1), which was not further assessed in this study. All samples were analysed together in one batch. For quality control (QC) samples with GFAp concentrations of 87.2 pg mL^{-1} and 465.2 pg mL^{-1}, coefficients of variation (CVs) were 7.3% and 3.2% respectively, for QC samples with NfL concentrations of 8.7 pg mL^{-1} and 46.2 pg mL^{-1} CVs were 9.2% and 3.7% and for quality control (QC) samples with tau concentrations of 1.0 pg mL^{-1} and 2.6 pg mL^{-1} CVs were 4.3% and 7.9%, respectively.

Statistics

Compilation of demographic data was performed using IBM SPSS® v24 (IBM, Armonk, NY, USA). Statistical analyses regarding absolute changes in GFAp, NfL and tau were performed by an independent statistical company using SAS® v9.4 (Cary, NC, USA). Concentrations of GFAp, NfL and tau (pg·mL^{-1}) were presented with both mean and median values with standard deviation (SD) and range (minimum and maximum values) stated. Fisher's non-parametric permutation test for paired observations was used in analyses within groups and Fisher's non-parametric permutation test in analyses between groups. Mean difference with 95% confidence interval (CI) was considered the main result. All significance tests were two-sided with a significance level of 5%. Spearman's rank correlation test was used to assess if there were any correlations between GFAp, NfL or tau concentrations and the presence of VGE. A positive or negative Spearman correlation coefficient greater than 0.8 was used as a limit to accept correlation between variables. KISS values and relative changes in GFAp, NfL and tau (%) were computed using Microsoft© Office Excel 2018 (Microsoft Corporation, Redmond WA, USA).

Missing data

One subject from the June 2015 cohort was excluded from the study due to dysbarism at the start of his first dive. His demographic data and Sample 1 results were omitted from all compilations and analyses. All remaining 32 divers completed two dives. There were no missing data for GFAp, NfL and tau. All VGE data were collected according to the protocol.

Results

Demographics

Among the 32 subjects that completed the study, 31 were males and one was female. Mean age was 37.9 years [standard deviation (SD) 8.1, range 26–55 years) and mean body mass index (BMI) was 25.4 (SD 1.7, range 21.4–29.2). Two subjects had experienced shoulder pain, possibly a sign of decompression sickness (DCS), earlier in their diving career but none had been treated in a hyperbaric oxygen chamber. One subject was prescribed antihypertensive medication with losartan, another for unknown reasons used diclofenac once between the two dives.

Biomarkers of neuronal injury

Effect of breathing oxygen after diving

To assess if breathing oxygen after diving had an effect on GFAp, NfL or tau concentrations, samples obtained after breathing oxygen (samples 2 and 3 or 5 and 6) were compared to matching samples after breathing air (samples 5 and 6 or 2 and 3, respectively). A potential period effect was adjusted for by using Fisher's non-parametric test to analyse changes in GFAp, NfL and tau concentrations between the first and second dive, comparing subjects who breathed air to subjects who breathed oxygen after the first dive. Oxygen breathing did not influence obtained NfL or tau values. No significant differences were found for these proteins at any point. At 30–45 min after diving there were no differences in GFAp results between divers breathing oxygen or air, but results for GFAp were higher 120 min after diving when breathing air compared to oxygen. Results for analyses of the effect of oxygen breathing on GFAp, NfL and tau are presented in Table 1.

Effect of prior diving (48 h before sample 4)

The difference between samples obtained before each dive (samples 1 and 4) was compared separately for subjects breathing oxygen and air after their first dive, to see if a residual effect was present. No significant differences in GFAp, NfL and tau concentrations immediately before the two dives were found, suggesting that neither diving nor oxygen breathing 48 h prior to the second dive had an effect on the results obtained. The results are presented in Table 2.

Effect of diving

GFAp, NfL and tau blood levels after diving were analysed linearly, comparing changes between sample 1 and samples 2–4 as well as between samples 4 and samples 5–6 in sequence (Table 3), and also according to breathing gas used, ignoring order of the dives (Table 4).

GFAp

When divers were analysed together irrespective of post-dive breathing gas, GFAp concentrations were significantly decreased at 30–45 min after the first dive but not at 120 min post-dive. No significant changes in GFAp concentrations were observed after the second dive (Table 3). In addition, there were no significant changes in GFAp after either dive when analysed based on post-dive breathing gas (Table 4).

NfL

Variations in mean NfL concentrations observed after the two dives did not reach statistical significance (Tables 3 and 4).

Tau

Mean protein tau concentrations increased after each dive. When all divers were analysed together, irrespective of

Table 1 Differences in GFAp, NfL and tau values after breathing oxygen compared to air-adjusted for period effect

Absolute change from sample 2 to sample 5 at 30–45 min after first or second dive					
	Breathing oxygen after first dive ($n=18$)	Breathing air after first dive ($n=14$)	*p*-value	Arithmetic mean difference between groups	Effect of breathing oxygen adjusted for period effect − mean difference between groups
GFAp (pg mL^{-1})	1.06 (9.15) 0.39 (−18.4; 14.05) (−3.46; 5.57)	−2.47 (11.98) −0.35 (−25.38; 19.47) (−9.42; 4.45)	0.35	3.53 (−4.15; 11.07)	1.76 (−2.08; 5.54)
NfL (pg mL^{-1})	−0.035 (1.061) 0.01 (−3.348; 1.424) (−0.563; 0.446)	0.249 (2.159) −0.233 (−2.477; 6.625) (−0.816; 1.503)	0.65	−0.284 (−1.429; 0.851)	−0.14 (−0.72; 0.43)
Tau (pg mL^{-1})	0.180 (0.920) −0.163 (−1.034; 2.315) (−0.273; 0.640)	0.275 (0.728) 0.147 (−0.657; 2.236) (−0.114; 0.700)	0.75	−0.095 (−0.695; 0.529)	−0.05 (−0.35; 0.26)
Absolute change from sample 3 to sample 6 at 120 min after first or second dive					
	Breathing oxygen after first dive ($n=18$)	Breathing air after first dive ($n=14$)	*p*-value	Arithmetic mean difference between groups	Effect of breathing oxygen adjusted for period effect − mean difference between groups
GFAp (pg mL^{-1})	4.92 (8.80) 3.93 (−9.31; 22.32) (0.58; 9.33)	−2.70 (11.22) 1.01 (−23.39; 17.42) (−9.21; 3.75)	0.039	7.62 (0.35; 14.78)	3.81 (0.18; 7.39)
NfL (pg mL^{-1})	−0.029 (0.780) −0.189 (−1.54; 1.985) (−0.404; 0.357)	−0.203 (0.796) −0.081 (−2.339; 0.621) (−0.664; 0.219)	0.54	0.175 (−0.394; 0.749)	0.09 (−0.20; 0.38)
Tau (pg mL^{-1})	−0.138 (1.509) −0.091 (−4.539; 3.702) (−0.855; 0.571)	0.285 (1.318) 0.233 (−1.792; 4.229) (−0.363; 1.047)	0.43	−0.423 (−1.459; 0.603)	−0.21 (−0.73; 0.30)

Mean (standard deviation)/median (range)/(95% CI for mean using the inversion of Fisher's non-parametric permutation test) are presented. For comparison between groups the Fisher's non-parametric permutation test was used. Mean difference between groups is presented with a 95% confidence interval. The arithmetic mean difference between groups equals twice the effect of given treatment (oxygen or air)

Table 2 Differences in GFAp, NfL and tau values before each dive—estimation of carryover effect

Absolute difference between samples 1 and 4						
	Subjects breathing oxygen after first dive ($n=18$)	*p*-value within group	Subjects breathing air after first dive ($n=14$)	*p*-value within group	Mean absolute difference between groups	*p*-value between groups
GFAp (pg mL^{-1})	−0.04 (12.77)	0.99	−6.88 (15.38)	0.12	6.83 (−3.14; 17.12)	0.18
	−4.52 (−20.05; 27.82)		−8.36 (−33.76; 15.47)			
	(−6.29; 6.39)		(−15.90; 2.06)			
NfL (pg mL^{-1})	0.02 (1.16)	0.95	0.60 (2.81)	0.62	−0.58 (−1.97; 0.80)	0.52
	−0.28 (−1.56; 3.47)		−0.22 (−1.33; 9.62)			
	(−0.520; 0.599)		(−0.65; 2.21)			
Tau (pg mL^{-1})	−0.04 (0.52)	0.75	0.01 (0.48)	0.96	−0.05 (−0.42; 0.31)	0.78
	−0.08 (−1.36; 0.86)		0.16 (−0.96; 0.56)			
	(−0.30; 0.21)		(−0.27; 0.29)			

Mean (standard deviation)/median (range)/(95% CI for mean using the inversion of Fisher's non-parametric permutation test) is presented. For comparison within groups Fisher's non-parametric permutation test for matched pairs was used. The confidence interval for the mean difference between groups was based on Fishers non-parametric permutation test

breathing gas used after a certain dive, the changes were statistically significant 120 min after both dives, and at 30–45 min after the second dive. Tau concentrations decreased between the dives with mean tau concentrations obtained prior to both dives being almost identical (Table 3). When changes in tau were analysed based on breathing gas used, mean levels were significantly increased at 30–45 min following oxygen breathing and at 120 min regardless of breathing gas used (Table 4).

Mean tau had increased by 29.1% (SD 44.7%) and 33.9% (SD 81.7%) at 120 min after the first and second dive, respectively. When all 64 dives were analysed together, the mean tau increase at 120 min after diving was 31.5% (SD 66.4%). In one diver, tau increased by 428% after the second dive. If this outlier value was omitted, the tau increase after the second dive was 21.2% (SD 41.5%), and was 25.2% (SD 43.7%) at 120 min for the remaining 63 dives taken together.

Correlation between markers of neuronal injury and venous gas emboli

Absolute values for GFAp, NfL and tau concentrations at 30–45 and 120 min after each dive as well as changes in GFAp, NfL and tau concentrations at these points were tested for correlation with matching VGE_{max}, and $KISS_{30min}$ or $KISS_{120min}$ values, respectively. No correlations were found. Results are presented in Appendices 1 and 2 (Figs. 1, 2, 3).

Discussion/conclusions

The present study found that tau levels in blood increased after a dive made to an equivalent of 42 msw depth. When an identical dive was performed 2 days later, the increases in tau observed after the first dive were reproduced. Tau changes seemed to be fast, with a measurable increase as soon as 30–45 min after diving, with yet higher tau levels found after 120 min. The design of the present study did not allow us to determine peak tau levels, or determine when they appeared, but after 48 h tau blood levels had returned to values obtained before the first dive.

Breathing normobaric oxygen after diving did not affect the tau blood levels obtained. Hence, tau changes could be analysed without regard to the breathing gas used after each dive. Nevertheless, the results were strengthened by the fact that increases in tau concentrations seen 120 min after diving also remained statistically significant for both groups when dives with oxygen and air breathing afterwards were analysed separately. There was no correlation between absolute tau concentrations or their changes and VGE loads, which indicates that tau release is not affected by the presence of VGE.

Tau blood levels rise after brain damage and neuronal cell death, with median concentrations of 49.5 pg mL^{-1} observed at 48 h after cardiac arrest among patients with poor neurological outcome (Mattson 2017), whereas in the present study, tau concentrations were 2.2 pg mL^{-1} 120 min after diving. The physiological mechanism that causes tau levels to increase, albeit to a lesser degree, after presumed neuronal stress without manifest injury to the CNS has not been identified. Breathing normobaric oxygen after diving

Table 3 Changes in GFAp, NfL and tau after diving

Sample 1: before first dive	Absolute value ($n=32$)	Absolute change compared to sample 1 ($n=32$)	*p*-value
GFAp (pg mL^{-1})	60.3 (26.2)		
	51.9 (31.2; 139.2)		
NfL (pg mL^{-1})	7.84 (7.72)		
	6.12 (3.4; 47.79)		
Tau (pg mL^{-1})	1.70 (0.89)		
	1.5 (0.4; 4.3)		
Sample 2: 30–45 min after first dive	Absolute value ($n=32$)	Absolute change compared to sample 1 ($n=32$)	*p*-value
GFAp (pg mL^{-1})	56.6 (24.0)	−3.69 (10.08)	0.045
	49.4 (29; 139)	−2.90 (−30.21; 20.22)	
		(−7.29; −0.06)	
NfL (pg mL^{-1})	7.75 (8.28)	−0.09 (0.92)	0.61
	5.66 (3.21; 51.01)	−0.24 (−1.29; 3.22)	
		(−0.40; 0.24)	
Tau (pg mL^{-1})	1.89 (0.84)	0.18 (0.73)	0.17
	1.86 (0.39; 3.68)	0.20 (−2.08; 2.22)	
		(−0.08; 0.44)	
Sample 3: 120 min after first dive	Absolute value ($n=32$)	Absolute change compared to sample 1 ($n=32$)	*p*-value
GFAp (pg mL^{-1})	56.5 (20.9)	−3.82 (12.88)	0.11
	55.4 (26.5; 128.5	−3.31 (−40.28; 15.25)	
		(−8.52; 0.87)	
NfL (pg mL^{-1})	8.11 (8.33)	0.271 (1.11)	0.18
	6.4 (3.05; 51.61)	0.11 (−1.68; 3.83)	
		(−0.13; 0.67)	
Tau (pg mL^{-1})	2.18 (1.47)	0.48 (1.03)	0.0008
	1.84 (0.45; 8.13)	0.27 (−1.03; 5.21)	
		(0.15; 0.82)	
Sample 4: before second dive (48 h after the first dive)	Absolute value ($n=32$)	Absolute change compared to sample 1 ($n=32$)	*p*-value
GFAp (pg mL^{-1})	57.3 (20.6)	−3.03 (14.16)	0.24
	58.3 (33; 135.7)	−5.83 (−33.76; 27.82)	
		(−8.18; 2.14)	
NfL (pg mL^{-1})	8.11 (8.43)	0.27 (2.03)	0.55
	6.02 (3.6; 51.25)	−0.28 (−1.56; 9.62)	
		(−0.38; 0.97)	
Tau (pg mL^{-1})	1.68 (0.81)	−0.02 (0.50)	0.81
	1.54 (0.58; 3.16)	0.01 (−1.36; 0.86)	
		(−0.20; 0.16)	
Sample 5: 30–45 min after second dive	Absolute value ($n=32$)	Absolute change compared to sample 4 ($n=32$)	*p*-value
GFAp (pg mL^{-1})	56.2 (19.5)	−1.14 (8.34)	0.45
	55 (31.5; 127.6)	−0.88 (−14.61; 13.69)	
		(−4.21; 1.91)	
NfL (pg mL^{-1})	7.84 (7.64)	−0.27 (2.07)	0.48
	6.16 (3.44; 47.66)	−0.11 (−7.54; 6.38)	
		(−1.01; 0.45)	

Table 3 (continued)

Sample 5: 30–45 min after second dive	Absolute value ($n=32$)	Absolute change compared to sample 4 ($n=32$)	p-value
Tau (pg mL^{-1})	2.11 (1.32) 1.89 (0.53; 5.27)	0.42 (0.92) 0.24 (−1.67; 3.09) (0.10; 0.76)	0.0098
Sample 6: 120 min after second dive	Absolute value ($n=32$)	Absolute change compared to sample 4 ($n=32$)	p-value
GFAp (pg mL^{-1})	58.1 (20.3) 57.3 (30.5; 138)	0.80 (10.06) 0.95 (−18.24; 22.63) (−2.81; 4.39)	0.65
NfL (pg mL^{-1})	8.01 (8.38) 6.12 (3.05; 51.95)	−0.10 (1.49) 0.19 (−7.31; 1.94) (−0.61; 0.34)	0.84
Tau (pg mL^{-1})	2.23 (1.56) 1.96 (0.6; 6.74)	0.54 (1.20) 0.12 (−0.90; 4.91) (0.14; 0.97)	0.0041

Mean (SD)/median (min; max)/(95% CI for mean using the inversion of Fisher's non-parametric permutation test) is presented. For comparison within groups the Fisher's non-parametric permutation test for matched pairs was used

did not affect tau blood levels in the present study, but it is still possible that exposure to supranormal partial pressures of oxygen might affect tau levels. Other possible causes of increased tau blood levels are increased ambient pressure per se, changes in cerebral perfusion during immersion, or compression stress. As there was no correlation between VGE and tau levels, decompression stress seems unlikely to have influenced the results.

Trimix breathing gas is used for deep dives and contains oxygen, helium and nitrogen. Tau increased by 98.8% in a small pilot study where ten divers performed repeated deep dives between 52–90 msw over 4 days using trimix (Rosén et al. 2019), with an oxygen partial pressure of 130 kPa during the dive and up to 160 kPa during decompression; nitrogen pressures at depth were around 176–193 kPa. In the present study the partial pressures of oxygen and nitrogen at depth were 109 kPa and 406 kPa, respectively. The trimix study found no correlation between VGE loads and tau concentrations, either in terms of their absolute values or their changes. The larger relative increase in tau blood levels after diving in the trimix study compared to the present study could be due to differences in dive depths, oxygen partial pressures, breathing gas, and number of dives between the two studies.

In another study, when submariners were exposed to a pressure equivalent to 30 msw (401 kPa) for 36 h in a dry hyperbaric chamber, then followed by slow decompression over a further 70 h, no significant change in tau blood concentration was seen (Rosén et al. 2020). The submariners experienced a lower ambient pressure than the divers in the present study, but their duration of exposure was longer. Tau was sampled before exposure, at 33–34 h of exposure, and after exposure had ended. No samples were obtained at either 30–45 or 120 min of exposure, making comparison between the present study and the submariner study difficult; it is possible that tau levels may have increased after initial pressurization to 30 msw (401 kPa) and then decreased, reaching baseline levels before a sample was obtained at 33–34 h. In the submariner study, the maximum oxygen partial pressure was 50 kPa, with a maximum nitrogen partial pressure of about 350 kPa, and the rate of decompression was 0.375–0.5 msw/h; this rate is much slower than that of the present study (9 msw/min).

It could be speculated that the changes in tau seen after diving were caused by oxidative stress and increased levels of ROS but the fact that tau increases were unaffected by normobaric oxygen breathing during 30 min after diving, an exposure three times longer and just slightly less hyperoxic than the dive itself, makes such a mechanism less plausible. Higher nitrogen partial pressures in this study did not coincide with larger tau increases, compared to the trimix study.

Different preconditioning techniques have been shown to decrease VGE load after diving though none of them were employed in this study, which makes it impossible to judge their potential effect on tau concentrations. Oxygen breathing before diving would probably have reduced VGE load but at the same time caused oxidative stress to the diver. As there was no association between VGE and tau, and the effect of oxidative stress on tau is uncertain, it is questionable if oxygen breathing before diving would have affected the results. Possible effects of other preconditioning techniques such as sauna-induced heat exposure or whole-body vibration remain to be elucidated.

Table 4 Changes in GFAp, NfL and tau—breathing air or oxygen after diving

Breathing oxygen after diving 30–45 min after diving	Absolute change ($n=32$)	p-value
GFAP (pg mL^{-1})	−1.76 (9.72) −1.26 (−22.73; 13.69) (−5.24; 1.75)	0.31
NfL (pg mL^{-1})	−0.24 (2.014) −0.28 (−7.54; 6.38) (−0.95; 0.452)	0.52
Tau (pg mL^{-1})	0.30 (0.77) 0.22 (−2.08; 2.22) (0.02; 0.57)	0.03
120 min after diving	Absolute change ($n=32$)	p-value
GFAP (pg mL^{-1})	−1.99 (10.65) −2.43 (−34.37; 15.25) (−5.81; 1.83)	0.31
NfL (pg mL^{-1})	−0.08 (1.637) 0.08 (−7.31; 3.83) (−0.64; 0.45)	0.83
Tau (pg mL^{-1})	0.60 (1.29) 0.24 (−1.03; 5.21) (0.16; 1.05)	0.0014
Breathing air after diving 30–45 min after diving	Absolute change ($n=32$)	p-value
GFAP (pg mL^{-1})	−3.07 (8.89) −2.41 (−30.21; 20.22) (−6.27; 0.11)	0.06
NfL (pg·mL^{-1})	−0.12 (1.03) −0.01 (−3.59; 1.83) (−0.49; 0.25)	0.53
Tau (pg·mL^{-1})	0.307 (0.903) 0.20 (−1.67; 3.09) (−0.01; 0.63)	0.06
120 min after diving	Absolute change (n = 32)	p-value
GFAP (pg mL^{-1})	−1.02 (12.82) 0.04 (−40.28; 22.63) (−5.68; 3.59)	0.66
NfL (pg mL^{-1})	0.24 (0.89) 0.27 (−1.27; 1.94) (−0.07; 0.56)	0.12
Tau (pg mL^{-1})	0.42 (0.90) 0.21 (−0.90; 4.18) (0.12; 0.74)	0.0034

For continuous variables mean (SD)/median (min; max)/(95% CI for mean using the inversion of Fisher's non-parametric permutation test) are presented. For comparison within groups the Fisher's non-parametric permutation test for matched pairs was used

A difference in GFAp blood concentrations 120 min after diving was observed when samples from divers breathing oxygen were compared to their paired air samples, which suggests that oxygen might have influenced the change in GFAp blood levels obtained at this point. Therefore, it was not prudent to analyse changes in GFAp blood levels without regard to breathing gas used after the dive. However, when GFAp samples were analysed in two separate groups based on breathing gas, GFAp blood levels were not significantly changed at any point, though a

decrease seen in GFAp 30–45 min after dives followed by air breathing almost reached significance ($p = 0.06$). When all dives were analysed irrespective of breathing gas post-dive, GFAp was significantly decreased at 30–45 min after

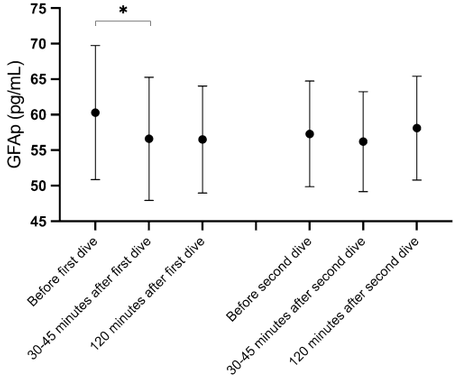

Fig. 1 GFAp concentrations after diving. Mean GFAp concentrations with 95% confidence intervals

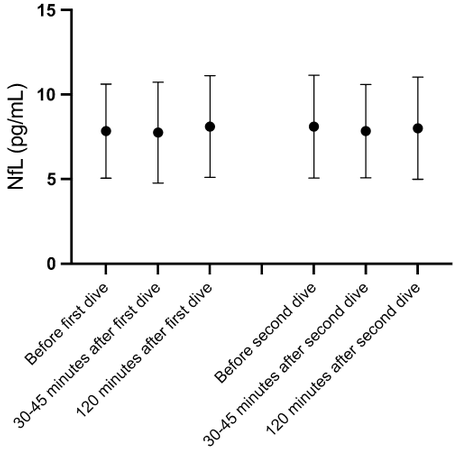

Fig. 2 NfL concentrations after diving. Mean NfL concentrations with 95% confidence intervals

the first dive but not at the same point after the second. There were no significant changes at 120 min after either dive. These disparate results regarding changes in GFAp blood levels after diving suggest that it would not be useful as a marker of dive-related, presumably neuronal, stress. There was no correlation between GFAp concentrations, or their changes and VGE loads.

NfL levels in blood did not change significantly in the present study, which is consistent with the aforementioned studies on deep repeated diving and prolonged hyperbaric exposure of submariners. The results do not support neuroaxonal injury, although the slow dynamics of NfL, with maximum increases seen later than seven days after an insult (Shahim et al. 2016), makes it a less well-suited marker in the setting of this study. NfL levels in blood were not influenced by oxygen breathing after diving or correlated to VGE load.

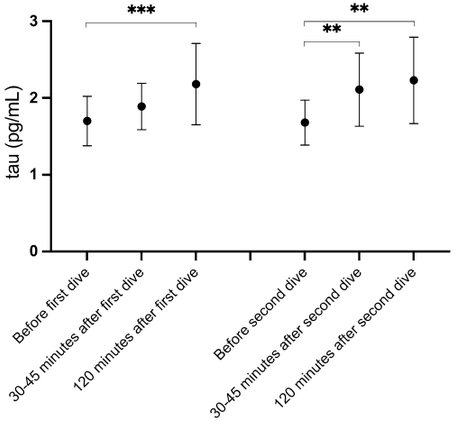

Fig. 3 Protein tau concentrations after diving. Mean tau concentrations with 95% confidence intervals

Dehydration is common after diving and could cause increased concentrations of proteins measured in blood but this parameter was not assessed, which is a shortcoming of this study. However, as tau increased while NfL remained unchanged and GFAp either remained unchanged or decreased, significant dehydration seems unlikely. Active hydration before diving is a preconditioning technique (Gempp et al. 2008) which theoretically may cause a decrease in venous protein concentrations after diving, but it was not employed in the present study.

Samples were taken only up to 120 min after each dive. Continued sampling may have yielded more detailed data on changes in tau concentration after diving, potentially making it possible to determine maximum tau values in blood after each dive.

In this study, tau blood levels increased after diving. The use of repeated, uniform dive exposures is a strength of the study and the fact that tau increases were similar after the first and the second dives makes the results convincing. Tau concentrations in blood were not correlated to VGE. Based on these results, as well as the aforementioned pilot study on deep trimix diving, tau seems to be a promising marker of dive-related, presumably neuronal, stress.

A larger study where subjects perform repeated dives to different depths and durations is necessary both to validate these results and to establish if there is a quantitative relationship between dive exposure and tau levels in blood. Blood sampling should ideally be frequent and continued for at least hours after each dive.

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Author contributions The project was conceived and designed by AR, NO and MG. AR, AK and NO collected the blood samples. HZ and JS were responsible for blood analyses. AR drafted the manuscript, which NO, AK, MG, GS, HSL, JS and HZ critically revised. All authors approved the final version before submission.

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Declarations

Conflict of interest HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

Availability of data and material The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval The study was approved by Swedish ethical review authorities as EPN Dnr 352–14 with supplementary approvals T847-15, T1032-18 and 202–05525.

Consent to participate Written consent to participate was obtained from all study subjects.

Consent for publication All authors have approved publication of the manuscript.

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Appendix 1

Correlations between VGE and tau, GFAp and NfL after diving when breathing oxygen or air

			VGE max	KISS
Treatment	**Sample**	**Variable**	**Spearmans correlation p-value n**	**Spearmans correlation p-value n**
Breathing oxygen after diving	**30–45 minutes after diving**	**GFAP, absolute value**	-0.05 0.78 n=32	-0.10 0.57 n=32
		NFL, absolute value	0.09 0.63 n=32	0.14 0.43 n=32
		tau, absolute value	0.25 0.17 n=32	0.21 0.25 n=32
		Absolute change of GFAP from before dive to sample	0.19 0.29 n=32	0.15 0.40 n=32
		Absolute change of NFL from before dive to sample	0.45 0.0095 n=32	0.43 0.013 n=32
		Absolute change of tau from before dive to sample	0.24 0.18 n=32	0.22 0.22 n=32
Breathing oxygen after diving	**120 minutes after diving**	**GFAP, absolute value**	-0.02 0.93 n=32	-0.01 0.94 n=32
		NFL, absolute value	0.02 0.91 n=32	-0.01 0.97 n=32
		tau, absolute value	-0.08 0.68 n=32	-0.08 0.65 n=32
		Absolute change of GFAP from before dive to sample	0.13 0.49 n=32	0.16 0.38 n=32
		Absolute change of NFL from before dive to sample	0.20 0.27 n=32	0.15 0.41 n=32
		Absolute change of tau from before dive to sample	-0.19 0.29 n=32	-0.22 0.23 n=32
Breathing air after diving	**30–45 minutes after diving**	**GFAP, absolute value**	0.29 0.11 n=32	0.27 0.14 n=32
		NFL, absolute value	0.32 0.073 n=32	0.33 0.064 n=32
		tau, absolute value	0.12 0.52 n=32	0.07 0.70 n=32
		Absolute change of GFAP from before dive to sample	-0.21 0.24 n=32	-0.20 0.28 n=32
		Absolute change of NFL from before dive to sample	-0.23 0.20 n=32	-0.25 0.16 n=32
		Absolute change of tau from before dive to sample	0.03 0.85 n=32	0.06 0.75 n=32
Breathing air after diving	**120 minutes after diving**	**GFAP, absolute value**	0.32 0.072 n=32	0.29 0.10 n=32
		NFL, absolute value	0.35 0.052 n=32	0.35 0.048 n=32
		tau, absolute value	0.15 0.41 n=32	0.16 0.38 n=32
		Absolute change in GFAP concentration from before dive to sample	-0.24 0.18 n=32	-0.17 0.34 n=32
		Absolute change of NFL from before dive to sample	-0.15 0.42 n=32	-0.21 0.25 n=32
		Absolute change of tau from before dive to sample	0.31 0.088 n=32	0.38 0.030 n=32

For each variable, the spearman's rank correlation coefficient is presented with corresponding p-value and number of observations

Appendix 2 Correlations between VGE and tau, GFAp and NfL after diving

Variable (pg·mL^{-1})	VGE_{max}	$KISS_{30\ min}$
GFAP absolute value sample 2	0.337 0.059 n=32	0.316 0.078 n=32
GFAP absolute change from sample 1 to sample 2	0.007 0.97 n=32	-0.028 0.88 n=32
NFL absolute value sample 2	0.417 0.017 n=32	0.433 0.013 n=32
NFL absolute change from sample 1 to sample 2	-0.066 0.72 n=32	-0.091 0.62 n=32
Tau absolute value sample 2	0.050 0.79 n=32	-0.044 0.81 n=32
Tau absolute change from sample 1 to sample 2	0.158 0.39 n=32	0.147 0.42 n=32

Variable (pg·mL^{-1})	VGE_{max}	$KISS_{120min}$
GFAP absolute value sample 3	0.376 0.034 n=32	0.361 0.043 n=32
GFAP absolute change from sample 1 to sample 3	-0.091 0.62 n=32	-0.082 0.66 n=32
NFL absolute value sample 3	0.456 0.0087 n=32	0.414 0.018 n=32
NFL absolute change from sample 1 to sample 3	-0.020 0.91 n=32	-0.103 0.57 n=32
Tau absolute value sample 3	0.038 0.84 n=32	0.071 0.70 n=32
Tau absolute change from sample 1 to sample 3	0.277 0.12 n=32	0.321 0.073 n=32

Variable (pg·mL-1)	VGE_{max}	$KISS_{30\ min}$
GFAP absolute value sample 5	-0.152 0.41 n=32	-0.127 0.49 n=32
GFAP absolute change from sample 4 to sample 5	0.021 0.91 n=32	0.024 0.90 n=32
NFL absolute value sample 5	0.040 0.83 n=32	0.090 0.62 n=32
NFL absolute change from sample 4 to sample 5	0.282 0.12 n=32	0.242 0.18 n=32
Tau absolute value sample 5	0.232 0.20 n=32	0.223 0.22 n=32
Tau absolute change from sample 4 to sample 5	0.157 0.39 n=32	0.196 0.28 n=32

Variable (pg·mL^{-1})	VGE_{max}	$KISS_{120min}$

Variable (pg·mL^{-1})	VGE_{max}	$KISS_{30\ min}$
GFAP absolute value sample 6	-0.036 0.84 n=32	-0.017 0.93 n=32
GFAP absolute change from sample 4 to sample 6	0.037 0.84 n=32	0.109 0.55 n=32
NFL absolute value sample 6	-0.067 0.72 n=32	-0.031 0.87 n=32
NFL absolute change from sample 4 to sample 6	0.021 0.91 n=32	-0.032 0.86 n=32
Tau absolute value sample 6	-0.007 0.97 n=32	-0.036 0.84 n=32
Tau absolute change from sample 4 to sample 6	-0.093 0.61 n=32	-0.059 0.75 n=32
For each variable, the spearman's rank correlation coefficient is presented with corresponding p-value and number of observations.		

Paper IV

Venous gas bubble load after immediate or delayed normobaric oxygen breathing post decompression

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Abstract

Introduction

Normobaric oxygen (NBO) is used as an adjunctive treatment for decompression illness before hyperbaric therapy. NBO has been shown to reduce the presence of venous gas emboli (VGE), but not as effectively as the combination of oxygen and hyperbaric pressure. The question arises whether NBO will be more efficient if administered before VGE appear.

Methods

Air-dives were conducted in a wet-chamber to 42 m (521 kPa). After decompression, the divers were given oxygen or air via a mask for 30 minutes. Divers and researchers were blinded as to which gas was delivered. In one series the oxygen breathing started immediately after decompression, while in the other oxygen breathing was delayed for 15 minutes. Presence of VGE were investigated using ultrasound Doppler.

Results

Immediate start of NBO significantly reduced the amount of VGE. After the NBO-period, only two of 13 divers exhibited any VGE compared to 11 of 13 divers after air breathing. The maximum bubble grades were significantly reduced. NBO after a 15 min delay also reduced the presence of VGE (nine of 24 divers with VGE after NBO vs 17 of 24 divers after air breathing). There was no difference in maximum bubble grades with delayed NBO, but the integrated bubble grade was significantly lower after NBO.

Conclusions

NBO helps to accelerate the removal of VGE. However, when NBO is applied before VGE emerge in the blood, the efficacy appears to be improved.

Key words: Decompression, Doppler, Hyperoxia, Venous gas embolism

INTRODUCTION

After a diver decompresses from a compressed air dive, bubbles consisting mainly of nitrogen may form in the tissues and venous blood. Breathing oxygen will increase the diffusion gradient of inert gas out of the bubbles, increasing the speed of resolution. This mechanism was first proposed and also illustrated in experiments by Paul Bert.[1]

In the 1970s, normobaric oxygen (NBO) was proposed as a method to alleviate symptoms of decompression sickness (DCS) before definite treatment by recompression could be administered.[2,3] Today, NBO is routinely given during transport of an injured diver to the site of recompression.[4,5] An epidemiological study has shown that NBO administered in the period between presentation of the initial symptoms and recompression treatment reduces the need for multiple recompressions to obtain optimal outcome.[6]

The same physical principle that causes oxygen breathing to increase the speed of bubble resolution also increases the speed of wash-out of inert gas remaining in solution. Thus, NBO could also be used to decrease the supersaturation period post decompression, diminishing the tendency of bubble formation and reducing the time with increased residual inert gas dissolved in the tissues. The latter would in principle allow the diver to perform repeated dives after a shorter surface interval, or remain at depth for a longer period during a second dive. Strangely enough, the use of NBO to increase the efficiency of repeated dives is not incorporated into most published decompression tables. The authors are aware of only one decompression table, the French Navy table Marine National 90,[7] where the effect of NBO breathing on residual nitrogen is included. However, it is unclear to what extent this is purely a theoretical construct or whether this part of the table has been extensively tested. NBO breathing to increase the efficiency of repeated dives is apparently not used often by French navy divers (Blatteau, personal communication).

Only a few papers in the peer-reviewed literature examine the effect of NBO breathing post-decompression.[8,9] The first of these compared the effects of air breathing with 30 min NBO, or 30 min at 6 m depth breathing oxygen after a 30 min dive to 30 m on the presence of venous gas emboli (VGE) after the treatments. NBO reduced the incidence of VGE significantly compared to control (air breathing) but the effect of a combination

of hyperbaric recompression and oxygen breathing (HBO) was significantly better.[8] The authors allowed an interval of 10 minutes between the end of decompression and start of treatment. No measurement of VGE was performed during this period or the treatment periods. Therefore, it is unclear whether the appearance of VGE had started before the treatments began. However, the fact that HBO was significantly more effective in removing VGE indicates that part of the effect of the recompression treatment was due to compression of bubbles. Given that the divers received oxygen in both treatments, the increase in inert gas diffusion gradient of the venous blood would be similar in the two conditions. If, however, bubbles had formed in tissues and blood, the diffusion distances would be increased and thus diffusion rates reduced. This could be alleviated immediately by the hyperbaric pressure.[10]

The second study considered awake goats exposed to a simulated submarine escape profile from 240 msw.[9] Even though NBO breathing started within five minutes after the end of decompression, bubbles were already evident in the central venous circulation. Administration of NBO significantly reduced the time to no bubbles remaining in the circulation, but it had no effect on the maximum bubble score, nor on the time with high bubble score, i.e., bubble load during the first 30 minutes post decompression.[9]

Both sets of results give rise to the question whether NBO breathing may be more effective if oxygen breathing is started before bubbles have started to form. This hypothesis was tested in the present study.

METHODS

This study was conducted at the Swedish Navy Diving and Medical Centre in Karlskrona Sweden. The dives were carried out in the diving simulator: a water filled pressure chamber fitted with a Lanphier-Morin barrier.[11]

Divers made two dives. After each dive they were given a mask to breathe compressed gas. After one dive the gas was air ('Control'), while after the other the gas was oxygen ('NBO'). Neither the divers nor the researchers knew which gas was given after any given dive. The selection of gas was carried out by the dive supervisor. The blinding code was not broken until all the data had been collected. The dives were balanced so

that roughly half of the divers breathed oxygen after their first dive. The minimum interval between dives was 48 hours. All dives were carried out according to the same profile: compression to 42 msw (521 kPa) using a compression speed of 20 msw/min. The exposure time was 10 minutes. This exposure allows a direct ascent to the surface according to the Swedish Navy air decompression tables. However, the initial dives were also part of table testing, and therefore a 'safety stop' of 3 min at 5 msw was added and then maintained in all the subsequent dives. The ascent rate was 9 msw/min.

The divers used open circuit scuba gear and wore a dry suit. The same equipment was used in both dives.

In the first dive series, the divers started to breathe from the mask as soon as they surfaced, and they continued to do so for the first 30 minutes after decompression. The masks were connected to long hoses, which allowed the divers to move into the neighbouring dry chamber and lie down on a gurney for Doppler ultrasound measurements. The divers sat still for the first 30 minutes apart from the measurement periods.

A second dive series was carried out using the same protocol except that the divers did not breathe from the mask until 15 minutes post-decompression, which they then continued for a further 30 minutes. As before, both the divers and the researchers were blinded to the gas breathed.

Ultrasound measurements were carried out according to the same methods as described previously.[12] In short, Doppler audio measurements were made at the precordial site using a Doppler Bubble Monitor (DBM9008; Techno Scientific Inc, Ontario, Canada). The subjects were asked to lie in the left lateral decubitus position during measurements. For the first 30 minutes after decompression, measurements were made every five minutes, then at 15-minute intervals until two hours had passed. At all time periods, measurements were made both with the subject at rest, and then after 'flex', where the subject kicked his or her feet away from the body vigorously three times. Venous gas emboli (VGE) were evaluated using the Kisman Masurel scale.[13] All measurements were made by one of the authors (SLB) who is an experienced operator. As Doppler grades are ordinal data, they are represented by Roman numerals in the

text. Kisman Integrated Severity Scores (KISS) were calculated from the KM bubble grades.[14] For statistical purpose and for calculation of the KISS the subdivisions of the bubble grades ('+' and '-') were given numeral values of 0.33 and 0.66. Thus, II+ was given the value 2.33, and III- was given the value 2.66. KISS were calculated for the time period 30–105 minutes in Series I and compared to the time period 45–120 minutes in Series II, i.e., for 75 min directly after the subjects finished breathing the test gases.

Subjects

Divers who took part in this study were volunteers from the navy or local coastguard. The study received ethical approval from the local ethics committee at Gothenburg university (EPN 352-14). All divers signed an informed consent form. Divers in the first series and the first half of the second series also took part in a study regarding brain injury markers. Blood was drawn from a venous puncture ~30-45 minutes and 120 minutes after the decompression.

In the first series, 16 divers took part (15 men, one female) with a mean age of 40 ±9.6 years (range: 25–55 years), height 181 ±7.1 cm (range: 169–192 cm), and weight 81.6 ±7.1 kg (range: 69–95 kg).

In the second series 32 divers took part (all male), with a mean age of 34 ±6.3 years (20–46 years), height 182 ± 8.7 cm (177–199 cm), and weight 86.2 ±10.3 kg (64–112 kg).

Statistics

Kisman Masurel grades are ordinal data and non-parametric tests should be used. To obtain the KISS score the original authors linearized the KM grades.[14] Often KISS values are expressed in averages and standard deviations, but, given that the KISS score is based on ordinal data the correct way to test such data is non-parametric test.[15] In this study both dive series were treated separately using Wilcoxon signed rank test for both KM grades and KISS. Comparison between the two series of experiments were made with the Mann Whitney U test. For comparisons of frequency of dives with high or low bubble grades Fischer's exact test was used. For all tests $p < 0.05$ was considered significant.

RESULTS

Series I

In Series I, a total of 16 two-man dives were performed. One dive had to be aborted when a diver had problems with pressure equilibration during the compression. This diver was replaced and the dive was completed successfully at a later date. No other complications were noted.

Three of the 16 divers had no measurable VGE at rest or after flex following both dives. These subjects were excluded from the rest of the analysis. Four of the divers had detectable bubbles during their NBO dives, and 12 of the 13 divers had bubbles during the Control dives ($p = 0.0037$, Fischer's exact test).

After the oxygen breathing period, VGE could only be found in two of the divers. In contrast, during the Control dive 11 of the 13 divers had bubbles present in the central venous circulation. ($p = 0.0021$, Fischer's exact test). Both the maximum KM grades and the KISS were significantly different between NBO and Control, both for the whole observation period and the period after mask-breathing (Table 1, Figure 1).

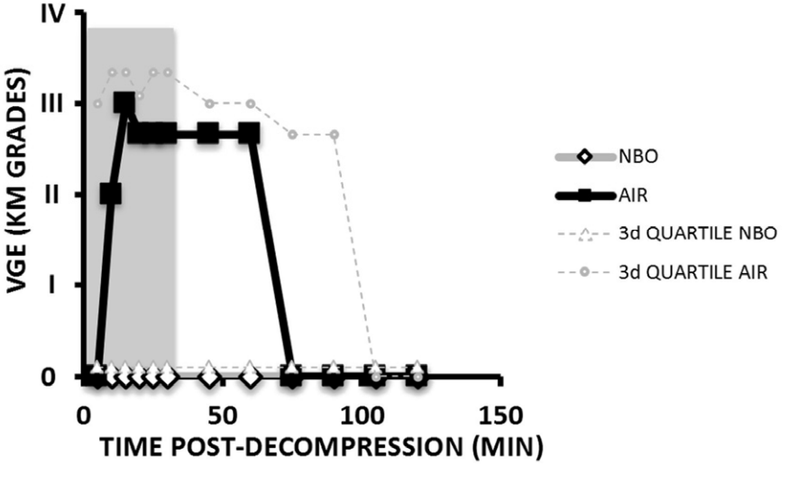

Figure 1*: Median maximum KM grades after dives to 42 m with 10 min exposure time. Mask breathing was started immediately after decompression and was maintained for 30 minutes (grey shaded area). AIR, air breathing; NBO, normobaric oxygen; N = 13.*

Series II

In Series II, 32 divers took part. Mask breathing started 15 minutes after end of decompression and was maintained for 30 minutes. All dives were performed without any complications. In this series of dives, 8 divers had no detectable bubbles in either of the dives, so only 24 divers were used for analysis.

During the 15 minutes prior to start of mask breathing there was no significant difference between Control dives and NBO dives neither with respect to KM grades nor KISS (Table 1). The KM grade time profiles are shown in Figure 2.

After NBO, VGE were detected in 9 of the 24 divers and in 17 of 24 divers after the air breathing period (p=0.042, Fischer's exact test). The maximum KM grades after the NBO-period were not significantly lower than the KM grades after air breathing but the KISS were significantly lower after NBO compared to Control (Table 1).

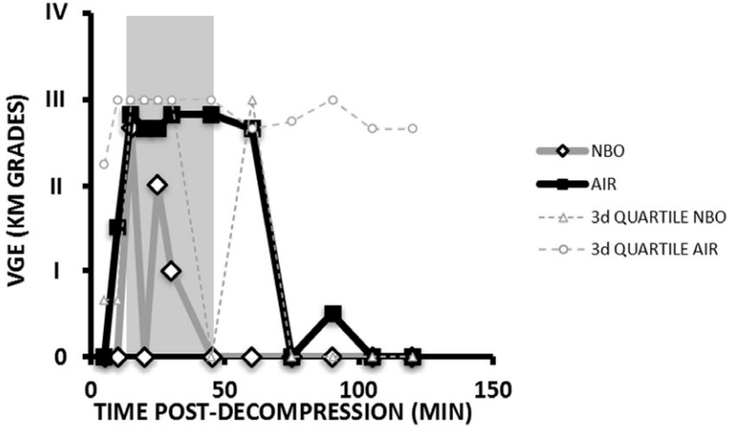

Figure 2*: Median maximum KM grades after dives to 42 m with 10 min exposure time. Mask breathing was started 15 min after decompression and was maintained for 30 min (grey shaded area). AIR, air breathing; NBO, normobaric oxygen; N = 24.*

Comparison between Series I and Series II

Both median maximum KM grades and KISS values were similar for the Control dives in the two experimental series (Table 1, Fig 1 & 2).

Overall, KM grades and KISS were lower for the NBO dives in Series I compared to those in Series II (Table 1). However, when only the period post-NBO breathing was considered, there was no difference in maximum KM grades or KISS between Series I and Series II. However, after the NBO period, 0 of 13 divers had KM grades equal to or above III in Series I, whereas 7 of 24 divers had such high grades in Series II (p = 0.034, one-sided Fischer's exact test).

			Complete post-dive observation period (0 – 120 min)			
			KM rest	**KM flex**	**KISS rest**	**KISS flex**
SERIES I	**CON**		III-	III	5.5 (11.8)	19.8 (19.8)
	NBO		0	0	0 (1.3)	0 (2.2)
	prob		0.022	0.016	0.0041	0,0022
SERIES II	**CON**		III-/III	III	6.4 (11.7)	17 (18.3)
	NBO		II*	III*	0.9* (5.4)	3.6** (9.4)
	prob		n.s.	n.s.	0.020	0,026

		Pre-NBO/air (0 – 15 min)		**Post- NBO/air (Series I: 30 – 105 min Series II; 45 – 120 min)**			
		KM rest	**KM flex**	**KM rest**	**KM flex**	**KISS rest**	**KISS flex**
SERIES I	**CON**	-	-	II	III	5.9 (11.4)	13.9 (20.1)
	NBO	-	-	0	0	0 (0.7)	0 (1.2)
	prob	-	-	0.0012	0.0034	0.005	0.0034
SERIES II	**CON**	I+	III-/III	0	III-	3.6 (8.6)	12.6 (15.9)
	NBO	I	II+	0	0	0 (2.5)	0 (6.1)
	prob	n.s.	n.s.	n.s.	n.s.	0.015	0.016

Table 1*: Median KM and KISS scores. KISS scores within parenthesis: arithmetic average. Prob: comparison between Control and NBO values. CON, control; KISS, Kisman integrated scoring system; KM, Kisman Masurel; NBO, normobaric oxygen;*

** Comparison between NBO Series I and Series II, p<0.05, ** Comparison between NBO Series I and Series II, p<0.01.*

DISCUSSION

Starting NBO immediately upon surfacing appears to have a clear effect on the presence of VGE. In this study, when the subjects began to breathe NBO within less than a minute after surfacing, only four subjects had any detectable bubbles during the whole observation period. In contrast, when breathing air for the whole post-decompression period, bubbles were detected in 12 of the 13 divers.

When the NBO period was delayed by 15 minutes, bubbles started to form before the start of the oxygen breathing. The number of VGE decreased during the oxygen breathing period, and the KISS post-NBO was significantly lower than that for the same time period during the Control dive on air. However, in nine of the 24 divers, bubbles remained in the circulation post NBO, and during the period post-mask breathing there was no significant difference in maximum KM grades between NBO dives and Control dives.

One should be aware that the experimental profile was not ideal, as the median bubble grade started to decrease at an hour post-decompression also for the Control dives. Therefore, the comparison between the delayed NBO dives and the air dives during the period after the mask breathing may underestimate the effect of NBO. Nevertheless, in the Series II Control dives, bubbles were still detected in 17 of the 24 divers during the period post-mask breathing and the median maximum KM score was as high as III-. In addition, after the delayed NBO period, seven of the 24 divers exhibited KM ≥III, which is considered a high bubble score. None of the 13 divers in Series I had such high scores after the NBO post decompression.

The hypothesis underlying this study was that once bubbles start to form, NBO breathing would be less efficient in terms of bubble removal, thus oxygen breathing would have to be maintained for longer periods. In the present study, six divers (both immediate and delayed NBO exposure) exhibited scores of III+ within minutes of surfacing during their NBO dives. In only one of those divers did the bubbles disappear during the oxygen period, and in four divers the bubble score was still equal to or greater than KM III after the oxygen period was finished.

Apart from the French study comparing NBO and HBO, there appears to be a dearth of human studies regarding the effect of NBO on decompression bubbles.[8] A few animal studies have been published: one, as mentioned previously, used goats as experimental subjects and showed a rather small effect of NBO on removal of bubbles after simulated deep submarine escape profiles.[9] These dives, which are in essence very deep and short air dives, load preferentially the fast tissues with nitrogen.[16] Thus in theory, even a short period of NBO would have a large effect on the gas wash-out. However, although oxygen breathing was initiated as soon as the animals were outside the chamber (< 5 minutes), in that short time large numbers of venous gas emboli had already been formed. Oxygen breathing did not have any effect on the early release of bubbles, but eventually shortened the time to no detectable bubbles. The result compared relatively well with some earlier animal studies, where sedated pigs had been exposed to aggressive decompressions.[17] Here the researchers waited until what appeared to be maximum bubble grades were present before treating the animals with either NBO or HBO or hyperbaric air. With NBO it took more than 15 minutes before an effect on the VGE grades was noted. However, NBO removed the bubbles much faster than normal air breathing.[17]

Formation of bubbles reduces the diffusion gradient of nitrogen between tissues and venous blood.[16] This is the reason that bubbles can remain in the venous circulation for very long periods, even in dives where only tissues with very short half-times have amassed increased nitrogen pressures.

Exchange of breathing gas has also been shown to affect the growth and resolution of microbubbles. Danish researchers injected microbubbles into aqueous tissues in the rat after decompression from 355 kPa and observed their resolution during air breathing, and when the breathing gas was exchanged for heliox or oxygen.[18] In two types of tissues (tendons and the anterior chamber of the eye) oxygen breathing caused a short-term growth before accelerating the resolution of the bubbles.[18] In the present experiment there was no evidence of an oxygen induced bubble growth. In Series II, a transient increase in KM grades was seen in six subjects during the delayed NBO, but the same number of subjects exhibited an increase in KM grades during mask breathing during the air control dives.

It was noted that after the NBO breathing in Series II, when the oxygen was exchanged for air, the KM grades started to increase in seven subjects. However, five subjects exhibited similar results during the Control dives. In Series I the KM grade increased in one subject as air breathing commenced after NBO, but the same pattern was seen in three subjects during the Control dives (where there was no change of breathing gas). Thus, it appears that time rather than change of breathing gas caused these shifts in KM grades.

The French decompression table MN90 contains a table with residual nitrogen calculations for oxygen breathing during whole or part of the surface interval.[7] Instead of using the '120-minute' tissue to calculate the excess nitrogen, the authors decided to calculate the nitrogen outflow using a 240-minute half-time compartment. The reason given for this safety feature is the fact that oxygen is vasoconstrictive. This is true, but during a surface interval, when the diver usually can maintain thermal comfort, it is doubtful how much effect oxygen will have on the peripheral blood flow. Anderson and co-workers measured a non-significant nitrogen washout reduction of 3.5% during oxygen breathing at atmospheric pressure.[19] The MN90-table allows the diver to start an NBO period at any time during the surface interval, and that period will count the same whether it is started directly after surfacing or much later. Given the results of the present study, it is questionable whether oxygen breathing started late after decompression when bubbles may have formed, will accelerate the nitrogen washout to the same extent as an early period of NBO. Technical divers who use rebreathers often allow the oxygen fraction in their breathing loops to reach 100% at the end of decompression[20] and often stay on the loop for some 10–15 minutes after surfacing (personal observation, MG). This appears to be a good way of reducing the risk of bubble formation.

Starting oxygen breathing later after the decompression may well act as a prophylactic against decompression sickness, but it appears that once bubbles have formed in the venous circulation, they will affect the vascular endothelium.[21] Brubakk and co-workers[22] showed that in a pig-model both NBO and HBO treatment 15–40 minutes post decompression appeared to prevent any deterioration of the endothelial function. However, in the same model, recompression to 160 kPa on oxygen one hour after decompression rapidly removed all bubbles but did not prevent endothelial damage.[22]

It seems that the endothelial loss of function is both dependent on the bubble load and the time with circulating bubbles. The faster the bubbles are removed from the circulation the less risk of deteriorating functions.

In conclusion, it appears that NBO is effective in removing gas bubbles from the venous circulation. A rapid onset of oxygen breathing seems to enhance the effect. Obviously, the length of time necessary for oxygen breathing to remove all excess nitrogen depends on which tissue compartments have been loaded during the dive. However, our results indicate that the effectiveness of oxygen breathing will also depend on the amount of bubbles that have formed before it commences.

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