Diving and the brain

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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.

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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.

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[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]

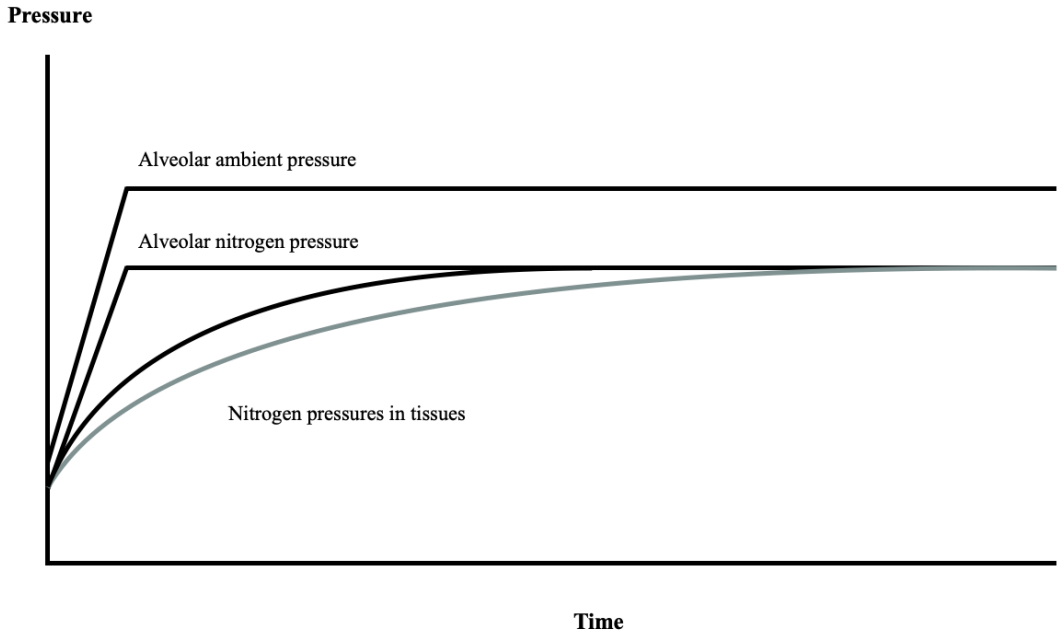

Figure 1 *Uptake of nitrogen in tissue. Theoretical depiction with two compartments*

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.

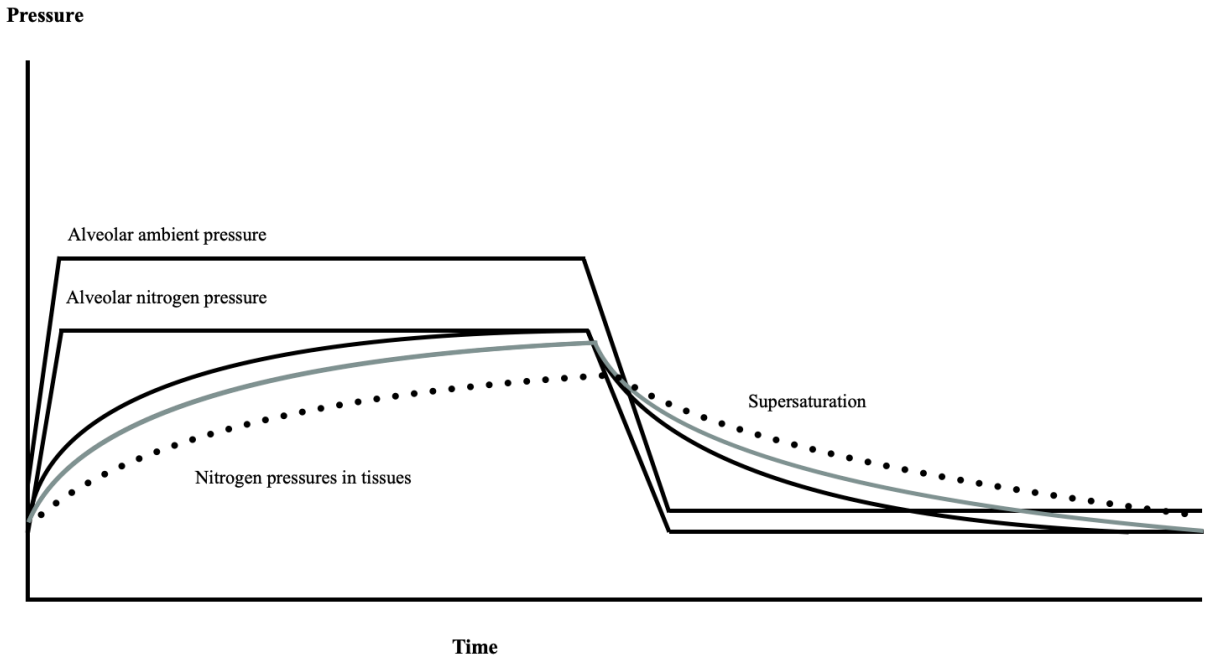

Figure 2 *Uptake and release of nitrogen in tissues with different saturation half-times. Theoretical depiction with three compartments.*

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.

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[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]

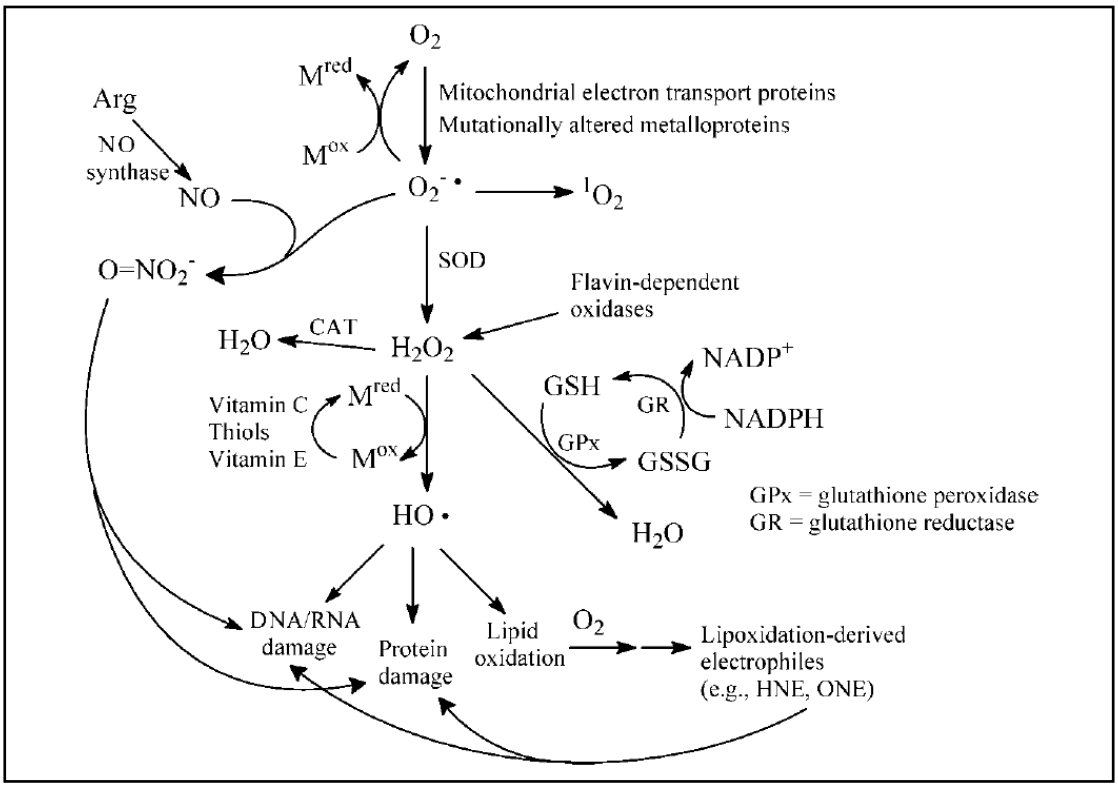

Figure 3 *Schematic depiction of formation and fate of ROS.[97] From Sayre LM, Perry G, Smith MA (2008) Oxidative stress and neurotoxicity. Chem Res Toxicol 21(1):172-88. doi:10.1021/tx700210j. Reproduced with permission from ACS Publications. Further permissions related to the material excerpted should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

[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)

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]

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.

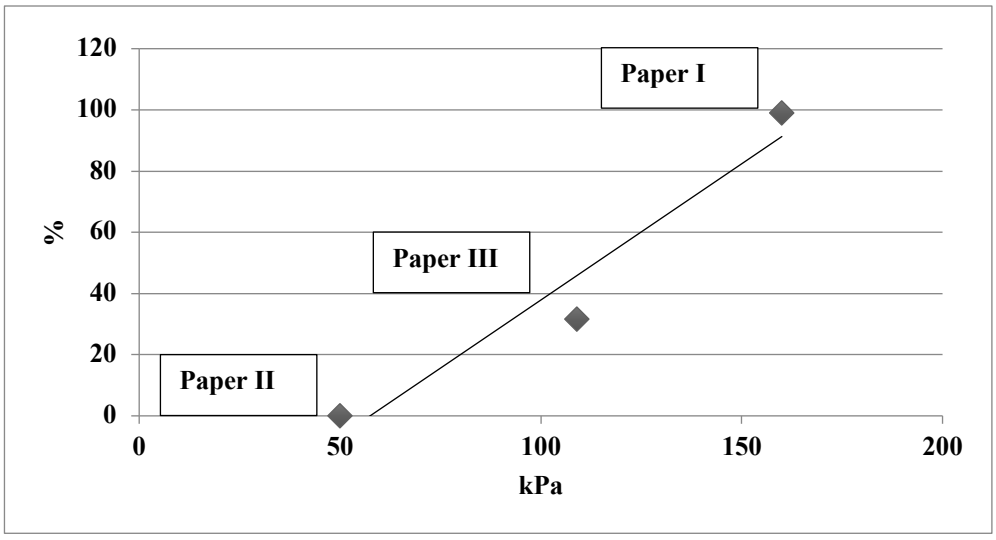

Figure 4 *Relationship between maximal oxygen partial pressure during exposure and relative change in tau, in Papers I–III.*

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.

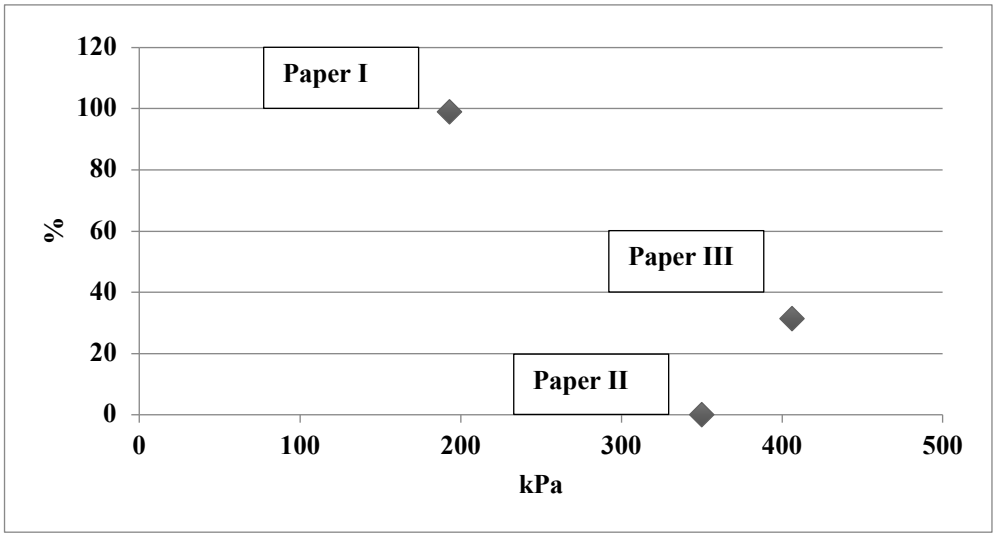

Figure 5 *Relationship between nitrogen partial pressure and relative change in tau, in Papers I–III.*

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.

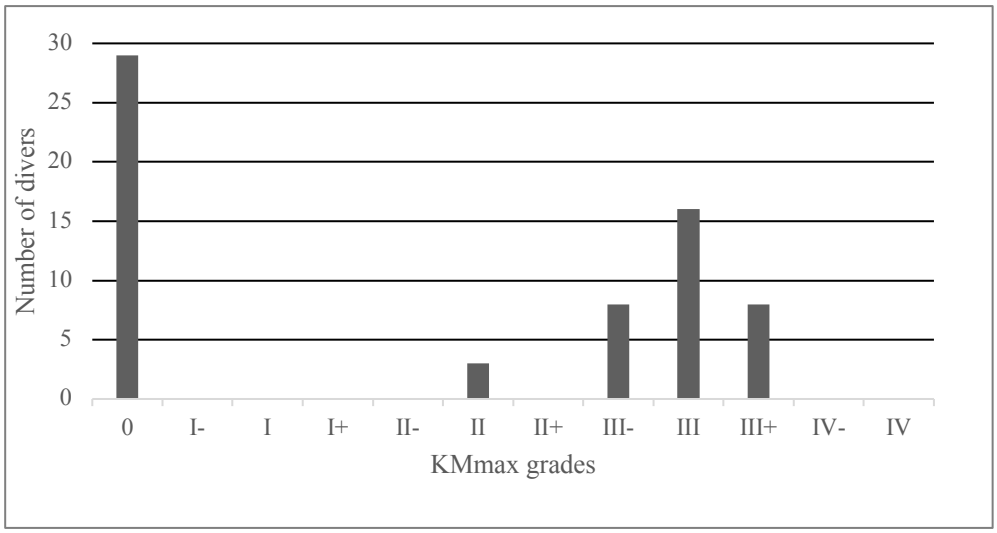

Figure 6 *Distribution of KM_{max} grades in Paper III*

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.

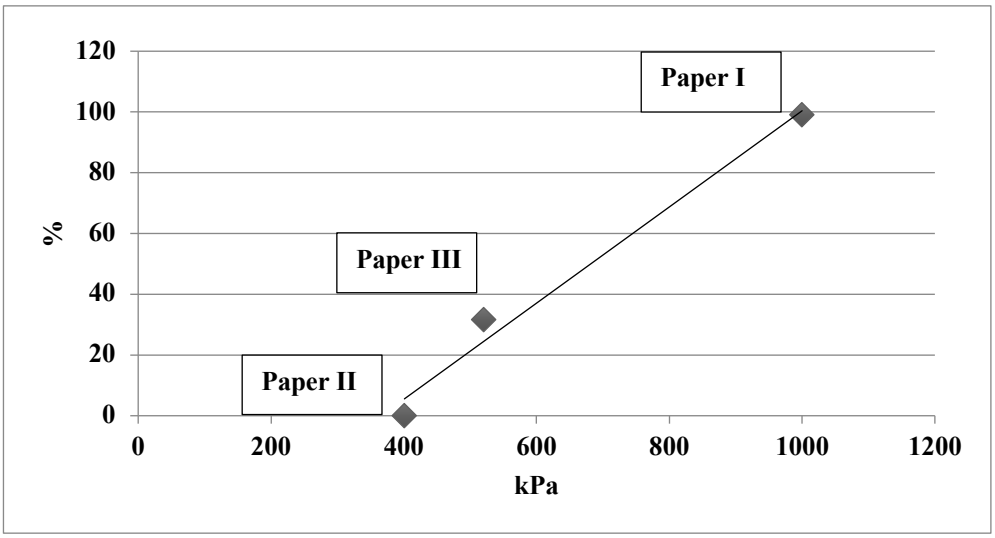

Figure 7 *Relationship between ambient pressure exposure and relative change in tau, in Papers I–III.*

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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