Serum tau concentration after diving – an observational pilot study

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Key words

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Abstract

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Introduction: Increased concentrations of tau protein are associated with medical conditions involving the central nervous system, such as Alzheimer's disease, traumatic brain injury and hypoxia. Diving, by way of an elevated ambient pressure, can affect the nervous system, however it is not known whether it causes a rise in tau protein levels in serum. A prospective observational pilot study was performed to investigate changes in tau protein concentrations in serum after diving and also determine their relationship, if any, to the amount of inert gas bubbling in the venous blood.

Methods: Subjects were 10 navy divers performing one or two dives per day, increasing in depth, over four days. Maximum dive depths ranged from 52–90 metres' sea water (msw). Air or trimix (nitrogen/oxygen/helium) was used as the breathing gas and the oxygen partial pressure did not exceed 160 kPa. Blood samples taken before the first and after the last dives were analyzed. Divers were monitored for the presence of venous gas emboli (VGE) at 10 to 15 minute intervals for up to 120 minutes using precordial Doppler ultrasound.

Results: Median tau protein before diving was 0.200 pg·mL⁻¹ (range 0.100 to 1.10 pg·mL⁻¹) and after diving was 0.450 pg·mL⁻¹ (range 0.100 to 1.20 pg·mL⁻¹; $P=0.016$). Glial fibrillary acidic protein and neurofilament light protein concentrations analyzed in the same assay did not change after diving. No correlation was found between serum tau protein concentration and the amount of VGE.

Conclusion: Repeated diving to between 52–90 msw is associated with a statistically significant increase in serum tau protein concentration, which could indicate neuronal stress.

Introduction

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

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

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

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

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

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

Neurofilament light protein (NfL) is a structural axonal protein which is found mainly in myelinated subcortical axons.⁸ Serum NfL levels correlate with outcome in patients with TBI, but their rise is slower than that for tau, reaching

a maximum beyond 10 days following the insult.¹⁹ Glial fibrillary acidic protein (GFAP) is expressed almost solely in astrocytes. Elevated blood serum levels of GFAP have been reported within 24 hours after TBI.⁸

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

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

Methods

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

SUBJECTS

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

DIVING PROTOCOL

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

Figure 1

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

Table 1

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

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

Median time spent at maximum depth during the first three days was 20 min (range 10 to 25 min). On the fourth day, time spent at maximum depth was 10 min for dives to 52 msw and 20 min for dives to 82–90 msw. All subjects used electronically controlled closed circuit rebreathers. Air was used as the diluent gas for dives less than 40 msw

and trimix was used for all dives deeper than 40 msw. For dives between 40–65 msw the trimix diluent gas contained 15% oxygen, 50% helium, 35% nitrogen. During dives deeper than 65 msw the diluent contained 10% oxygen, 70% helium and 20% nitrogen. The rebreather equipment maintained a constant oxygen partial pressure of 130 kPa while the divers descended and were at depth. During the final decompression phase, an oxygen partial pressure of 160 kPa was allowed. Decompressions were planned according to the VPM-B algorithm with conservatism factor 2.²⁶ Immediately after dives deeper than 60 msw 100% oxygen was breathed for 10 minutes.

DATA COLLECTION

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

Table 2

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

Sample 1 (pg·mL ⁻¹) <i>n</i> = 9		
Mean (SD)	0.322 (0.315)	
Median (range)	0.200 (0.10–1.10)	
Sample 2 (pg·mL ⁻¹) <i>n</i> = 10		
Mean (SD)	0.500 (0.337)	
Median (range)	0.450 (0.10–1.20)	
Delta-tau (pg·mL ⁻¹) <i>n</i> = 9	0.211 (0.145)	<i>P</i> = 0.016
Mean (SD)	0.300 (0.0–0.40)	
Median (range)		
Delta-tau (%) <i>n</i> = 9	98.8 (96.0)	<i>P</i> = 0.016
Mean (SD)	100 (0.0–300)	
Median (range)		

Figure 2

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

Table 3

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

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

four biomarkers were obtained. For QC samples, with NfL concentrations of 101.2 pg·mL⁻¹, 8.0 pg·mL⁻¹ and 14.8 pg·mL⁻¹, CVs were 5.0%, 9.5% and 3.5%, respectively and for QC samples, with GFAP concentrations of 75.3 pg·mL⁻¹, 95.6 pg·mL⁻¹ and 118.9 pg·mL⁻¹, CVs were 2.2%, 9.4% and 4.9%. The results of UCHL-1 analyses were discarded due to an unacceptably high level of imprecision as CVs were 44.9% and 121.0% for QC samples with UCHL-1 concentrations of 8.4 pg·mL⁻¹ and 9.7 pg·mL⁻¹ respectively.

Within 20 minutes after surfacing, each diver was monitored for the presence of VGE, at 10 to 15 minute intervals for

up to 120 minutes, using precordial Doppler ultrasound (DBM9008; Techno Scientific Inc., Ontario, Canada). VGE load was assessed while the subjects lay in the left lateral decubitus position at rest and measurements were also made following movement (knee bends made whilst still lying down) and graded according to the KM scale.

The Kisman integrated severity score (KISS) algorithm²⁷ was used to convert KM grade measurements collected during the four-day study period into one mean score for each diver (VGE-KISS).

Figure 3

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

Figure 4

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

STATISTICS

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

Primary outcome

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

Secondary outcome

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

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

GFAP and NfL

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

Missing data

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

Results

PRIMARY OUTCOME

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

SECONDARY OUTCOME

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

GFAP AND NfL

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

Discussion

In this prospective pilot study, diving over a four-day period was associated with a statistically significant rise in serum

tau concentration. The median tau value increased 2.5 times. This serum tau change is comparable to changes in plasma tau and CSF tau observed in earlier studies in athletes and after mild concussion injuries.^{8,9} Causality between diving and serum tau concentrations is still uncertain, due to the lack of a control group and the small number of observations. Yet, as the divers' tau values after diving were compared to values obtained shortly before the first dive, the results are consistent with causation.

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

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

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

The lack of a control group is a shortcoming of this study. There was a difference between the time of day when samples 1 and 2 were taken. Studies show no diurnal variation in CSF tau levels,^{23–25} making it improbable that

they should fluctuate in the blood significantly during the day. Nevertheless, a representative control group could have ensured that no confounding factors, such as diurnal variation, were responsible for changes in serum tau. Ideally in future studies, tau should be sampled at the same times of day and the results compared to a representative control group. In the context of hypoxic brain injury, studies have shown that the increase in serum tau levels reach a maximal elevation within 24 hours, though sometimes there is a delayed peak at about 72 hours.¹⁴ The change in serum tau levels after a far milder but prolonged impact, such as repeated diving, are unknown. Additional sampling of venous blood at other points might have yielded even higher serum tau values.

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

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

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

Conclusion

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

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Conflicts of interest

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

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