Serum tau concentration after diving – an observational pilot study

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Authors´ contributions

The project was conceived and designed by AR and MG. AR and NO collected the blood samples. KB and HZ were responsible for blood analyses. AR drafted the manuscript. All authors participated in data interpretation and writing of the final article.

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Abstract

Introduction: A prospective observational pilot study was performed to investigate change in tau protein concentration in serum after diving. The association between serum tau protein concentration and the amount of inert gas bubbles in venous blood was also assessed. 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 diving causes a rise in tau protein levels in serum.

Methods: The study group consisted of 10 navy divers performing one or two dives per day, increasing in depth, over four days. Maximum dive depths ranged from 52 - 90 meters of sea water. 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.

Results: Median tau protein concentration before diving was 0.200 pg/mL (range 0.100 to 1.10 pg/mL) and after diving was 0.450 pg/mL (range 0.100 to 1.20 pg/mL; $p = 0.016$). No correlation was found between serum tau protein concentration and the amount of inert gas bubbles.

Conclusion: Repeated diving to between 52 - 90 meters is associated with a statistically significant increase in serum tau protein concentration, which could indicate neuronal stress. Further studies are needed to validate these results and establish the use of tau protein as a marker of dive-related 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 abates 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, which disrupts 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 regarding 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 meter 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 manifest seizures.[5] A 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 pressure of oxygen and nitrogen and their effects at greater depths, gas mixtures containing nitrogen, oxygen and helium are used, commonly being referred to as 'trimix'.

Exposure to high ambient pressure, equivalent to diving depths of more than 150 msw, can cause neuromuscular dysfunction. The condition is called high-pressure neurological syndrome (HPNS). Nausea, dizziness and tremors are common symptoms. With increasing depths myoclonic episodes appear. Generalized seizures are rare in humans. 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, but it can also to a lesser extent be detected in the liver, kidneys and testes.[8, 19] Increased tau levels are found in blood in conjunction with dementia, traumatic brain injury (TBI), cerebral concussion, boxing,[9, 10, 11, 12] and hypoxic brain injury, where it correlates with outcome.[13, 14] Tau levels in blood rise early, within 24 hours, after cerebral damage. A delayed second peak of elevated tau in blood appearing a few days after hypoxic injury has been reported.[14, 19] A recent study on patients undergoing surgery and general anesthesia showed a transient rise of tau levels in the blood.[15] High intensity interval training (HIIT) can also lead to increased tau levels in the bloodstream; however, a two-week period of HIIT 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.[19] NFL level in blood correlates with outcome in patients with TBI, but it rises slower than tau, reaching its maximum beyond 10 days following the insult.[20] Glial fibrillary acidic protein (GFAp) is expressed almost solely in astrocytes. Elevated blood levels of GFAp has been reported within 24 hours after TBI.[19] The potential influence of diurnal variation on neuronal fluid biomarker results has been a subject of scientific discussion.[21, 22, 23] However, a study including patients with Alzheimer's disease and older healthy volunteers concluded that there was no circadian pattern for tau in CSF.[24] Another study on neurosurgical patients showed no diurnal variation in CSF tau levels.[25] Most likely tau levels in blood reflect those in CSF. Consequently, diurnal variation in blood tau values seems improbable. It is not known whether a hyperbaric exposure alone, without hypoxia, is associated with a rise in protein tau levels in serum.

Hypothesis

Diving, by way of an elevated ambient pressure, affects the central nervous system and causes a rise in tau protein concentration in blood.

Objectives

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

Study

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).

Study subjects

Ten male military divers participating in professional naval dive training on the Swedish west coast from June 12th - 15th 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. Median time spent at maximum depth during the first three days was 20 minutes (range 10 to 25 minutes). On the fourth day, time spent at maximum depth was 10 minutes for dives to 52 msw and 20 minutes for dives to 82 - 90 msw. All subjects used electronically controlled closed circuit rebreathers. Air was used as the breathing gas for dives less than 40 msw and trimix (nitrogen/oxygen/helium) was used for all dives deeper than 40 msw. For dives between 40-65 msw the 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. Decompressions were planned according to the VPM-B algorithm with conservatism factor 2.[26] During the later stages of the dive, during the final decompression phase, an oxygen partial pressure of 160kPa was allowed. 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, 12th June 2017 between 11:30 - 12:50) and approximately two to three hours after the last dive (Sample 2, 15th June 2017 between 15:35 - 17:05). Samples were collected in gel tubes (Vacuette no. 454420, Hettish Labinstrument AB, Sweden) and immediately centrifuged for 10 minutes at 2200 rpm and 20 degrees centigrade (Sorvall ST 8 / 8R Centrifuge, Thermo Scientific, Germany). Directly afterwards, aliquots of 500 µL serum were frozen on dry ice and then stored at -78 degrees centigrade 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, 1.4 pg/mL and 24.1 pg/mL, coefficients of variation (CVs) were 8.1%, 11.8% and 6.2%, respectively. The N4PA assay is designed to measure four biomarkers, namely tau, GFAp, NfL and ubiquitin carboxy-terminal hydrolase L1 (UCHL-1). Therefore, results for all these four biomarkers were obtained. For QC samples, with NfL concentrations of 101.2 pg/mL, 8.0 pg/mL and 14.8 pg/mL, CVs were 5.0%, 9.5% and 3.5%, respectively and for quality QC samples, with GFAp concentrations of 75.3 pg/mL, 95.6 pg/mL and 118.9 pg/mL, 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.

Within 20 minutes after the dives, each diver was monitored for the presence of VGE, at 10 to15 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 KM scale.

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

Statistics

Results for tau and its association with VGE, were compiled by an independent statistical company (Statistiska Konsultgruppen, Gothenburg, Sweden) using SAS® v9.3 (Cary, NC, USA). Statistical analyses for GFAp and NfL were performed using IBM SPSS® v24 (IBM, Armonk, NY, USA) and Spearman's correlation tests involving VGE KISS was 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 objective

Tau levels before and after diving were 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) and a relative change (%). Statistical significance was tested using the Wilcoxon signed-rank test.

Secondary objective

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 Spearman´s correlation test and again presented in scatter plots.

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) 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 all dives performed and no data was collected for the pair of divers who were diving no deeper than 52 msw.

Results

Primary Objective

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 (pg/mL) and the relative (%) changes in serum tau concentration between sample 1 and sample 2 were statistically significant. The results are shown in *table 2* and *figure 2*.

Secondary Objective

Eight 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 distribution of results no statistical correlation was found between serum tau protein concentration and maximum VGE load after diving. The results are shown in *figure 3* and *figure 4*.

There was no statistically significant correlation between the VGE-KISS scores and sample 2 serum tau concentration ($R^2 = 0.1483$, $t = 1.022$) nor between VGE-KISS and delta-tau ($R^2 = 0.00222$, $t = 0.116$).

GFAp and NfL

Neither GFAp nor NfL concentrations changed significantly after diving. The results are shown in *table* 3.

Discussion

In the present 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 the Ultrasound 2015 concensus,[28] but it is subjective and non-linear. Furthermore, all categorization results in a loss of information and reduced precision. A majority of KM grades after diving were III at rest and III or III+ following knee bends. No statistical correlation between serum tau levels and VGE load was found in this study, but the accumulation of data around only two 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 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. GFAp, a protein highly expressed in astrocytes, appears to have similar kinetics in blood as tau. 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 160kPa 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 its relationship with 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 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, making it improbable that tau blood levels should fluctuate 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 each 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 any 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. Prior to the study there was a 40% prevalence of DCS among the participants. 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, preferably 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 through 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 at 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, a GU Ventures-based platform company at the University of Gothenburg. No other authors have reported any conflicts of interest.

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Legends/ captions

Table 1

For categorical variables n (%) is presented.

For continuous variables Mean (SD) / Median (Min;Max) is presented.

Table 2

For continuous variables Mean (SD) / Median (Min;Max) is presented.

For comparison the Wilcoxon signed-rank test was used.

Table 3

For continuous variables Mean (SD) / Median (Min;Max) is presented.

For comparison the Wilcoxon signed-rank test was used.

Figure 1

Diving protocol

Divers 7-10 performed two 65-66 msw dives on the 13th June. Divers 1 and 2 performed two 52 msw dives on the 15th June. Diver 10 did not dive on the 14th June.

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 (0.2pg/mL–0.5pg/mL) was identical. They are represented by one line.

Figure 3

Serum tau protein after the last dive versus maximal KM grade at rest

n = 8. Spearman's 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's rank correlation coefficient: -0.4. p-value: 0.33