

Blood biomarkers indicate increased amyloid β production and mild neuroaxonal injury after transient hypoxia during breath-hold diving

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ABSTRACT

Processing of amyloid precursor protein by β - and γ -secretase to form fragments of amyloid β ($A\beta$) which aggregate into extracellular plaques is thought to be a key pathological process in the development of Alzheimer's disease (AD). Little is known about the mechanism behind the disequilibrium of production and clearance of $A\beta$ in sporadic cases of AD. Animal and human studies have implicated hypoxia as a factor behind increased production of $A\beta$, the relevance of this is supported by the frequent comorbidity of AD and vascular dementia. The development of highly sensitive assays enables analysis of neurochemical markers in blood instead of in cerebrospinal fluid. This opens a window of discovery into brief and dynamic physiological processes of the brain, previously beyond reach. In the current paper we studied effects of transient hypoxia on amyloid metabolism and neuronal damage in human subjects. Sixteen divers competing in the national Swedish championship in breath-hold diving were enrolled. Blood was sampled at baseline and after each of three competitions and analyzed for markers of amyloid metabolism ($A\beta_{42}$) and neuronal damage (T-tau, NFL, S100 β). During the first competition, divers held their breath for a median of five minutes and we saw increased $A\beta_{42}$ ($p = 0.0006$) and T-tau ($p = 0.001$) compared to baseline. The levels of T-tau correlated with the length of the apneic time during two of the competitions. We conclude that transient hypoxia in healthy adults may stimulate $A\beta$ production, with implications in AD molecular pathogenesis, and cause mild transitory neuronal dysfunction or damage.

INTRODUCTION

Processing of amyloid precursor protein by β - and γ -secretase to form amyloid β ($A\beta$) that aggregates into insoluble extracellular plaques is thought to be a key pathological process in the development of Alzheimer's disease (AD) (3). Little is known about the mechanism behind the disequilibrium of production and clearance of $A\beta$ in sporadic cases of AD (3). Both animal (21) and human (8, 15, 25) studies have implicated hypoxia as a factor leading to increased production of $A\beta$. The relevance of this is supported by the frequent comorbidity of AD and vascular dementia (10, 16), as well as the observation that those afflicted by sleep-disordered breathing run a higher risk of developing dementia (2, 14, 24). Further, $A\beta$ appears to be a potent vasoconstrictor and accumulates in vessel walls, and its overabundance could have a discordant effect on the cerebral circulation (1, 13, 22). Development of highly sensitive assays capable of detecting proteins at subfemtolar levels of concentration allow for analysis of brain derived proteins in blood instead of in cerebrospinal fluid (18). This opens a window of discovery into brief and dynamic physiological processes of the brain, previously beyond reach.

Static apnea and dynamic apnea with or without fins are disciplines of breath-hold diving in the sport of freediving. Divers are timed while holding their breath and remaining motionless, static, or while trying to swim, dynamic, as far as they can underwater.

Studies have confirmed the intuition that holding one's breath will decrease oxygen saturation (SaO_2) in a time-dependent fashion, and that trained divers are able to

lower their SaO₂ further compared with untrained individuals (5). In addition, oxygen consumption and thus decrease in SaO₂ is more pronounced during physical exercise compared with rest (4). Therefore, subjects trained in breath-hold diving present an interesting opportunity to study the effect of hypoxia on various physiological systems.

In the present study, we test the hypothesis that hypoxia during breath-hold diving may affect blood biomarkers reflecting amyloid metabolism and structural brain injury.

METHODS

Ethical approval & consent

The study was approved by the ethics committee at the University of Gothenburg and written informed consent was obtained from all participants.

Study population

Sixteen subjects (14 male, 2 female, median [range] 36 [16-51] years old) participating in the national Swedish championship in breath-hold diving were included. Five age and gender matched subjects (4 male, 1 female, 32 [27-38] years old) volunteered as controls (C) (Table 1).

Execution

The divers and the matched healthy controls were sampled for baseline (B) values approximately 3 hours prior to the start of competitions. Divers were then sampled after the first competition (static apnea, STA), following the remaining competitions after an additional 21 hours (dynamic apnea without fins, DYN1) and finally after an additional 16 hours (dynamic apnea with fins, DYN2). Samples were collected within an hour of the competitions ending.

Biochemical analyses

Blood samples were collected by peripheral venipuncture in gel-separator tubes for serum and EDTA tubes for plasma. Samples were centrifuged within 20 to 60 minutes and then aliquoted and stored at -80°C.

S-100 β was analyzed in serum on a Modular E170 instrument (Roche Diagnostics, Basel, Switzerland) with reagents from the same manufacturer. Plasma T-tau and A β 42 were measured using immunoassay digital array technology (Quanterix Corp, Boston, MA, USA) as described previously (12, 18). Serum NFL levels were determined using the NF-Light kit from UmanDiagnostics (UmanDiagnostics, Umeå, Sweden), transferred onto the Simoa platform using a homebrew kit (Quanterix Corp, Boston, MA, USA). The lower limits of quantification were 5 pg/mL for S-100 β , 0.007 pg/mL for T-tau, 0.032 pg/mL for A β 42 and 0.26 pg/mL for NFL. Intra-assay coefficients of variation were 3.1% for S-100 β and 5-10% for T-tau, A β 42 and NFL.

All samples were analyzed at the same time using the same batch of reagents by board-certified laboratory technicians who were blinded to clinical information.

Statistical analyses

For comparison of biomarker levels after competition versus their baseline levels or the control group, the Mann-Whitney U-test (unpaired analyses) or the Wilcoxon matched-pairs signed rank test (paired analyses) was used. The Spearman's rank correlation coefficient (ρ) was used for analyses of correlation between changes in various biomarker levels after competition and breath-holding time. Statistical significance was determined at $P < 0.05$. All statistical calculations were performed using GraphPad Prism version 6.05 (GraphPad Inc., San Diego, California).

RESULTS

Biomarker reflecting amyloid metabolism

There was no difference in the plasma level of A β 42 between the divers at baseline and the control group (Table 1). Comparing divers' baseline vs. STA biomarker results, there was a significant increase in the levels of A β 42 after the first event of static apnea ($p = 0.0006$, Figure 1). However, there was no significant change in the levels of A β 42 when comparing divers' baseline vs. the DYN1 and DYN2 time points (Figure 1). A β 42 did not correlate to the length of apneic time (Figure 2A).

Biomarkers reflecting structural brain injury

Divers had significantly higher baseline T-tau than controls ($p = 0.04$, Table 1). Comparing divers' baseline vs. STA biomarker results, we found a significant increase in the levels of T-tau after the first competition ($p = 0.001$) and a significant decrease after one of the dynamic events, DYN2 ($p = 0.03$, Figure 1). Further, T-tau correlated with the length of apneic time during STA ($\rho = 0.72$, $p = 0.004$) and during DYN1 ($\rho = 0.66$, $p = 0.01$) but not during DYN2 ($\rho = 0.42$, $p = 0.14$, Figure 2D). There were no significant changes in the levels of serum NFL and S-100 β between competition events (Figure 1). Also, the levels of NFL and S100 β did not correlate with apneic time (Figure 2B-C).

DISCUSSION

The first finding was that plasma levels of A β 42 increased following the transient hypoxia induced by breath-hold diving. Divers held their breath longer during STA compared with DYN1 or DYN2 (Table 1), which may have resulted in a more severe hypoxia and thus explain why the rise of A β 42 was only seen during STA. As expected, the magnitude of the rise was much smaller than what we have previously seen after the severe failure of cerebral circulation during cardiac arrest (17, 25). After ischemic damage or severe traumatic brain injury, a significant portion of the efflux of brain-derived proteins likely occur across a damaged, leaky blood-brain barrier (BBB) (11). Transient hypoxia only partially mimics the physiological disruption induced by global cerebral ischemia and the absence of a concomitant increase of S100 β and NFL in our study makes BBB damage less likely to explain the increase of A β 42. Another potential explanation to the increase of A β 42 may be that we unknowingly measured A β 42 derived from peripheral tissues. However this is less likely considering the absence of increase after DYN1 and DYN2, during which the stress on peripheral tissues was arguably greater. It is also necessary to consider how A β 42 reaches the circulation rapidly in the absence of evident BBB disruption. It is possible that the translocation of A β 42 from brain interstitial fluid to blood occurs through the recently described glymphatic system, which enables a rapid turnover of the brain extracellular fluid and its contents (9).

We suggest that our findings indicate an increased amyloidogenic processing of APP as a result of transient hypoxia, which might have implications in AD molecular

pathogenesis and resonates well with epidemiological studies showing an association between sleep-disordered breathing and dementia (2, 14, 24).

The second finding was that levels of T-tau increased after STA (to 196% of baseline levels) and that T-tau correlated to apneic time during STA and DYN1. T-tau is predominantly expressed in unmyelinated cortical axons, abundant in the central nervous system and less common elsewhere (23). The implication is that transient hypoxia may result in mild cortical neuronal dysfunction or injury. It is interesting that this occurs below the physiological breaking point of drawing breath, i.e. before the prolonged hypoxia results in a forced breath. While we studied divers trained in breath-hold diving, a similar physiological situation may regularly occur in subjects afflicted by sleep-disordered breathing.

T-tau decreased after DYN2 compared to baseline values, however the statistical significance of this was weak compared to our other findings ($p = 0.03$) and additionally the diver baseline T-tau was already slightly elevated compared to controls ($p = 0.04$). To place this in a context, albeit speculative, we note that studies on mouse models of traumatic brain injury have shown a 60% reduction of glymphatic pathway post-injury, most pronounced 3 to 7 days and persisting at least a month, the effect likely mediated through altered localization of the astroglial water channel aquaporin-4 (7). Thus mild neuronal injury divers during STA may have caused impaired clearance of T-tau during the subsequent competitions. However, this line of thought is not entirely consistent with recent findings on ice hockey players who suffered concussion, and where T-tau was increased within 1 hour of

injury, followed by an initial decline during the first 12 hours and then a trend towards a secondary increase peaking at about 36 hours (20).

The third finding was that neither NFL nor S-100 β showed any change in concentration or correlation to apneic time. This may be due to a different distribution pattern of these biomarkers in the brain. NFL is predominately expressed in subcortical myelinated axons while S-100 β is predominately expressed in astrocytes (6, 19). Theoretically, this could render NFL and S-100 more resilient to a transient hypoxic state.

The main limitation of this study was the small sample size, which restricts the generalizability of the results. We also did not directly measure SaO₂, and thus we cannot be certain if the level of hypoxia correlated to apneic time, in particular during the dynamic events.

Conclusion

In conclusion, transient hypoxia may acutely increase levels of A β 42 and T-tau in healthy adults thus further supporting that general hypoxia may stimulate A β production, with potential implications in AD molecular pathogenesis, and cause mild transitory neuronal dysfunction or damage.

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DISCLOSURES

MG, PS, UA and RL report no conflicts of interest. NN is an employee at UmanDiagnostics. DHW is an employee at Quanterix Corporation. KB and HZ are co-founders of Brain Biomarker Solutions, a University of Gothenburg-based platform company.

FIGURES

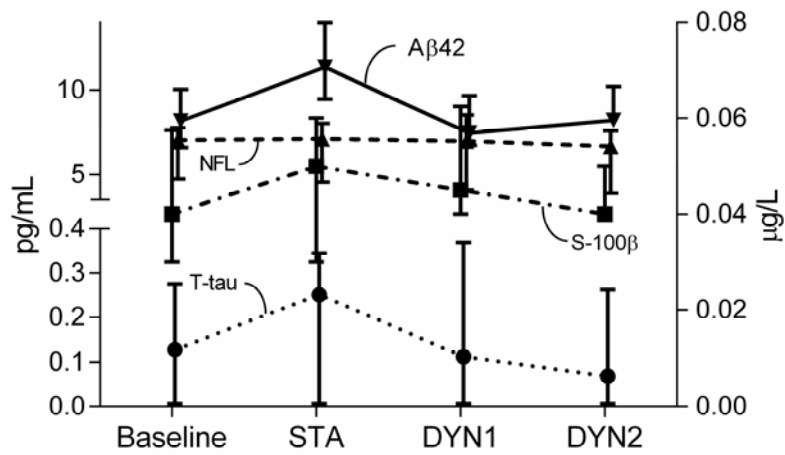


Fig. 1. Biomarkers for structural brain injury and amyloid metabolism amongst divers across all events, median value and interquartile range. Plasma T-tau, serum NFL and plasma Aβ42 measured in pg/mL (left Y-axis), serum S-100β measured in μg/L (right Y-axis). Significant elevations were seen at STA compared to baseline regarding Aβ42 ($p = 0.006$) and T-tau ($p = 0.001$).

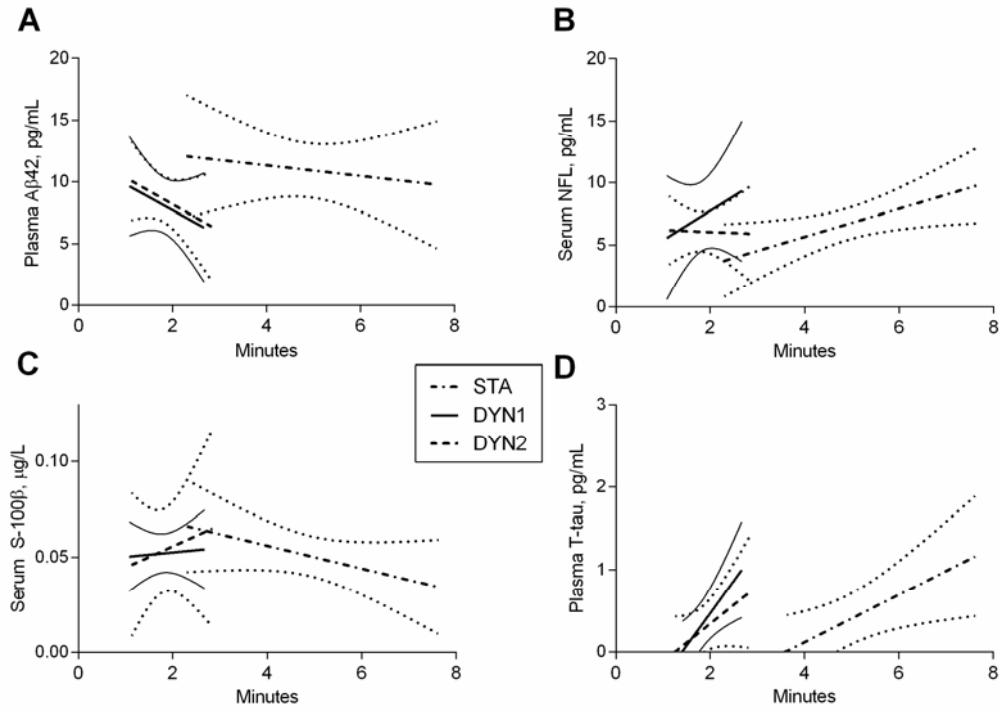


Fig. 2. Linear regression and 95% confidence bands of biomarker levels vs. apneic time. A-C, No significant correlation of apneic time with A β 42, NFL or S-100 β concentrations. D, Apneic time correlated to levels of T-tau at STA ($\rho = 0.72$, $p = 0.004$) and during DYN1 ($\rho = 0.66$, $p = 0.01$).

TABLES

Table 1. Demographics and descriptive statistics of blood biomarkers and apneic time at baseline and across competitions

Measurement	Median (Range) [n]				
	Baseline	STA	DYN1	DYN2	
	Controls	Divers			
Age, years	32 (27-38) [5]	36 (16-51) [16]			
Sex, M/F	4/1 [5]	14/2 [16]			
A β 42, pg/mL	11.9 (5.07-20.2) [5]	8.16 (0.62-12.0) [16]	11.3 (0.57-16.6) [15]	7.45 (0.32-15.7) [13]	8.21 (0.92-14.8) [14]
T-tau, pg/mL	0.007 (0.007-0.007) [5]	0.128 (0.007-0.253) [16]	0.251 (0.007-2.57) [15]	0.112 (0.007-2.37) [14]	0.069 (0.007-2.00) [14]
S-100 β , μ g/L	0.04 (0.03-0.08) [5]	0.04 (0.02-0.08) [16]	0.050 (0.03-0.09) [15]	0.045 (0.03-0.09) [14]	0.04 (0.03-0.16) [14]
NFL, pg/mL	5.8 (2.21-9.08) [5]	7.03 (2.42-14.7) [16]	7.11 (3.72-14.3) [15]	6.98 (3.15-21.6) [14]	6.66 (2.85-12.6) [14]
Apneic time, minutes	-	-	5.03 (2.30 – 7.63) [15]	1.83 (1.08 – 2.67) [16]	1.78 (1.13 – 2.83) [14]

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