

Longitudinal performance of plasma neurofilament light and tau in professional fighters: The Professional Fighters Brain Health Study

Running title: Plasma biomarkers in professional fighters

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Abstract

The objective of this study is to evaluate longitudinal change in plasma neurofilament light (NF-L) and tau levels in relationship to clinical and radiological measures in professional fighters. Participants (active and retired professional fighters and control group) underwent annual blood sampling, 3 Tesla MRI brain imaging, computerized cognitive testing, and assessment of exposure to head trauma. Plasma tau and NF-L concentrations were measured using Simoa assays. Multiple linear regression models were used to compare the difference across groups in regard to baseline measurements, while mixed linear models was used for the longitudinal data with multiple measurements for each participant. Plasma samples were available on 471 participants. Baseline NF-L measures differed across groups ($F_{3,393} = 6.99$, $p = 0.0001$), with the active boxers having the highest levels. Higher NF – L levels at baseline were correlated with lower baseline MRI regional volumes and lower cognitive scores. The number of sparring rounds completed by the active fighters was correlated with NF – L (95% CI 0.0116 – 0.4053, $p = 0.0381$), but not tau, levels. Among 126 subjects having multiple yearly samples, there was a significant difference in average yearly percentage change in tau across groups ($F_{3,83} = 3.87$, $p = 0.0121$). We conclude that plasma NF-L and tau behave differently in a group of active and retired fighters; NF-L better reflects acute exposure whereas the role of plasma tau levels in signifying chronic change in brain structure over time requires further study.

Key words: mild traumatic brain injury, tau, neurofilament light

Introduction

Repetitive head impacts (RHI) can result in long term neurological injury and are a risk factor for chronic traumatic encephalopathy (CTE) (1, 2). A vulnerable neural element to RHI is the axon (2). Neurofilament light (NF-L) and tau are two well established biochemical markers of axonal injury (3). Both NF-L and tau levels have been reported to rise after concussion (4-6). However, more chronic elevations of NF-L levels have been reported in individuals with prolonged post concussive symptoms and increased tau levels in those with self-reported history of mild traumatic brain injury (7, 8).

The use of these markers to follow or predict progressive neurological injury has not been investigated as thoroughly. In cross sectional studies, higher levels of NF-L have been associated with several neurodegenerative conditions including progressive supranuclear palsy, amyotrophic lateral sclerosis and Alzheimer's disease (9-11). Moreover, while tau deposits in the form of neurofibrillary tangles are considered the pathological hallmark of CTE, there are no known peripheral indicators of brain tau accumulation in the blood of those at increased risk of CTE.

There has yet to be any published reports of NF-L and tau levels in blood or CSF over multiple years in those who have been exposed to RHI. Utilizing a well characterized cohort of professional fighters, both active and retired, from the Professional Fighters Brain Health study, we explore the longitudinal performance of plasma NF-L and tau and their relationship to clinical and radiological evidence of impairment

Methods

The Professional Fighters Brain Health study (PFBHS) is a cohort study of active and retired professional fighters (boxers and mixed martial artists), along with age and education matched controls. Active fighters were required to have at least 1 professional fight within 2 years of enrollment and be training with the intent to compete; information about the study was disseminated by the Nevada Athletic Commission, fight promoters and local training facilities. Retired fighters were included if they had been boxers, had a minimum of 10 professional fights, no sanctioned fights for at least two years and did not intend to return to competition (there were too few retired MMA fighters to include as a separate

group). Control subjects were recruited from outreach efforts in the community and could not have any prior history of neurological disorders, head trauma, military service, or participation at a high school level or higher in a combat sport or a sport in which head trauma can be anticipated to occur, such as football, wrestling, hockey, rugby, soccer, or rodeo. Enrollment in the PFBHS began in 2011 and has been continuous since then. Each participant is seen on an annual basis, and for active fighters, not sooner than 45 days from a sanctioned fight. The PFBHS was approved by the Cleveland Clinic Institutional Review Board and written informed consent was obtained from all participants. Methods of recruitment and study procedures have been described previously (12).

At each visit, blood sampling is obtained, along with a battery of other tests including MRI brain imaging, computerized cognitive testing, and exposure information. At the baseline visit, participants answer questionnaires with the assistance of the study coordinator that collect information on demographics; educational attainment; medical history including concurrent illnesses and prescribed medications; previous head trauma, both related and unrelated to athletic activities; and prior involvement in other contact sports. Number of professional fights was ascertained by review of commonly recognized databases (boxrec.com for boxers, sherdog.com for MMA fighters). Information on the amount of sparring the participant has engaged in, as well as whether there has been any concussions or head injuries within the 2 weeks prior to the study visit is obtained through self-report.

Cognitive function was assessed by a computer based battery that consists of four subtests of the CNS Vital Signs (CNS Vital Signs, North Carolina) including verbal memory, symbol digit coding, Stoop and a finger tapping test. CNS Vital Signs offers robust and reliable measurements of cognition which are computerized but are supervised by a technician (13). Results from these tests are used to make up scores in various clinical domains: verbal memory, processing speed, psychomotor speed and reaction time.

A high resolution T1-weighted anatomical MRI was obtained on all fighters at each visit. A 3-T MR imaging unit (Verio; Siemens) with a 32-channel head coil was used to acquire structural three-dimensional T1-weighted magnetization prepared rapid acquisition

gradient echo images (repetition time msec/echo time msec, 2300/2.98; resolution, 1 X 1 X1.2 mm³ (14,15). Volumes of the hippocampus and amygdala and subcortical grey matter including thalamus, caudate and putamen, along with corpus callosum, were calculated using the automated full brain segmentation process in FreeSurfer software (version 5.3.0; <http://surfer.nmr.mgh.harvard.edu>). These regions have been shown in pathological series and our prior work to be effected in those with extensive RHI (1, 14). The volumes of each structure were measured in both hemispheres separately and adjusted for total intracranial volume.

The blood samples were collected in EDTA tubes and centrifuged at 3200 rpm for 10 minutes to separate plasma from blood cells. The supernatant was aliquoted in 2 ml portions that were immediately frozen and stored at -80 degrees pending analysis. Plasma tau and NF-L concentrations were measured using ultrasensitive Single molecule array (Simoa) assays as previously described in detail (16,17). For NF-L, two quality control (QC) levels were run in duplicates in the beginning and the end of each run. For QC with concentration 11.8 pg/mL, repeatability was 8.1 % and intermediate precision was 14.7 %. For QC with concentration 108.4 pg/mL, repeatability was 7.0 % and intermediate precision was 13.5 %. For Tau, two QC levels were run in duplicates in the beginning and the end of each run. For QC with concentration 2.5 pg/mL, repeatability was 9.0 % and intermediate precision was 10.1 %. For QC with concentration 28.7 pg/mL, repeatability was 7.9 % and intermediate precision was 9.9 %. The lower limits of quantification for tau and NF-L were 1.22 pg/mL and 6.7 pg/mL, respectively.

All analyses were performed by board-certified laboratory technicians who were blinded to clinical data

Statistical analysis: Descriptive statistics were computed for continuous outcomes (e.g., age) with mean and standard deviation and dichotomized outcomes (e.g., sex) with proportions. Multiple linear regression models were used to compare the difference across groups with regards to the baseline measurement after controlling for the confounding factors in the study: age, race, and education. The total intracranial volume was additionally controlled for volumetric analysis. For the post hoc test to compare the

pairwise group differences, the Tukey-Kramer approach was used to adjust the multiple comparisons. For the longitudinal data with multiple measurements for each participant, a mixed linear model was used in this repeated measures design. This model is able to capture the dependence of the measurements from the same participant. The model included the main effects of group, and time, and the confounding factors as aforementioned. All tests were two-sided at the significant level of 0.05. Statistical software, SAS (Version 9.4; SAS Institute Inc., Cary, NC) was used for data analysis.

Results

Plasma samples were available on 471 participants, with 126 having two or more measurements spanning an average of 1.64 years (range 1-5 years). The characteristics of the study group are described in Table 1. Baseline results reflect the entire group of participants, whereas the longitudinal results only include those with two or more measurements.

Baseline NF-L or Tau measures across the four study groups were compared by using the multiple linear regression model with group as the primary variable of interest while controlling for other covariates: age and race. For the post hoc test to compare the pairwise group differences, the Tukey-Kramer approach was used to adjust the multiple comparisons for the p-value and the confidence interval (18). There was a significant difference in baseline NF-L measures across groups ($F_{3,393} = 6.99$, $p=0.0001$), with the active boxer fighters having higher levels than the active MMA fighters (95% CI 2.27-12.85, $p=0.0015$), and controls (95% CI 3.91-16.87, $p=0.0002$) (figure 1a). The mean baseline NF-L levels for the active boxers was 21.55 pg/ml (SE=1.85), active MMA 14.58 pg/ml (SE= 0.86), retired fighters 15.12 pg/ml (SE= 3.30), and controls 11.27 pg/ml (SE=1.40). Tau levels at baseline were slightly higher in active fighters than controls but did not reach statistical significance (figure 1b).

There was a significant relationship between the number of sparring rounds completed by the active fighters within the 2 weeks prior to drawing the plasma sample with NF-L (95% CI 0.0116-0.4053, $p=0.0381$), but not tau, after controlling for age and race. This was particularly prominent in the active boxers (95% CI 0.0696-0.8003, $p=0.0206$).

Similarly, higher levels of baseline NF-L were associated with lower baseline volumes of thalamus, hippocampus and central and posterior corpus callosum on MRI imaging, after controlling for age, race, education, number of fights, and total intracranial volume (TIV) (Table 2). Higher baseline NF-L levels were associated with lower baseline performance in the domains of psychomotor speed ($r = -0.1219$, p value = 0.0203) and processing speed ($r = -0.1097$, p value = 0.0378) on computerized cognitive testing after controlling from age, race, education, and number of fights. However, no correlation was seen between baseline tau level and performance on baseline cognitive testing.

A multiple linear model was used to assess the average yearly percentage change of Tau or NFL among the four groups, by controlling for age, race, education, and baseline measurement. Tau and NFL levels remained relatively constant over 2 years in the controls and retired fighters; in the control subjects with at least 2 measurements the average change between baseline and last measurement was 0.30 pg/ml (-1.38 -0.84 pg/ml). The active MMA fighters as a group showed a rise in tau over time which was not seen as prominently with the boxers (figure 2). There was a significant difference in average yearly percentage change in Tau across groups ($F_{3,83} = 3.87$, $p = 0.0121$), with the active MMA fighters having greater increases in Tau levels than controls (95% CI 0.0811-1.0369, $p = 0.0152$). However, there was no relationship between increasing tau levels and change in either MRI volumetric measures or performance on cognitive testing.

Discussion

There are currently no validated biomarkers that are known to reliably reflect underlying neuronal injury from repetitive head impacts. One potential method would be to measure a constituent that is released from damaged neurons in the brain and make its way into the blood.

Due to advances in ultrasensitive assays, it is now possible to detect brain derived substances in blood; literature is accumulating on two markers of axonal injury, NF-L and tau (3-4, 6-8). However, previous reports have evaluated these markers either in a cross sectional manner or pre and post-concussion.

From a longitudinal cohort study of active and retired professional fighters, we now report the behavior of plasma tau and NF-L over multiple years. In individuals no longer, or never, exposed to repetitive head impacts, both of these markers remain relatively stable over several years. On the other hand, active fighters have elevated NF-L levels, possibly related to acute exposure to repetitive head impacts, as suggested by the correlation between the amount of sparring done in close proximity to when the plasma sample was obtained. Tau levels were not correlated to amounts of sparring, and were found to increase primarily in MMA fighters, over a few years.

Before discussing how our findings fit into the current body of literature on NF-L and tau, it is important to note the characteristics of the cohort. The active professional fighters in the study are exposed to repetitive head impacts (RHI) to variable degrees depending on the amount and intensity of sparring they are doing with others and in preparation for a fight; the amount of activity for each individual fighter may vary from year to year.

Furthermore, active boxers and MMA fighters, while both exposed to RHI, likely differ in the amounts and type of RHI. Thus, while we record self-reports of sparring amounts over the time between annual visits and within 2 weeks of blood sampling, as well as verify the number of professional fights they had, we are unable to ascertain the absolute amount of RHI a participant is exposed to. The retired professional fighters in the cohort have, by criteria, had a minimum of 10 professional fights and not had any sparring or fights for at least 2 years. Many of the retired fighters had numerous professional fights and are quite symptomatic, with 28% scoring at least 2 standard deviations below average for their age on baseline cognitive testing. However, there is no way to know what, if any, neuropathological process they may have.

We, like others, found that NF-L and tau levels obtained cross-sectionally, were, as a group, higher in active fighters than controls (6). Whereas other studies have reported long term elevations in plasma tau in individuals exposed to RHI, our retired fighters had measurements that were very similar to controls (7).

The observation that NF-L, but not tau, levels at baseline were strongly correlated with the amount of sparring the participant reported in the 2 weeks prior to sampling underscores

the differences in these two markers. Neurofilament light is highly expressed in large-caliber myelinated axons of the white matter (3, 19, 20). Tau is a CNS-enriched protein with greater expression in unmyelinated cortical axons (21, 22). Blood NF-L levels have been shown to correlate better with CSF concentrations than tau (6, 23, 24). Previous studies in amateur boxers have reported relatively higher increases in CSF levels of NF-L than tau, with only NF-L levels remaining elevated over a 14 day rest period (5). In addition, over a season of collegiate football, there was no significant change seen in plasma tau levels in a small group of players sampled serially (25). Thus, plasma measures of NF-L may be more sensitive than tau to detect damage associated with recent repetitive sub concussive impacts. In addition, the strong relationships between baseline NF-L levels and various subcortical regional volumes, may reflect accumulated chronic injury to white matter tracts and resultant inflammation or wallerian degeneration of the structures they innervate (26).

The role of blood biomarkers to monitor cumulative neuronal brain injury over time has not been previously examined; the results of this study shed some initial light on the subject. Plasma tau and NF-L are generally stable in not only individuals never exposed to RHI but also in our retired boxers, many of whom had extensive exposure in the past. And because a significant number of retired boxers were expressing symptoms and signs of cognitive, mood, and motoric impairment, it could be speculated that these plasma markers may not be very useful in identifying individuals that may be harboring CTE pathology (though post mortem diagnostic confirmation would be needed). Other forms of tau may perform differently for longitudinal monitoring of trauma related brain disorders. Aggregation of hyper-phosphorylated tau (P-tau) in cortical areas is a characteristic of CTE; a recent report indicated the P-tau and the P-tau/total tau ratio outperformed total tau levels as a diagnostic marker in acute traumatic brain injury, as well as showing sustained elevations up to 176 days after injury (27). In addition, exosomal tau measurement in plasma has been suggested as a potential biomarker for CTE (28), but these results need validation in independent cohorts.

On the other hand, a number of active professional fighters showed an increase in tau levels over time. The significance of this finding is uncertain; one could speculate that

there may be a window of time in those exposed to RHI where there is either increased expression or reduced clearance of tau occurring in the brain. Whether these individuals are at higher risk of developing a progressive neurodegenerative condition down the line is uncertain and awaits longitudinal follow up of this group.

Longitudinal measures of both NF-L and tau levels, were not associated with cognitive performance in the active fighters or the number of professional fights they had. It may be that the computerized cognitive tests utilized in the study were not sensitive enough to detect what at best may be small changes in a young, healthy cohort. Moreover, simply considering number of fights may not adequately capture the amount of sub concussive exposure each fighter is sustaining while training. Finally, why MMA fighters were more likely than boxers to show longitudinal elevations of tau is unclear. If anything, boxers generally are on the receiving end of more punches than MMA fighters. Further exploration of other potential factors that differentiate the boxers and MMA fighters is needed to explain this finding.

The strength of this study is the relatively large number of well characterized subjects that are, or have been exposed, to numerous repetitive head impacts. Moreover, this is the first study to our knowledge to measure plasma tau and NF-L serially over a period of years. On the other hand, several limitations need to be mentioned. For one, we cannot be certain that all of the tau and NF-L we measure in plasma is from brain origin. Tau can be detected in the liver, kidney and testis (29). One could imagine that fighters, particularly MMA fighters that often sustain blows to the unprotected body by punches and kicks, could sustain blunt trauma to these organs. However, samples were not obtained close to a sanctioned competition and we did find a correlation between increases in tau and structural brain changes in active fighters which may argue against extracerebral sources. In addition, a previous report showed that plasma tau did not increase on participation in an ice hockey game without incident concussions (4). NF-L is also expressed in peripheral nerves, although the robust correlation of plasma with CSF NF-L concentration seen in numerous studies suggests that most NF-L in plasma is CNS-derived (30, 31). Further, blood NF-L levels correlate with severity of head impacts during bout in amateur boxers (6). Another limitation is that we cannot be certain about the precise amount of exposure

to RHI each active fighter experienced; sparring amounts prior to blood sampling are self-reported and the intensity of sparring is variable between subjects. Finally, since the cohort in the PFBHS and more specifically, those for whom longitudinal blood sampling was obtained, is not a random sample, it is possible that they may differ from the general population of fighters in some yet to be determined way. Because we have rolling enrollment in the study, and given the transient nature of active fighters, many participants have yet to return for follow up visits.

In conclusion, plasma NF-L and tau levels have slightly different behaviors in a group of active and retired fighters. While both measures are relatively stable over time in individuals not actively exposed to head trauma, NF-L may better reflect the neural effects of acute exposure. Further longitudinal follow up is needed to understand the implications of increasing plasma tau levels over time.

Authorships and Disclosures

Charles Bernick, MD, MPH:

Study concept and design, analysis and interpretation of data, acquisition of data, study supervision, drafting and revising of manuscript for content

Receives funding support for the Professional Fighters Brain Health Study from UFC, Bellator/Spike TV, Haymon Boxing, Top Rank Promotions, UCLA Dream fund. Speaker for Allergan pharmaceuticals

Henrik Zetterberg, MD, PD:

Study concept and design, analysis and interpretation of data, drafting and revising of manuscript for content, contribution of vital reagents, study supervision

Has served at advisory board meetings for Eli Lilly and Roche Diagnostics, has received travel support from TEVA and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg.

Guogen Shan, PhD:

Statistical analyses, analysis and interpretation of data, drafting and revising manuscript for content

No disclosures to report

Sarah Banks, PhD:

Drafting and revising manuscript for content, analysis and interpretation of data

No disclosures to report

Kaj Blennow, MD, PhD

Drafting and revising manuscript for content, analysis and interpretation of data, contribution of vital reagents

Has served as a consultant or at advisory boards for Alzheon, BioArctic, Biogen, Eli Lilly, Fujirebio Europe, IBL International, Merck, Pfizer, and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg.

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Table and Figure Legends

Table 1 Characteristics of study cohort

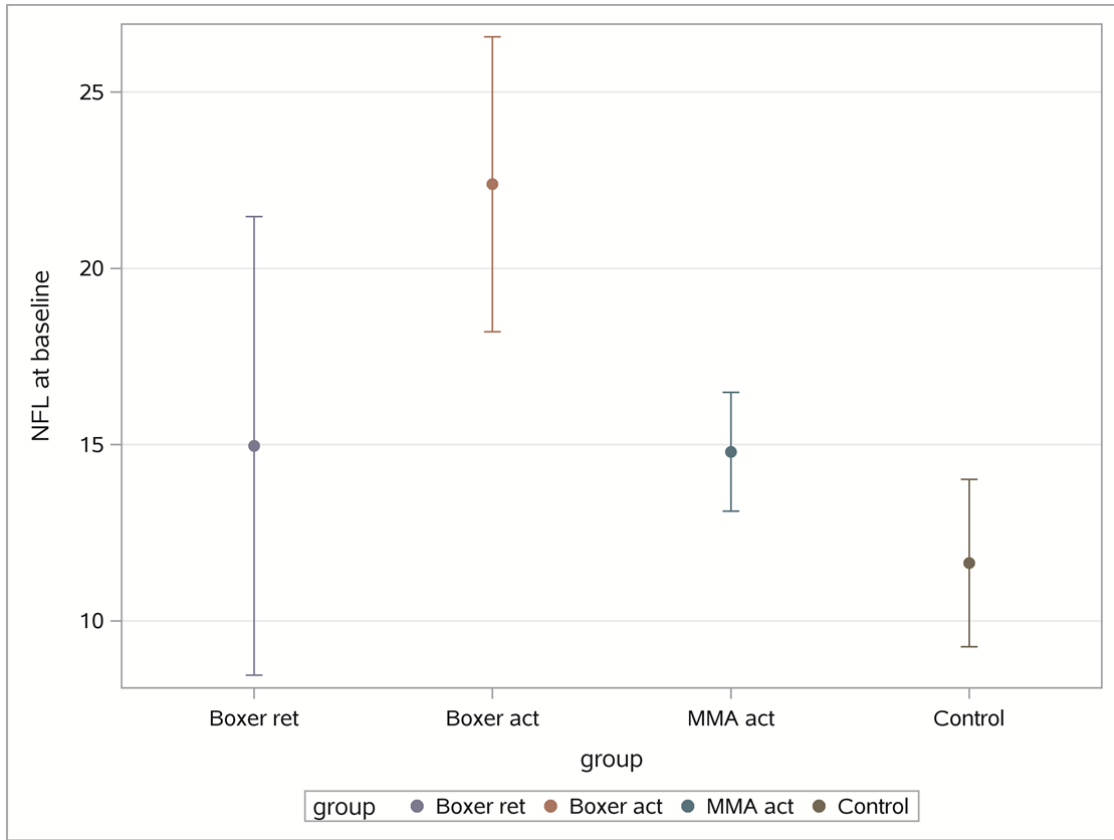
Characteristics of the study cohort including retired boxers (Boxer ret), retired MMA fighters (MMA ret), active boxers (Boxer act), active MMA fighters (MMA act), and control subjects.

	Boxer ret	Boxer act	MMA act	Control
Male	50(96%)	110(94%)	152(90%)	69(87%)
Female	2(4%)	7(6%)	17(10%)	10(13%)
Education	13.00(2.60)	12.92(2.42)	14.24(2.46)	13.63(2.60)
Age	48.00(10.26)	30.38(6.86)	29.59(4.77)	30.78(10.01)
Ethnicity				
African American	13(25%)	32(27%)	42(25%)	7(9%)
White	34(65%)	68(58%)	105(63%)	59(76%)
Asian	1(2%)	4(3%)	6(4%)	5(6%)
Other	4(8%)	13(11%)	14(8%)	7(9%)
Number fights	38.15(20.23)	14.66(12.86)	12.53(12.29)	0
Years of fights	11.70(5.27)	5.94(4.52)	5.61(4.22)	0

Table 2 Baseline NF-L and Regional Volumes

Correlations between baseline neurofilament light and tau levels with baseline MRI based regional volumes adjusted for age, education, number of professional fights. (cc central = central corpus callosum; cc posterior = posterior corpus callosum)

MRI regional volumes	NFL at Baseline		Tau at Baseline	
	Partial Correlation	P-value	Partial Correlation	P-value
left_thalamus_proper	-0.153	0.005	0.014	0.805
right_thalamus_proper	-0.138	0.012	0.006	0.908
left_hippocampus	-0.125	0.022	0.002	0.978
right_hippocampus	-0.115	0.036	-0.053	0.333
cc_central	-0.109	0.047	-0.029	0.592
cc_posterior	-0.110	0.045	-0.053	0.331



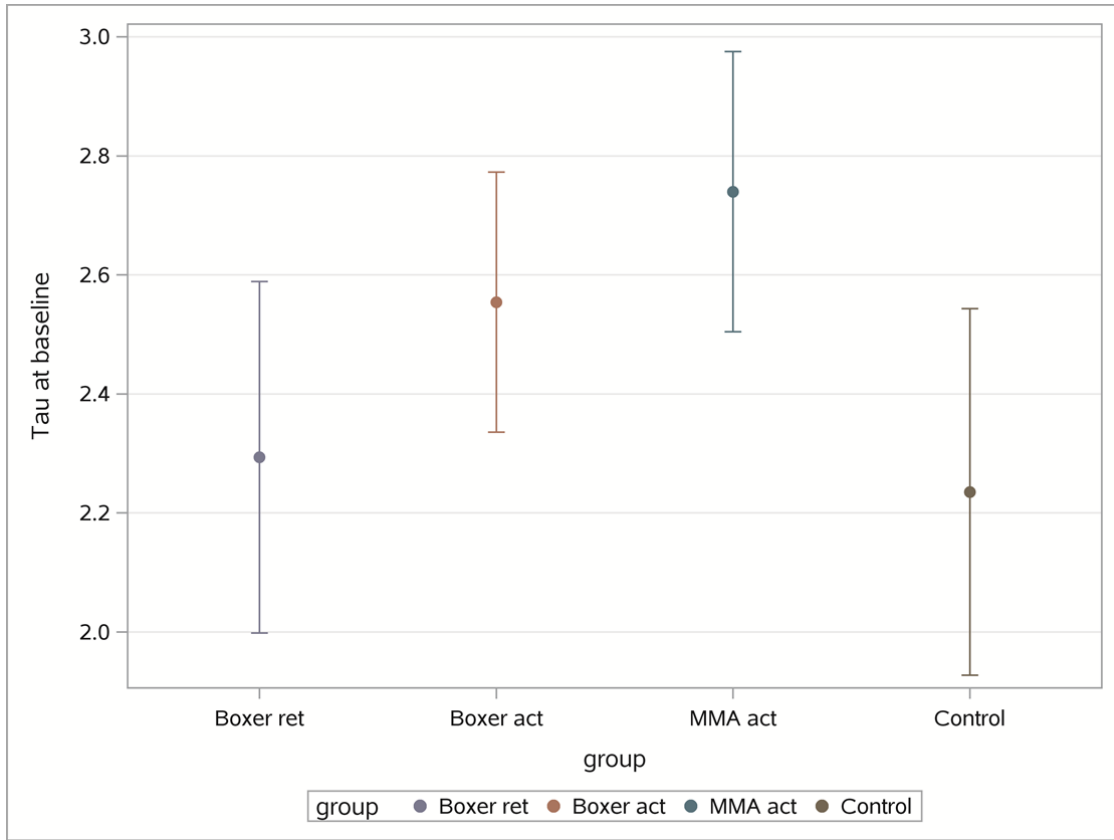


Figure 1 Baseline NF-L and Tau levels

Baseline (a) neurofilament light and (b) tau levels (pg/mL) among retired fighters, active boxers, active mma fighters and controls (mean and 1 standard error of mean)

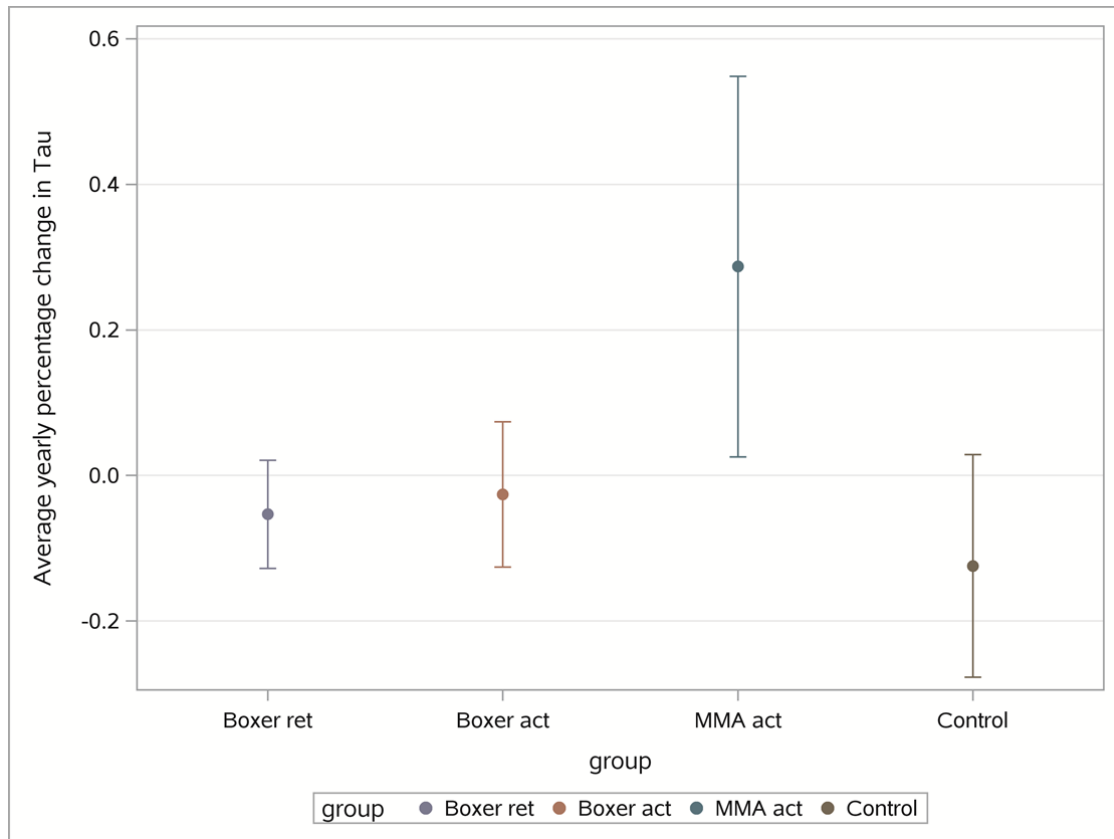


Figure 2 Longitudinal Tau levels

Average yearly percentage change in plasma tau levels (pg/mL) among controls, retired fighters, active mma fighters, active boxers (mean and 1 standard error of mean).