Elevated Axonal Protein Markers Following Repetitive Blast Exposure in Military Personnel

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Blast exposures that occur during training are common in military personnel; however, the biomarkers that relate to these subtle injuries is not well understood. Therefore, the purpose of this study is to identify the acute biomarkers related to blast injury in a cohort of military personnel exposure to blast-related training. Thirty-four military personnel who participated in the training program were included in this study. Blood samples were collected before and after repetitive blast-related training on days 2 (n = 19) and days 7 (n = 15). Serum concentration (pg/mL) of tau, glial fibrillary acidic protein (GFAP), neurofilament light chain (NfL), and phosphorylated tau181 (p-tau181) were measured using an ultrasensitive immunoassay platform. We observed that serum p-tau181 concentrations were elevated after exposed to repetitive blast on days 2 (z = −2.983, p = 0.003) and days 7 (z = −2.158, p = 0.031). Serum tau (z = −2.272, p = 0.023) and NfL (z = −2.158, p = 0.031) levels were significantly elevated after exposure to repetitive blasts on days 7. Our findings indicate that blast exposure affects serum biomarkers indicating axonal injury.

Keywords: concussion, biomarker, axonal, repetitive blast, low level blast

INTRODUCTION

Blast exposures are a prominent feature of injury in military training and combat related due to the use of improvised explosive devices (Ritenour and Baskin, 2008). The impact of blast can be divided into four classification categories: primary, secondary, tertiary, and quaternary based on the mechanism of blast injury such as impact of the overpressure wave with body surfaces, penetrating fragmentation or blunt injury, and/or direct exposure to toxic inhalant, burn, and asphyxia (Wolf et al., 2009). History of blast traumatic brain injuries (bTBI) of all severities can be associated with long-term neurobehavioral sequelae (Lippa et al., 2020) and have been linked to a risk for neurodegenerative processes (McKee et al., 2013; Barnes et al., 2018). The primary bTBIs (mild TBI or concussion) are account for 80% of the bTBI in military population, yet the biological mechanism of blast affects the brain physiological function are limited. Most common concussion or mild TBI occur in athletes, military training,
To date, there is little known about the impact of repetitive low-level blast exposures on fluid biomarkers, and the management of subconcussive impacts is based primarily on clinical symptom presentation. Therefore, a better understanding of temporal changes in proteins that are related to neuronal injuries is important to determine impacts of these exposures, and for future clinical management.

In contrast to low-level blast exposures, blunt TBI blood-based biomarkers have been relatively well investigated, including glial fibrillary acidic protein (GFAP), neurofilament light chain (NfL), tau, and phosphorylated tau (p-tau) (Bogoslovsky et al., 2017; Rubenstein et al., 2017; Wang et al., 2018). Elevated blood concentrations of GFAP, and NfL level have been observed following a TBI and sports concussion, a component of the cytoskeleton of astrocytes, and neuro-axonal damaged membrane varied with the charges. Group 1 participants (n=19) were exposed to 4 light blasts during training on day 1 and exposed to a single heavy blast on day 2. Group 2 participants (n=15) were exposed to 4 light blasts on day 5 and a single heavy blast on day 7 of the training program. Participants have experienced the same types of explosive charges, and the light charges were less than 1 pound (0.03, 0.07, 0.11, and 0.15) of net explosive weight (NEW) and the heavy charges were 10.44 pounds of NEW. The heavy charges in this study were similar to 4.35 pounds per square inch (psi) blast overpressure in the previous study (Boutté et al., 2019). The length of the detonation cord varied with the charges. Group 1 participants (n=19) were exposed to 4 light blasts during training on day 1 and exposed to a single heavy blast on day 2. Group 2 participants (n=15) were exposed to 4 light blasts on day 5 and a single heavy blast on day 7 of training. Blood was collected before and after training within 30 min of blast exposure. The representative figure of this study design is presented in Figure 1.

**FIGURE 1** Summary of blast exposure schedule in both group Participants were exposed to four light charges which less than 1 pound (0.03, 0.07, 0.11, 0.15) of net explosive weight (NEW) and a single heavy charges (10.44 pounds of NEW).

| Group 1  | Blast exposure events | Day 1 | Day 2 | Day 5 | Day 7 |
|----------|-----------------------|-------|-------|-------|-------|
| n=19     | Blood sampling        | Pre-blast exposure | within 30 mins after blast |
| Group 2  | Blast exposure events | Blood sampling    | Pre-blast exposure    | within 30 mins after blast |
| n=15     |

**TABLE 1** | Sample characteristics of the study participants.

|                | Group 1 (N = 19) | Group 2 (N = 15) |
|----------------|------------------|------------------|
| Age, year, mean (SD) | 31.21 (4.49)    | 35.40 (8.16)    |
| Male, no. (%)      | 19 (100)         | 15 (100)        |
| Race, no. (%)      |                  |                  |
| White              | 17 (85.5)        | 13 (86.7)       |
| Missing            | 2 (10.5)         | 2 (13.3)        |
| Previous concussion, no (%) | 0 (0.0) | 1 (6.7) |
| 0                  | 19 (100.0)       | 14 (93.3)       |
| 5                  | 0 (0.0)          | 1 (6.7)         |

Blast exposure on peripheral biomarkers of axonal damage is not well understood. Specifically, the effect of acute blast exposure on serum p-tau181 level is not known in the clinical cohort. Therefore, the purpose of this study was to investigate the feasibility of acute biomarkers changes after blast-related training exposure in military personnel.

**MATERIALS AND METHODS**

**Study Participants**

This study protocol was reviewed and approved by Institutional Review Board Committee at Washington University and U.S Army Fort Leonard Wood. Written informed consents were received prior to enrolling in this study. All participants were male military personnel (n = 34) who participated in the breaching training program at Fort Leonard Wood. The blood samples were collected on day 1 (baseline) and after post-training on day 2 and day 7 of the training program. Participants have experienced the same types of explosive charges, and the light charges were less than 1 pound (0.03, 0.07, 0.11, and 0.15) of net explosive weight (NEW) and the heavy charges were 10.44 pounds of NEW. The heavy charges in this study were similar to 4.35 pounds per square inch (psi) blast overpressure in the previous study (Boutté et al., 2019). The length of the detonation cord varied with the charges. Group 1 participants (n = 19) were exposed to 4 light blasts during training on day 1 and exposed to a single heavy blast on day 2. Group 2 participants (n = 15) were exposed to 4 light blasts on day 5 and a single heavy blast on day 7 of training. Blood was collected before and after training within 30 min of blast exposure. The representative figure of this study design is presented in Figure 1.

| Previous concussion, no (%) | Group 1 | Group 2 |
|-----------------------------|---------|---------|
| 0                           | 19 (100.0) | 14 (93.3) |
| 5                           | 0 (0.0) | 1 (6.7) |
Protein Quantification
Venipuncture blood was collected, centrifuged (15 min, 1,500 g, room temperature), and frozen (−80°C) in aliquots within 60 min of sample collection. Samples were shipped on dry ice to the National Institutes of Health for protein quantification. Serum samples were analyzed in duplicate using the Single Molecule Array (SIMOA) Assay (Quanterix, Lexington, MA) for measurement of tau, GFAP, NfL, and p-tau181 concentration on a HD-X Analyzer™. Samples were diluted 4-fold for measurement. Briefly, four distinct, dye-encoded bead populations presented with analyte-specific capture antibodies were first incubated with samples and biotinylated detector antibodies. The target molecule present within each sample was captured by capture beads and labeled with the corresponding detector antibodies. The bead-conjugated immunocomplex was thoroughly washed and labeled with streptavidin-conjugate β-galactosidase. Following a final wash, resorufin β-D-galactopyranoside was added. The bead-conjugated immunocomplexes were loaded on the SIMOA array disc, which is designed to enable imaging of each bead via their encoded dyes and fluorescent substrate generated signals. The number of bead-containing wells producing positive signals was proportional to the number of target molecules within the sample for eachplex. The average number of enzymes per bead (AEB) of each sample fit into a four-parameter logistic curve plotted using the known concentration of the calibrators. The correlation was confirmed for the accuracy of fit and for the conversion of AEB values to concentrations. The average coefficient of variation of biomarkers were no higher than 25%; and the lower limits of quantifications (LLOQs) for tau, GFAP, NfL, and p-tau181 were 0.212, 1.868, 0.964, and 1.352 pg/ml, respectively.

Statistical Analysis
Statistical analyses were conducted using Statistical Package for the Social Science (SPSS) version 28 (Armonk, NY, IBM Corp.). GraphPad Prism version 9.3 was used to generate a graph in this study (GraphPad Software, La Jolla, CA). Wilcoxon signed-rank test was used to assess changes in proteins concentration before and after blast-related training. Statistical significance was considered with \( p < 0.05 \).

RESULTS
All participants in this study were male with a mean age of 31.21 years (\( SD = 4.49 \)) and with a range of 24–38 years of age for group 1. The mean age of group 2 was 35.40 years (\( SD = 8.16 \)) with a range of 26–52 years of age. The majority of them were White for both groups (85.5 and 86.7%). Demographic characteristics of the study participants are shown in Table 1.

In group 1, serum tau concentration was not significantly different after day 2 blast exposure (\( z = -1.415, \ p = 0.157 \))
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**FIGURE 3** | Dot plots of serum tau (A), p-tau181 (B), GFAP (C), and NfL (D) concentration before and after blast exposure at day 7. The horizontal line in each box represents the median, with the error bars representing the interquartile range. Wilcoxon signed-rank tests were used to analyze the differences before and after blast exposure. p-tau181, phosphorylated tau-181; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain.

(Figure 2A). The median concentration of tau at day 1 was 0.19 pg/mL (25–75th percentile, 0.12–0.39) and day 2 blast exposure was 0.33 pg/mL (0.23–0.47). Serum p-tau181 concentration was significantly elevated after day 2 blast exposure (z = −2.983, p = 0.003) (Figure 2B). The median concentration of p-tau181 at day 1 was 0.81 pg/mL (0.62–1.05) and 1.13 pg/mL (0.92–1.53) in day 2. Concentration of GFAP level was trending significant after day 2 blast exposure (z = −1.807, p = 0.071) (Figure 2C). The level of NfL was not significantly different after day 2 blast exposure (z = −0.631, p = 0.528) (Figure 2D).

In group 2, serum tau concentration was significantly different after day 7 blast exposure (z = −2.272, p = 0.023) (Figure 3A). The median concentration of tau at day 1 was 0.25 pg/mL (0.23–0.35) and day 7 blast exposure was 0.32 pg/mL (0.25–0.40). Serum p-tau181 concentration was significantly elevated after day 7 blast exposure (z = −2.158, p = 0.031) (Figure 3B). The median concentration of p-tau181 at baseline was 0.89 pg/mL (0.79–1.26) and 1.14 pg/mL (0.99–1.21) in day 7 blast exposure. Concentration of GFAP level was not significant after day 7 blast exposure (z = −0.057, p = 0.955) (Figure 3C). The levels of NfL was significantly different after day 7 blast exposure (z = −2.158, p = 0.031) (Figure 3D). The median concentration of NfL at day 1 was 5.96 pg/mL (4.43–6.89) and day 7 blast exposure was 6.13 pg/mL (5.34–7.11).

**DISCUSSION**

Our study investigated the impact of blast-related training on serum biomarkers in military personnel. These findings show similar trends in the reduction of serum GFAP levels after exposure to repetitive low-level blasts during the training program on day 2 (Boutté et al., 2019; Tschiffely et al., 2020). Here, we report that blast-related training results in acute changes in the axonal markers of tau, p-tau181, and NfL. Specifically, the concentration of tau and NfL levels were significantly elevated after day 7 blast exposure compared to pre-blast. Notably, serum p-tau181 level was significantly elevated at days 2 and 7 following blast exposure. Therefore, these proteins may serve as a potential candidate biomarker of blast-related injury in military training.

We observed an elevated level of tau in the acute and subacute timepoints following sports injury (McCrea et al., 2020; Giza et al., 2021). The concentration of tau and NfL has been shown higher in concussion and predicted symptom resolution following concussion (Shahim et al., 2018; Pattinson et al., 2020a). Proteins tau and NfL levels are more than acute markers of axonal injury but persistently elevated in chronic repetitive TBI, which is associated with neurobehavioral outcomes (Olivera et al., 2015; Pattinson et al., 2019). Further, we observed p-tau to be higher after blast, which is a novel finding. Tau phosphorylation...
plays an important role in the physiological function of tau protein regulation in the maintenance of the homeostasis of microtubules and the pathogenesis of neurodegenerative disorders (Rajmohan and Reddy, 2017; Katsumoto et al., 2019). In previous studies level of tau, p-tau181, and NfL proteins were significantly higher in chronic mTBI with persistent symptoms of post-traumatic stress disorder and depression (Kenney et al., 2018; Pattinson et al., 2019, 2020b). Recently, we have shown that extracellular vesicle tau and NfL proteins were associated with behavioral outcomes in military personnel (Edwards et al., 2021; Guedes et al., 2021). Elevation of p-tau181 was observed in many different brain regions and abnormal p-tau accumulation in astroglial, that may associate with behavioral changes following chronic repetitive blast exposure in preclinical model (Dickstein et al., 2021). Abnormal aggregation of p-tau causes synaptic impairment, neuronal dysfunction, and the formation of NFTs and is a key pathological feature of CTE (Rajmohan and Reddy, 2017; Katsumoto et al., 2019). Therefore, changes in these biomarker levels reflect axonal damage or regeneration that induced brain function impairment after repetitive low-level blast exposure. Additional studies of longitudinal change of these biomarkers are needed to determine the implication of these findings.

Our study has some limitations, including a small sample size and lack of gender and racial diversity, as well as lack of clinical symptoms over-time. Despite these limits, this study indicates that blast exposure results in changes in serum biomarkers of axonal injury. Notably, the level of p-tau181 protein was significantly elevated after blast exposures in both cohorts, which suggested that phosphorylated tau at threonine 181 may serve as an early biomarker of axonal injury in low-level blast exposures. However, the level of tau and NfL were not significant differences after immediate blast exposure, unlike the other cohort with 2 days intervals. We observed that 12 out of 19 participants were elevated in tau level and only 9 out of 19 participants were elevated in the NfL level in this group. Our plausible explanation is that is some of these individuals may expose to low severity events of blast compared to the other participants. Previous studies showed that proteins biomarkers of GFAP and inflammatory cytokines were strongly associated with blast exposure levels (Gill et al., 2017; Tschiffely et al., 2020). We suggested that these serum biomarkers changes in our study may also impact by the level of blast exposure during training. However, we did not measure the blast exposure levels in this cohort. Additional larger studies with equal populations are needed to confirm these findings. In addition, a longitudinal study of these serum biomarkers changes is needed to evaluate the effect of blast exposure over time. These findings suggest that blast exposure is associated with acute changes of serum tau, p-tau181, and NfL level in military personnel. In conclusion, blast exposures in military personnel induced axonal damage which may serve as potential biomarkers of blast injury.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board Committee at Washington University and U.S Army Fort Leonard Wood. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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REFERENCES

Barbier, P., Zejneli, O., Martinho, M., Lasorsa, A., Belle, V., Smet-Nocca, C., et al. (2019). Role of tau as a microtubule-associated protein: structural and functional aspects. Front. Aging Neurosci. 11:204. doi: 10.3389/fnagi.2019.00204

Barnes, D. E., Byers, A. L., Gardner, R. C., Seal, K. H., Boscardin, W. J., and Yaffe, K. (2018). Association of mild traumatic brain injury with and without loss of consciousness with dementia in US military veterans. JAMA Neurol. 75, 1055–1061. doi: 10.1001/jamaneurol.2018.0815

Bogoslovsky, T., Wilson, D., Chen, Y., Hanlon, D., Gill, J., Jeromin, A., et al. (2017). Increases of plasma levels of gial fibrillary acidic protein, tau, and amyloid $\beta$ up to 90 days after traumatic brain injury. J. Neurotrauma 34, 66–73.

Bouté, A. M., Thangavelu, B., LaValle, C. R., Nemes, J., Gilsdorf, J., Shear, D. A., et al. (2019). Brain-related proteins as serum biomarkers of acute, subconcussive blast overpressure exposure: a cohort study of military personnel. PLoS One 14:e0221036. doi: 10.1371/journal.pone.0221036

Dickstein, D. L., De Gasperi, R., Gama Sosa, M. A., Perez-Garcia, G., Short, J. A., Sosa, H., et al. (2021). Brain and blood biomarkers of tauopathy and neuronal...
injury in humans and rats with neurobehavioral syndromes following blast exposure. *Mol. Psychiatry* 26, 5940–5954. doi: 10.1038/s41380-020-0674-z

Dikmen, S., Machamer, J., and Temkin, N. (2017). Mild traumatic brain injury: longitudinal study of cognition, functional status, and post-traumatic symptoms. *J. Neurotrauma* 3, 1524–1530. doi: 10.1089/neu.2016.4618

Edwards, K. A., Greer, K., Leete, J., Lai, C., Devoto, C., Qu, B. X., et al. (2021). Neuronally-derived tau is increased in experienced breachers and is associated with neurobehavioral symptoms. *Sci. Rep.* 11:19527. doi: 10.1038/s41598-021-97913-0

Edwards, K. A., Leete, J. J., Tschiffely, A. E., Moore, C. Y., Dell, K. C., Statz, J. K., et al. (2020). Blast exposure results in tau and neurofilament light chain changes in peripheral blood. *Brain Inj.* 34, 1213–1221. doi: 10.1080/02699052.2020.1797171

Frieden, T. R., Houry, D., and Baldwin, G. (2015). Traumatic brain injury in the united states: epidemiology and rehabilitation. *CDC NIH Rep. Congr.* 2015, 1–74.

Gill, J., Latour, L., Diaz-Arrastia, R., Motamed, V., Turtzo, C., Shahim, P., et al. (2018). Glial fibrillary acidic protein elevations relate to neuroimaging abnormalities after mild TBI. *Neurology* 91, e1385–e1389. doi: 10.1212/WNL.0000000000006321

Gill, J., Motamed, V., Osier, N., Dell, K., Arcurio, L., Carr, W., et al. (2017). Moderate blast exposure results in increased IL-6 and TNFα in peripheral blood. *Brain Behav. Immun.* 65, 90–94. doi: 10.1016/j.bbi.2017.02.015

Giza, C. C., McCrea, M., Huber, D., Cameron, K. L., Houston, M. N., Jackson, J. C., et al. (2011). Assessment of blood biomarker profile after acute concussion during combative training among US military cadets. *JAMA Neurol.* 4:e20137731. doi: 10.1001/jama-neuro.2020.37731

Guedes, V. A., Lai, C., Devoto, C., Edwards, K. A., Mithani, S., Sass, D., et al. (2021). Extracellular vesicle proteins and micromonas are linked to chronic post-traumatic stress disorder symptoms in service members and veterans with mild traumatic brain injury. *Front. Pharmacol.* 12:745348. doi: 10.3389/fphar.2021.745348

Katsumoto, A., Takeuchi, H., and Tanaka, F. (2019). Tau pathology in chronic traumatic encephalopathy and alzheimer's disease: similarities and differences. *Front. Neurol.* 10:980. doi: 10.3389/fneur.2019.00980

Kenny, K., Qu, B. X., Lai, C., Devoto, C., Motamed, V., Walker, W. C., et al. (2018). Higher exosomal phosphorylated tau and total tau among veterans with combat-related repetitive chronic mild traumatic brain injury. *Brain Inj.* 32, 1276–1284. doi: 10.1080/02699052.2018.1483530

Lehman, E. J., Hein, M. J., Baron, S. L., and Gercis, C. M. (2012). Neurodegenerative causes of death among retired national football league players. *Neurology* 79, 1970–1974. doi: 10.1212/WNL.0b013e31826dadaf

Lippa, S. M., French, L. M., Bell, R. S., Brickell, T. A., and Lange, R. T. (2020). United States military service members demonstrate substantial and heterogeneous long-term neuropsychological dysfunction after moderate, severe, and penetrating traumatic brain injury. *J. Neurotrauma* 37, 608–617. doi: 10.1080/08977175.2019.166969

McAllister, T., and McCrea, M. (2017). Long-Term cognitive and neuropsychiatric consequences of repetitive concussion and head-impact exposure. *J. Athl. Train.* 5, 309–317. doi: 10.4085/1062-6050-52.1.14

McCrea, M., Broglio, S. P., McAllister, T. W., Gill, J., Giza, C. C., Huber, D. L., et al. (2020). Association of blood biomarkers with acute sport-related concussion in collegiate athletes: findings from the NCAA and department of defense CARE consortium. *JAMA Neurol.* 3:e1919771. doi: 10.1001/jamanetworkopen.2019.19771

McKee, A. C., and Robinson, M. E. (2014). Military-related traumatic brain injury and neurodegeneration. *Alzheimers Dement.* 10(3 Suppl.), S242–S253. doi: 10.1016/j.jalz.2014.04.003

McKee, A. C., Stein, T. D., Nowinski, C. J., Stern, R. A., Daneshvar, D. H., Alvarez, V. E., et al. (2013). The spectrum of disease in chronic traumatic encephalopathy. *Brain* 136, 43–64. doi: 10.1093/brain/aws307

Meier, T. B., Huber, D. L., Bohorquez-Montoya, L., Nitta, M. E., Savitz, J., Teague, T. K., et al. (2020). A prospective study of acute blood-based biomarkers for sport-related concussion. *Ann. Neurol.* 87, 907–920. doi: 10.1002/ana.25725

Olivera, A., Lejbman, N., Jeromin, A., French, L. M., Kim, H. S., Cashon, A., et al. (2015). Peripheral total tau in military personnel who sustain traumatic brain injuries during deployment. *JAMA Neurol.* 72, 1109–1116. doi: 10.1001/jamaneurol.2015.1383