

THE EFFECT OF DIFFERENT EXERCISE MODES ON SERUM CONCENTRATIONS  
OF NF-L AND BLP

A Thesis  
By  
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## **Abstract**

### **THE EFFECT OF DIFFERENT EXERCISE MODES ON SERUM CONCENTRATIONS OF NF-L AND BLBP**

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Sports-related concussion is difficult to diagnose due to subjective symptoms, and because diagnosis relies on athletes to accurately report their condition. Protein biomarkers have garnered interest as a diagnostic aid as some studies have shown that blood serum protein concentration increases in subjects with mild traumatic brain injuries. However, some biomarker serum concentrations may rise after physical exertion, diminishing their diagnostic usefulness in a sport setting. The objective of this study was to determine if sport participation increases serum concentrations of brain lipid binding protein (BLBP) and neurofilament light (NF-L) in the absence of concussion. Serum concentrations were measured in rugby players, runners, cyclists, and non-athlete controls. In the athlete groups, serum was collected before exercising as well as 0 hours, 0.5 hours, 1 hour, 24 hours, 48 hours, and 72 hours post-exercise. Athlete serum concentrations of BLBP and NF-L were compared between athlete groups, within athlete groups, and to non-athlete controls. Serum

BLBP concentrations were not significantly altered within the cycling ( $p = 0.573$ ) or running ( $p = 0.275$ ) group at any time point. Rugby players showed a significant BLBP increase at the 72-hour time point compared to their pre-match baseline ( $p = 0.004$ ). Rugby players also had significantly higher BLBP concentrations compared to runners at the 72-hour time point ( $p = 0.021$ ). There were no significant sex differences for BLBP concentration, and BLBP was not significantly elevated in the athletes at any time point compared to controls. Serum NF-L concentrations were not significantly ( $p > 0.05$ ) altered within the rugby, running, or cycling groups at any time point. NF-L concentration was not significantly different among athlete groups and there was no significant concentration difference between athletes and controls at any time point. Female runners had significantly higher serum NF-L concentration at 0.5 hours post-exercise compared to male runners ( $p = 0.035$ ). This study showed that serum BLBP concentration does not appear to be affected by running or cycling, but our results show that BLBP was significantly elevated 72 hours after playing a contact sport even without a concussion. Serum NF-L concentration does not appear to be affected by sport participation, although significant concentration differences were found between male and female runners 0.5 hours post-exercise.

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I would like to sincerely thank Dr. Rogatzki, Dr. Triplett, Dr. Fasczewski, and Dr. Kappus. Their support and advice have made this process more manageable, and they are greatly appreciated.

## **Dedication**

I would like to dedicate this work to my friends and family who have always been supportive of my academic endeavors.

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## INTRODUCTION

Sports related concussion (SRC) is difficult to diagnose due to subjective symptoms utilized in the diagnostic process such as headache, dizziness, or confusion.<sup>1</sup> SRC diagnosis also relies on athletes to accurately report the symptoms they experience,<sup>2,3</sup> but athletes may not always recognize their symptoms or they may try to hide their symptoms in order to stay in the game and not disappoint their team.<sup>4</sup> For these reasons, there is a need for objective diagnostic tools that can discriminate between concussed and non-concussed athletes.<sup>5</sup> Blood-based biomarkers may aid in concussion diagnosis as they can be objectively measured and have shown potential to indicate the presence of a brain injury, determine concussion severity, and monitor recovery.<sup>2,3,6,7</sup>

A confounding factor regarding the use of blood-based biomarkers for SRC diagnosis is that their levels may increase after physical activity in the absence of concussion. Potential brain injury biomarkers like S100B, BLBP, and tau have been shown to increase after running, participating in contact sports, and high intensity interval training.<sup>7-11</sup> This phenomenon is not yet fully understood, but it has been speculated that inflammation and oxidative stress resulting from rigorous exercise increases the permeability of the blood-brain barrier, allowing biomarkers to leak into the bloodstream.<sup>1,10,12</sup> If biomarker levels in the blood are significantly altered by exercise, their diagnostic utility will be diminished. Therefore, we need a better understanding of how protein concentrations fluctuate in blood serum following different modes of physical activity.

In this study, we measured blood serum levels of two potential SRC biomarkers, brain lipid binding protein (BLBP) and neurofilament light (NF-L) in non-concussed rugby players, runners, and road cyclists. BLBP is a fatty-acid binding protein expressed by radial

glial cells with functions that include intracellular fatty-acid transport, organizing immature migrating neuronal cells, and the proliferation of astrocytes.<sup>13-15</sup> Serum BLBP has been shown to distinguish concussed male rugby athletes from healthy non-athletes, however serum BLBP was also shown to significantly increase in non-injured match-control subjects one hour post-match compared to non-athletes.<sup>11</sup> Additionally, there is a lack of information showing how BLBP fluctuates after playing non-contact sports.

NF-L is a protein within the central nervous system (CNS) that is expressed by myelinated axons and contributes to the structural strength of the axon skeleton.<sup>2,3,16,17</sup> Thus far, previous research has focused on the ability of NF-L to diagnose concussion. Research has shown NF-L concentration to be increased in the blood after a concussion and it has been able to distinguish concussed athletes from non-concussed athletes.<sup>7,18,19</sup> Furthermore, NF-L levels may remain elevated several days post-concussion in athletes who require more than 10 days to return to play or athletes who experience loss of consciousness or post-traumatic amnesia.<sup>7,20</sup> Researchers have also investigated how NF-L levels fluctuate in contact sport athletes in response to head impacts that do not result in concussion.<sup>3,16</sup> However, there is a lack of information showing if physical exertion alone can affect NF-L levels. Serum NF-L was shown to increase in concussed hockey players but remain unchanged after a “friendly” game where no concussions were reported.<sup>7</sup> Serum NF-L levels were also unchanged in football players who experienced high acceleration head impacts but no concussions.<sup>3</sup> These are desirable results suggesting that NF-L may be a promising biomarker to aid in concussion diagnosis, but there is a need for a more thorough investigation into how NF-L levels respond to different exercise activities in the absence of concussion. Since many studies have been conducted with contact sport athletes like football or hockey players, it is unknown how NF-L levels are

affected by sports like running and cycling. To fill in these gaps, we wanted to observe NF-L levels in non-concussed contact sport and non-contact sport athletes before and after exercise.

The goal of this study was to provide insight into how BLBP and NF-L levels fluctuate in blood serum before and after playing rugby, running, and cycling, and compare athlete BLBP and NF-L levels to healthy non-exercised controls. Since road cycling is a low impact exercise, we hypothesized that this activity would have no impact on serum BLBP and NF-L levels. We further hypothesized that running, a moderate-impact sport, would not affect serum BLBP and NF-L levels. Given that rugby is a high-impact sport that often involves head impacts we hypothesized that we would likely see increases in serum BLBP and NF-L within one hour following the match.

## METHODS

### **Sample size**

G\*power analysis (G\*Power 3.1.9.6)<sup>21</sup> determined that 10 subjects in each group were needed to obtain a power of 0.80 and an effect size of 0.25. There were 8 subjects in the rugby group, 9 subjects in the running group, 7 subjects in the cycling group, and 8 subjects in the control group. Although we did not obtain 10 subjects in each group, we felt that this research was still important to present. To conduct the G\*power analysis, we used the mean (SD) athlete baseline serum BLBP and NF-L concentrations presented by Rogatzki et al. (2021) and Joseph et al. (2018) respectively<sup>3,11</sup>.

### **Study design and population**

The Appalachian State University (Boone, NC, USA) Institutional Review Board for the Protection of Human Subjects approved this study (IRB # 21-0286). Prior to participation, written informed consent was obtained by all subjects. Subjects were recruited by asking athletes and non-athletes to participate in the study (Figure 1). Three rugby athletes did not

play in the game and one runner withdrew from the study. The data from these individuals were not included in our analyses. The rugby group included four males and four females [mean age (SD)= 21.8(2.53) years old]. The running group included five males and four females [mean age (SD) = 19.8(0.667) years old]. The road cycling group included three males and four females [mean age (SD)] = 24.3(11.0) years old]. The control group included three males and five females [mean age (SD) = 22.1(2.17) years old].

Male rugby players played two 40-minute halves and female rugby players played two 20-minute halves. The number of head impacts experienced by each rugby player were visually counted. This was due to a malfunction in our BioStamp nPoint equipment as the devices did not collect data throughout the entire match. Cross-country runners ran 6.44 kilometers. Mean run time for males was 34.9 minutes (SD = 7.58 minutes) and mean run time for females was 33.4 minutes (SD = 0.71 minutes). Cyclists road 39 kilometers. The mean cycling time for males was 60.4 minutes (SD = 0.21 minutes), and mean cycling time for females was 57.7 minutes (SD = 5.69). Due to inclement weather, five out of the nine runners ran indoors on a treadmill and six out of seven road cyclists rode indoors on a stationary bike. During the study, the athlete groups were allowed to adhere to their normal training schedules. The control group was a mixture of sedentary and recreationally active individuals who had not exercised within the past 24 hours.

### **Blood collection and processing**

Seven four-milliliter blood samples from athlete subjects and one four-milliliter blood sample from control subjects were obtained from a prominent vein in the antecubital space by standard venipuncture. Blood collection took place in a research tent located on the sideline of rugby matches, and in the phlebotomy lab of the Health Sciences building for runners, cyclists, and controls. Blood samples were taken before physical activity as well as 0 hours

[mean (SD) = 0.23 (0.12) hours], 0.5 hours mean (SD) = 0.67 (0.10) hours], 1 hour [mean (SD) = 1.14(0.138) hours], 24 hours [mean (SD) = 23.5(2.31) hours], 48 hours [mean (SD) = 48.1(2.18) hours], and 72 hours [mean (SD) = 72.6(2.24) hours] post physical activity. The blood was drawn into a silicone-coated serum tube and allowed to clot at ambient temperature for at least 30 minutes but no more than 60 minutes. After the ambient temperature incubation period the blood was centrifuged at 3,500 RPM (2,630xg) for 10 minutes at 4°C (ThermoScientific Sorvall Legend RT+ refrigerated centrifuge, Thermo Fisher Scientific, Inc., Waltham, MA USA) to separate the serum from red blood cells. Serum was then removed using a pipettor and placed in a cryotube which was then stored in a permanent -80°C freezer. The samples remained frozen at -80°C until biomarker analysis.

### **Biomarker analysis**

Serum BLBP concentration ([BLBP]) was measured using a human FABP7 ELISA96-well plate assay kit (OKCD02536, Aviva Systems Biology Corporation, San Diego, CA USA). The detection range of the assay was 0.47-30 ng·mL<sup>-1</sup>, with a lower limit of detection (LOD) less than 0.19 ng·mL<sup>-1</sup>. The inter-assay coefficient of variation was 16.9% and the average intra-assay coefficient of variation was 22.4%. Serum NF-L concentration ([NF-L]) was measured using a human NEFL ELISA-96-well plate assay kit (OKCD08589, Aviva Systems Biology Corporation, San Diego, CA USA). The detection range of the assay was 15.6-1000 pg·mL<sup>-1</sup>, with a LOD less than 6.2 pg·mL<sup>-1</sup>. The inter-assay coefficient of variation was 17.5% and the average intra-assay coefficient of variation was 20.7%. Absorbance for all ten assays were read at 450 nm using an Eon spectrophotometer (BioTek Instruments, Inc., Winooski, VT USA).

## **Statistical analysis**

Normality of data was determined using a Shapiro-Wilk test and homogeneity of variance was determined using Levene's test. Missing data was accounted for by performing a multiple imputation analysis using the mean from five imputations to replace four missing data points. Two data points were missing due to scheduling conflicts that prevented the subjects from providing a blood sample. The remaining two data points were missing due to one athlete who provided five out of seven blood samples before withdrawing from the remainder of the study. To analyze significant difference within subjects for changes in mean serum concentration of BLBP and NF-L over time a repeated measures ANOVA (RMANOVA) was used. When the assumption for sphericity was not met a GreenhouseGeisser correction factor was used. A Friedman test was used to compare medians when the assumption of multivariate normality was not met. To compare mean data between groups and between sexes, a 2 (sexes) x 3 (exercise groups) x 7 (time points) multivariate ANOVA was used. If the assumption of normality as assessed by Levene's test for homogeneity of variance was not met a non-parametric Kruskal-Wallis test was used to compare medians. When statistical analysis showed significance a Bonferroni correction factor was used for pairwise comparisons. Partial eta squared ( $\eta^2$ ) was used to calculate effect size. Statistical significance was determined at  $p < 0.05$ . A Pearson correlation was used to determine the relationship between biomarker concentration and the number of hits experienced by rugby players. All statistical analyses were generated using Statistical Package for the Social Sciences (SPSS) version 28 (SPSS Inc., Chicago, IL USA).

## RESULTS

Subject demographics for each athlete group can be seen in Table 1. We had one subject in the cycling group who was 19 years older than the average age of the other six participants, but this subject was not removed from the data set as their biomarker concentration did not differ from the other participants. Results are reported as median, quartile 1 (Q1), and quartile 3 (Q3) when nonparametric statistics were used, and are reported as mean, standard deviation (SD) when parametric statistics were used. Rugby players experienced an average of 9 head impacts (SD = 6) during their match. There was no correlation between serum BLBP concentration and the number of hits experienced by the rugby players at any time point (pre-match:  $r^2 = 0.01$ ,  $p = 0.829$ , 0 hours:  $r^2 = 0.00$ ,  $p = 0.997$ , 0.5 hours:  $r^2 = 0.05$ ,  $p = 0.580$ , 1 hour:  $r^2 = 0.02$ ,  $p = 0.734$ , 24 hours:  $r^2 = 0.16$ ,  $p = 0.332$ , 48 hours:  $r^2 = 0.06$ ,  $p = 0.557$ , 72 hours:  $r^2 = 0.01$ ,  $p = 0.886$ ).

Median (Q1, Q3) BLBP serum concentrations can be seen in Table 2. Median serum BLBP fluctuations over time for each group are depicted in Figure 2. There was no statistical difference among all time points for serum BLBP concentration in the runner ( $p = 0.275$ ,  $\eta^2 = 0.063$ ,  $1 - \beta = 0.119$ ) and cycling ( $p = 0.573$ ,  $\eta^2 = 0.083$ ,  $1 - \beta = 0.123$ ) subjects. There was a statistical significance among time points for the rugby group ( $p = 0.015$ ,  $\eta^2 = 0.211$ ,  $1 - \beta = 0.945$ ) where serum BLBP was significantly higher at the 72-hour time point (median = 11.7 ng·mL<sup>-1</sup>, Q1 = 8.20 ng·mL<sup>-1</sup>, Q3 = 18.0 ng·mL<sup>-1</sup>) compared to the pre-match time point (median = 0.00 ng·mL<sup>-1</sup>, Q1 = 0.00 ng·mL<sup>-1</sup>, Q3 = 4.88 ng·mL<sup>-1</sup>) ( $p = 0.004$ ).

Serum concentration of BLBP was not significantly different among groups ( $p > 0.05$ ,  $\eta^2 = 0.285$ ,  $1 - \beta = 0.631$ ) except at the 72-hour time point where the rugby group had a significantly greater serum concentration (median = 11.7 ng·mL<sup>-1</sup>, Q1 = 8.2 ng·mL<sup>-1</sup>, Q3 =

18.0 ng·mL<sup>-1</sup>) than the runner group (median = 3.2 ng·mL<sup>-1</sup>, Q1 = 3.3 ng·mL<sup>-1</sup>, Q3 = 14.4 ng·mL<sup>-1</sup>) ( $p = 0.021$ ). There were no significant differences in serum concentration of BLBP between sexes at all time points ( $p > 0.05$ ,  $\eta^2 = 0.169$ ).

Mean (SD) serum NF-L concentrations for each group can be seen in Table 3. Average serum fluctuations over time for each group are depicted in Figure 3. There was no correlation between serum NF-L concentration and the number of hits experienced by the rugby players at any time point (pre-match:  $r^2 = 0.09$ ,  $p = 0.467$ , 0 hours:  $r^2 = 0.01$ ,  $p = 0.862$ , 0.5 hours:  $r^2 = 0.08$ ,  $p = 0.484$ , 1 hour:  $r^2 = 0.04$ ,  $p = 0.635$ , 24 hours:  $r^2 = 0.10$ ,  $p = 0.450$ , 48 hours:  $r^2 = 0.04$ ,  $p = 0.649$ , 72 hours:  $r^2 = 0.02$ ,  $p = 0.748$ ). Serum NF-L concentration was not found to be significantly different among time points for the rugby group ( $p = 0.378$ ,  $\eta^2 = 0.854$ ,  $1 - \beta = 0.945$ ), the running group ( $p = 0.238$ ,  $\eta^2 = 0.835$ ,  $1 - \beta = 0.226$ ), or the cycling group ( $p = 0.649$ ,  $\eta^2 = 0.855$ ,  $1 - \beta = 0.710$ ).

Serum concentration of NF-L was not found to be significantly different between groups ( $p = 0.144$ ,  $\eta^2 = 0.354$ ,  $1 - \beta = 0.809$ ). There was found to be a group\*sex interaction effect ( $p = 0.031$ ,  $\eta^2 = 0.420$ ). Significant sex differences were found in the runner group between the males and females at the 0.5 hour blood draw (males: mean = 27.6 pg·mL<sup>-1</sup>, SD = 38.4 pg·mL<sup>-1</sup>, females: mean = 84.5 pg·mL<sup>-1</sup>, SD = 62.8 pg·mL<sup>-1</sup>,  $p = 0.035$ ).

## DISCUSSION

In our study, serum BLBP levels were not altered at any time point after cycling or running. This finding supports our hypothesis that these activities would not lead to a significant increase in serum BLBP concentration. Previously conducted biomarker studies have similarly shown no changes to biomarker levels after cycling. Schulte et al. (2011), Otto et al., (2000) and Stocchero et al. (2014) found that cycling did not cause an increase in serum



levels of the biomarker S100B (S100B), which is another protein that has been studied for its use in indicating brain injury.<sup>9,22,23</sup> Interestingly, Hasselblatt et al. (2004), and Stocchero et al. (2014) saw a significant increase in serum S100B after running, but it is important to note that unlike BLBP, S100B is not specific to the brain and can be released from muscle and adipose tissue.<sup>8,9,22,23</sup> In the Hasselblatt and Stocchero studies, serum S100B increase correlated with the increase in serum creatine kinase suggesting a release from damaged muscle rather than the brain.<sup>8,9</sup> Since BLBP is a brain-specific protein, we anticipated no significant changes in serum levels as neither the runners nor the cyclists experienced any head injuries, therefore our results met our expectations.

The rugby players showed no significant elevations in serum BLBP compared to the control group at any time point. This is contrary to the findings of Rogatzki et al. (2021) who observed a significant elevation in serum BLBP in non-injured male rugby players one hour after a match when compared to healthy non-athletes.<sup>11</sup> This difference in our results may be because the BLBP concentration seen in our control group is more comparable to our rugby group. In our study the non-athlete controls had a mean (SD) BLBP serum concentration of 4.69 (4.03) ng·mL<sup>-1</sup> and the rugby players had a mean (SD) concentration of 5.70 (2.61) ng·mL<sup>-1</sup> at the 1-hour post-match time point. In the Rogatzki study, non-athlete controls had a mean (SD) BLBP concentration of 0.96 (0.37) ng·mL<sup>-1</sup> while the match-control subjects had a mean (SD) concentration of 5.01 (3.08) ng·mL<sup>-1</sup>. The rugby players in our study did show a significant increase in serum BLBP concentration at the 72-hour time point compared to their pre-match baseline. This could possibly mean that roughly 72 hours are required for serum BLBP to significantly increase after playing a contact sport where no head injuries are reported.

At the 72-hour blood draw rugby players had a significantly higher median BLBP concentration than runners. This could mean that BLBP kinetics are affected by different modes of exercise. Rugby athletes are typically exposed to more head impacts than runners; therefore, we would expect rugby players to have a greater serum concentration than runners at all time points post-match, but BLBP may require roughly 72 hours to rise to a significantly greater concentration than that seen in runners. Interestingly, the rugby player's BLBP concentration was not significantly higher than the cyclists at this time point. This may be because the cyclists exercised for roughly twice as long as the runners. The extended exercise time may have increased BLBP levels in the cyclists comparable to the rugby players.

According to our results, serum BLBP concentration was not affected by physical exertion within our running and cycling groups. However, playing rugby resulted in significant serum BLBP concentration at 72 hours post-match even in the absence of concussion. The time it took for BLBP to increase following a match, and the result of BLBP increasing in the absence of concussion are two factors that should be considered if BLBP is to be used to aid in concussion diagnosis for contact sport athletes.

Serum NF-L levels were not significantly affected by any of the exercise modes in our study. These results are consistent with the findings of Joseph et al. (2018) who saw no significant changes in serum NF-L levels in male football players after experiencing high acceleration head impacts [mean (SD) = 87.7 (9.5) minutes post-impact].<sup>3</sup> Similarly, in a study by Shahim et al. (2018) conducted with male hockey players, there was no observed change to serum NF-L levels one hour after a "friendly" game, where no concussions were reported, compared to pre-game levels.<sup>7</sup> Additionally, in a study by Cornali et al. (2022), serum NF-L levels did not change over the course of two seasons in professional male soccer players

compared to pre-season levels or compared to healthy controls.<sup>24</sup> However, in a study by Wallace et al. (2018) conducted with male soccer players, serum NF-L significantly increased one hour after each subject performed 40 soccer headers within 20 minutes with soccer balls launched at [mean (SD)] 77.4 (2.7) km/hr.<sup>17</sup> The protocol of the Wallace study involved deliberate and repeated head impacts. In our study, the rugby athletes experienced an average of nine head impacts while the runners and cyclists experienced no head impacts. The lower number of head impacts in our study may explain why we saw no changes to serum NF-L.

Female runners had a significantly higher mean NF-L concentration at the 0.5 hour blood draw compared to male runners. These results could possibly be due to differences in neck strength between males and females. Compared to males, females typically have smaller neck girth and a lower head-neck segment mass.<sup>25,26</sup> Females also tend to have less neck flexor and extensor strength than males.<sup>26</sup> This indicates that females likely experience greater force to the head during physical activity while males may be more protected.<sup>26</sup>

Therefore, ground reaction forces associated with running may have resulted in the serum NF-L levels of females in our study to surpass those of the male runners. Another potential cause of the higher serum NF-L concentration in females may be due to sex differences in running stride and cadence. Female runners have been shown to have a shorter stride and an increased stride frequency when compared to male runners.<sup>27</sup> Additional steps taken by females compared to males when running the same distance may expose the female brain to more jostling leading to an increase in serum NF-L. As speculated by Otto et al. (2000), running may expose the brain to axial vibrations resulting in serum biomarker increases.<sup>23</sup> Interestingly, we did not see any sex differences in serum NF-L between the male and female

rugby players. This could possibly be because the female athletes played for half the time as the males and therefore experienced fewer head impacts.

Our results are consistent with previous research showing that sport participation does not affect serum NF-L levels. Furthermore, our results suggest that NF-L levels are not significantly different between athlete groups, however, there may be significant concentration differences between male and female runners at early post-exercise time points.

## LIMITATIONS

Our study is not without limitations. We had relatively small samples sizes, therefore our findings may not accurately represent the biomarker concentration ranges of different athlete groups in larger studies. Our small samples sizes also contributed to our study being under powered in some cases. Also, the athletes adhered to their normal training schedules during the length of our study. Because of this, the observed biomarker levels at the 24, 48, and 72 hour blood draws may vary from the levels that may be seen if the athletes had abstained from exercise during the study. Furthermore, the head impacts experienced by the rugby players were visually counted instead of being monitored using a wearable device. Finally, we did not assess our subjects using the Sport Concussion Assessment Tool (SCAT5), a currently used and accepted tool for diagnosing concussion. Particularly in the rugby players, there is the chance that a subject may have had a concussion without our knowledge. Future studies should consider the use of equipment to accurately count the number of impacts and measure impact magnitude, and use of the SCAT5 to accurately identify concussed and non-concussed individuals.

## CONCLUSION

Physical exertion in the context of running or cycling may not affect serum BLBP levels, but concentration increases may be seen at later time points after playing a contact sport like rugby. Sport participation does not appear to cause fluctuations in serum NF-L levels, but there may be significant concentration differences between male and female runners at early post-run time points. Importantly, serum NF-L was not affected by playing a contact sport which is consistent with previous research. This suggests that NF-L may be a good candidate biomarker to aid in sideline concussion diagnosis.

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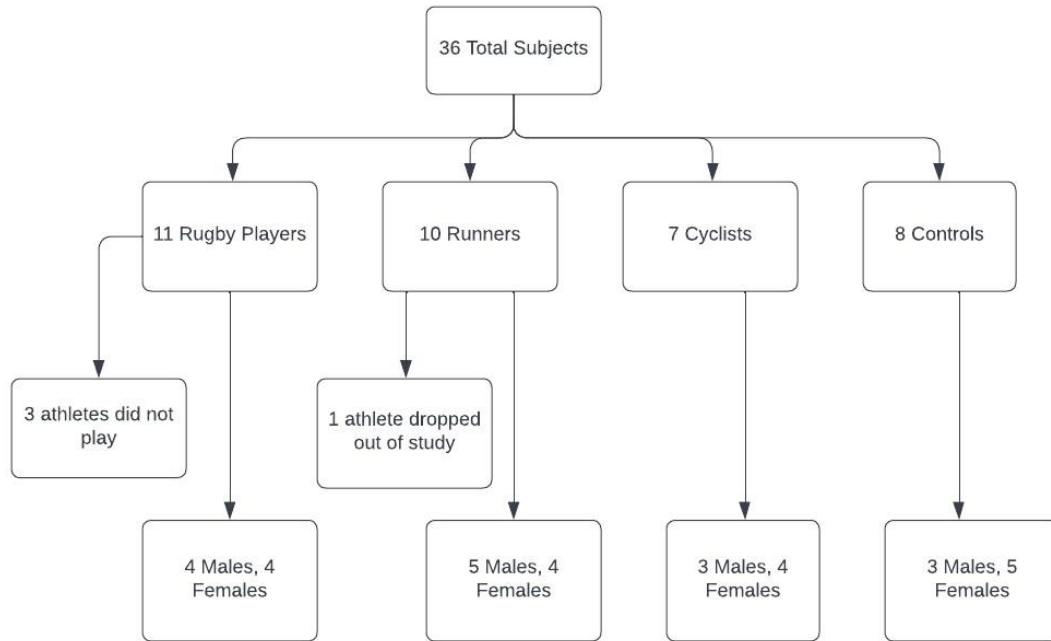
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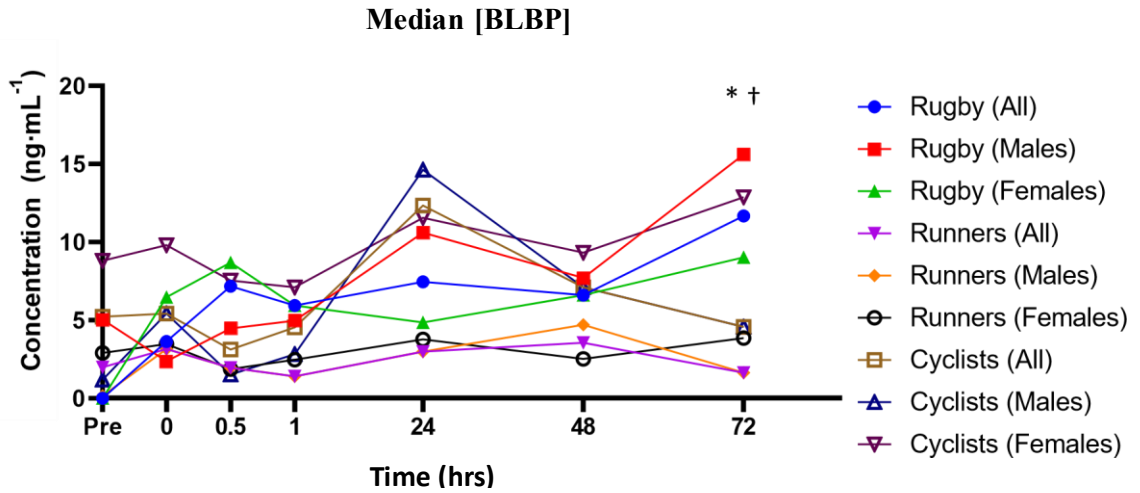
**Figure 1:** Schematic explanation of subject groups. Three rugby athletes did not play in their match and one runner dropped out of the study. These subjects were not included in the analyses.

	N	Age (Yrs)	Height (cm)	Mass (kg)	AVG HR (BPM)	Event Time (min)	Yrs Experience	Concussion Hx (n)
<b>Rugby</b>	8	21.9 (2.52)	169.5 (12.8)	79.5 (20.3)	NA	NA	3.6 (3.85)	1.5 (1.77)
Males	4	23.0 (1.41)	180.3 (8.03)	86.9 (18.8)	NA	80	5.00 (4.83)	1.50 (2.38)
Females		20.3 (3.10)	158.8 (2.54)	72.1 (21.5)	NA	40	2.25 (2.50)	1.50 (1.29)
<b>Runners</b>	9	19.8 (0.67)	177.2 (11.7)	73.3 (14.3)	165.6 (13.6)	34.2 (5.44)	8.00 (4.24)	0.22 (0.44)
Males	5	19.6 (0.89)	185.4 (8.98)	80.9 (15.6)	160.4 (14.5)	34.9 (7.58)	6.20 (3.96)	0.00 (0.00)
Females	4	20.0 (0.00)	167.0 (2.43)	63.9 (2.60)	172 (6.53)	33.4 (0.71)	10.3 (3.86)	0.50 (0.58)
<b>Cyclists</b>	7	24.3 (11.0)	177.4 (7.39)	69.5 (8.40)	155.3 (13.8)	58.9 (4.28)	9.71 (9.79)	0.57 (1.13)
Males	3	20.3 (1.53)	184.6 (1.47)	77.3 (5.71)	147 (17.5)	60.4 (0.21)	4.00 (5.20)	0.00 (0.00)
Females	4	27.3 (14.5)	172.1 (4.34)	63.2 (3.81)	161.5 (7.33)	57.7 (5.69)	14.0 (10.8)	1.00 (1.41)
<b>Controls</b>	8	22.1 (2.17)	173.7 (10.4)	72.6 (10.1)	NA	NA	NA	0.63 (1.06)
Males	3	24.0 (2.65)	182.0 (8.92)	80.9 (5.74)	NA	NA	NA	1.33 (1.53)
Females	5	21.0 (0.71)	168.7 (8.15)	67.6 (8.96)	NA	NA	NA	0.20 (0.45)

**Table 1:** Subject demographics for each group. Values are presented as mean (SD). Yrs = years, AVG = average, HR = heart rate, BPM = beats per minute, min = minutes, Hx = history, SD = standard deviation.

Median (Q1, Q3) concentration of serum BLBP (ng·mL <sup>-1</sup> )								
	N	Pre	0 Hours	0.5 Hours	1 Hour	24 Hours	48 Hours	72 Hours
<b>RUGBY</b>	8	0.00 (0.00, 4.88)	3.64 (2.36, 9.72)	7.19 (4.34, 10.8)	5.94 (3.47, 7.10)	7.45 (2.88, 13.2)	6.61 (4.34, 11.9)	11.7 (8.20, 18.0)*†
Males	4	5.04 (3.53, 8.29)	2.37 (0.85, 5.48)	4.48 (1.54, 8.61)	4.98 (3.47, 7.10)	10.6 (6.37, 14.5)	7.71 (3.20, 14.0)	15.6 (10.5, 19.5)
Females	4	0.0 (0.0, 0.0)	6.47 (3.36, 14.0)	8.69 (6.67, 10.8)	5.94 (4.90, 6.86)	4.85 (2.88, 8.43)	6.61 (5.43, 9.00)	9.02 (8.20, 12.1)
<b>RUNNERS</b>	9	1.99 (0.16, 3.70)	3.16 (1.77, 3.92)	1.95 (1.05, 2.38)	1.40 (0.91, 4.41)	2.99 (1.81, 5.73)	3.55 (1.97, 5.16)	1.64 (0.52, 7.09)
Males	5	0.16 (0.04, 1.99)	3.16 (1.16, 3.77)	1.95 (0.98, 2.38)	1.40 (0.91, 4.41)	2.99 (1.97, 4.57)	4.71 (3.33, 5.16)	1.64 (0.72, 1.68)
Females	4	2.91 (2.05, 4.01)	3.47 (2.71, 5.34)	1.88 (1.37, 3.21)	2.48 (1.03, 4.26)	3.77 (1.80, 6.06)	2.52 (1.29, 4.38)	3.85 (0.40, 7.83)
<b>CYCLISTS</b>	7	5.22 (1.06, 8.82)	5.42 (3.66, 11.4)	3.12 (1.68, 8.44)	4.54 (1.92, 11.9)	12.3 (6.46, 16.2)	7.10 (3.03, 12.7)	4.58 (3.33, 14.4)
Males	3	1.19 (1.06, 3.22)	5.42 (3.24, 6.74)	1.53 (1.35, 3.22)	2.82 (1.86, 8.46)	14.6 (8.40, 16.2)	7.10 (3.59, 9.32)	4.58 (4.06, 5.36)
Females	4	8.80 (4.08, 17.0)	9.80 (4.22, 15.7)	7.54 (2.80, 12.1)	7.10 (3.66, 11.5)	11.6 (8.26, 15.4)	9.32 (3.94, 14.0)	12.9 (2.83, 24.6)
<b>CONTROLS</b>	8	3.43 (2.41, 5.28)						
Males	3	4.80 (3.43, 9.22)						
Females	5	3.27 (2.52, 3.58)						

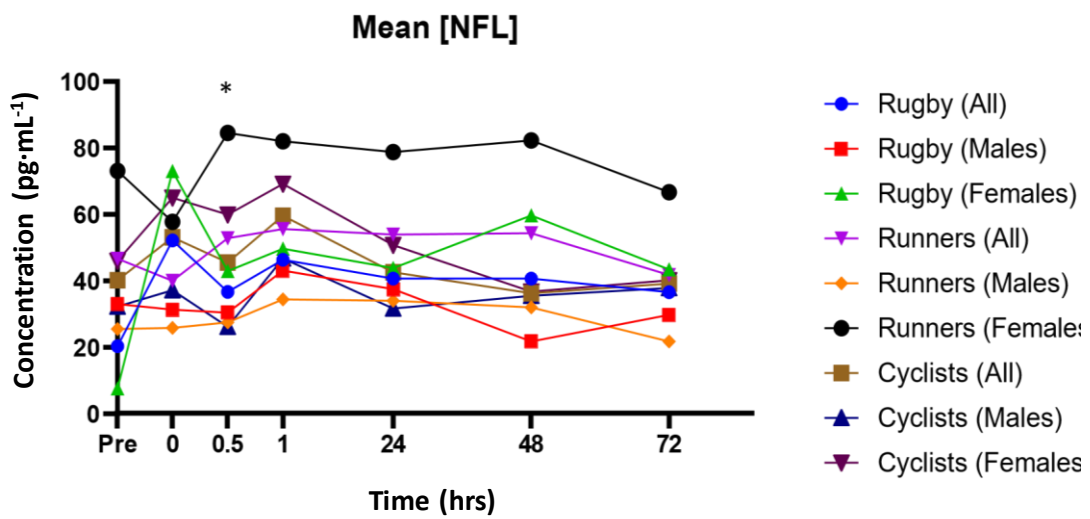
**Table 2:** Median serum BLBP concentration. Values are presented as median (Q1, Q3). BLBP = brain lipid binding protein, Q1 = 1<sup>st</sup> quartile, Q3 = 3<sup>rd</sup> quartile. \* = statistically significant compared to pre-match levels (p = 0.004), † = statistically significant compared to running group at 72 hours (p = 0.021).



**Figure 2:** Median BLBP concentration for all groups and sexes. \* = rugby group (all) statistically different compared to pre-match levels ( $p = 0.004$ ), † = Rugby group (all) statistically different from the running group (all) at 72 hours ( $p = 0.021$ ).

Mean (SD) concentration of serum NF-L (pg·mL <sup>-1</sup> )								
	N	Pre	0 Hours	0.5 Hours	1 Hour	24 Hours	48 Hours	72 Hours
<b>RUGBY</b>	8	20.3 (23.5)	52.2 (40.2)	36.7 (28.6)	46.4 (21.5)	40.7 (25.4)	40.7 (37.3)	36.6 (21.6)
Males	4	33.1 (26.0)	31.4 (19.9)	30.5 (20.7)	43.1 (17.9)	37.6 (22.2)	21.8 (24.6)	29.8 (17.0)
Females	4	7.61 (13.5)	73.1 (47.1)	43.0 (37.1)	49.7 (27.1)	44.0 (31.4)	59.7 (41.0)	43.4 (26.0)
<b>RUNNERS</b>	9	46.7 (55.9)	40.0 (58.5)	52.9 (55.8)	55.6 (57.1)	54.0 (66.2)	54.4 (56.7)	41.8 (51.5)
Males	5	25.6 (37.5)	25.8 (46.2)	27.6(38.4)	34.5 (46.1)	34.1 (46.9)	32.1 (40.0)	21.8 (28.7)
Females	4	73.1 (69.2)	57.8 (74.4)	84.5 (62.8)*	82.0 (64.8)	78.8 (85.2)	82.3 (67.5)	66.7 (66.9)
<b>CYCLISTS</b>	7	40.2 (32.0)	53.1 (40.5)	45.5 (35.2)	59.6 (46.8)	42.6 (41.5)	36.3 (21.3)	39.2 (28.7)
Males	3	32.3 (33.4)	37.2 (42.8)	26.2 (27.8)	46.8 (55.5)	31.7 (44.6)	35.6 (32.0)	37.9 (42.5)
Females	4	46.1 (34.7)	65.1 (40.2)	60.0 (36.2)	69.2 (45.2)	50.8 (43.6)	36.8 (14.8)	40.2 (21.1)
<b>CONTROLS</b>	8	38.5 (32.6)						
Males	3	22.0 (19.5)						
Females	5	48.4 (36.7)						

**Table 3:** Mean serum NF-L concentration. Values are presented as mean (SD). NF-L = neurofilament light, SD = standard deviation. \* = female runners statistically different compared to male runners at 0.5 hours (p = 0.035).



**Figure 3:** Mean serum NF-L concentration for all groups and sexes. \* = female runners statistically different from male runners at 0.5 hours ( $p = 0.035$ ).

## **Vita**

Jazmin Olivia Harrell was born in Durham, North Carolina, to Raymond Harrell and Lisa Smith. She graduated from Christian Liberty Academy in Etowah in July 2011. The following autumn, she entered Asheville-Buncombe Technical Community College to study Science, and in May 2013 she was awarded the Associate's of Science degree. In the fall of 2013 she attended Western Carolina University to study Biology with a Chemistry minor and was awarded the Bachelor of Science Degree in May of 2016. In the fall of 2020, she accepted a research assistantship in Exercise Science at Appalachian State University and began study toward a Master of Science degree. The M.S. was awarded in December 2022. In January 2023, Ms. Harrell commenced work toward her D.C. in Chiropractic at Logan University. She currently resides in Chesterfield, Missouri.