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Article

Concussion-Based ApoE4 and Lipid Prodromal Biomarkers: The UK Rugby Health Study

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Abstract

Neurodegenerative diseases such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) have been associated with long-term effects of concussions, particularly in contact sport athletes. Lipid dysregulation has emerged as a potential contributor to neuronal damage and could serve as an early biomarker of brain pathology. This study aimed to investigate serum lipid biomarkers in retired rugby players with a history of concussion to explore their diagnostic relevance and potential link to neurodegenerative disease risk. A comparative analysis was conducted comparing serum levels of ApoE4, 24-hydroxycholesterol (24-HC), 25-HC, 27-HC, total triglycerides, and ceramide between concussed retired rugby players and non-contact healthy controls. ELISA-based quantification and statistical comparisons were used to identify significant group differences. Retired rugby players exhibited significantly lower serum 24-HC and significantly higher levels of ApoE4, 27-HC, triglycerides, and ceramide compared to controls. No significant difference was found in 25-HC, though some concussed individuals showed elevated levels. These findings align with previous reports in AD, PD, and ALS populations, supporting lipid dysregulation as a hallmark of neurodegenerative progression. Alterations in lipid profiles among concussed athletes suggest potential roles for ApoE4, 24-HC, 27-HC, ceramide, and triglycerides as early biomarkers for concussion-induced neurodegeneration. These findings may guide future diagnostics and post-care strategies for at-risk populations.

Keywords: lipids; biomarkers; concussion; brain injury; sport; neurodegenerative diseases

1. Introduction

Rugby, a well-known contact sport, carries a notably high risk of physical injury—particularly concussions—due to its inherently aggressive nature, which includes frequent and repeated tackles and collisions [1]. Concussions are a form of traumatic brain injury resulting from rapid head movements involving acceleration and deceleration. They often manifest as brief loss of consciousness, impaired reflexes, or amnesia [2]. Typical symptoms include headaches, dizziness, sleep irregularities, balance issues, disorientation, cognitive disturbances, and mood changes [3]. These injuries are particularly concerning because of their association with long-term deficits in cognition, motor abilities, and mental health [4–6].

Research has shown that repeated concussions can have cumulative effects, increasing the likelihood of future brain injuries and deteriorating neurological health. Tsushima *et al.* [7] reported that athletes with a prior concussion were three to five times more likely to sustain another compared to those without such a history. Moreover, experiencing concussions can significantly impair cognitive functions such as memory, executive functioning, and motor coordination. Retired athletes across various sports who have experienced concussions tend to report greater cognitive difficulties

[8]. In a study by Kerr *et al.* [9] involving 204 former collegiate football players (84.3% with a history of concussion), the researchers explored the relationship between previous concussions and negative health outcomes. Their findings revealed associations between concussion history and several adverse effects, including poor general physical and mental health, depression, and increased risk of alcohol dependence.

While physical activity generally promotes overall health, there are instances where the potential risks may surpass the advantages of engaging in exercise. In this regard, there is growing concern about the health and well-being of former contact sport athletes, particularly in relation to the potential long-term effects of concussions and other brain injuries. For example, former professional and collegiate athletes in sports such as soccer and American football have been found to experience significantly higher rates of amyotrophic lateral sclerosis (ALS) [10]. In a large-scale mortality study involving 3,439 National Football League (NFL) players, Lehman *et al.* [11] reported that deaths due to neurodegenerative diseases—including Alzheimer's disease and ALS—occurred at a rate nearly three times higher than in the general population. An earlier investigation by Guskiewicz *et al.* [12] involved administering a general health questionnaire to 2,552 retired professional football players, 61% of whom reported experiencing at least one concussion, and 24% reported three or more during their careers. The study found that players with a greater history of repeated concussions were at increased risk for developing dementia-related conditions. Additional research has consistently shown a strong association between traumatic brain injury (TBI) and elevated risk for cognitive decline and neurodegenerative disorders, including Alzheimer's disease. Regrettably, most neurodegenerative diseases that lead to dementia remain undiagnosable and untreatable during their early, prodromal stages due to the lack of clinically validated biomarkers [13]. In addition, there is currently a gap in research, as no studies have specifically explored potential concussion-related biomarkers that may be associated with neurodegenerative conditions in retired athletes with a history of sports-related concussions (see the exceptional Alanazi *et al.*, 2024) [14].

The current study aims to investigate lipid biomarkers, originally associated with cardiovascular diseases, as potential indicators of neurodegeneration in a well-defined cohort of retired rugby players with a history of multiple concussions (chronic state), compared to healthy controls. The lipid biomarkers examined include Apolipoprotein E4 (ApoE4), 24S-hydroxycholesterol (24-HC), 25-hydroxycholesterol (25-HC), 27-hydroxycholesterol (27-HC), ceramide, and total triglycerides.

ApoE is a central transporter of extracellular lipids and cholesterol within the brain, facilitating cholesterol exchange between neuronal and glial cells [15] (Posse de Chaves and Narayanaswami, 2008). Under normal physiological conditions, ApoE is primarily synthesized by astrocytes, though its expression can be induced in neurons during stress. It is most abundantly expressed in the midbrain and peripheral liver [16] (Konings *et al.*, 2021). The role of ApoE—particularly the E4 isoform (ApoE4)—in concussion and traumatic brain injury (TBI) has been extensively explored, as recovery outcomes vary significantly among individuals. Notably, the ApoE4 genotype has been linked to poorer long-term outcomes post-TBI and neurodegenerative disease [17,18] (Huang and Mahley, 2014; McFadyen *et al.*, 2021) and an increased risk of developing chronic traumatic encephalopathy (CTE) [19] (Atherton *et al.*, 2022). ApoE4 is also a well-established genetic risk factor for early-onset Alzheimer's disease (AD) [20] (Fernández-Calle *et al.*, 2022), largely due to its impaired capacity to clear amyloid-beta ($A\beta$) compared to ApoE2 and ApoE3 isoforms [21] (Castellano *et al.*, 2011). In neurons, ApoE4 is associated with the formation of neurofibrillary tangles (NFTs) and mitochondrial dysfunction, while astrocyte-derived ApoE4 may drive $A\beta$ accumulation [22] (Zhang *et al.*, 2023). These pathological changes promote neuronal injury and neuroinflammation, disrupting memory and learning [22] (Zhang *et al.*, 2023). Astrocytic overexpression of ApoE4 has also been shown to enhance tau phosphorylation and aggregation [23] (Jablonski *et al.*, 2021). Furthermore, ApoE4-related disruptions in cholesterol homeostasis may impair glucose metabolism, further contributing to AD pathology [24,25] (Wu *et al.*, 2018; Sun *et al.*, 2023). Animal studies reveal that ApoE4 delays blood-brain barrier (BBB) repair after injury [26] (Main *et al.*, 2018) and compromises BBB integrity [27] (Bell *et al.*, 2012), consistent with human

findings showing increased BBB permeability in older ApoE4 carriers compared to ApoE3 homozygotes, irrespective of cognitive function [28] (Montagne et al., 2020). In military veterans with TBI history, ApoE4 was associated with poorer memory, slower processing speed, broader cognitive deficits [29] (Merritt et al., 2018), and damaging lipid droplets in Alzheimer's disease microglia [30] (Haney et al., 2024). These findings suggest that post-TBI ApoE4 alterations may underlie molecular vulnerabilities that increase neurodegeneration risk [31] (Muza et al., 2019). Therefore, the present study will evaluate serum ApoE4 levels in retired rugby players with and without a history of concussions to assess its utility as a potential diagnostic biomarker.

Lipids are a diverse group of organic molecules that play essential roles in cellular structure, energy storage, and signaling pathways (Fahy et al., 2009) [32]. Remarkably, the brain contains one of the highest concentrations of lipids in the human body—second only to adipose tissue—with lipids accounting for over 50% of its dry weight (Bruce et al., 2017) [33]. These molecules are fundamental to key neurological functions such as axonal insulation, neurite outgrowth, and synapse formation. Consequently, disruptions in lipid metabolism have been increasingly linked to neurodegenerative processes and brain disorders (Cermenati et al., 2015) [43]. Additionally, brain lipid regulation is closely intertwined with oxidative stress, neuroinflammation, and the maintenance of energy balance. Dysregulated lipid metabolism in neuroglial cells can adversely affect normal neuronal function and signaling (Yang et al., 2022) [35]. Following traumatic brain injury (TBI), measurable alterations in lipid profiles have been detected in peripheral fluids such as blood, plasma, and serum, highlighting their potential as accessible biomarkers (Nessel et al., 2021) [36]. In this context, a deeper understanding of lipid dynamics may not only enhance the diagnosis of concussion-related injuries but also inform early intervention strategies for mitigating long-term neurodegenerative risks.

Oxysterols—oxidized derivatives of cholesterol such as 24-HC, 25-HC, and 27-HC—play pivotal roles in both physiological regulation and pathological progression, particularly in the context of neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) (de Freitas et al., 2021) [37]. These metabolites arise via distinct enzymatic pathways: 24-HC is predominantly synthesized in the brain through the action of cholesterol 24-hydroxylase (CYP46A1) (Sun et al., 2016) [38], 25-HC is produced in macrophages and glial cells via cholesterol 25-hydroxylase (CH25H) in response to Toll-like receptor 4 (TLR-4) activation (Romero et al., 2024) [39], and 27-HC is generated by CYP27A1, which is widely expressed across various human tissues (Marwarha and Ghribi, 2015) [40]. These distinct hydroxylation sites on the cholesterol molecule contribute to their varying cytotoxic effects on neural cells and are believed to play critical roles in the pathogenesis of neurodegeneration (Nakazawa et al., 2017) [41]. Elevated levels of 24-HC in serum have been associated with TBIs and linked to neurodegenerative conditions such as multiple sclerosis and PD. Conversely, reduced 24-HC concentrations may reflect neuronal loss and brain atrophy, as observed in AD and multiple sclerosis (Leoni et al., 2002; [42] Björkhem et al., 2013) [43]. Due to its enhanced ability to cross biological membranes, including the blood-brain barrier, 24-HC holds promise as a serum biomarker for brain injury (Lu et al., 2021) [44]. Likewise, 25-HC has been shown to induce apoptosis in cultured motor neurons, with high levels negatively impacting neuronal viability (Odnoshivkina et al., 2022) [45]. Clinical findings by Kim et al. (2017) [46] revealed significantly elevated 25-HC levels in both serum and cerebrospinal fluid (CSF) of ALS patients compared to controls, implicating its role in disease pathology. Regarding 27-HC, elevated levels have been observed in patients with mild cognitive impairment, suggesting a link to early AD development (Zhang et al., 2019) [47]. However, contrasting evidence from Wuolikainen et al. (2014) [48] showed decreased 27-HC plasma levels in ALS patients relative to controls, indicating complex and disease-specific patterns in oxysterol metabolism.

Ceramide, a bioactive lipid composed of a sphingosine backbone linked to a fatty acid, is a central component of sphingolipid metabolism and plays a critical role in cellular signaling and homeostasis (Mencarelli and Martinez-Martinez, 2013) [49]. The regulation of intracellular ceramide levels is vital, as alterations in ceramide and sphingolipid profiles have been implicated in the pathophysiology of various neurological, neuroinflammatory, and age-associated disorders

(Mencarelli & Martinez–Martinez, 2013) [49]. Elevated ceramide concentrations have been linked to mitochondrial dysfunction, impaired autophagic processes, and disrupted amyloid- β clearance, all of which are recognized contributors to the development and progression of Alzheimer's disease (AD) (Chowdhury et al., 2022 [50]; Custodia et al., 2022 [51]; Tringali and Giussani, 2022) [52]. Notably, Han et al. (2002) [53] observed increased ceramide levels during the early stages of AD, with a subsequent decline as the disease advanced, suggesting a dynamic role in disease progression. Post-mortem analysis by He et al. (2010) [54] further confirmed elevated ceramide concentrations in brain tissues of AD patients compared to controls. Similarly, Fernández-Irigoyen et al. (2021) [55] reported significantly higher ceramide levels in the plasma of patients with Parkinson's disease (PD) relative to healthy individuals. In preclinical research, Barbacci et al. (2017) [56] utilized mass spectrometry imaging to examine ceramide distribution in a rat model of controlled cortical impact (CCI), revealing elevated ceramide in damaged brain regions. In parallel, Ojo et al. (2019) [57] analyzed phospholipid alterations, including ceramide dysregulation, in mouse models of repetitive mild TBI and AD. Their findings identified both shared and unique lipidomic signatures, highlighting ceramide's potential role in the transition from TBI to neurodegenerative disease. More recently, Liu et al. (2024) [58] demonstrated that mice with increased serum ceramide exhibited poorer cognitive performance, as evidenced by reduced discrimination indices, suggesting that circulating ceramide may serve as a viable biomarker for cognitive impairment.

Triglycerides are a class of lipids primarily involved in the storage and transport of energy. They originate from two main sources: dietary absorption through the gastrointestinal tract and endogenous synthesis by the liver. Structurally, triglycerides are composed of three fatty acids bound to a glycerol molecule, and their diversity is vast, with an estimated 6,000 distinct forms based on variations in fatty acid composition and configuration (Bernath et al., 2020) [59]. Triglycerides are fundamental constituents of triglyceride-rich lipoproteins, such as chylomicrons and very low-density lipoproteins (VLDL), and also exist in remnant particles that persist following lipoprotein catabolism (Do et al., 2013). The relationship between serum triglyceride levels and cognitive function has been explored in several human studies. Parthasarathy et al. (2017) [60], in a cohort of 251 older adults, reported an inverse correlation between serum triglyceride levels and executive function, although no association was found with memory performance. These findings suggest that elevated triglyceride levels may selectively impair certain cognitive domains. Similarly, Bernath et al. (2020) [59] conducted a cross-sectional analysis involving 689 participants, including both Alzheimer's disease (AD) patients and healthy controls. Their results indicated that lower triglyceride levels were associated with better cognitive outcomes and reduced brain atrophy, implying a potential protective role. Complementing this, Zhao et al. (2019) [61] assessed serum lipid profiles in a large sample of 1,762 individuals aged 40 to 85. They found that higher triglyceride levels were significantly associated with cognitive decline in middle-aged men (≤ 55 years), further supporting a link between dyslipidemia and cognitive impairment. Despite these associations, human studies specifically examining serum triglycerides in individuals with a history of concussion or traumatic brain injury (TBI) remain lacking. However, animal models provide preliminary insights. For instance, Kuo et al. (2020) [62] demonstrated that rats subjected to TBI and fed a high-fat diet exhibited elevated triglyceride levels, which correlated with increased injury severity. Additionally, Hahnefeld et al. (2022) [63] reported that triglyceride accumulation contributed to behavioral changes post-TBI, highlighting a potential connection between altered lipid metabolism and neurobehavioral outcomes following brain injury.

Given their association with neuronal injury and degeneration, 24-HC, 25-HC, 27-HC, ceramide, and total triglycerides represent promising biomarker candidates for the early detection of neurodegenerative risk. This is particularly relevant for retired rugby players, who are frequently exposed to repeated head trauma. Early identification of oxysterol and other lipid imbalances in this population could inform timely therapeutic strategies aimed at reducing long-term cognitive and neurological burden.

2. Results

A total random selection of 45 males (with a history of concussions, n=26, and control n=19) participated in this study. Participants’ information is given in **Table 1** below.

Table 1. Descriptive characteristics.

	Concussed Group N = 26	Control Group N = 19
Mean ages	39.32±6.44	47.19±12.11
Mean ages at retirement	26.56±4.89	33.77±9.24
Mean years since retirement from the sport	7.04±5.23	8.33±4.29
Playing position	4 prop, 3 hooker, 1 forward, 2 s row, 1 fly half, 4 center, 4 wing, 3 backward, 1 openside flanker, 1 blindside flanker, 2 lock.	1 blindside flanker, 2 backwards, 1 wing, 1 number 8, 1 prop, 1 standoff, 12 non-athletes.
Mean weight	100.2±11.14	86.53±15.47
Mean height	183.81±7.09	178.5±6.33
Rugby league (RL) or union (RU)	12(RL), 14 (RU)	5(RU), 2(RL), 12 (N/A)

2.1. Serum Biomarker Levels in Concussed and Control Groups

A series of related serum biomarkers was explored (**Figure 1**). Concentrations of serum 24-HC were significantly lower in the retired rugby group compared to the healthy control group (**p* < 0.01). There was a highly significant elevation of serum ApoE4, ceramide, and total triglycerides concentrations in the serum of retired rugby players who had suffered multiple concussions when compared to the non-contact control group (***p* < 0.01). Moreover, there was a significant difference in 27-HC levels between the concussed and control groups (**p* < 0.05), while there was no difference between the levels of 25-HC in serum between the two groups.

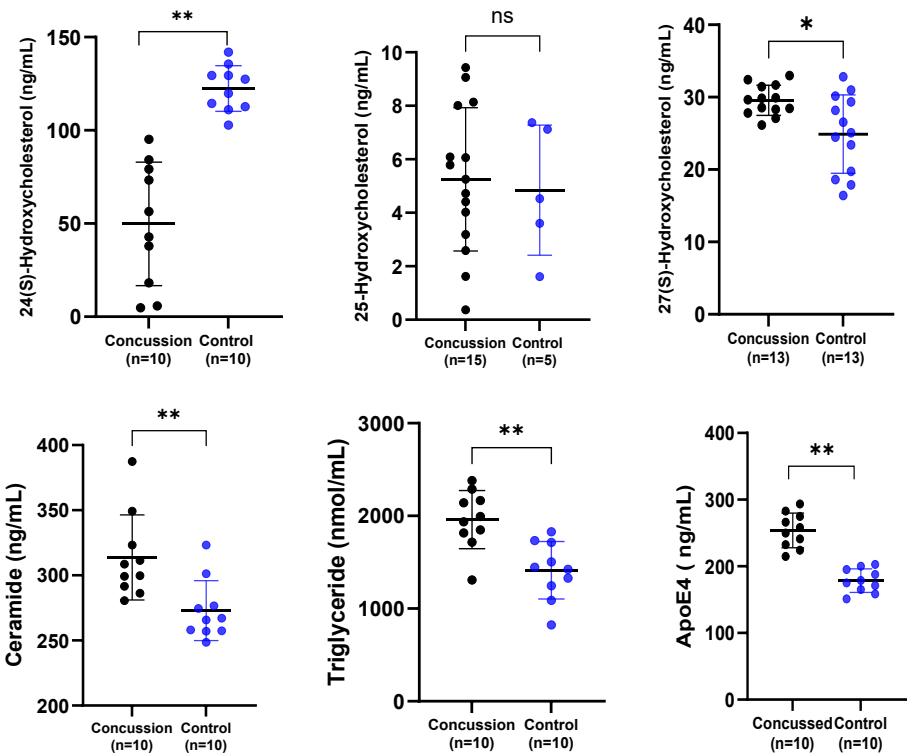
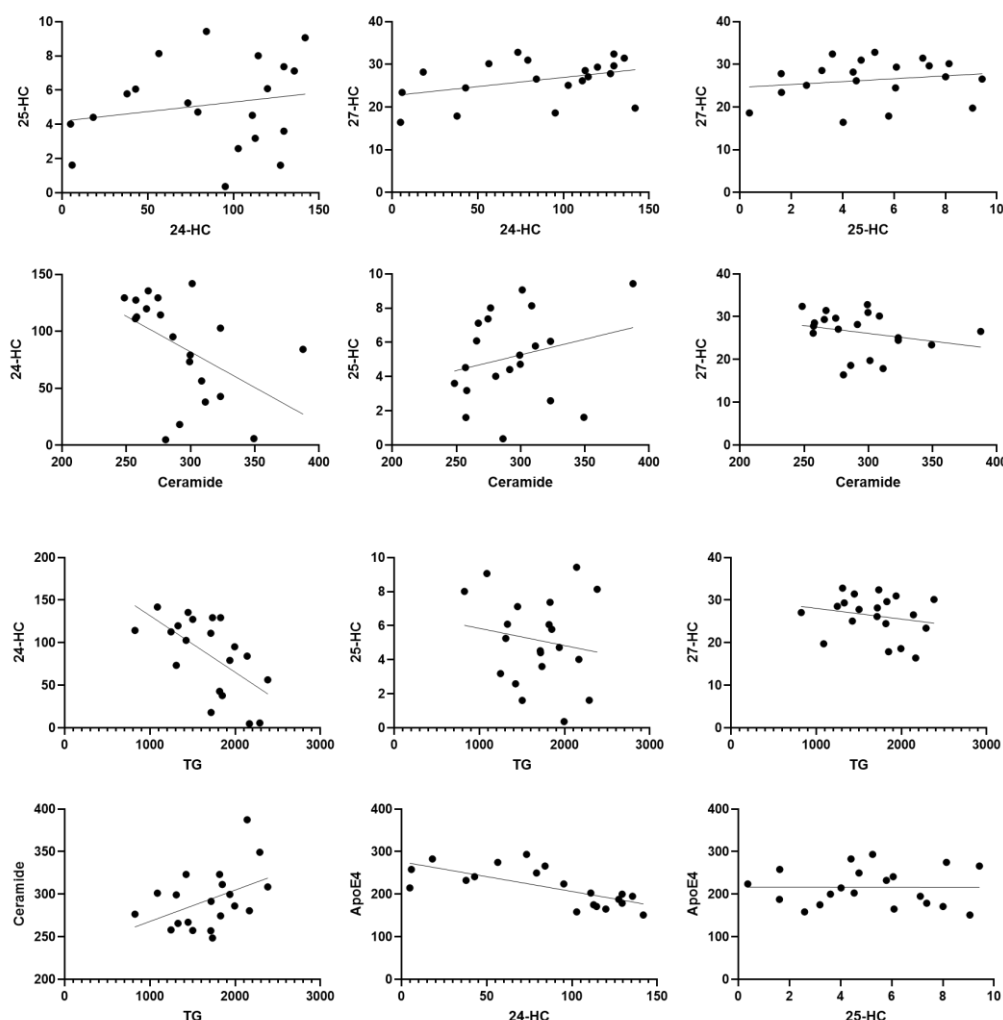


Figure 1. Comparison of the serum concentration of 24-HC, 25-HC, 27-HC, ceramide, total triglycerides, and ApoE4 in a concussion vs. control group. Each dot represents an individual data point, with the bars representing the median and the range. Blue dots = the control group, and black dots = the concussion group. * $p < 0.05$; ** $p < 0.01$; ns not significant.

2.2. Correlations Among Biomarkers

A standard regression Pearson's correlation analysis was performed between all the markers. Unexpectedly, there were insignificant correlations between 24-HC levels and total 25-HC ($R^2 = 0.03717$, $p = 0.41$); 24-HC and 27-HC ($R^2 = 0.1508$, $p = 0.09$); 25-HC and 27-HC ($R^2 = 0.03090$, $p = 0.45$); 25-HC and ceramide ($R^2 = 0.06039$, $p = 0.2963$); 27-HC and ceramide ($R^2 = 0.06558$, $p = 0.27$); 25-HC and triglycerides ($R^2 = 0.02654$, $p = 0.49$); 27-HC and triglycerides ($R^2 = 0.04482$, $p = 0.37$); ceramide and triglycerides ($R^2 = 0.1939$, $p = 0.052$); ApoE4 and 25-HC ($R^2 = 0.00003048$, $p = 0.98$); ApoE4 and 27-HC ($R^2 = 0.01551$, $p = 0.6009$);

Interestingly, there was a significant correlation between reduced 24-HC levels and higher total triglycerides ($R^2 = 0.38$, $p = 0.003$) and between lower 24-HC and elevated ceramide levels ($R^2 = 0.2354$, $p = 0.03$). Moreover, there was a highly significant correlation between 24-HC and ApoE4 ($R^2 = 0.4902$, $p = 0.0006$). There were also strong positive correlations between ApoE4 and ceramide ($R^2 = 0.2447$, $p = 0.0266$) and between ApoE4 and total triglycerides ($R^2 = 0.3693$, $p = 0.0045$) (**Figure 2**).



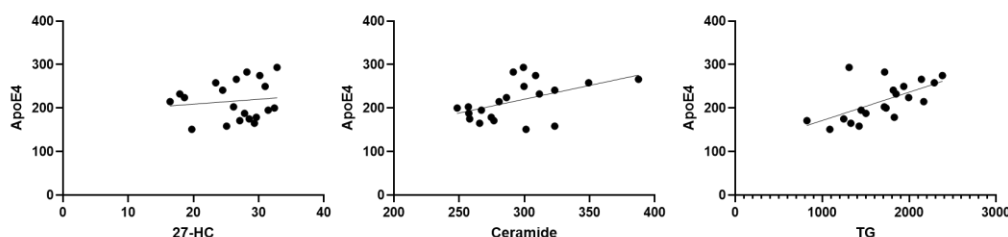


Figure 2. Scatter plots to highlight the correlation between lipid biomarkers. Dots are individual data points for the concentration of the substances specified on graphs of specific serum samples ($n = 20$ or more).

3. Discussion

Given their established links to neurodegenerative conditions such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS), this paper assesses the concentrations of key lipid biomarkers: ApoE4, 24-HC, 25-HC, 27-HC, triglycerides, and ceramide between retired rugby players with a history of concussion and matched healthy controls. These markers were examined to determine whether measurable differences exist that may serve a diagnostic purpose. This paper highlights that several lipid species may contribute meaningfully to the early detection of concussion-related pathology and the long-term risk of neurodegeneration, with potential implications for revising concussion protocols in high-impact sports such as rugby.

We found a significant increase in serum ApoE4 concentrations in concussed individuals compared to the control group (Figure 1). This finding aligns with previous research, indicating that the presence of the ApoE4 allele is associated with a heightened risk of both neurodegenerative diseases and concussion-related injuries such as traumatic brain injuries [17,64]. (Zhou et al., 2008; Huang & Mahley, 2014). Supporting this, Hellström et al. (2022) [65] reported that individuals carrying the ApoE4 allele exhibited significantly greater verbal memory impairments following TBI compared to non-carriers. Additionally, ApoE4 is believed to contribute to AD pathology through mechanisms involving oxidative stress, mitochondrial dysfunction, and disrupted sleep patterns (Sun et al., 2023) [25]. Yang et al. (2015) [66] also found a notably higher frequency of the ApoE4 allele among patients with mild TBI, and comorbid dementia compared to healthy controls. Similarly, Merritt et al. (2018) [29] observed that ApoE4 was associated with more pronounced cognitive deficits in military veterans with a history of mild TBI than in non-carriers. While these studies demonstrate a clear association between ApoE4 and adverse outcomes in both TBI and AD, limited research has directly assessed ApoE4 serum concentrations in individuals with a history of concussion compared to non-concussed controls. This study addresses that gap, introducing a novel approach that enhances the understanding of ApoE4's diagnostic potential in the context of sports-related brain injury and neurodegenerative disease.

The findings revealed significantly reduced serum 24-HC concentrations in concussed individuals compared to the control group (Figure 1). This observation corresponds with previous studies in neurological disorders. For instance, lower 24-HC levels have been documented in older patients with multiple sclerosis (MS), Parkinson's disease (PD), and ALS, likely reflecting neuronal loss or impaired metabolic activity in cholesterol catabolism (Lee et al., 2009; [67] La Marca et al., 2016) [68]. Conversely, studies such as Weiner et al. (2008) [69] did not report a difference in 24-HC following TBI, although their sample involved younger individuals, which may account for this discrepancy. Overall, our findings support the hypothesis that decreased 24-HC may indicate reduced neuronal viability and suggest its potential utility as a biomarker for concussion-induced neural damage.

Regarding 25-HC, no significant group differences were observed; however, several concussed individuals exhibited elevated levels relative to controls (Figure 1). Prior research on ALS patients showed mixed outcomes—Kim et al. (2017) [46] reported increased serum and CSF 25-HC, whereas Wuolikainen et al. (2014) [48] found no significant alterations. The lack of statistical significance in

our study may be attributed to the small sample size, the variability of ELISA assays, or undiagnosed head injuries in the control cohort.

Serum levels of 27-HC were found to be elevated in both the concussed and control groups (Figure 1). These findings partially mirror reports of increased 27-HC in CSF of ALS patients (Kim et al., 2017) [46], possibly due to impaired clearance or upregulated synthesis. In contrast, reduced plasma 27-HC was observed by Wuolikainen et al. (2014) [48] in ALS, suggesting divergent peripheral and central regulation mechanisms. This divergence emphasizes the need to distinguish between systemic and CNS-specific oxysterol pathways in neurodegenerative risk.

Total serum triglyceride levels were also significantly elevated in the retired rugby players compared to controls (Figure 1). This novel finding aligns with studies linking triglyceride dysregulation to brain atrophy and reduced cognitive function (Bernath et al., 2020 [59]; Zhao et al., 2019) [61]. Furthermore, elevated triglyceride concentrations have been associated with a heightened risk of dementia and AD (Tian et al., 2023) [70]. Animal studies further substantiate these associations—Kuo et al. (2020) [62] and Hahnefeld et al. (2022) [46] both reported that increased triglycerides exacerbated injury and behavioral deficits post-TBI.

Ceramide levels were notably higher in the concussed group relative to controls (Figure 1), representing another novel contribution. While ceramide has been extensively studied in AD and PD, its association with sports-related concussion has not previously been investigated. Prior work demonstrated elevated ceramide in early AD and PD cases (Han et al., 2002 [53]; He et al., 2010 [54]; Fernández-Irigoyen et al., 2021) [55]. Animal models similarly reveal ceramide accumulation in brain regions impacted by injury (Barbacci et al., 2017 [56]; Ojo et al., 2019) [57]. Most recently, Liu et al. (2024) [58] linked elevated ceramide with cognitive impairment in mice, suggesting serum ceramide as a promising marker of neural dysfunction.

With reference to the correlation between biomarkers, we found a negative correlation between 24-HC and triglycerides. Previous studies discussed the association between 24-HC and triglycerides. For instance, Dunk *et al.* [71] (2024) found that there is an association between 24-HC and triglycerides, suggesting that plasma oxysterols may be a modifiable risk factor for dementia. Lin et al (2018) [72] observed that the plasma 24-HC was correlated with total plasma cholesterol ($R = 0.659$, $p = 0.001$), affecting the risk of age-related macular degeneration (AMD) development and progression. Though not a direct measure of triglycerides, total cholesterol correlation often implies a broader relationship across lipid fractions, including TG. Furthermore, key circulating oxysterols, including 24-HC, were found to be positively correlated with triglycerides, indicating systemic lipid interactions (Passarelli et al., 2022) [73]. These studies suggest that 24-HC levels are significantly associated with systemic lipid markers, including triglycerides. They reinforce the biological link between brain cholesterol metabolism (via 24-HC) and peripheral lipid status. We also observed a negative correlation between 24-HC and ceramide. Previous research suggested that 24-HC is substantially reduced in the presence of neurodegenerative disease. Because the final effect of a neurodegenerative process is a loss of active neural cells, a reduction in 24-hydroxylase activity, with subsequent decline in the formation of 24-HC and its lower efflux from the brain into circulation, is likely to be the outcome in these disorders (Leoni et al., 2013) [74]. Prior studies also denoted that higher levels of ceramide are associated with increased AD pathology and inflammation (Teitsdottir et al., 2021) [75]. Currently, no studies have reported direct correlations between 24-HC and ceramide levels in neurological patients or within the context of concussion/TBI. This highlights the novelty of the present study, which explores lipid biomarkers and their interrelationships as potential tools for diagnosing concussions and predicting neurodegenerative disease. We also observed a negative correlation between ApoE4 and 24-HC. This finding is inconsistent with a study on AD patients that reported no effect of ApoE4 positivity on serum 24-HC levels in AD subjects (Roy et al., 2019 [76]. Furthermore, there were also strong positive correlations between ApoE4 and ceramide and between ApoE4 and total triglycerides. In brain tissues from AD patients, especially those carrying the ApoE4 allele (Bandaru et al. 2009) [77] observed significantly elevated ceramide levels were observed compared to ApoE3 carriers, along with disrupted sphingomyelin metabolism. Similarly,

Taken together, these results suggest that lipids—particularly 24-HC, 27-HC, triglycerides, and ceramide—warrant further investigation as potential biomarkers of concussion-related neurodegeneration. This study represents one of the first attempts to explore lipidomics in the context of repeated head trauma in contact sport athletes and contributes valuable evidence for refining diagnostic strategies and long-term monitoring.

4. Materials and Methods

4.1. Study Design and Setting

The current research analyzed a range of selected biomarkers in retired rugby players with a history of concussion and non-contact sports controls from the UK Rugby Health Project [6]. The UK Rugby Health Project was initiated in 2016 as an extension to the inaugural New Zealand Rugby Health Project [78], and the methods and results so far have been published elsewhere [1,6,78,79].

4.2. Study Participants

Former male rugby players and non-contact sport athletes took part in the study and were recruited from September 2016 to December 2018 using past player/athlete associations, printed and televised media reports, word of mouth, and social media. Participants in the current study were those who had attended an in-person clinical appointment and provided a fasted blood sample. The group of concussed in this study were retired rugby players who reported 5 or more diagnosed concussions during their sporting career ($n = 26$), and the control group was both retired rugby players and retired non-contact sports participants with no reported concussions ($n = 19$). The number of replicate analyses reflected the limited availability of sample volumes for randomly selected age-matched groups of participants, which reflects a limitation of this present study. No significant differences were seen in participant ages or mean years at and since retirement from sport ($p > 0.05$). The severity of the individual concussions was not monitored, and only the group with over 5 concussions was explored in this initial study to explore the extremes in the first instance. Further studies will follow to meet these limitations.

A general health questionnaire was used to gather information regarding engagement in rugby, injuries, including concussions, present health and well-being, and height and weight measurements [1,6]. The questionnaire was accessible online from September 2016 to December 2018.

4.3. Biomarker Assays

This project investigated concentration levels of serum (ApoE4, 24-HC, 25-HC, 27-HC, ceramide, and total triglycerides) for the highly concussed cohort and the non-concussed UK athletes [78–82].

4.4. ELISA Assays

Assays performed using serum followed manufacturer instructions for 24-HC (abcam, catalog no: ab204530), 25-HC (BT lab, catalog no: EA0143Hu), 27-HC (abbexa, catalog no: abx257403), ceramide (AFG Scientific, catalog no: EK710698), and total triglycerides (abbexa, catalog no: abx257659).

4.5. Statistical Analyses

Data were processed in Microsoft Excel 2023 and GraphPad Prism software version 10 was used for all statistical analyses, including the calculation of serum and exosome concentrations, means, standard deviations (SD), coefficient of determination (R^2), and p values (where * significant $p < 0.05$; ** highly significant $p < 0.01$; n.s. denotes non-significant). The test carried out was a Mann–Whitney U test. All quantitative data are expressed as mean values \pm SD of the mean for individual cases.

5. Conclusions

This study highlights the potential of lipid biomarkers, particularly 24-HC, 27-HC, ceramide, and triglycerides, as indicators of concussion-related neurodegenerative risk in retired rugby players. The observed alterations in lipid levels mirror patterns reported in neurodegenerative diseases such as AD, PD, and ALS, reinforcing the biological relevance of these markers. While 25-HC showed no significant difference, its elevation in some concussed individuals warrants further exploration. These findings support the use of lipid profiling as a non-invasive approach to detect early neurodegenerative changes, offering valuable insight for clinical monitoring, early intervention, and refining concussion management protocols in high-impact sports.

6. Patents

There are no patents resulting from the work reported in this manuscript.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization, P.C., K.H.; methodology, N.A.; validation, P.C. formal analysis, N.A,P.C.; investigation, N.A., T.R., I.E.; resources, P.C., K.H.; data curation, N.A., P.C.; writing—original draft preparation, N.A.; writing—review and editing, P.C., K.H., I.E.; supervision, P.C.,K.H.; project administration, P.C., K.H., I.E.; funding acquisition, P.C.,K.H. All authors have read and agreed to the published version of the manuscript.” Please turn to the [CRediT taxonomy](#) for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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