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Original Article

Concussion Acutely Decreases Plasma Glycerophospholipids in Adolescent Male Athletes

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Abstract

Concussions are frequent in sports and can contribute to significant and long-lasting neurological disability. Adolescents are particularly susceptible to concussions, with accurate determination of the injury challenging. Our previous study demonstrated that concussion diagnoses could be aided by metabolomics profiling and machine learning, with particular weighting on changes in plasma glycerophospholipids (PCs). Here, our aim was to report directional change of PCs after concussion and develop a diagnostic concussion panel utilizing a minimum number of plasma PCs. To this end, we enrolled 12 concussed male athletes at our academic Sport Medicine Concussion Clinic, as well as 17 sex-, age-, and activity-matched healthy controls. Blood was drawn and 71 plasma PCs were measured for statistically significant changes within 72 h of injury, and individual PCs were further analyzed with receiver operating characteristic (ROC) curves. Our data demonstrated that 26 of 71 PCs measured were significantly decreased after sports-related concussion ($p < 0.01$). None of the PCs increased in plasma after concussion. ROC curve analyses identified the top four PCs with areas under the curve (AUCs) ≥0.86 for concussion diagnosis: PCaeC36:0 (0.92; $p < 0.001$); PCaaC42:6 (0.90; $p < 0.001$); PCaeC36:2 (0.86; $p = 0.001$), and PCaaC32:0 (0.86; $p = 0.001$). Cut-off values in μM were ≤0.31, 0.22, 5.07, and 4.63, respectively. Importantly, combining these four PCs produced an AUC of 0.96 for concussion diagnoses ($p < 0.001$; 95% confidence interval, 0.89, 1.00). Our data suggest that as few as four circulating PCs may provide excellent diagnostic potential for adolescent concussion. External validation is required in larger cohorts.

Keywords: athlete; biomarker; concussion; diagnosis; glycerphospholipid

Introduction

Concussions remain a major global healthcare problem.[1] Approximately half of all adolescent concussions occur in sporting activities.[2,3] Accurate diagnosis of concussion is essential to optimize medical care, provide timely interventions, and prevent repeat injury before healing. Diagnosis of concussion relies on an injury event, which may be a direct blow to the head or as a result of transmitted forces from a blow to the body, as well as standardized clinical testing with concussion tools.[4] However, concussion diagnoses are often uncertain given that self-reporting of symptoms can be inaccurate,[5] and the contribution of other factors, such as chronic pain, can exacerbate symptoms.[6]

Adolescents are particularly susceptible to concussions[3] and their potentially long-lasting neurological effects,[7,8] making accurate diagnoses in this age group critically important. To date, neither a single nor cluster of symptoms accurately predict concussions in adolescents, although self-reported headache, head pressure, fatigue, and/or noise and light sensitivity have been identified as useful discriminators of injury.[9,10] A number of blood protein biomarkers for concussion diagnoses have also been investigated[4,11,12]; however, current concussion guidelines do not recommend the use of any blood protein biomarkers for diagnosis in children because of insufficient evidence,[4,13] illustrating the need for additional biomarker studies.

We recently demonstrated that a wide variety of blood metabolites also change after concussion in adolescent athletes, and that these injury-induced patterns can be accurately identified with machine learning and advanced analytics.[14] After reduction techniques, we then determined that the most widely relied-upon metabolites for concussion diagnoses were from a single class of choline-containing metabolite, the glycerophospholipids (PCs). Subsequently, magnetic resonance (MR) spectroscopy demonstrated persistently decreased brain choline in these same adolescent

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concussion patients, supporting a role for PCs as a potential biomarker.[15] PCs make up 20–26% of human brain dry weight[16] and are critical for membrane structure, generation of second messengers, and regulation of neuronal apoptosis, transporter activities, and membrane-bound enzymes.[17]

In this study, we hypothesized that the plasma concentrations of individual blood PCs after adolescent concussion would be significantly depressed. We report the directional changes in 71 PCs and provide a simplified diagnostic model utilizing the leading four PCs with maximal change.

Methods

This study was approved by the Western University Human Ethics Review Board. Male adolescent ice hockey athletes (Bantam Division; 12–14 years of age) participated in this study. Patients suspected to have suffered a concussion were clinically evaluated at our academic Sports Medicine Clinic within 72 h of the injury, a time frame to account for weekend injuries. They were either referred by other healthcare providers, including emergency physicians, family physicians, coaches, and/or trainers, or they had booked an appointment by self-referral. Control patients were recruited by posters in hockey arenas or by coaches and/or trainers who provided athletes and their families with a study information package. To be considered as controls, the athletes were non-injured hockey players who were age and sex matched and who had not suffered a diagnosed concussion in the past 6 months.

All patients with a suspected concussion, as well as non-injured control athletes, including their parents/guardians, completed a Sports Concussion Assessment Tool–3rd Edition (SCAT3[18]; 13–14 years of age) or a Child-SCAT3[19] (a modified tool recommended for children ≤12 years of age that considers developmental differences in performance). A complete history, physical, and neurological examination was also conducted by an experienced sport medicine physician. All prescription medications were recorded. Our patient evaluation was in keeping with the Berlin Consensus guidelines.[4] Any subject with a reported neurological disease was excluded. All injured athletes were provided with standardized concussion care.

All athletes on their first clinic visit had 20 mL of blood drawn into EDTA Vacutainer tubes. No restrictions were placed on the time-of-day collection by intent and design, to better represent the natural state of the athlete. Blood was centrifuged and plasma aliquoted into cryovials at a volume of 500 μL and stored at −80°C. Freeze/thaw cycles were avoided. Plasma was collected by strict standard operating procedures, with equal processing times between cohorts.

A targeted quantitative approach was applied to analyze plasma samples using a combination of direct injection mass spectrometry (AbsoluteIDQTM Kit) with a Liquid Chromatography tandem mass spectrometry Kit (BIOCRATES Life Sciences AG, Innsbruck, Austria), as previously described.[14] We report the quantitative measurements of 71 plasma PCs.

Statistical analysis

Demographic data, concussion tool data, and PC concentrations are reported as median (interquartile range; IQR). Statistical significance for demographic data was determined with p values <0.05. Given the number of PCs analyzed and the risk of false positives, a p value <0.01 was used as our standard of statistical significance. Receiver operating characteristic (ROC) curves were conducted to determine sensitivity and specificity of all individual PCs for predicting concussion. Area under the curve (AUC) was calculated for each PC, with an AUC >0.7 considered as acceptable.[20] The coordinates of the curves were then analyzed to identify cut-off values based on the highest sensitivity and specificity for predicting concussion. Logistic regression analyses were also conducted with concussion as the outcome and the top four PCs with AUC >0.85 entered as predictors; the predicted values from the logistic regression models were then saved for use in ROC curve analyses to determine the most parsimonious combination of PCs with the greatest combined AUC. All analyses were conducted using SPSS software (version 25; IBM Corp., Armonk, NY).

Results

Subject demographic and injury data are presented in Table 1. We investigated a total of 12 concussed athletes (median years of age, 13; IQR, 13, 14) and 17 age-, sex-, and activity-matched athlete controls (median years of age, 13; IQR, 12, 14; $p = 0.431$). The predominant mechanism of injury was a body check. One concussion patient had brief loss of consciousness, whereas 3 concussion patients reported amnesia. Headache was the most prevalent self-reported symptom, occurring in 91% of concussion patients. Self-reported symptom evaluation as per SCAT3 ($n = 11$) revealed a median total symptom score and a median total symptom severity of 14 (IQR, 7, 16) and 23 (IQR, 10, 43), respectively. In contrast, the non-concussed athletes had a median total symptom score and a median total symptom severity of 0 (IQR, 0, 0). One concussed athlete was evaluated with the Child SCAT, with a total symptom score of 6 and a total symptom severity of 12 (the parent score indicated a total symptom score of 3 and a total symptom severity of 8).

Medical and medication history are also reported in Table 1. Three concussion patients reported at least one previous concussion,

Table 1. Subject Demographics and Clinical and Injury Data

	Concussion patients (n = *12*)	*Control subjects* (n = *17*)	p *value*
Age, years	13 (13, 14)	13 (12, 14)	0.431
Sex, male (M), female (F)	12M:0F	17M:0F	1.000
Medical history			
Concussion(s)	3 (25)	4 (24)	1.000
Anxiety	1 (8)	0	0.414
Depression	1 (8)	0	0.414
Mood disorder	1 (8)	0	0.414
Other	4 (33)	2 (12)	0.198
Medications	3 (25)	1 (6)	0.279
Mechanism of injury			
Body checked	6 (50)	—	—
Tripped/fell	3 (25)	—	—
Head into boards	1 (8)	—	—
Elbowed	1 (8)	—	—
Unknown	1 (8)	—	—
Injury details			
Loss of consciousness	1 (8)	—	—
Amnesia	3 (25)	—	—
SCAT3 results			
Total no. of symptoms	14 (7, 16)	0 (0, 0)	**<0.001**
Symptom severity score	23 (10, 43)	0 (0, 0)	**<0.001**

Continuous data are presented as medians (IQR), and categorical data are presented as frequency (percent).

SCAT3, Sports Concussion Assessment Tool–3rd Edition; IQR, interquartile range.

Table 2. Plasma Glycerophospholipid (PC) Concentrations and Areas Under the Curve (AUCs) on ROC Analyses

PC	*Concussion patients* (n = *12*) *median (IQR)*	*Control subjects* (n = *17*) *median (IQR)*	p *value*	*AUC*
PC aa C24:0	0.06 (0.04, 0.07)	0.06 (0.05, 0.08)	0.330	0.61
PC aa C28:1	0.63 (0.55, 0.85)	0.85 (0.67, 1.08)	0.046	0.72
PC aa C30:0	0.78 (0.67, 0.96)	1.09 (0.86, 1.35)	0.017	0.77
PC aa C30:2	0.09 (0.07, 0.13)	0.13 (0.07, 0.19)	0.156	0.66
PC aa C32:0	**4.00 (3.43, 4.57)**	**5.08 (4.77, 6.83)**	**0.001***	**0.86**
PC aa C32:1	3.46 (2.02, 5.04)	5.07 (3.96, 6.35)	0.027	0.75
PC aa C32:2	0.95 (0.77, 1.22)	1.46 (1.17, 1.94)	0.009*	0.79
PC aa C32:3	0.16 (0.13, 0.20)	0.23 (0.19, 0.29)	0.002*	0.84
PC aa C34:1	64.47 (49.41, 71.48)	73.99 (68.34, 105.53)	0.017	0.77
PC aa C34:2	143.50 (126.12, 160.18)	181.84 (156.67, 233.46)	0.002*	0.84
PC aa C34:3	4.98 (4.03, 5.91)	6.78 (5.70, 9.06)	0.007*	0.80
PC aa C34:4	0.44 (0.32, 0.72)	0.72 (0.54, 0.85)	0.042	0.73
PC aa C36:0	0.55 (0.43, 0.69)	0.74 (0.58, 0.98)	0.034	0.74
PC aa C36:1	14.02 (11.95, 16.72)	19.64 (16.59, 22.77)	0.009*	0.79
PC aa C36:2	80.34 (73.29, 99.81)	112.96 (92.97, 138.41)	0.005*	0.81
PC aa C36:3	43.59 (40.94, 52.57)	56.67 (45.10, 71.54)	0.034	0.74
PC aa C36:4	57.56 (47.13, 70.87)	74.84 (59.88, 91.82)	0.034	0.74
PC aa C36:5	4.09 (3.11, 5.62)	6.44 (5.09, 9.08)	0.006*	0.80
PC aa C36:6	0.16 (0.14, 0.24)	0.29 (0.23, 0.37)	0.007*	0.80
PC aa C38:0	0.66 (0.52, 1.04)	0.92 (0.76, 1.00)	0.063	0.71
PC aa C38:1	0.22 (0.17, 0.37)	0.29 (0.20, 0.48)	0.352	0.60
PC aa C38:3	14.59 (12.92, 18.86)	17.10 (14.20, 23.85)	0.101	0.68
PC aa C38:4	33.94 (31.30, 40.97)	43.28 (31.91, 54.02)	0.184	0.65
PC aa C38:5	16.63 (13.60, 19.20)	21.47 (16.40, 29.13)	0.030	0.74
PC aa C38:6	17.89 (15.49, 23.64)	24.11 (19.14, 27.19)	0.084	0.69
PC aa C40:2	0.09 (0.08, 0.10)	0.11 (0.09, 0.14)	0.015	0.77
PC aa C40:3	0.17 (0.12, 0.18)	0.16 (0.13, 0.22)	0.565	0.56
PC aa C40:4	1.16 (0.90, 1.38)	1.33 (1.12, 1.77)	0.069	0.70
PC aa C40:5	3.05 (2.60, 3.76)	3.86 (3.31, 4.71)	0.057	0.71
PC aa C40:6	5.79 (4.90, 7.90)	7.57 (6.12, 8.77)	0.069	0.70
PC aa C42:0	0.16 (0.13, 0.21)	0.18 (0.16, 0.20)	0.215	0.64
PC aa C42:1	0.07 (0.06, 0.12)	0.10 (0.08, 0.11)	0.232	0.63
PC aa C42:4	0.06 (0.05, 0.07)	0.07 (0.06, 0.08)	0.042	0.73
PC aa C42:5	0.12 (0.08, 0.14)	0.13 (0.11, 0.15)	0.232	0.63
PC aa C42:6	**0.18 (0.16, 0.21)**	**0.24 (0.22, 0.30)**	**<0.001***	**0.90**
PC ae C30:0	0.11 (0.10, 0.16)	0.18 (0.14, 0.21)	0.003*	0.83
PC ae C30:1	0.04 (0.03, 0.08)	0.07 (0.06, 0.10)	0.013	0.78
PC ae C32:1	0.80 (0.61, 0.98)	1.07 (0.94, 1.25)	0.006*	0.80
PC ae C32:2	0.19 (0.16, 0.26)	0.28 (0.22, 0.34)	0.006*	0.80
PC ae C34:0	0.34 (0.28, 0.46)	0.57 (0.48, 0.73)	0.001*	0.85
PC ae C34:1	2.87 (2.58, 3.30)	3.91 (3.33, 4.79)	0.001*	0.86
PC ae C34:2	3.25 (2.77, 3.83)	4.94 (3.62, 5.45)	0.001*	0.85
PC ae C34:3	2.44 (2.14, 3.15)	4.05 (3.42, 4.30)	0.002*	0.85
PC ae C36:0	**0.27 (0.17, 0.29)**	**0.38 (0.33, 0.53)**	**<0.001***	**0.92**
PC ae C36:1	2.15 (1.82, 2.54)	3.11 (2.48, 4.39)	0.001*	0.85
PC ae C36:2	**4.22 (3.66, 4.98)**	**6.05 (5.05, 7.61)**	**0.001***	**0.86**
PC ae C36:3	2.32 (2.18, 2.57)	3.36 (2.71, 3.95)	0.003*	0.83
PC ae C36:4	5.42 (4.39, 6.84)	7.45 (6.29, 8.52)	0.019	0.76
PC ae C36:5	3.55 (2.81, 4.85)	4.81 (3.87, 5.63)	0.057	0.71
PC ae C38:0	0.54 (0.42, 0.64)	0.66 (0.60, 0.91)	0.010*	0.78
PC ae C38:1	0.28 (0.15, 0.30)	0.38 (0.24, 0.57)	0.024	0.75
PC ae C38:2	0.69 (0.57, 0.83)	1.01 (0.79, 1.29)	0.003*	0.83
PC ae C38:3	1.15 (1.07, 1.31)	1.71 (1.29, 2.15)	0.003*	0.83
PC ae C38:4	4.26 (3.69, 5.02)	5.64 (4.48, 6.83)	0.017	0.77
PC ae C38:5	5.80 (5.07, 8.40)	7.49 (6.57, 8.00)	0.111	0.68
PC ae C38:6	2.02 (1.57, 2.52)	2.63 (2.22, 2.89)	0.037	0.73
PC ae C40:1	0.33 (0.25, 0.45)	0.41 (0.36, 0.51)	0.084	0.69
PC ae C40:2	0.38 (0.34, 0.44)	0.44 (0.41, 0.60)	0.008*	0.79
PC ae C40:3	0.32 (0.24, 0.35)	0.39 (0.33, 0.49)	0.003*	0.83
PC ae C40:4	0.73 (0.64, 0.90)	0.89 (0.71, 1.02)	0.063	0.71
PC ae C40:5	1.09 (0.98, 1.29)	1.36 (1.13, 1.54)	0.012	0.78

(*continued*)

Table 2. (Continued)

PC	*Concussion patients* (n = *12*) *median (IQR)*	*Control subjects* (n = *17*) *median (IQR)*	p *value*	*AUC*
PC ae C40:6	1.11 (0.93, 1.45)	1.56 (1.31, 1.82)	0.015	0.77
PC ae C42:1	0.14 (0.12, 0.18)	0.16 (0.14, 0.20)	0.046	0.72
PC ae C42:2	0.13 (0.12, 0.15)	0.16 (0.14, 0.21)	0.051	0.72
PC ae C42:3	0.18 (0.16, 0.23)	0.21 (0.18, 0.26)	0.101	0.68
PC ae C42:4	0.23 (0.21, 0.25)	0.26 (0.24, 0.31)	0.034	0.74
PC ae C42:5	0.84 (0.72, 0.97)	0.88 (0.75, 0.93)	0.790	0.53
PC ae C44:3	0.04 (0.03, 0.04)	0.04 (0.04, 0.05)	0.077	0.70
PC ae C44:4	0.13 (0.11, 0.14)	0.14 (0.12, 0.16)	0.232	0.63
PC ae C44:5	0.59 (0.46, 0.69)	0.59 (0.52, 0.66)	0.894	0.49
PC ae C44:6	0.41 (0.31, 0.50)	0.42 (0.39, 0.50)	0.452	0.58

PC concentrations are in μM. The PCs in bold were those used in the final diagnostic model.
*p values that represent significant differences between concussed patients and non-concussed controls.
ROC, receiver operating characteristic; IQR, interquartile range.

whereas 4 control subjects had suffered previous concussions ($p = 1.000$). There were no significant differences between groups with respect to anxiety, depression, mood disorders, or other medical conditions. Three concussion patients were prescribed medications, including salbutamol, methylphenidate, and fluoxetine, whereas 1 control subject had been prescribed methylphenidate ($p = 0.279$). None of the patients had been prescribed anti-inflammatories or analgesics.

Median time from concussion occurrence to blood draw at the first clinic visit was 2.0 days (IQR, 1, 3). We then examined 71 blood PCs, of which 26 had a statistically significant decrease in concentration after concussion ($p < 0.01$; Table 2). None of the PCs measured increased after concussion. A ROC curve analysis was completed for each PC; the top four PCs with a combination of the lowest p value and highest AUC are shown in Figure 1 (PCaeC36:0, PCaaC42:6, PCaeC36:2, and PCaaC32:0; $p \leq 0.001$). Cut-off

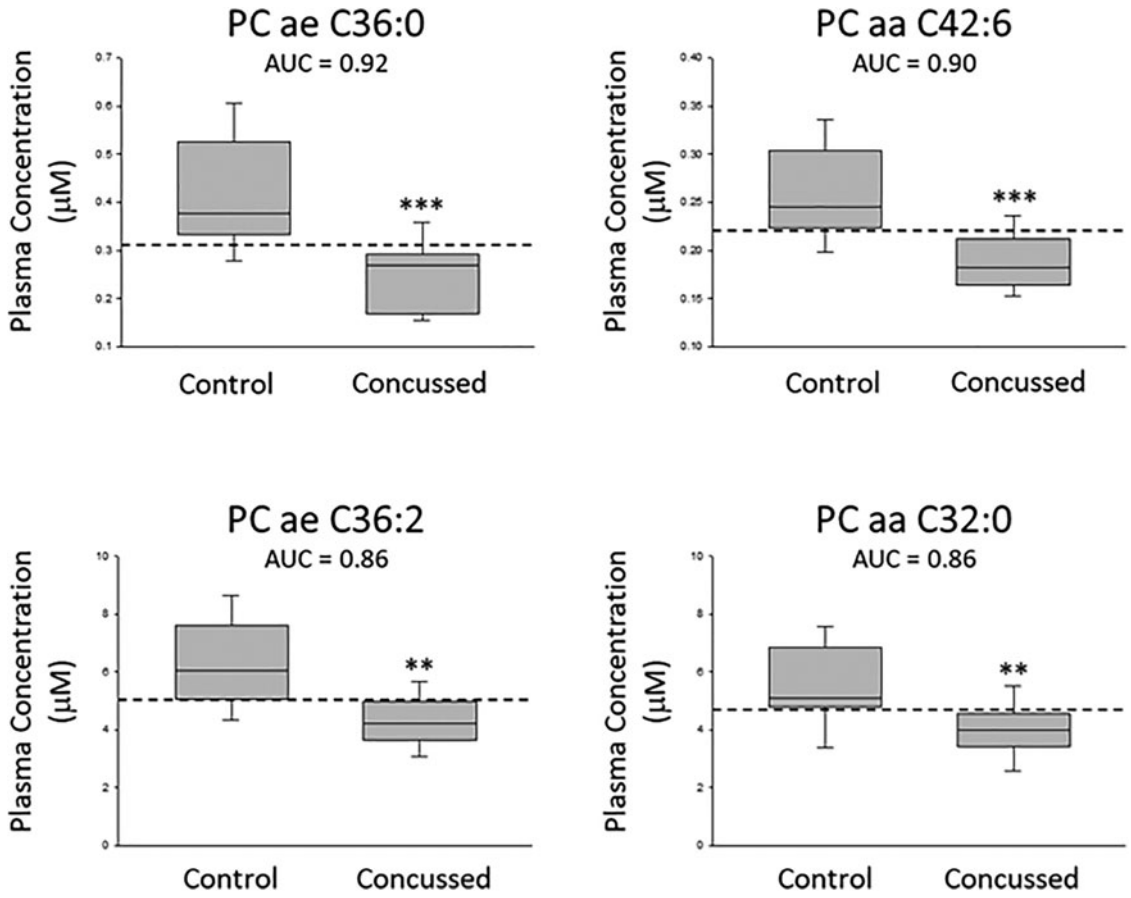

FIG. 1. Box plots demonstrating the significant reductions in four individual PCs after concussion (median with IQR and 95% CIs, ***$p < 0.001$; **$p < 0.005$). The y-axis is plasma concentration in μM. The AUCs on ROC curve analyses are listed for each PC. The dotted horizontal lines on each plot reflect the calculated cut-off values. AUCs, areas under the curve; PC, plasma glycerophospholipid; ROC, receiver operating characteristic.

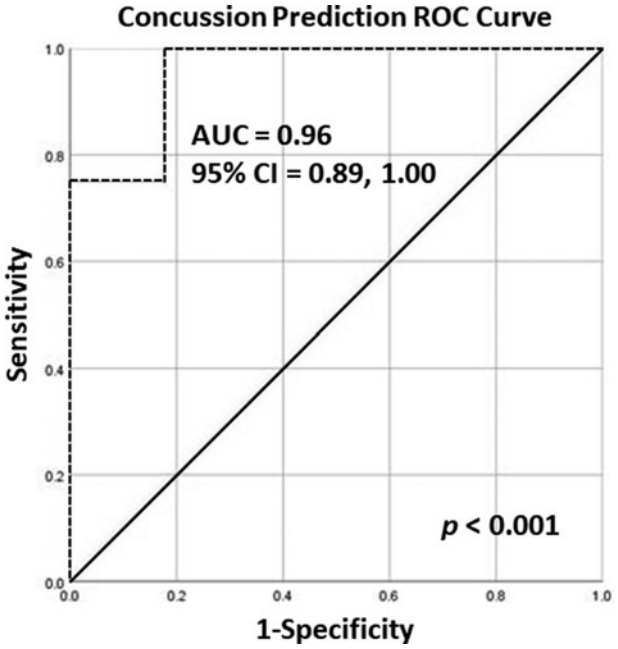

FIG. 2. A final ROC curve analysis for the combination of four PCs. The AUC, 95% CIs, and *p* value are indicated. The solid line reflects an AUC of 0.5 attributed to chance. AUC, area under the curve; CIs, confidence intervals; IQR, interquartile range; PC, plasma glycerophospholipid; ROC, receiver operating characteristic.

values in μM were ≤0.31 for PCaeC36:0, ≤0.22 for PCaaC42:6, ≤5.07 for PCaeC36:2, and ≤4.63 for PCaaC32:0. When predicted values for the four PCs, as determined by regression analyses, were combined, the AUC increased to 0.96 (Fig. 2; $p < 0.001$). The addition or subtraction of other PCs failed to significantly improve the model.

Discussion

In this study, we show that a large number of blood PCs are decreased after concussion, with four individual PCs having very good to excellent diagnostic potential (AUC, >0.85). Combining the four PCs resulted in a potentially excellent diagnostic test with an AUC of 0.96.

Concussion diagnosis remains problematic. Patients often have difficulty in recognizing injury-induced deficits and in quantifying their symptoms. Concussion diagnostics are further complicated by patient and injury heterogeneity. Indeed, every athlete and injury are different. Moreover, injury symptoms may be under-reported, or denied altogether, for secondary gain (e.g., an athlete who wants to continue play).[2122] Concussion diagnosis relies on clinical criteria alone, but clinical diagnostics tools are imperfect. The SCAT has good diagnostic accuracy,[9] but certain self-reported symptoms are unhelpful with initial concussion diagnosis in adolescents, including sleep disturbances, anxiety, irritability, emotional level, and sadness.[9,10]

Once a concussion has occurred, a graded return to activities with symptom resolution is recommended.[23] Medical clearance requires resolution in self-reported symptoms and clinical signs. If return to sport occurs prematurely for any reason, the athlete is at increased risk of injury, with potentially serious consequences, including second impact syndrome.[24] Our previous study on these same adolescent athletes, utilizing MR spectroscopy, demonstrated persistently decreased choline at 3 months post-injury, a finding consistent with the depression of the four choline-containing PCs measured here.[15]

The consistent depression in blood PC concentrations reported here is novel and intriguing, but the underlying pathophysiology is unknown. Glycerophospholipids are liberated from cellular membranes by receptor-mediated degradation by phospholipase activation, with the most predominant in brain being phospholipase A2 (PLA2). Second messengers are liberated and include fatty acids, such as arachidonic acid, eicosanoids, platelet-activating factor, and diacylglycerol. Downstream actions attributed to glycerophospholipid-generated second messengers include inflammation, membrane trafficking, cellular differentiation and proliferation, and apoptotic processes. With concussion, one can only speculate that depressed PCs may indicate injury-induced inflammation, increased demand for membrane repair or, alternatively, less cellular signaling activity and/or turnover. Of the four PCs utilized in our prediction model, two PCs were plasmalogens that are present in significant amounts in myelin and may play an additional role as an antioxidant because of their vinyl ether bond.[25]

A medication history revealed limited prescription drugs in our study cohorts; those medications identified would be unlikely to interfere with PC metabolism. A detailed medication history to identify non-steroidal anti-inflammatories, including over-the-counter medications, could be important. Indeed, non-steroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenase enzymes, which are important for arachidonic acid metabolism to prostaglandins. Although PLA2 is not inhibited by NSAIDs, depressed prostaglandin formation might alter upstream PC metabolism by feedback mechanisms.

Whereas we demonstrated a reduction in blood PCs after concussion, other brain injury biomarkers increase after concussion.[11] Protein brain injury biomarkers are released after injury and are relatively specific to a variety of brain cells, including neurons (ubiquitin carboxy-terminal hydrolase L1 [UCH-L1], neurofilament light chain, Tau, neuron-specific enolase, and αll-spectrin N-terminal fragment), astrocytes (glial fibrillary acidic protein [GFAP], S100 calcium-binding protein B), and oligodendrocytes (myelin basic protein). To date, only a handful of brain injury protein biomarkers have shown some degree of diagnostic accuracy, such as a combination of GFAP and UCH-L1 yielding an AUC of 0.71.[26] These latter two biomarkers are U.S. Food and Drug Administration approved specifically in adults to identify concussion patients in need of computerized tomography scanning. The specificity of these biomarkers precludes their use in adolescent concussion patients.

Our PC measurements may provide diagnostic accuracy in isolation, or as part of a multi-modal concussion diagnostic model. Indeed, multi-modal approaches have been used previously and may include electroencephalography, neurocognitive tests, and standard concussion assessment tools,[27] as well as multi-modal advanced imaging.[28] Adolescent concussion consequences[29,30] and recovery[31] have been improved with multi-modal approaches.

Our data raised an additional question related to potential therapeutics: Could dietary supplementation of PCs improve or hasten concussion recovery? In both animal and human studies, PC supplementation has been demonstrated to slow cerebral structure decline with age and support cognitive functioning.[32] Also, PCs positively influence brain health by decreasing reactive oxygen species, depressing proinflammatory cytokines, and reducing homocysteine.

Our study has several limitations. First, our study evaluated a limited number of adolescent athletes, and we were unable to

correlate PC concentrations with clinical outcomes. Despite this caveat, to identify such a strong predictive model with high statistical significance illustrates the potential of PCs for diagnostic utility. Second, we did not have baseline measurements from each athlete, and therefore we compared concussed athletes to a control cohort who were age-, sex-, and activity-matched athletes. Third, our matched control group was uninjured; further studies should add an additional control group consisting of matched athletes with musculoskeletal injuries. Fourth, we are unclear of the anatomical origin of the PC changes; however, given that MR spectroscopy on these same injured athletes demonstrated reduced brain choline,[15] a major constituent of PCs, we suggest that the PC changes have a brain origin. Fifth, although none of the reported prescription drugs are known to interfere with PC metabolism, it is imperative in future studies to also accurately record over-the-counter analgesics and NSAIDs.

Conclusions

In summary, we show that a combination of four plasma PCs accurately diagnose concussion in adolescent athletes. These data suggest that decreased plasma PCs may be novel biomarkers of mild traumatic brain injury, but external validation in larger, more-diverse cohorts is required. Ultimately, future studies should endeavor to have a larger cohort of athletes with measurements at baseline, post-injury, and multiple intervals during recovery.

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Author Disclosure Statement

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