

**The effects of cytomegalovirus on brain structure following sport-related concussion**

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**Supplementary Material**

## Supplementary Results – Analyses Without Propensity Weighting

Sensitivity analyses were conducted to determine if similar results were observed without the use of inverse probability of treatment weighting. Generalized linear models were run as described in the main manuscript without the inclusion of the propensity weights. As in the primary analyses, Bonferroni correction was used to account for multiple comparisons within each level of analyses (primary analyses:  $p < 0.017$  for 3 tests; secondary analyses:  $p < 0.0125$  for 4 tests; exploratory analyses:  $p < 0.017$  for 3 tests). Descriptive statistics for all metrics are provided in the **Supplementary Tables**.

Primary Analyses - Diffusion Kurtosis Metrics: There was a significant effect of CMV on axial kurtosis in athletes with SRC ( $t(158)=2.49$ ,  $p=0.014$ , mean difference(standard error)(MD)=0.009 (0.004), 95% confidence interval (CI) [.002, .017]), but not in controls ( $t(154)=0.28$ ,  $p=0.78$ , MD=0.001 (0.004), 95%CI[-.007, .010]). Similarly, there was a significant effect of CMV on radial kurtosis in athletes with SRC ( $t(166)=2.81$ ,  $p=0.006$ , MD=0.051 (0.018), 95%CI[.015, .087]), but not controls ( $t(159)=0.75$ ,  $p=0.46$ , MD=0.015 (0.021), 95%CI[-.025, .056]). For both axial and radial kurtosis, CMV+ athletes with SRC had elevated kurtosis relative to CMV- athletes with SRC. CMV status did not affect kurtosis fractional anisotropy levels in either athletes with SRC ( $t(164)=-1.04$ ,  $p=0.30$ , MD=-0.008 (0.008), 95%CI[-.023, 0.007]) or controls ( $t(157)=-1.61$ ,  $p=0.11$ , MD=-0.014 (0.009), 95%CI[-.031, .003]).

Secondary Analyses – Gray Matter Structure: There was a significant effect of CMV status on right hemisphere mean cortical thickness in athletes with SRC ( $t(159)=-2.78$ ,  $p=0.006$ , MD=-0.051 (0.018) mm, 95%CI[-.087, -.015]), but not in controls ( $t(156)=-1.72$ ,  $p=0.09$ , MD=-0.036 (0.021)

mm, 95%CI[-.077, .005]). As in the analyses with propensity weighting, a similar effect of CMV in athletes with SRC was observed on left hemisphere mean cortical thickness though it did not survive strict multiple comparison correction ( $t(160)=-2.40$ ,  $p=0.017$ , MD=-0.042 (0.018) mm, 95%CI[-.077, -.008]). There was no association of CMV with left hemisphere thickness in controls ( $t(157)=-1.65$ ,  $p=0.10$ , MD=-0.033 (0.020) mm, 95%CI[-.073, .007]).

For total surface area, there was no significant CMV effect for either athletes with SRC or controls for the left hemisphere ( $t(155)=1.37$ ,  $p=0.17$ , MD=1204.42 (877.72) mm<sup>2</sup>, 95%CI[-529.40, 2938.24]; ( $t(155)=0.57$ ,  $p=0.57$ , MD=585.95 (1021.31) mm<sup>2</sup>, 95%CI[-1431.54, 2603.43]) or right hemisphere ( $t(155)=1.44$ ,  $p=0.15$ , MD=1265.70 (880.69) mm<sup>2</sup>, 95%CI[-473.98, 3005.39];  $t(155)=0.78$ ,  $p=0.44$ , MD=793.97 (1024.73) mm<sup>2</sup>, 95%CI[-1230.27, 2818.20]).

Exploratory Analyses – C-reactive Protein and Symptoms: The effect of CMV seropositivity on SCAT symptom severity scores was not significant in athletes with SRC ( $\chi^2=0.027$ ,  $p=0.87$ , MD=-0.80 (4.85), 95%CI[-10.30, 8.70]) or controls ( $\chi^2=0.058$ ,  $p=0.81$ , MD=0.13 (0.54), 95%CI[-0.92, 1.18]). Similarly, the effect of CMV status on BSI-GSI scores was not significant in SRC ( $\chi^2=0.044$ ,  $p=0.83$ , MD=-0.42 (1.98), 95%CI[-4.31, 3.47]) or controls ( $\chi^2=0.60$ ,  $p=0.44$ , MD=0.42 (0.55), 95%CI[-.65, 1.49]). Finally, there was no significant effect of CMV on log-transformed serum CRP levels (mg/L) in SRC ( $\chi^2=0.54$ ,  $p=0.46$ , MD=0.24 (0.32), 95%CI[-.40, .87]) or controls ( $\chi^2=2.38$ ,  $p=0.12$ , MD=-0.65 (0.42), 95%CI[-1.47, 0.18]).

In summary, results from sensitivity analyses without propensity weighting show similar patterns of differences in athletes with concussion due to CMV seropositivity.

## Supplementary Results – Self-Reported Medical Conditions

Additional sensitivity analyses were conducted to determine if the rates of various medical conditions collected upon enrollment differed based on CMV serostatus in either athletes with concussion or controls. **Supplementary Table 5** shows self-reported medical conditions (current or prior) collected upon enrollment. The proportion of athletes with and without CMV that reported the various collected diagnoses/conditions did not differ for either athletes with SRC or controls, even when collapsing across all conditions (Fisher's Exact Tests,  $p > 0.05$ ). Nevertheless, to explore any potential role of these conditions in our current findings, we conducted sensitivity analyses in which a combined variable accounting for the presence of any medical condition was included as a covariate in our analyses, with propensity weighting applied. As demonstrated below, the inclusion of this variable had no impact on observed results.

Primary Analyses - Diffusion Kurtosis Metrics: There was a significant effect of CMV on axial kurtosis in athletes with SRC ( $t(154)=2.65, p=0.009$ , mean difference (standard error)(MD)=0.009 (0.004), 95% confidence interval (CI) [.002, .017]), but not in controls ( $t(152)=-0.49, p=0.63$ , MD=-0.002 (0.004), 95%CI[-.010, .006]). Similarly, there was a significant effect of CMV on radial kurtosis in athletes with SRC ( $t(163)=2.57, p=0.011$ , MD=0.042 (0.016), 95%CI[.010, .074]), but not controls ( $t(160)=0.54, p=0.59$ , MD=0.010 (0.018), 95%CI[-.026, .045]). For both axial and radial kurtosis, CMV+ athletes with SRC had elevated kurtosis relative to CMV- athletes with SRC. CMV status did not affect kurtosis fractional anisotropy levels in either athletes with SRC ( $t(161)=-0.39, p=0.69$ , MD=-0.003 (0.007), 95%CI[-.017, 0.011]) or controls ( $t(158)=-1.36, p=0.18$ , MD=-0.010 (0.008), 95%CI[-.025, .005]).

Secondary Analyses – Gray Matter Structure: There was a significant effect of CMV status on right hemisphere mean cortical thickness in athletes with SRC ( $t(158)=-2.57, p=0.011, MD=-0.043$  (0.017) mm, 95%CI[-.076, -.010]), but not in controls ( $t(156)=-1.04, p=0.30, MD=-0.019$  (0.018) mm, 95%CI[-.055, .017]). A similar effect of CMV in athletes with SRC was observed on left hemisphere mean cortical thickness though it did not survive strict multiple comparison correction ( $t(160)=-2.02, p=0.045, MD=-0.032$  (0.016) mm, 95%CI[-.063, -.001]). There was no association of CMV with left hemisphere thickness in controls ( $t(157)=-0.97, p=0.33, MD=-0.017$  (0.017) mm, 95%CI[-.050, .017]).

For total surface area, there was no significant CMV effect for either athletes with SRC or controls for the left hemisphere ( $t(154)=1.52, p=0.13, MD=1222.67$  (805.08) mm<sup>2</sup>, 95%CI[-367.75, 2813.08]; ( $t(154)=0.94, p=0.35, MD=839.81$  (889.95) mm<sup>2</sup>, 95%CI[-918.27, 2597.89]) or right hemisphere ( $t(154)=1.70, p=0.09, MD=1364.44$  (802.41) mm<sup>2</sup>, 95%CI[-220.70, 2949.57];  $t(154)=1.23, p=0.22, MD=1093.79$  (886.96) mm<sup>2</sup>, 95%CI[-658.40, 2845.98]).

Exploratory Analyses – C-reactive Protein and Symptoms: The effect of CMV seropositivity on SCAT symptom severity scores was not significant in athletes with SRC ( $\chi^2=0.13, p=0.72, MD=1.44$  (4.04), 95%CI[-6.47, 9.35]) or controls ( $\chi^2=0.01, p=0.94, MD=0.03$  (0.36), 95%CI[-.67, .73]). Similarly, the effect of CMV status on BSI-GSI scores was not significant in SRC ( $\chi^2=0.09, p=0.77, MD=0.55$  (1.84), 95%CI[-3.06, 4.15]) or controls ( $\chi^2=0.01, p=0.93, MD=0.03$  (0.37), 95%CI[-0.68, 0.75]). Finally, there was no significant effect of CMV on log-transformed serum CRP levels (mg/L) in SRC ( $\chi^2=2.34, p=0.13, MD=0.46$  (0.30), 95%CI[-0.13, 1.04]) or controls ( $\chi^2=3.14, p=0.08, MD=-0.66$  (0.37), 95%CI[-1.40, 0.07]).

**Supplementary Table 1: Estimated marginal means from a priori contrasts of metrics of interest.**

<b>Metric</b>	<b>Group</b>	<b>CMV Status</b>	<b>Mean</b>	<b>SE</b>	<b>95%CI Lower</b>	<b>95%CI Upper</b>
KAX	SRC	+	0.771	0.002	0.766	0.776
KAX	SRC	-	0.761	0.003	0.756	0.766
KAX	CC	+	0.750	0.003	0.744	0.756
KAX	CC	-	0.752	0.003	0.747	0.757
KRAD	SRC	+	1.223	0.011	1.201	1.245
KRAD	SRC	-	1.180	0.012	1.157	1.203
KRAD	CC	+	1.246	0.013	1.219	1.272
KRAD	CC	-	1.237	0.012	1.214	1.260
KFA	SRC	+	0.460	0.005	0.451	0.470
KFA	SRC	-	0.464	0.005	0.454	0.474
KFA	CC	+	0.436	0.006	0.425	0.447
KFA	CC	-	0.445	0.005	0.435	0.455
LH CT (mm)	SRC	+	2.531	0.011	2.509	2.552
LH CT (mm)	SRC	-	2.564	0.011	2.542	2.587
LH CT (mm)	CC	+	2.546	0.013	2.521	2.571
LH CT (mm)	CC	-	2.560	0.011	2.538	2.583
RH CT (mm)	SRC	+	2.512	0.011	2.490	2.535
RH CT (mm)	SRC	-	2.557	0.012	2.533	2.581
RH CT (mm)	CC	+	2.530	0.014	2.503	2.557
RH CT (mm)	CC	-	2.547	0.012	2.523	2.571
LH SA (x 10 <sup>4</sup> mm <sup>2</sup> )	SRC	+	9.591	0.055	9.483	9.699
LH SA (x 10 <sup>4</sup> mm <sup>2</sup> )	SRC	-	9.472	0.058	9.357	9.587
LH SA (x 10 <sup>4</sup> mm <sup>2</sup> )	CC	+	9.468	0.066	9.338	9.599
LH SA (x 10 <sup>4</sup> mm <sup>2</sup> )	CC	-	9.380	0.058	9.265	9.495
RH SA (x 10 <sup>4</sup> mm <sup>2</sup> )	SRC	+	9.651	0.055	9.543	9.758
RH SA (x 10 <sup>4</sup> mm <sup>2</sup> )	SRC	-	9.518	0.058	9.403	9.633
RH SA (x 10 <sup>4</sup> mm <sup>2</sup> )	CC	+	9.519	0.066	9.389	9.649
RH SA (x 10 <sup>4</sup> mm <sup>2</sup> )	CC	-	9.404	0.058	9.290	9.519
SCAT sym. sev.	SRC	+	22.46	2.89	16.80	28.12
SCAT sym. sev.	SRC	-	21.96	3.06	15.97	27.95
SCAT (binary)	CC	+	0.405	0.265	-0.114	0.923
SCAT (binary)	CC	-	0.413	0.230	-0.037	0.863
BSI-GSI T-score	SRC	+	48.62	1.33	46.02	51.23
BSI-GSI T-score	SRC	-	48.55	1.41	45.79	51.30
BSI-GSI (binary)	CC	+	0.194	0.260	-0.316	0.705
BSI-GSI (binary)	CC	-	0.337	0.228	-0.110	0.784
CRP (ln mg/L)	SRC	+	0.026	0.203	-0.373	0.424
CRP (ln mg/L)	SRC	-	-0.410	0.220	-0.841	0.021
CRP (ln mg/L)	CC	+	-1.096	0.281	-1.648	-0.545
CRP (ln mg/L)	CC	-	-0.402	0.240	-0.872	0.068

*Note:* SRC = sport-related concussion, CC = contact control, CMV = cytomegalovirus. SE = standard error, CI = confidence interval, KAX = axial kurtosis, KRAD = radial kurtosis, KFA = kurtosis fractional anisotropy, LH = left hemisphere, RH = right hemisphere, CT = cortical thickness, SA = surface area, mm = millimeter, SCAT sym. sev. = Sport Concussion Assessment Tool symptom severity, BSI-GSI = Brief Symptom Inventory-18 Global-Severity Index, CRP = c-reactive protein, ln = natural log-transformed, mg/L = milligram per liter.

**Supplementary Table 2:** Descriptive statistics of diffusion kurtosis metrics in athletes based on injury and cytomegalovirus status.

Primary	Status	SRC				CC			
		1d	8d	15d	45d	1d	8d	15d	45d
Axial Kurtosis	CMV+	0.772 (0.022), 29	0.776 (0.021), 27	0.766 (0.022), 22	0.765 (0.023), 19	0.753 (0.018), 20	0.750 (0.016), 18	0.750 (0.016), 17	0.756 (0.018), 16
	CMV-	0.761 (0.018), 55	0.763 (0.017), 50	0.760 (0.020), 45	0.757 (0.017), 46	0.752 (0.018), 51	0.749 (0.016), 52	0.753 (0.015), 47	0.749 (0.016), 44
Radial Kurtosis	CMV+	1.226 (0.097), 29	1.232 (0.107), 27	1.192 (0.108), 22	1.256 (0.118), 19	1.221 (0.083), 20	1.251 (0.075), 18	1.261 (0.059), 17	1.261 (0.066), 16
	CMV-	1.187 (0.097), 55	1.178 (0.113), 50	1.155 (0.118), 45	1.176 (0.121), 46	1.220 (0.103), 51	1.232 (0.096), 52	1.237 (0.093), 47	1.233 (0.071), 44
Kurtosis FA	CMV+	0.457 (0.037), 29	0.459 (0.036), 27	0.469 (0.045), 22	0.457 (0.042), 19	0.446 (0.044), 20	0.432 (0.024), 18	0.431 (0.031), 17	0.426 (0.026), 16
	CMV-	0.464 (0.045), 55	0.462 (0.046), 50	0.478 (0.057), 45	0.469 (0.050), 46	0.453 (0.043), 51	0.451 (0.043), 52	0.451 (0.041), 47	0.444 (0.031), 44

*Note:* Mean (standard deviation), N shown in each cell. SRC = sport-related concussion, CC = contact control, CMV = cytomegalovirus. 1d = 1 day visit, 8d = 8 day visit, 15d = 15 day visit, 45d = 45 day visit.



**Supplementary Table 3:** Descriptive statistics of gray matter structural metrics in athletes based on injury and cytomegalovirus status.

Secondary	Status	SRC				CC			
		1d	8d	15d	45d	1d	8d	15d	45d
Left Hemi Thickness (mm)	CMV+	2.515 (0.084), 29	2.533 (0.096), 27	2.534 (0.099), 20	2.525 (0.109), 19	2.548 (0.071), 20	2.530 (0.075), 18	2.517 (0.086), 17	2.543 (0.069), 15
	CMV-	2.567 (0.096), 53	2.560 (0.076), 48	2.559 (0.083), 41	2.572 (0.087), 43	2.574 (0.075), 50	2.572 (0.083), 51	2.569 (0.086), 46	2.562 (0.087), 42
Right Hemi Thickness (mm)	CMV+	2.499 (0.089), 29	2.514 (0.106), 27	2.516 (0.099), 20	2.516 (0.105), 19	2.532 (0.069), 20	2.528 (0.075), 18	2.523 (0.081), 17	2.524 (0.083), 15
	CMV-	2.561 (0.099), 53	2.552 (0.079), 48	2.554 (0.081), 41	2.565 (0.075), 43	2.562 (0.086), 50	2.559 (0.087), 51	2.562 (0.089), 46	2.553 (0.082), 42
Left Hemi Area (x 10 <sup>4</sup> mm <sup>2</sup> )	CMV+	9.559 (0.870), 29	9.664 (0.906), 27	9.588 (1.007), 20	9.693 (0.914), 19	9.269 (0.808), 20	9.207 (0.842), 18	9.344 (0.819), 17	9.300 (0.836), 15
	CMV-	9.444 (0.767), 53	9.515 (0.766), 48	9.426 (0.777), 41	9.409 (0.730), 43	9.584 (0.614), 50	9.570 (0.619), 51	9.588 (0.609), 46	9.650 (0.620), 42
Right Hemi Area (x 10 <sup>4</sup> mm <sup>2</sup> )	CMV+	9.607 (0.865), 29	9.712 (0.893), 27	9.629 (1.000), 20	9.741 (0.909), 19	9.309 (0.807), 20	9.249 (0.837), 18	9.387 (0.845), 17	9.316 (0.841), 15
	CMV-	9.487 (0.773), 53	9.554 (0.764), 48	9.468 (0.772), 41	9.451 (0.731), 43	9.605 (0.622), 50	9.596 (0.611), 51	9.607 (0.612), 46	9.671 (0.615), 42

*Note:* Mean (standard deviation), N shown in each cell. SRC = sport-related concussion, CC = contact control, CMV = cytomegalovirus. 1d = 1 day visit, 8d = 8 day visit, 15d = 15 day visit, 45d = 45 day visit. Hemi = hemisphere. 3 1d, 3 8d, 7 15d, 6 45 day.

**Supplementary Table 4:** Descriptive statistics of c-reactive protein and symptom measures in athletes at the 1-day visit based on injury and cytomegalovirus status.

		<b><u>SRC</u></b>	<b><u>CC</u></b>
SCAT symp sev.	CMV+	23.04 (22.99), 28, 100%	1.80 (2.86), 20, 40%
	CMV-	23.84 (20.16), 55, 98%	1.69 (3.03), 51, 43%
BSI-GSI T- score	CMV+	48.89 (9.41), 28, 82%	39.65 (6.15), 20, 35%
	CMV-	49.31 (8.25), 55, 91%	40.31 (5.39), 51, 45%
CRP (mg/L)	CMV+	2.32 (3.76), 28	1.06 (2.32), 19
	CMV-	1.35 (1.58), 53	2.98 (9.25), 50

*Note:* Mean (standard deviation), N shown in each cell. For symptom measures, the percentage of athletes reporting any symptoms is also shown. SRC = sport-related concussion, CC = contact control, CMV = cytomegalovirus. 1d = 1 day visit, SCAT symp sev. = Sport Concussion Assessment Tool symptom severity, BSI-GSI = Brief Symptom Inventory-18 Global-Severity Index. CRP at 1d was not available for 4 participants and CRP for 1 participant was above detection limits. Clinical data at 1d was not available for 1 participant.

**Supplementary Table 5:** *Number of participants self-reporting medical and other conditions upon enrollment by CMV serostatus in athletes with concussion and controls.*

<b>Self-reported History</b>	<b><u>SRC</u></b>			<b><u>CC</u></b>		
	<b>CMV+</b>	<b>CMV-</b>	<b>p-value</b>	<b>CMV+</b>	<b>CMV-</b>	<b>p-value</b>
Vision Problems	1	1	1.0	0	1	1.0
Hearing Problems	1	1	1.0	2	2	0.57
Stroke	0	0	NA	0	0	NA
Diabetes	0	1	1.0	0	0	NA
Confusion	0	0	NA	0	0	NA
Memory Diff.	1	1	1.0	1	1	0.50
Learning Disorder	0	1	1.0	0	0	NA
ADHD	1	7	0.26	2	4	1.0
Autism Spectrum Disorder	0	0	NA	0	0	NA
Meningitis	0	1	1.0	0	0	NA
Balance Disorder	0	0	NA	0	0	NA
Psychiatric Disorder	0	0	NA	0	0	NA
Sleep Disorder	0	0	NA	0	0	NA
Non-Migraine Headaches	1	6	0.42	1	1	0.50
Migraines	1	5	0.66	1	2	1.0
Any Condition	5	20	0.09	5	10	0.75

*Note:* N shown in each cell. SRC = sport-related concussion, CC = contact control, CMV = cytomegalovirus, Diff = difficulties, ADHD = attention deficit hyperactivity disorder.