Crosstalk between hemostasis inhibitors and cholesterol biomarkers in multiple sclerosis

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ABSTRACT

The individual roles of cholesterol pathway biomarkers (CPB) and hemostasis inhibitors with neuroimaging outcomes were previously investigated in multiple sclerosis (MS). The purpose of this extension study was to investigate potential crosstalk between plasma CPB (total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and apolipoproteins (Apo) ApoA-I, ApoA-II, ApoB, ApoC-II and ApoE) and hemostasis inhibitors [heparin cofactor-II (HCII), protein C (PC), protein S (PS), thrombomodulin, ADAMTS13 and PAI-1] in a cohort of 127 MS patients, and 40 healthy individuals (HI). The associations were assessed with regressions. In MS patients, HCII was positively associated with TC, LDL-C, HDL-C and ApoA-I (p=0.028, 0.027, 0.002 and 0.027, respectively) but negatively associated with ApoC-II (p=0.018). PC was positively associated with ApoC-II (p=0.001) and ApoB (p=0.016) whereas PS was associated with TC (p=0.024) and ApoE (p=0.003) in MS. The ApoC-II associations were not observed in HI. The negative association between ApoC-II and HCII was an exception amongst other positive associations between CPB and hemostasis inhibitors in MS. CPB do not modulate the PC associations with neurodegeneration in MS.

INTRODUCTION

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system (CNS) that causes progressive accumulation of disabilities. Accumulating evidence suggests that cardiovascular risk factors can contribute MS disease progression. Among the cardiovascular risk factors linked to MS progression, the cholesterol pathway biomarkers (CPB), which can cause sustained inflammation at the vascular endothelium, have been linked to poorer clinical prognosis. Cholesterol is an essential component of myelin and cellular membranes of the CNS and it plays a role in cell-
lular signaling, synapse development, and neurogenesis. Low-density lipoprotein-cholesterol (LDL-C) particles transport cholesterol to tissues but also mediate proatherogenic mechanisms by supplying cholesterol to arterial walls. High-density lipoprotein-cholesterol (HDL-C) particles, which mediate cholesterol reverse transport from tissues and inhibit LDL-C oxidation, have anti-atherogenic properties. In MS, higher LDL-C and total cholesterol (TC) have been associated with disability. LDL-C increases were associated with a greater number of new lesions whereas increases in HDL-C and its signature apolipoprotein (Apo) ApoA-I were associated with less gray matter (GM) and cortical atrophy. Evidence for smaller LDL-C particles and an altered HDL-C structure has been reported in relapsing-remitting (RR) MS. ApoC-II, which is found on triglyceride-rich lipoproteins such as chylomicrons, is exchanged between lipoproteins and is essential for removal of cholesterol from tissues. ApoC-II and ApoE are induced by oxysterol signaling and exhibit adverse associations with MS progression.

There is an increasing scientific rationale for exploring the inter-dependencies between cholesterol pathway dysregulation and the coagulation cascade in order to explain clinical progression in MS. The extrinsic and intrinsic coagulation pathways merge at thrombin and lead to fibrinogen activation that results in fibrin clots. Fibrinogen deposition, which is capable of activating microglia leading to macrophage recruitment and activation, is frequently found in MS lesions. Previous work from our group on the hemostasis inhibitors heparin cofactor II (HCII), plasminogen activator inhibitor-1 (PAI-1), protein C (PC), protein S (PS), a disintegrin and metalloproteinase with a thrombospondin type 1 motif-13 (ADAMTS13) and thrombomodulin (TM), identified potential dysregulation of hemostasis markers in MS. These hemostasis inhibitors are key regulators of the coagulation cascade. HCII inhibits thrombin at sites of vascular injury and is associated with reduced arterial plaque thickness in elderly individuals with atherosclerotic disease. These sites are enriched with dermatan sulfate (DS), a type of glycosaminoglycan (GAG), that is synthesized and secreted by smooth muscle cells. DS and heparin are the main GAGs that enhance the inhibitory function of HCII. PC inhibits factors Va and VIIIa in plasma, and this mechanism is enhanced in the presence of its cofactor PS. Thrombin bound to TM activates PC by proteolysis. PAI-1 inhibits tissue-type plasminogen activator, and thus fibrinolysis.

Elevated levels of lipids and/or apolipoproteins are known to have procoagulant and atherogenic effects. The progression of arterial plaques occurs at a confluence of inflammatory cells, lipid accumulation and the extracellular matrix and can impede blood flow to tissues. In MS, greater levels of HDL-C and the HDL-associated lipoproteins ApoA-I and A-II are associated with better global brain perfusion; ApoC-II and ApoE were associated with lower GM perfusion. A hyper-coagulable state combined with perturbed fibrinolysis can contribute to neurodegeneration in MS.

The pathophysiological mechanisms by which CPB modulate MS disease progression are not well understood. The purpose of this study was to extend prior work on the individual roles of CPB and hemostasis biomarkers to include potential crosstalk and interdependence between them. In this study, we hypothesize that both CPB and hemostasis inhibitors might act in concert to promote MS disease progression in a well-characterized cohort of MS patients with both classes of biomarkers.

MATERIALS AND METHODS

Study population

The study protocol was approved by the University at Buffalo Human Subjects and Institutional Review Board. Written informed consent was collected from all participants.

The clinical data for this observational, cross-sectional sub-study was obtained from CEG study at the MS Center at the State University of New York at Buffalo. Patients with MS and healthy individuals (HI) were assessed and provided the blood samples. This sub-study involved MS patients and HI between 18 and 75 years of age with CPB and hemostasis biomarkers available.

All MS patients were assessed by a neurologist and their disability was ascertained on the Expanded Disability Status Scale (EDSS) scale. RRMS and progressive MS (PMS) disease was assigned based on disease history and clinical characteristics of patients and disease course guidelines. The primary-progressive MS (PPMS) and secondary-progressive MS (SPMS) patients were combined into a progressive MS (PMS) group.

Trained project coordinators utilized a structured questionnaire and collected information regarding use of disease modifying treatment (DMT), presence of cardiovascular comorbidities such as hypertension and hyperlipidemia and use of cardiovascular-based medications. Body mass index (BMI) was calculated as the ratio of weight in kilograms to the square of the height in meters.

Cholesterol and hemostasis biomarkers

CPB, apolipoprotein and hemostasis components measured were made on ethylenediaminetetraacetic acid (EDTA) plasma samples from individuals in the non-fasted state. Study samples were collected within 30 days of the clinical visit. Plasma samples were stored at -80°C until use.
The assay methods for the CPB: cholesterol (TC, HDL-C and LDL-C), apolipoprotein (ApoA-I, ApoA-II, ApoB, ApoC-II and ApoE) and hemostasis inhibitors (HCII, PC, PS, TM, ADAMTS13 and PAI-1) have been described in publications from our group.14,15,25

MRI-derived measures of brain volume

The associations of PC with quantitative MRI-derived measures of brain parenchymal volume (BPV), gray matter volume (GMV) and neocortical volume (NCV) were assessed. The MRI acquisition and analysis protocols previously described.26

Statistical analyses

All analyses were conducted on SPSS (IBM Inc., Armonk, USA, version 26.0) statistical program. A p-value of less than 0.05 was considered statistically significant.

The Fisher exact test was used to assess differences in the frequency of binary categorical variable such as gender and statin use in MS vs HI and RRMS vs PMS. The Student’s t-test was performed to assess differences in age, BMI, and disease duration in years and Mann-Whitney U test was used to assess differences in EDSS.

The hemostasis biomarker levels were log transformed to reduce skew. Multiple linear regression was performed with the hemostasis biomarkers assessed (either HCII, PC, PS, TM, or PAI-1) as the dependent variable. The individual CPB biomarkers (TC, HDL-C and LDL-C, ApoA-I, ApoA-II, ApoB, ApoC-II and ApoE), age, gender, BMI, and MS disease status were considered predictors of interest. The slope and p-values from regression analyses were shown in table. To avoid false positives, bootstrap analyses (1000 sample size), which is more robust to outliers, was used to confirm significance of findings.

The BPV, GMV and NCV MRI measures in MS patients were analyzed as dependent variables in multiple regression analyses that included PC and adjusted for age, gender, BMI, and MS disease status. To assess whether the PC associations were abrogated in the presence of CPB, regression analyses were conducted with individual CPB as an additional predictor.

RESULTS

Demographic and clinical characteristics

The demographic and clinical characteristics of the MS, RRMS, PMS and HI groups are displayed in Table 1. The demographic characteristics were similar in the MS (n=127) and HI (n=40) groups. The MS patients had a mean age of 54 years (SD=11.6), disease duration of 21 years (SD=10.8), and median EDSS score of 3.5 (IQR=2.0–6.0).

The majority of MS patients were prescribed disease-modifying therapy (DMT); 16% (n=20) were not on any DMT. Among the DMT, interferon-β (n=39, 31.2%) was most frequent, followed by glatiramer acetate (n=37, 29.6%), oral DMTs (n=16, 12.8%), natalizumab (n=7, 5.6%) and off-label medication (n=6, 4.8%).

The RRMS (n=78) and PMS (n=49) groups had similar gender ratio (70.5% vs 73.5% females, p=0.84), BMI (27.9 kg/m² vs 27.3 kg/m², p=0.50), and statin use (14.1% vs 18.5% patients, p=0.62).

Table 1. Demographic, clinical and characteristics of healthy individuals and multiple sclerosis patients.

<table>
<thead>
<tr>
<th></th>
<th>HI</th>
<th>All MS</th>
<th>RR-MS</th>
<th>P-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size, n</td>
<td>40</td>
<td>127</td>
<td>78</td>
<td>49</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>30 (75.0)</td>
<td>91 (71.7)</td>
<td>55 (70.5)</td>
<td>36 (73.5)</td>
</tr>
<tr>
<td>Age, years</td>
<td>49.2±15.3</td>
<td>54.0±11.6</td>
<td>49.6±11.7</td>
<td>60.9±7.20</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.6±6.95</td>
<td>27.7±5.97</td>
<td>27.9±6.25</td>
<td>27.3±5.55</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>–</td>
<td>21.0±10.8</td>
<td>16.9±9.04</td>
<td>27.5±10.1</td>
</tr>
<tr>
<td>EDSS, score</td>
<td>–</td>
<td>3.50 (2.0–6.0)</td>
<td>2.00 (1.5–3.0)</td>
<td>6.00 (4.0–6.5)</td>
</tr>
<tr>
<td>Statin use, n (%)</td>
<td>4 (10.0%)</td>
<td>20 (15.7%)</td>
<td>11 (14.1%)</td>
<td>9 (18.4%)</td>
</tr>
<tr>
<td>DMT use, n (%)</td>
<td>–</td>
<td>20 (16.0)</td>
<td>9 (11.7)</td>
<td>11 (22.9)</td>
</tr>
<tr>
<td>No treatment</td>
<td>–</td>
<td>39 (31.2)</td>
<td>26 (33.8)</td>
<td>13 (27.1)</td>
</tr>
<tr>
<td>Interferon beta</td>
<td>–</td>
<td>37 (29.6)</td>
<td>21 (27.3)</td>
<td>16 (33.3)</td>
</tr>
<tr>
<td>Glatiramer Acetate</td>
<td>–</td>
<td>7 (5.60)</td>
<td>5 (6.50)</td>
<td>2 (4.20)</td>
</tr>
<tr>
<td>Natalizumab</td>
<td>–</td>
<td>6 (4.80)</td>
<td>4 (5.20)</td>
<td>2 (4.20)</td>
</tr>
<tr>
<td>Other</td>
<td>–</td>
<td>16 (12.8)</td>
<td>12 (15.6)</td>
<td>4 (8.30)</td>
</tr>
</tbody>
</table>

All continuous variables (age, BMI, disease duration) are mean ± standard deviation. For the ordinal EDSS, the median (inter-quartile range) is given. HI: healthy individuals; MS: multiple sclerosis; RR-MS: relapsing-remitting multiple sclerosis; P-MS: progressive multiple sclerosis; BMI: body mass index; EDSS: Expanded disability status scale; DMT: Disease-modifying therapy. Dimethyl fumarate, fingolimod and teriflunomides were categorized as Orals. Interferon-beta includes AVONEX®, REBIF®, BETASERON®, EXTAVIA® and PLEGRIDY™.
Associations of CPB with hemostasis biomarkers

Supplementary Table S1 summarizes the CPB and hemostasis biomarkers in HI and MS groups. Table 2 summarizes the regression results of the hemostasis biomarkers on CPB after adjusting for age, gender, BMI, and MS disease status.

In MS patients, HCII displayed the majority of significant associations with CPB (Table 2). HCII was positively associated with TC (Partial correlation coefficient $r_p=0.20$, $p=0.028$), LDL-C ($r_p=0.21$, $p=0.027$), HDL-C ($r_p=0.28$, $p=0.002$) and ApoA-I ($r_p=0.20$, $p=0.027$). HCII was negatively associated with ApoC-II ($r_p=-0.21$, $p=0.018$). Figure 1, which graphically summarizes HCII levels vs quartiles of TC, LDL-C, HDL-C and ApoA-I in RRMS and PMS, shows that the association patterns in the PMS group were qualitatively similar to the RRMS subgroup.

ApoC-II was negatively associated with HCII (see above) but positively associated to PAI-1 ($r=0.30$, $p=0.001$) and PC ($r_p=0.30$, $p=0.001$), and displayed a trend towards significance for PS and TM (Table 2). Figure 2 shows that the association patterns of hemostasis in-

Table 2. Associations between hemostasis biomarkers and cholesterol biomarkers in multiple sclerosis. The partial correlation ($r_p$) and p values from multiple linear regression are shown.

<table>
<thead>
<tr>
<th></th>
<th>HCII</th>
<th>PAI-1</th>
<th>PC</th>
<th>PS</th>
<th>ADAMTS13</th>
<th>TM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>0.20 (0.028)*</td>
<td>-0.026 (0.78)</td>
<td>0.10 (0.26)</td>
<td>0.21 (0.024)*</td>
<td>-0.057 (0.55)</td>
<td>0.083 (0.38)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.28 (0.002)*</td>
<td>-0.092 (0.32)</td>
<td>-0.12 (0.20)</td>
<td>0.14 (0.14)</td>
<td>-0.19 (0.039)</td>
<td>-0.13 (0.15)</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.21 (0.027)*</td>
<td>-0.15 (0.12)</td>
<td>0.037 (0.69)</td>
<td>0.13 (0.19)</td>
<td>-0.048 (0.61)</td>
<td>0.033 (0.73)</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>0.20 (0.027)*</td>
<td>-0.12 (0.18)</td>
<td>-0.099 (0.28)</td>
<td>0.17 (0.059)</td>
<td>-0.12 (0.207)</td>
<td>-0.071 (0.44)</td>
</tr>
<tr>
<td>ApoA-II</td>
<td>0.071 (0.44)</td>
<td>0.085 (0.35)</td>
<td>-0.061 (0.51)</td>
<td>0.10 (0.26)</td>
<td>-0.090 (0.33)</td>
<td>-0.023 (0.81)</td>
</tr>
<tr>
<td>ApoB</td>
<td>-0.14 (0.13)</td>
<td>0.066 (0.47)</td>
<td>0.022 (0.016)*</td>
<td>0.052 (0.58)</td>
<td>0.036 (0.70)</td>
<td>0.20 (0.026)*</td>
</tr>
<tr>
<td>ApoC-II</td>
<td>-0.21 (0.018)*</td>
<td>0.30 (0.001)*</td>
<td>0.30 (0.001)*</td>
<td>0.18 (0.054)</td>
<td>-0.040 (0.66)</td>
<td>0.17 (0.060)</td>
</tr>
<tr>
<td>ApoE</td>
<td>-0.13 (0.17)</td>
<td>0.10 (0.26)</td>
<td>0.038 (0.68)</td>
<td>0.27 (0.003)*</td>
<td>0.087 (0.34)</td>
<td>0.10 (0.27)</td>
</tr>
</tbody>
</table>

The partial correlation ($r_p$) and p values for the cholesterol biomarker from multiple linear regression analyses are shown for log transformed values FXII, HCII, PAI-1, PC, PS, ADAMTS13, and TM. The multiple linear regression analyses are adjusted for age, gender, body mass index, type of MS (RR vs PMS). The p-values for each cholesterol level that were significant after performing bootstrap analyses are marked with an asterisk.

Table 3. Associations of protein C with magnetic resonance imaging measures when each cholesterol pathway biomarkers was included as a predictor.

<table>
<thead>
<tr>
<th></th>
<th>GMV $r_p$ (p-value)</th>
<th>BPV $r_p$ (p-value)</th>
<th>NCV $r_p$ (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein C</td>
<td>-0.29 (0.002)*</td>
<td>-0.25 (0.008)*</td>
<td>-0.27 (0.004)*</td>
</tr>
<tr>
<td>TC</td>
<td>0.066 (0.50)</td>
<td>0.010 (0.92)</td>
<td>0.063 (0.52)</td>
</tr>
<tr>
<td>Protein C</td>
<td>-0.30 (0.002)*</td>
<td>-0.25 (0.010)*</td>
<td>-0.27 (0.006)*</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.015 (0.87)</td>
<td>-0.085 (0.37)</td>
<td>0.031 (0.75)</td>
</tr>
<tr>
<td>Protein C</td>
<td>-0.30 (0.001)*</td>
<td>-0.25 (0.008)*</td>
<td>-0.27 (0.005)*</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.043 (0.66)</td>
<td>-0.003 (0.98)</td>
<td>0.041 (0.68)</td>
</tr>
<tr>
<td>Protein C</td>
<td>-0.30 (0.002)</td>
<td>-0.25 (0.009)*</td>
<td>-0.26 (0.007)*</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>0.11 (0.25)</td>
<td>0.018 (0.85)</td>
<td>0.11 (0.23)</td>
</tr>
<tr>
<td>Protein C</td>
<td>-0.30 (0.001)</td>
<td>-0.25 (0.008)*</td>
<td>-0.27 (0.004)*</td>
</tr>
<tr>
<td>ApoA-II</td>
<td>0.11 (0.25)</td>
<td>0.098 (0.30)</td>
<td>0.13 (0.17)</td>
</tr>
<tr>
<td>Protein C</td>
<td>-0.31 (0.001)</td>
<td>-0.25 (0.007)*</td>
<td>-0.27 (0.003)*</td>
</tr>
<tr>
<td>ApoB</td>
<td>-0.13 (0.16)</td>
<td>0.17 (0.08)</td>
<td>0.13 (0.17)</td>
</tr>
<tr>
<td>Protein C</td>
<td>-0.32 (0.001)</td>
<td>-0.28 (0.002)*</td>
<td>-0.29 (0.002)*</td>
</tr>
<tr>
<td>ApoC-II</td>
<td>0.12 (0.21)</td>
<td>0.15 (0.11)</td>
<td>0.13 (0.18)</td>
</tr>
<tr>
<td>Protein C</td>
<td>-0.32 (0.001)</td>
<td>-0.29 (0.002)*</td>
<td>-0.29 (0.002)*</td>
</tr>
<tr>
<td>ApoE</td>
<td>0.047 (0.63)</td>
<td>0.039 (0.68)</td>
<td>0.050 (0.60)</td>
</tr>
<tr>
<td>Protein C</td>
<td>-0.30 (0.001)</td>
<td>-0.25 (0.007)*</td>
<td>-0.27 (0.004)*</td>
</tr>
</tbody>
</table>

The partial correlation ($r_p$) and p-values for Protein C (logarithm transformed) and cholesterol pathway biomarkers from multiple linear regression analyses are shown for GMV, BPV, and NCV. BPV: Whole brain volume; GMV: Gray matter volume; NCV: Cortical volume; PC: Protein C. The multiple linear regression analyses are adjusted for age, gender, body mass index, type of MS (RR vs PMS). The p-values that were significant are marked with an asterisk.
hibitors with ApoC-II quartiles were generally similar in the PMS and RRMS groups.

ApoB levels were positively associated with PC ($r_p=0.22$, $p=0.016$) and TM ($r_p=0.20$, $p=0.026$) whereas TC ($r_p=0.21$, $p=0.024$) and ApoE were associated with PS.

The hemostasis-cholesterol biomarker associations that were significant in MS patients were not observed in HI (Supplementary Table S2).

Effects CPB on the associations of protein C with MRI measures of brain volume

In our earlier report, we found that greater PC was associated with lower BPV and GMV as assessed by quantitative MRI. We therefore investigated whether or not the associations of PC with BPV, GMV and NCV were abrogated by including CPB as predictors. Table 3 summarizes the regression results for PC without CPB and for PC and individual CPB when both were included as predictors. The inclusion of CPB did not affect the strength of any of the associations of PC with BPV, GMV and NCV, which is consistent with the possibility that the PC associations do not contain mediating influences from the CPB investigated.

DISCUSSION AND CONCLUSIONS

In this research, we investigated the associations between CPB and a panel of hemostasis inhibitors in MS patients. The effects of CPB on the associations of protein C with MRI measures of brain volume were examined. Our results suggest that the associations of PC with brain volume measures are not influenced by CPB. This finding is consistent with the hypothesis that the PC associations do not contain mediating influences from the CPB investigated.

Figure 1. Dependence of heparin cofactor II (HCII) plasma concentrations on cholesterol pathway biomarker level quartiles in relapsing-remitting multiple sclerosis (RR-MS) and progressive MS (P-MS) patients.

A: HCII levels in the lowest, 2nd, 3rd, and highest quartiles of total cholesterol in RR-MS (left) and P-MS (right). B: HCII levels in the lowest, 2nd, 3rd, and highest quartiles of Apolipoprotein AII (ApoA-I) in RR-MS and P-MS. C: HCII levels in the lowest, 2nd, 3rd, and highest quartiles of low-density lipoprotein cholesterol (LDL-C) in RR-MS and P-MS. D: HCII levels in the lowest, 2nd, 3rd, and highest quartiles of high-density lipoprotein (HDL-C) in RR-MS and P-MS. The bars represent mean values and the error bars are standard errors of the mean.
patients. We found that HCII was positively associated with TC, LDL-C, HDL-C, and ApoA-I but negatively associated with ApoC-II. ApoC-II was also associated with PC and PAI-1 and showed association trends with PS and TM. Additional hemostasis biomarker-CBP associations included: PC with ApoB, TM with ApoB, and PS with TC, and ApoE. The associations found in MS were not observed in HI. Notably, the strength of the associations of PC with BPV, GMV and NCV were not weakened when Apo-CII, ApoB and other CPB were included as a predictor. These results build on and substantially extend our earlier work on hemostasis markers in MS by including the clinically relevant focus on CPB.

In the context of cardiovascular disease, hypercholesterolemia and apolipoproteins including ApoC-II and Apo-E are associated with shorter coagulation times and higher levels of procoagulant proteins. From this simplistic vantage point, we expected greater CPB levels to be associated with lower levels of hemostasis inhibitors. We were therefore surprised to find positive associations for TC and LDL-C with HCII, which is a hemostasis inhibitor and has anticoagulant activity. Given our results, we were particularly interested in studies of lipid associations of HCII, in HI and other diseases but there was a paucity of information. It is also noteworthy that PC antigen has negative rather than positive associations with MRI measures of brain volume. This raises the possibil-

![Figure 2](image-url)  
**Figure 2.** Dependence of hemostasis biomarker concentrations on apolipoprotein C-II level quartiles in relapsing-remitting multiple sclerosis (RR-MS) and progressive (P-MS) patients.
ity that MS neurodegeneration can be facilitated when there is dysregulation of a key anticoagulation biomarkers such as PC. Our results do not support a role for CPB in the PC associations with MRI measures.

However, studies have investigated the lipid associations of PS, PC and PAI-1 in HI, which provided a framework for comparative assessment of our findings in MS. In a study of HI from south-eastern England, TC was found to be associated with PS (and PC) levels. In comparison, we found an association between TC and PS but did not find evidence for the TC-PC association. In Japanese women, PS was found to be associated with TC, LDL-C, ApoA-II, ApoC-II and ApoE; in our MS group, we found associations of PS with TC and ApoE and a trend with ApoC-II. HDL-C and ApoA-I, the signature apolipoprotein of HDL particles, increase PS and PC anticoagulant activities. We could not estimate PS or PC activity because the plasma samples available were anticoagulated with EDTA. We found a trend (p=0.059) between PS levels and ApoA-I. Kim et al. found associations of PC levels with TC and LDL-C in a sample of Korean HI. We identified an association of PC with ApoB, the signature apolipoprotein of LDL, but did not find evidence for the association between PC with TC or LDL-C in MS patients. PAI-1 was increased in active MS compared to stable MS and PAI-1 levels in Japanese HI were found to be associated with TC, ApoB and ApoC-II. In our MS group, we found the association between ApoC-II with PAI-1. We surmise that our PS, PC and PAI-1 results are partially concordant with findings reported in the literature for HI.

While our study provides information on the associations of CPB with hemostasis inhibitors, it has limitations. For example, we did not measure oxidized LDL-C which is important in both atherogenesis and also in MS. While the associations of CPB levels with HC-II were not statistically significant, the plots of HC-II vs CPB quartiles (Supplementary Figure S1) were qualitatively similar to those observed in MS. The ApoC-II associations with hemostasis inhibitors found in MS were not present in HI (Supplementary Table S2 and Supplementary Figure S2). Our study had a cross-sectional design and only a limited number of HI, which may have limited the statistical power to detect associations between CPB and hemostasis inhibitors. While the findings are consistent with the possibility that CPB can contribute to dysregulated levels of hemostasis inhibitory biomarkers, the possibility that the associations are simply correlations unrelated to causation cannot be formally precluded. We did not have follow-up hemostasis inhibitor measurements, which could have enabled us to assess whether changes in PC (and other hemostasis inhibitors) abrogated the associations of HDL-C biomarkers changes with GMV and NCV atrophy over 5-years follow-up.

We postulate that the nexus of interactions between lipoprotein lipase (LPL) function, GAG and CPB could be a potential biomolecular pathway that could plausibly explain many of our results, particularly those related to HCII and ApoC-II. LPL is present in the vascular endothelium of brain capillaries and also in several CNS tissues including the hippocampus, cortical areas and the dentate gyrus, which is an area of neurogenesis. ApoC-II is a cofactor that activates triglyceride hydrolysis by LPL. LPL also interacts with ApoE-rich lipoproteins of the brain and its interactions with circulating HDL, LDL and VLDL at the vascular endothelium facilitate lipoprotein particle uptake. GAGs are important for tethering LPL in the brain because the glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (GPI-HBP1) that tethers LPL in heart, skeletal muscle and adipose tissue capillaries is absent on brain capillaries. The anticoagulant activities of HCII are enhanced as a result of interaction with GAGs such as heparan sulfate and dermatan sulfate that tether LPL. LDL-C stimulates GAG secretion by vascular smooth muscle cells but this effect is antagonized by HDL-C. The positive associations between LDL-C and ApoB, which are proatherogenic, with HCII and PC respectively, in MS patients may represent a hemostatic regulatory mechanism for slowing vascular changes caused by prothrombotic and atherosclerotic pathophysiological process.

The pathogenic role of macrophages, which express LPL, in the inflammation at the atherosclerotic lesion is well established. However, macrophages are a particularly important immune cell type in MS pathogenesis because they are capable of extravasation across the blood-brain barrier; they modulate immune milieu in active lesions toward inflammatory phenotypes by presenting antigens and toward anti-inflammatory phenotypes by phagocytosing myelin debris. Macrophages express LPL and also upregulate the LPL cofactor ApoC-II in response to activation of the liver X receptor transcription factor. Pathological evidence indicates activation of LXR by macrophages in active MS lesions. In the experimental allergic encephalitis model of MS, PC activation resulted in a reduction in activated macrophages and increases in the inflammatory markers tumor necrosis factor-a and intercellular adhesion molecule-1. In MS, PAI-1 was found to be localized in perivascular mononuclear cells and foamy macrophages in lesions. We hypothesize that CPB aggravate interactions between activated endothelial cells and macrophages/monocytes in the inflammatory MS pathophysiological milieu that can contribute to hemostasis and endothelial barrier dysfunction.

In conclusion, our results of CPB and hemostasis inhibitors in MS provide evidence for crosstalk between these important homeostatic pathways.
REFERENCES