Increased Complement Factor H with Decreased Factor B Determined by Proteomic Differential Displays as a Biomarker of Tai Chi Chuan Exercise

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BACKGROUND: Exhaustive exercise can be associated with short-term immune suppression, but moderate exercise such as tai chi chuan (TCC) has been shown to have beneficial effects on immunity. The mechanisms for the health benefits of exercise remain to be determined, and no potential biomarkers for these beneficial health effects have yet been identified. In the present investigation of the differential display of serum proteomes before and after a 12-week TCC exercise program, we have demonstrated and validated a unique proteomic profile of TCC exercise.

MATERIALS AND METHODS

STUDY DESIGN AND SAMPLES STUDIED
In this study, we used a single-group paired pre- and posttest research design, as previously described (6–8). Healthy adults without prior experience in practicing TCC were enrolled in this study. The study protocol was approved by the Institutional Review Board, and informed consent was obtained from all participants. Participants learned to perform 37 standardized movements (tai chi 37 forms), as described previously (6–8). These movements incorporate elements of balance, postural alignment, and concentration (9). Each TCC session was designed to last 60 min with a 10-min warm-up, a 40-min practice, and a 10-min cooldown. Sessions were given 3 times a week for 12 weeks. Peripheral blood was collected before and after the TCC exercise program for analyses of complete blood.

RESULTS: We identified 39 protein spots for 18 proteins with a significant increase or decrease after TCC exercise. Validation of the differentially displayed proteins with 20 paired pre- and postexercise samples revealed a significant increase in complement factor H (P = 0.0034) associated with decreases in C1q esterase inhibitor (P = 0.0038) and complement factor B (P = 0.0029).

CONCLUSIONS: In this first study of proteomic biomarkers of TCC exercise, we found an increase in complement factor H associated with a decrease in complement factor B. Complement factor H is involved in protection from microangiopathy and macular degeneration and may represent a useful marker of the health effects of exercise.
counts, high-sensitivity C-reactive protein, and serum proteomic profiles.

**CyDye™ Labeling and Differential Display of Serum Proteins Before and After TCC Exercise**

First, we enriched the proteins in serum samples by depleting albumin and IgG with an Albumin and IgG Removal Kit, followed by a 2-D Clean-Up Kit (GE Healthcare). Protein (50 μg) from serum samples obtained from participants before and after TCC exercise were labeled with Cy5 and Cy3 (GE Healthcare), respectively, and were placed on ice in the dark for 30 min. A mixture of equal amounts (25 μg each) of pre-TCC exercise serum protein and post-TCC exercise serum protein was labeled with Cy2 dye as an internal control (10). The Cy5-, Cy3-, and Cy2-labeled protein samples were applied onto each immobilized pH gradient strip (Immobiline DryStrips; GE Healthcare) for isoelectric focusing at 3000 V/h for a total of approximately 30,000 V · h. The Immobiline DryStrips were then loaded with SDS equilibration buffer and subjected to SDS-PAGE on a 100 g/L polyacrylamide gel at 20 mA/gel and 4 °C. The 2-dimensional fluorescence difference gel electrophoresis (2D-DIGE) gels were scanned with a Typhoon Trio imaging system (GE Healthcare) and analyzed with the aid of DeCyder 6.0 software (GE Healthcare).

**In-Gel Trypsin Digestion for MALDI-TOF/TOF Analysis of Peptide Fingerprints**

After imaging analysis, we silver-stained the 2D-DIGE gels and used a pipet tip (Labcon) to excise the spots from the gels that had a significantly different display before and after the TCC exercise program. The excised gel spots were then chopped into pieces of 1.5–2 mm, washed, destained, and digested with 20 g/L trypsin. The trypsin-digested peptides were extracted twice with 10 g/L trifluoroacetic acid in 100% acetonitrile and spotted onto an AnchorChip (Bruker-Franzen Analytik) that had been prewaxed with the matrix (2 g/L α-cyano-4-hydroxy-trans-cinnamic acid) for MALDI-TOF/TOF analysis of mass spectra (Bruker-Franzen Analytik). The autoproteolysis products of trypsin (m/z 842.51, 1045.56, 2211.10) were used as internal calibrators. The imprecision of molecular weight estimation was <0.5 Da. Identification of peptide and protein matches was performed with Mascot software (Swiss-Prot).

**Validation of Protein Concentration by Western Blotting**

Serum samples (30 μg) were subjected to SDS-PAGE for western blotting. The protein blots were transferred to nitrocellulose paper for immunoblotting with anti-human factor H, antihuman factor B, antihuman α-1B-glycoprotein, antihuman protease C1 inhibitor, or antihuman β-actin antibody (Abcam). The blots were then treated with horseradish peroxidase–conjugated goat antimouse immunoglobulin antibody (Santa Cruz Biotechnology) and visualized with an enhanced-chemiluminescence kit (Pierce) as previously described (11).

**Statistical Methods and Data Analysis**

In the initial screening study, we used 3 replicate pre- and post-TCC exercise serum samples for the 2D-DIGE analysis. The other 20 pairs of pre- and post-TCC exercise serum samples were used to validate the differentially displayed serum proteins, on the basis of a power of 0.8, an α level of 0.05, and an effect size of 0.45 (45% difference in the protein concentration). The proteins that were differentially displayed in pre- and post-TCC exercise samples and had specific antibodies commercially available were selected for further validation by western blot analysis. SPSS software (version 13.0 for Windows; SPSS) was used for statistical analysis.

**Results**

**Demographic Data of Participants Before and After the TCC Exercise Program**

Of the 32 healthy adult participants, 23 [11 women and 12 men; mean (SD) age, 52.1 (2.2) years] completed the 12-week TCC exercise program and supplied samples before and after taking part in the program. As shown in Table 1 in the Data Supplement that accompanies the online version of this Brief Communication at http://www.clinchem.org/content/vol56/issue1, post–TCC exercise serum samples for the 2D-DIGE analysis. The other 20 pairs of pre– and post–TCC exercise serum samples for the 2D-DIGE analysis. The other 20 pairs of pre– and post–TCC exercise serum samples were subjected to MALDI-TOF/TOF analysis of mass spectra (Bruker-Franzen Analytik). The autoproteolysis products of trypsin (m/z 842.51, 1045.56, 2211.10) were used as internal calibrators. The imprecision of molecular weight estimation was <0.5 Da. Identification of peptide and protein matches was performed with Mascot software (Swiss-Prot).

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**Serum Protein Profiling Before and After the TCC Exercise Program**

We used the DeCyder program to compare differences in serum protein fluorescence patterns before and after the TCC exercise program (mean ratio >1.2 or a P value in the Student t-test of <0.05 in the 3 replicate experiments). Thirty-nine protein spots comprising 18 proteins with significant increases or decreases after the TCC exercise program were identified and subjected to mass spectrometry and peptide sequence matches (see Fig. 1 in the online Data Supplement). Of the 18 differentially displayed proteins, 12 proteins (α-1B-...
glycoprotein, keratin type II, neurofilament triplet L protein, vitamin D–binding protein precursor, protease C1 inhibitor precursor, keratin type I cytoskeleton, complement factor B, complement C1r subcomponent precursor, transthyretin precursor, zinc finger protein 792, kininogen-1 precursor, and PRAME family member 7) decreased in concentration after TCC exercise; 4 proteins (complement factor H, apolipoprotein C-III precursor, complement C3 precursor, and /H9251 -1B-glycoprotein) increased in concentration after TCC exercise; and 2 proteins (apolipoprotein A-I precursor and serotransferrin precursor) exhibited an increase or a decrease in the concentrations of their different isoforms.

**Validation of Differentially Displayed Proteins by Western Blotting**

Four of the 18 proteins differentially displayed in pre- and post-TCC exercise serum samples (complement factor H, complement factor B, protease C1 inhibitor, and /H9251 -1B-glycoprotein) have specific antibodies commercially available. The differential protein displays of these 4 proteins were validated by western blotting with 20 paired samples. As shown in Fig. 2 in the online Data Supplement, western blotting showed that complement factor H was increased after the TCC exercise program and that complement factor B, protease C1 inhibitor, and /H9251 -1B-glycoprotein decreased after the TCC exercise program. Fig. 1 shows that the mean (SD) concentration ratio of complement factor H to /H9252 -actin increased from 0.85 (0.07) to 1.48 (0.05) after the TCC exercise program (P = 0.0034). In addition, the mean ratio of the complement factor B concentration to that of /H9252 -actin decreased significantly from 1.41 (0.7) to 0.86 (0.13) (P = 0.0038), and the mean concentration ratio of /H9251 -1B-glycoprotein to /H9252 -actin decreased from 1.65 (0.03) to 1.10 (0.02) after the TCC exercise program (P = 0.0005; see Fig. 3 in the online Data Supplement).

**Discussion**

Exercise has long been recognized as beneficial to health, but exhaustive exercise can cause immunosuppression (3). Our study, which used 2D-DIGE differential displays of serum proteomes before and after TCC exercise, is the first to demonstrate that an increase in complement factor H associated with a decrease in complement factor B may be a biomarker of TCC exercise.

Complement factor H, a regulator of complement activation, is involved in protection from thrombotic microangiopathies and advanced macular degeneration. Individuals with a congenital deficiency of factor H are susceptible to microangiopathies (12, 13), and those with senile deficiency are susceptible to early development of advanced macular degeneration (14, 15). An increase in complement factor H after TCC exercise may prevent vascular insults and macular degeneration.

In contrast to the increase in complement factor H, we also found that some serum inflammatory markers, such as protease C1 inhibitor, /H9251 -1B-glycoprotein, and complement factor B, decreased after the TCC exercise program. Complement factor B is an enhancer of an alternative pathway of complement activation and is increased and augmented in many inflammatory diseases. Studies with factor B knockout mice have demonstrated...
that factor B deficiency may limit inflammation of the lung (16) and brain (17). Further studies to investigate whether TCC exercise decreases inflammation in patients with cancer or infection seem to be warranted.

We applied proteomic differential displays, a tool that has been used for biomarker discovery in a variety of diseases (1, 2, 10, 18–20), to identify potential serum proteomic biomarkers of the beneficial effect of TCC exercise. Whether these biomarkers are associated only with TCC exercise or with all kinds of moderate exercise remains to be determined. A limitation of the 2D-DIGE analysis of exercise biomarkers in this study is that such analysis mainly detects proteins with molecular weights $>10$ kDa. As is shown in Table 1, we detected no protein of $<10$ kDa that had a differential display before and after the TCC exercise program.

**Table 1. Differential displays of serum proteins before and after the TCC exercise program.**

<table>
<thead>
<tr>
<th>Proteins identified</th>
<th>Accession</th>
<th>Spots with differential display, n</th>
<th>DIGE index, mean fluorescence ratio</th>
<th>MOWSE score</th>
<th>Protein MW, Da</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Down-regulated proteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 PRAME family member 7</td>
<td>PRAM7_HUMAN</td>
<td>1</td>
<td>$-2.31$</td>
<td>60</td>
<td>53 617</td>
</tr>
<tr>
<td>2 $\alpha$1B-glycoprotein precursor</td>
<td>A1BG_HUMAN</td>
<td>1</td>
<td>$-2.04$</td>
<td>124</td>
<td>54 239</td>
</tr>
<tr>
<td>3 Keratin, type II cytoskeletal 1</td>
<td>K2C1_HUMAN</td>
<td>1</td>
<td>$-1.65$</td>
<td>62</td>
<td>65 847</td>
</tr>
<tr>
<td>4 Neurofilament triplet L protein</td>
<td>NFL_HUMAN</td>
<td>1</td>
<td>$-2.74$</td>
<td>123</td>
<td>61 348</td>
</tr>
<tr>
<td>5 Vitamin D–binding protein precursor</td>
<td>VTDB_HUMAN</td>
<td>2</td>
<td>$-1.5$; $-5.22$</td>
<td>58; 76</td>
<td>52 929</td>
</tr>
<tr>
<td>6 Protease C1 inhibitor precursor</td>
<td>IC1_HUMAN</td>
<td>2</td>
<td>$-2.08$; $-2.58$</td>
<td>63; 55</td>
<td>55 119</td>
</tr>
<tr>
<td>7 Keratin, type I cytoskeletal 10</td>
<td>K1C10_HUMAN</td>
<td>3</td>
<td>$-1.76$; $-1.93$; $-3.36$</td>
<td>74; 98; 104</td>
<td>59 483</td>
</tr>
<tr>
<td>8 Complement factor B precursor</td>
<td>CFAB_HUMAN</td>
<td>2</td>
<td>$-1.6$; $-1.65$</td>
<td>86; 72</td>
<td>85 479</td>
</tr>
<tr>
<td>9 Complement C1r subcomponent precursor</td>
<td>C1R_HUMAN</td>
<td>1</td>
<td>$-2.41$</td>
<td>80</td>
<td>80 122</td>
</tr>
<tr>
<td>10 Transthyretin precursor</td>
<td>TTHY_HUMAN</td>
<td>2</td>
<td>$-1.36$; $-1.79$</td>
<td>118; 52</td>
<td>15 877</td>
</tr>
<tr>
<td>11 Zinc finger protein 792</td>
<td>ZN792_HUMAN</td>
<td>1</td>
<td>$-1.68$</td>
<td>58</td>
<td>64 015</td>
</tr>
<tr>
<td>12 Kininogen-1 precursor</td>
<td>KNG1_HUMAN</td>
<td>2</td>
<td>$-3.25$; $-3.08$</td>
<td>61; 84</td>
<td>71 900</td>
</tr>
<tr>
<td><strong>Up-regulated proteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1 Complement factor H precursor</td>
<td>CFAH_HUMAN</td>
<td>1</td>
<td>5.14</td>
<td>82</td>
<td>138 979</td>
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<tr>
<td>2 Apolipoprotein C-III precursor</td>
<td>APOC3_HUMAN</td>
<td>1</td>
<td>2.38</td>
<td>97</td>
<td>10 846</td>
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<tr>
<td>3 $\alpha$2-Macroglobulin precursor</td>
<td>AZMG_HUMAN</td>
<td>1</td>
<td>1.48</td>
<td>91</td>
<td>163 175</td>
</tr>
<tr>
<td>4 Complement C3 precursor</td>
<td>CO3_HUMAN</td>
<td>1</td>
<td>1.22</td>
<td>67</td>
<td>187 030</td>
</tr>
<tr>
<td><strong>Mixed up- and down-regulated proteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Apolipoprotein A-I precursors</td>
<td>APOA1_HUMAN</td>
<td>5</td>
<td>2 up, 3 down</td>
<td>66–149</td>
<td>30 759</td>
</tr>
<tr>
<td>2 Serotransferrin precursors</td>
<td>TRFE_HUMAN</td>
<td>12</td>
<td>3 up, 9 down</td>
<td>116–263</td>
<td>77 000</td>
</tr>
</tbody>
</table>

$^a$ UniProt entry name (http://www.uniprot.org).
$^b$ DIGE index.
$^c$ MOWSE score.
$^d$ MW, molecular weight.

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References


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