Biochemical and clinical effects of Whey protein supplementation in Parkinson's disease: A pilot study

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A R T I C L E   I N F O

Article history:
Received 26 November 2015
Received in revised form 20 May 2016
Accepted 30 May 2016
Available online 31 May 2016

Keywords:
Parkinson's disease
Whey protein
Soy protein
Oxidative stress
Plasma amino acid
Homocysteine
Unified Parkinson's disease rating scale

A B S T R A C T

Background: Parkinson's disease (PD) is an oxidative stress-mediated degenerative disorder. Elevated plasma homocysteine (Hcy) is frequently found in the levodopa-treated PD patients, is associated with disease progression and is a marker of oxidative stress. Whey protein is a rich source of cysteine, and branched-chain amino acids (BCAA). It has been shown that supplementation with Whey protein increases glutathione synthesis and muscle strength.

Objectives and methods: In this study, we conducted a placebo-controlled, double-blind study (NCT01662414) to investigate the effects of undenatured Whey protein isolate supplementation for 6 months on plasma glutathione, plasma amino acids, and plasma Hcy in PD patients. Clinical outcome assessments included the unified Parkinson's disease rating scale (UPDRS) and striatal L-3,4-dihydroxy-6-(18)F-fluorophenylalanine (FDOPA) uptake were determined before and after supplementation. 15 patients received Whey protein, and 17 received Soy protein, served as a control group.

Results: Significant increases in plasma concentration of reduced glutathione and the ratio of reduced to oxidized glutathione were found in the Whey-supplemented patients but not in a control group. This was associated with a significant decrease of plasma levels of Hcy. The plasma levels of total glutathione were not significantly changed in either group. Plasma BCAA and essential amino acids (EAA) were significantly increased in the Whey-supplemented group only. The UPDRS and striatal FDOPA uptake in PD patients were not significantly ameliorated in either group. However, significant negative correlation was observed between the UPDRS and plasma BCAA and EAA in the pre-supplemented PD patients.

Conclusion: This study is the first to report that Whey protein supplementation significantly increases plasma reduced glutathione, the reduced to oxidized glutathione ratio, BCAAs and EAA's in patients with PD, together with a concomitant significant reduction of plasma Hcy. However, there were no significant changes in clinical outcomes. Long-term, large randomized clinical studies are needed to explore the benefits of Whey protein supplementation in the management of PD patients.

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1. Introduction

Parkinson's disease (PD) is characterized by bradykinesia, rigidity, resting tremor and postural instability [1]. Its pathogenesis involves progressive loss of dopaminergic neurons in the substantia nigra (SN) leading to depletion of striatal dopamine [2]. This degenerative condition is known to be mediated by oxidative stress [3]. A thiol tripeptide called glutathione (GSH) is well recognized as a key antioxidant, which prevents the oxidative damage of dopaminergic neurons [4]. Since the level of reduced GSH in the SN of PD patients is depleted, replenishment of GSH is considered a possible therapeutic option for PD [5–8]. However, oral supplementation with glutathione will not be beneficial due to its instability in the gut and intravenous infusions [9]. As GSH is endogenously synthesized in cells and its rate of synthesis depends critically upon a supply of cysteine (Cys), there is the reason to try Whey protein supplementation, which is a by-product of cheese
production that contains a number of Cys-rich proteins, for example lactalbumin (alpha-lactalbumin, beta-lactalbumin, serum albumin, lactoferrin, and immunoglobulins) [10].

Whey protein is an excellent dietary source of Cys. Hepatic GSH of CCl₄-intoxicated rats (inducing high oxidative stress) is markedly reduced but is replenished after feeding with Whey protein for 3 weeks [11]. Likewise, oral intake of Whey protein causes increased hepatic GSH and decreased malondialdehyde in rats with nonalcoholic fatty liver disease (NAFLD) [12]. Clinically, oral supplementation with Whey protein for 2 weeks is capable of increasing plasma GSH levels in patients with advanced HIV-infection [13]. These data suggest that Whey protein is capable of boosting GSH synthesis and reducing oxidative stress. Undenatured Whey protein contains more bonded Cys than denatured protein and therefore is a better source of the GSH precursor [14]. In NAFLD patients, supplementation with undenatured Whey protein significantly increases plasma GSH and total antioxidant capacity [15].

The unified Parkinson’s disease rating scale (UPDRS) is a widely utilized tool to evaluate the severity of PD symptoms [16]. A higher score indicates greater severity. The association of the UPDRS with serum levels of nitric oxide and peroxynitrite is demonstrated in PD patients, indicating a correlation between oxidative stress and progression of PD [17]. A sophisticated positron emission tomography (PET) scan with [62Cu] diacetyl-bis (N4-methylthiosemicarbazone) shows that striatal oxidative stress in living PD patients is increased relative to healthy controls, and increased oxidative stress is associated with increased UPDRS scores [18]. Therefore, anti-oxidative treatment may decelerate the progression of PD. The authors are not aware of any previous investigation of the effect of PD patients of supplementation with Whey protein.

Although there is no cure for PD, medical treatment tailored to each individual patient is able to relieve many PD symptoms. Levodopa, which is rapidly converted into dopamine by dopa decarboxylase in the brain, is the drug of choice for treating PD. Elevation of plasma homocysteine (Hcy) is commonly found in levodopa-treated patients [19]. Furthermore, an association of hyperhomocysteinemia with cognitive dysfunction and dementia in patients with PD has been demonstrated [20]. It has not been determined if supplementation with Whey protein is helpful in reducing the plasma Hcy levels in PD patients.

In this study, we conducted a placebo-controlled, double-blind study to investigate the effects of Whey protein supplementation on plasma glutathione, plasma amino acids, plasma Hcy and UPDRS in patients with PD (NCT01662414). HMS 90®/Immunocal® was used as it has been shown in a recent study to provide a significant amount of Cys in a form of Whey protein supplement [21]. Soy protein was used as a placebo control because we also wanted to determine if Whey protein had a more positive medicinal quality for PD treatment than Soy protein. Soybean isoflavone genistin has been shown to be helpful in preventing PD development in ovariecotomized rats [22]. In a subgroup of patients, PET imaging was performed to see if supplementation with Whey protein was able to improve the L-3,4-dihydroxy-6-(18)F-fluorophenylalanine (FDOPA) uptake within the striatum.

2. Patients and methods

2.1. Patients

We initially recruited 38 patients with PD between May 2011 and September 2013. Patients were randomly assigned into two arms to receive Whey protein (n = 19) and Soy protein (n = 19) supplementation. Both physicians and patients were blinded to the supplement options. The trial period was 6 months. 2 (5.3%) discontinued and 4 (10.5%) were lost to follow-up. Therefore, a total of 32 patients completed the 6-months trial (Table 1). Fifteen patients received Whey protein (HMS 90®/Immunocal® Undenatured Cysteine-Rich Whey Protein Isolate). Seventeen patients received Soy protein (GNC Soy Pro) and served as controls. Proteins were re-packed into aluminium sachets (10 g/sachet) without any labels. Patients were instructed to disperse the proteins in water and ingest twice a day (2 sachets/day to reach a dose of 20 g/day); once in the morning and once in the evening. The dose of 20 g/day was based on previous published studies demonstrating that the supplementation of Whey protein at this dosage was capable of significantly increasing plasma and lymphocyte GSH and reducing related oxidative stress biomarkers [15,23]. Patients were advised to drink the protein solution at approximately 2 h after taking their PD medication and to control the daily consumption of proteins. Heparinized blood was collected from all participants at 0 (baseline), 3 and 6 months. Plasma samples were separated and kept at −20 °C until analysis.

The severity of the disease in each patient at baseline, 3 and 6 months was evaluated using the UPDRS (part I-IV), the modified Hoehn and Yahr (HY) scale, Clinical Global Impressions (CGI) scale and the Parkinson’s Disease Questionnaire (PDQ-39). The HY scale, developed to assess the severity of PD based on clinical findings and functional disability, was used to evaluate the patients at the baseline and 6-months visits [24]. The CGI Scale, designed to assess disease severity and progression of the disease during the treatment, was used to assess symptoms at the baseline, 3-months and 6-months visits [25]. The PDQ-39, a scale to assess patients’ health and well-being, was also used at the baseline, 3-months and 6-months visits [26].

The research protocol was reviewed and approved by the Ethics Committee, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Informed consent was obtained from all participants prior to inclusion in the study.

2.2. Glutathione measurement

Total glutathione and reduced glutathione were measured in the plasma samples using the HT Glutathione Assay Kit (Trevigen®). Plasma samples were treated with 5% (w/v) metaphosphoric acid for 15 min to precipitate proteins prior to measurement. Subsequent procedures were performed according to the manufacturer’s instruction. For
oxidized glutathione (GSSG), 4-vinylpyridine was used to block the re-action of reduced GSH with 5,5’-dithiobis-2-nitrobenzoic acid. Concentration of reduced GSH was calculated from total GSH–GSSG. The ratio of reduced GSH to oxidized GSSG (GSH/GSSG) was also calculated as a measure of redox potential that is widely used as marker of oxidative stress.

2.3. Amino acid analysis

Amino acids including alanine (Ala), arginine (Arg), aspartic acid (Asp), citrulline (Citr), Cys, glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), ornithine (Orn), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), and valine (Val) in plasma were analyzed using an amino acid analyzer (Biochrom 30, UK). The procedure was performed according to the manufacturer’s instructions. Plasma samples were treated with 10% sulfosalicylic acid at 4 °C for 30 min to pellet proteins. The supernatant was mixed with an equal volume of LiOH buffer, and the mixture was filtered through a 0.2 μm membrane prior to injection. Norleucine was used as an internal standard. The concentration of each amino acid was calculated from known concentrations of the respective amino acid standards and normalized by norleucine concentration. Branched-chain amino acids (BCAA) concentration was calculated as the sum of the concentrations of Ile, Leu and Val. Essential amino acids (EEA) concentration was calculated as the sum of the concentrations of Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp and Val. Aromatic amino acids (AAA) concentration was calculated as the sum of the concentrations of Phe, Tyr, Trp and His.

2.4. Homocysteine determination

Plasma samples were sent to the central laboratory, King Chulalongkorn Memorial Hospital, Bangkok for analysis. The concentration of Hcy was determined using an automated fluorescence polarization immunoassay (Abbott Diagnostics, USA). This laboratory routinely measures Hcy concentration in clinical specimens.

2.5. PET imaging

18 F-FDOPA PET/CT scan was performed on 8 PD patients who were randomly selected from the two groups (4 received Whey and 4 received Soy) to assess FDOPA uptake in the striatum. Subjects fasted for 6 h prior to the PET/CT scanning and all anti-parkinsonian medications were discontinued for at least 12 h prior to the procedure. PET/CT studies were performed using the Siemens/Biograph 16 scanner in 3D mode with 90 min scanning after a 2 MBq/kg bolus injection of 18 F-FDOPA. The pre-processing of PET images was conducted using Statistical Parametric Mapping (SPM8; Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm/) software running in Matlab (version R2011a, MathWorks Inc., Massachusetts, USA). The inter-frame motion was corrected using a two-pass procedure: first, all frames were realigned to the first frame of the baseline FDOPA scan; second, the frames were realigned to the mean image of the first round. The individual structural image was coregistered with the FDOPA images and used to estimate the normalization parameters to the Montreal National Institute (MNI) standard space with the clinical toolbox applying age-specific (mean 65 years old) CT templates [27]. Then, a study of specific FDOPA templates was created by averaging the summed normalized images across all subjects. Finally, the FDOPA images were normalized to the created template in MNI space. The same parameters were used for both baseline (0 month) and follow-up (6 months) scans of each subject to avoid any misregistration between the two time points. The regions of interest (ROIs) were delineated to the normalized images on the left and right side of the caudate, anterior putamen and posterior putamen, and bilaterally to the occipital cortex using PMOD (version 3.3; PMOD technologies Ltd., Zürich, Switzerland) to extract the time-activity-curves (TACs). Modelling was performed using the Patlak plot with occipital cortex as the reference region to obtain the Ki′ values reflecting the dopamine synthesis capacity in the striatum [28].

2.6. Statistical analysis

Data were presented as mean (standard deviation, SD) or median (interquartile range, IQR). Differences of variables between time points were tested using the Wilcoxon signed rank test. Two-way ANOVA was used to assess the difference between Whey and Soy supplementations. For PET scan data, the differences between the ROI data at baseline and after 6 months supplementation were investigated with the paired t-test or Wilcoxon signed rank test, as appropriate. Due to skewed distribution of variables, correlations between the UPDRS and blood parameters (reduced GSH, Hcy, BCAA and EAA) were evaluated using Spearman’s rank correlation test. GraphPad Prism version 5 (La Jolla, CA) and Stata version 12 (College Station, TX) were used for graphs and statistical analyses. P values less than 0.05 were considered statistically significant.

3. Results

3.1. PD patients demographics

Thirty-two patients completed the supplement trial: 15 in the Whey group and 17 in the Soy group. Demographic and clinical data of the studied cohorts are shown in Table 1. The mean age and male-to-female ratio between groups were not significantly different. Age of PD onset and duration of the disease between the two groups were also not significantly different. Likewise, severity of the disease at the baseline between the Whey and Soy groups was comparable. A majority of patients (11 (73%) in the Whey group, 12 (71%) in the Soy group) were prescribed with levodopa as the primary medication. Total daily levodopa equivalent doses (LED) for the Whey (669.06 ± 281.01 mg/day) and Soy (762.96 ± 662.40 mg/day) groups were not significantly different. These data ensured that clinical characteristics of the two groups were similar at the beginning of the trial.

3.2. Reduced GSH plasma levels increased in PD patients supplemented with Whey protein

Plasma levels of total and reduced GSH were measured at baseline, 3 months and 6 months. Total GSH Plasma levels tended to increase after the supplementation in both Whey and Soy groups, but the level of increase was not statistically significantly (Fig. 1a). In contrast, reduced GSH plasma levels significantly increased in patients supplemented with Whey protein for 6 months compared with the baseline (p < 0.05) (Fig. 1b) but not in the Soy supplemented group.

Plasma GSSG levels significantly decreased in the Whey-supple-mented patients at 6 months, as compared with the baseline (Supplementary Fig. 1a). Furthermore, the ratio of reduced to oxidized glutathione increased significantly in the patients supplemented with Whey protein for 6 months (Supplementary Fig. 1b). Significant changes of plasma GSSG and GSH/GSSG ratio were not observed in the Soy-supplemented patients.

3.3. BCAA and EAA plasma levels increased in PD patients supplemented with Whey protein

Plasma amino acid concentrations were measured to see whether supplementation with Whey or Soy protein was able to boost amino acid levels in the PD patients. In both groups, no significant increases in plasma levels of Ala, Arg, Asp, Citr, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Orn, Phe, Pro, Ser, Thr, Trp, Tyr, and Val were found after protein supplementation for 3- and 6-months compared with the baseline (Table 2). However, when these amino acids were categorized into BCAA (Ile, Leu and Val) and EAA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr,
Trp and Val), we found that BCAA (Fig. 2a) and EAA (Fig. 2b) concentrations in the Whey-supplemented patients after 6 months were significantly increased compared with baseline. There was no significant change of BCAA and EAA plasma levels in the Soy-supplemented patients. Plasma Cys tended to increase in both groups after supplementation but did not reach statistical significance (Fig. 2c).

AAA plasma levels in Whey-supplemented patients significantly increased after 3 months, but not after 6 months, as compared to baseline (Supplementary Fig. 2a). No significant change of plasma AAA in the Soy-supplemented patients was observed. In pre-supplemented patients (baseline), we found a significant positive correlation between plasma BCAA and AAA (Spearman’s rho = 0.3233, \( P = 0.0355 \)) (Supplementary Fig. 2b).

3.4. Hcy plasma levels decreased in PD patients supplemented with Whey protein

Plasma levels of Hcy were also determined at baseline and after 3 months and 6 months protein supplementation. Plasma Hcy was significantly decreased at 6 months compared with the baseline in the Whey-supplemented patients (Fig. 2d). In contrast, there was no significant change of plasma Hcy in the Soy-supplemented patients.

3.5. The UPDRS and striatal FDOPA uptake in PD patients supplemented with Whey protein

We also assessed clinical outcomes of PD patients following supplementation with Whey or Soy protein. Unfortunately, no significant changes of the UPDRS scores between baseline and after protein supplementation for 3 and 6 months were observed in both the Whey and Soy-supplemented groups (Fig. 3). Likewise, modified Hoehn and Yahr scale, CGI scale and PDQ-39 were not significantly different between baseline and after supplementation for 3 and 6 months in both groups.

To quantify the integrity of dopamine function, 18 F-FDOPA PET scans were performed on a subgroup of patients. All scanned patients had reduced striatal FDOPA uptake consistent with the diagnosis of PD. Compared with baseline, we found no significant difference in the rate of change in FDOPA uptake in any of the striatal sub-regions after

![Fig. 1. Plasma total glutathione (T-GSH) and reduced glutathione (R-GSH) in PD patients supplemented with Whey and soy proteins for 3 months (3 M) and 6 months (6 M). 0 M indicates a baseline or pre-supplementation. a: There were no significant increases in plasma T-GSH levels in PD patients after the protein supplementation in both Whey and soy groups. b: Plasma R-GSH level was significantly increased in PD patients supplemented with Whey protein for 6 months. Data are presented as median. Error bars indicate IQR. *: \( P < 0.05 \) vs. 0 M.]

<table>
<thead>
<tr>
<th>Plasma amino acids (μmol/L)</th>
<th>Whey 0 M</th>
<th>Whey 3 M</th>
<th>Whey 6 M</th>
<th>Soy 0 M</th>
<th>Soy 3 M</th>
<th>Soy 6 M</th>
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</thead>
<tbody>
<tr>
<td>Ala</td>
<td>190.2 ± 49.3</td>
<td>194.7 ± 55.2</td>
<td>221.0 ± 60.4</td>
<td>163.8 ± 38.9</td>
<td>171.5 ± 33.6</td>
<td>201.4 ± 40.2</td>
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<tr>
<td>Arg</td>
<td>80.4 ± 27.3</td>
<td>93.2 ± 32.8</td>
<td>87.9 ± 20.7</td>
<td>67.3 ± 19.1</td>
<td>82.3 ± 34.5</td>
<td>77.3 ± 25.6</td>
</tr>
<tr>
<td>Asp</td>
<td>7.7 ± 0.9</td>
<td>14.9 ± 5.4</td>
<td>nd</td>
<td>11.3 ± 0.4</td>
<td>11.6 ± 1.2</td>
<td>nd</td>
</tr>
<tr>
<td>Citr</td>
<td>20.5 ± 6.3</td>
<td>21.0 ± 3.7</td>
<td>22.2 ± 7.2</td>
<td>19.8 ± 6.3</td>
<td>18.9 ± 6.8</td>
<td>20.4 ± 7.3</td>
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<td>Cys</td>
<td>31.2 ± 15.4</td>
<td>30.8 ± 14.8</td>
<td>47.6 ± 24.6</td>
<td>29.2 ± 9.6</td>
<td>24.7 ± 10.8</td>
<td>40.4 ± 18.7</td>
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<tr>
<td>Glu</td>
<td>nd</td>
<td>nd</td>
<td>738.4 ± 206.3</td>
<td>nd</td>
<td>620.1 ± 81.0</td>
<td>725.0 ± 0</td>
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<tr>
<td>Gly</td>
<td>388.8 ± 104.6</td>
<td>388.3 ± 88.2</td>
<td>422.0 ± 78.4</td>
<td>366.2 ± 95.4</td>
<td>343.3 ± 95.1</td>
<td>371.8 ± 84.7</td>
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<tr>
<td>His</td>
<td>64.4 ± 14.7</td>
<td>69.8 ± 16.3</td>
<td>66.9 ± 11.7</td>
<td>61.1 ± 10.8</td>
<td>62.8 ± 13.6</td>
<td>62.3 ± 9.5</td>
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<tr>
<td>Ile</td>
<td>26.5 ± 10.2</td>
<td>40.9 ± 30.1</td>
<td>34.0 ± 13.0</td>
<td>21.6 ± 7.6</td>
<td>26.6 ± 15.6</td>
<td>25.5 ± 9.0</td>
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<td>Leu</td>
<td>120.6 ± 33.1</td>
<td>158.2 ± 83.1</td>
<td>142.9 ± 40.0</td>
<td>105.0 ± 34.9</td>
<td>119.8 ± 45.3</td>
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<td>Lys</td>
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<td>235.4 ± 98.3</td>
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<td>172.4 ± 58.9</td>
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<td>Met</td>
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<td>orn</td>
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<td>Phe</td>
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<td>Pro</td>
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<td>165.5 ± 160.3</td>
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<td>131.6 ± 176.6</td>
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<td>Ser</td>
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<td>107.9 ± 44.6</td>
<td>52.8 ± 7.4</td>
<td>68.7 ± 14.6</td>
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<td>Thr</td>
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<td>35.5 ± 9.5</td>
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<td>25.8 ± 7.7</td>
<td>48.1 ± 39.7</td>
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<td>Trp</td>
<td>44.2 ± 9.3</td>
<td>54.3 ± 17.3</td>
<td>51.6 ± 9.3</td>
<td>39.4 ± 8.1</td>
<td>44.8 ± 14.9</td>
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<td>Tyr</td>
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<td>90.4 ± 21.3</td>
<td>84.6 ± 18.9</td>
<td>102.2 ± 68.7</td>
<td>98.3 ± 42.2</td>
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<tr>
<td>Val</td>
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<td>102.8 ± 73.0</td>
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<td>87.0 ± 69.0</td>
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<td>BCAA</td>
<td>224.0 ± 82.3</td>
<td>302.0 ± 176.3</td>
<td>303.1 ± 136.7*</td>
<td>170.9 ± 81.4</td>
<td>222.5 ± 125.5</td>
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<tr>
<td>EAA</td>
<td>583.8 ± 167.9</td>
<td>735.4 ± 336.6</td>
<td>704.7 ± 225.8*</td>
<td>507.4 ± 150.0</td>
<td>561.9 ± 202.7</td>
<td>579.9 ± 135.2</td>
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</table>

Data presented as mean ± SD. nd: not detectable. M: months.

* \( p < 0.05 \) vs. 0 M.

Table 2 Plasma amino acid concentrations in patients supplemented with whey and soy proteins compared between pre-supplementation (0 M) and post-supplementation (3 and 6 months).
6 months protein supplementation between patients receiving Whey \((n = 4)\) and patients receiving Soy \((n = 4)\) (Fig. 4).

**3.6. Association of the UPDRS with plasma levels of reduced GSH, Hcy, BCAA and EAA in PD patients**

In order to elucidate a connection between biochemical parameters and severity of PD, Spearman’s rank correlation test was performed to look for correlations between the UPDRS and plasma concentrations of reduced GSH, Hcy, BCAA and EAA (Fig. 5a-e) in PD patients at the baseline. We found significant negative correlations between the UPDRS and plasma levels of BCAA \((p = 0.049)\) and EAA \((p = 0.047)\) (Fig. 5d-e). A significant and positive correlation was observed between plasma BCAA and EAA (Fig. 5f) \((p < 0.0001)\). Significant correlation between plasma AAA and UPDRS at baseline was not observed (Supplementary Fig. 2c). Plasma levels of AAA between PD patients with early and advanced stages were not significantly different (Supplementary Fig. 2d). In summary, we observed that decreases in plasma BCAA and EAA were associated with an increase in the clinical severity of PD as demonstrated by increased UPDRS.

**3.7. Effect of levodopa on biochemical parameters in PD patients before and after Whey protein supplementation**

11 out of 15 (73%) patients in the Whey-supplemented group and 12 out of 17 (71%) patients in the Soy-supplemented group were taking levodopa during the trial. Although there were no significant differences in the dosage of levodopa in both groups, we performed additional analyses to determine if levodopa had any significant impact on the biochemical parameters measured in this study. At baseline before supplementation of PD patients in both groups, there were no significant differences in the plasma levels of total GSH, reduced GSH, and GSSG between patients who were on levodopa and those who were not treated (Supplementary Fig. 3, \(p > 0.05\)). In addition, no significant differences were observed in plasma GSH/GSSG ratio and Hcy in these two groups \((p > 0.05)\). Furthermore, plasma levels of BCAA, AAA, and EAA were not significantly different in patients with and without levodopa treatment \((p > 0.05)\). To clarify if there were any effects of levodopa during trial, we performed additional analyses to compare if there were any significant changes on biochemical parameters between PD patients with and without levodopa before (baseline), and after Whey protein supplementation (3 and 6 M) (Supplementary Fig. 4). No differences in the modulation of plasma levels of reduced GSH, Hcy, BCAA, and EAA in Whey-supplemented patients treated with levodopa or untreated, after 3 and 6 M protein supplementation, from their respective baseline \((0 \text{ M})\) were observed \((p > 0.05)\). Similar observations were also demonstrated in the Soy-supplemented patients \((p > 0.05)\). Based on these findings, we can conclude that there were no significant effects of levodopa treatment on biochemical parameters monitored.

**4. Discussion**

Oxidative stress is a key factor in the pathogenesis of PD. It is well recognized that GSH, which is primarily synthesized in astrocytes and
that can naturally increase the synthesis of GSH in many tissues [29].

Whey protein contains a high level of Cys, and it is one of the dietary regimens (others such as raw milk, meat, alpha-lipoic acid, curcumin, fresh fruits and vegetables) that can naturally increase the synthesis of GSH in many tissues [29].

Whey protein supplementation has been demonstrated to increase GSH synthesis effectively both in experimental models [11,12], and clinical studies [13,15]. In this study, we found that Whey protein supplementation for 6 months was capable of increasing reduced GSH plasma levels in PD patients. Concomitantly, the plasma level of GSSG was significantly reduced and the ratio of reduced to oxidized glutathione increased. This suggests that Whey protein supplementation helps to improve antioxidant capacity in PD patients. To our knowledge, this is the first report showing a significant change in plasma GSH levels in PD patients supplemented with Whey protein. Increases in plasma total GSH in the Whey-supplemented patients were observed but these were not statistically significant. A study by Zavorsky et al. who examined lymphocyte GSH levels following supplementation with Whey protein [30] found that healthy subjects taking 15 g/day of Whey protein for 14 days did not cause a significant increase in lymphocyte GSH, whereas those supplemented with 45 g/day of Whey protein the lymphocyte GSH level was significantly increased by 24%. Similar findings were also observed in patients with advanced-HIV infection who demonstrated a significant increase in plasma GSH level after Whey supplementation at the dosage of 45 g/day for 2 weeks. Consequently, a higher daily amount of Whey protein isolate may be used in subsequent studies.

Another interesting finding was that BCAA and EAA concentrations were significantly increased in PD patients after 6 months supplementation with Whey protein. BCAAs, in particular Leu, are known to be potent inducers of muscle protein synthesis [31,32] as well as mitochondrial biogenesis [33]. Furthermore, EAA has been shown to stimulate muscle protein synthesis in healthy volunteers [34]. Supplementation with BCAA has been shown to have beneficial effects in various physiological and pathological conditions such as chronic liver diseases [31,35,36]. Dietary sources of BCAAs include meat, legumes and dairy products. Whey protein also contains high amount of BCAAs. Supplementation with Whey protein has been shown to significantly increase plasma BCAAs in both normal and diabetic mice [37].

Whey protein supplementation has been demonstrated to stimulate muscle protein synthesis in young men [38], elderly men [39], and cancer patients [40]. Since PD patients are commonly presented with loss of appetite (and malnutrition) [41–43] and weak muscle strength (impaired physical performance) [44,45], supplementation with Whey protein might deliver health benefits in improving PD patient’s nutritional status and muscle strength. However, this potential benefit needs to be confirmed in large-scale prospective clinical trials.

The other beneficial effect of supplementation with Whey protein in PD patients was a significant reduction of plasma Hcy levels which was not observed in the Soy-supplemented patients. It has been known that elevation of plasma Hcy is commonly found in PD patients treated with levodopa [19], and hyperhomocysteinemia is associated with cognitive dysfunction and dementia in patients with PD [20]. On a contrary, no association has been demonstrated between hyperhomocysteinemia and hyperkinetic movements, fluctuations, and freezing of gait [46]. Additionally, elevated plasma Hcy is an independent risk factor for cardiovascular disease [47]. The mechanism of increase of plasma Hcy in levodopa-treated patients is due to the methylation of levodopa to 3-O-methylmetyldopa. This methylation reaction is catalyzed by catechol-O-methyltransferase (COMT), and requires S-adenosymethylionine (SAM) as a methyl donor. SAM is converted to S-adenosylhomocysteine (SAH) and SAH is further converted to Hcy, eventually resulting in hyperhomocysteinemia. Levodopa treatment combined with a COMT inhibitor has been shown to effectively reduce plasma Hcy levels in PD patients compared with the levodopa treatment alone [48]. Over 70% of our studied cohorts in each group were treated with levodopa, and hyperhomocysteinemia was expected. In Whey-supplemented patients at both 3 and 6 months, plasma Hcy was significantly lower than at baseline. The clinically meaningful effect of the reduction of Hcy following the supplementation with Whey protein should be evaluated in clinical trials with objective outcomes before adopting this approach in daily clinical practice.

The clinical parameters that we measured in this study (the UPDRS and striatal FDOPA uptake) did not show a significant improvement after Whey or Soy supplementation, in spite of the fact that there were significant biochemical changes (improvements) in the Whey-supplemented group. There are several possible explanations. Nevertheless, sensitive measures of clinical parameters of PD patients will be included in subsequent trials as the objective of this program is help with holistic management of this disease for which improvements in these measurements would be a validation of the approach. First, the number of subjects in this study is too small for both the Whey supplementation and FDOPA PET study, the supplemented dosage was probably too low, and the trial duration was probably too short to reveal significant clinical and imaging improvement. Despite the clinical findings, both Whey and Soy proteins were safe. There were no reports of dopaminergic neurons, is depleted in the PD pathogenic region, SN. Therefore, regimens to restore GSH levels have been widely investigated as therapeutic alternatives for PD treatment. Whey protein contains a high level of Cys, and it is one of the dietary regimens (others such as raw milk, meat, alpha-lipoic acid, curcumin, fresh fruits and vegetables) that can naturally increase the synthesis of GSH in many tissues [29].

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adverse effects in either supplement groups. Although epidemiological data suggested that consumption of whole milk may increase the risk for PD, the mechanistic explanation is probably not a direct effect of the milk proteins, but rather a reduction of serum uric acid following consumption of a high volume of dairy products [49].

We evaluated a clinical link between biochemical markers measured in this study and the severity of PD (as indicated by the UPDRS). We found that an increase in the UPDRS was significantly associated with low levels of plasma BCAA and EAA. Whether decreased plasma BCAA and EAA are cause or consequence of PD progress is not known. Further experimental study is required to verify if there is a causal relationship. It is well known that oxidative stress is an important underlying cause of PD, and increased oxidative stress is associated with the disease severity. Serum levels of nitric oxide and peroxynitrite are positively correlated with the UPDRS in PD patients [17]. Erythrocyte antioxidant enzyme activities (catalase, superoxide dismutase and GSH peroxidase) are negatively correlated with the UPDRS in patients with PD [50]. Hcy is a known factor that mediates neurotoxicity. It induces oxidative stress in neurons and triggers neuronal cell death both in vitro and in vivo PD models [51,52]. A positive correlation between hyperhomocysteinemia and the HY scale was also demonstrated in PD patients treated with levodopa [53]. Taken together, both increased oxidative stress and elevated Hcy are likely to be associated with the severity of PD. In this study, we have shown that Whey protein supplementation for six months effectively increased plasma reduced GSH, and decreased plasma Hcy in PD patients. Whether supplementation with Whey protein may have a role in managing the progression of PD is still unclear and needs to be confirmed in well-designed prospective clinical trials involving a larger number of patients.

Limitations of the study should be mentioned. The sample size of the present trial was limited as it is a pilot study and it is not the authors’ intent to develop Whey protein as a drug, but to investigate if supplementation with Cys may have a beneficial effect on PD patients as part of a holistic approach to PD care. We have not correlated hyperhomocystinemia with various clinical outcomes as well as cardiovascular risk factors since it is not under the scope of our study. The elevation of plasma BCAA and EAA in the Whey-supplemented patients may be a temporary finding due to a direct replacement of amino acids from Whey protein isolate. Striatal FDOPA uptake was not performed in all cases and in the limited number of patients undergoing PET study may have been too few to demonstrate significant differences. There was no direct measurement of muscle protein synthesis or muscle strength to determine the clinical impacts of Whey protein supplementation on physical activity and performance. The biochemical measurements in this study were all made in plasma. The relationship between these parameters in plasma and those in the brain has not been studied and should be considered in future trials.

5. Conclusion

The present study is the first to report that daily supplementation of Whey protein for 6 months in PD patients at the dose of 20 g/day was
capable of elevating reduced GSH plasma levels, the ratio of reduced to oxidized glutathione, plasma BCAA and plasma EAA, and decreasing plasma Hcy. These biochemical changes may be beneficial for improving oxidative stress status, stimulating muscle protein synthesis, and reducing the risk for cognitive impairment and dementia. Large-scale prospective randomized, double-blind clinical trials are needed to evaluate further the potential of Whey protein supplementation as part of the holistic management of PD patients.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jns.2016.05.056.

Acknowledgement

The present study was supported by the Research Unit Grant (GRU-58-010-30-001), and Ratchadapisekompjoo grant from Chulalongkorn University, and Immunothai, Limited. Gratitude is hereby expressed to the staff at Chulalongkorn Center of Excellence for Parkinson’s Disease and Related Disorders, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Thai Red Cross Society, and the Biochemistry and Molecular Biology of Metabolic Diseases Research Unit for research facilities and assistants.

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