Effects of soya oligosaccharides and soya oligopeptides on lipid metabolism in hyperlipidaemic rats

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Abstract

In the present study, we aimed to examine the effects of soya oligosaccharides (SOS) and soya oligopeptides (SOP) on blood lipid levels, release of vasoactive substances, antioxidant activity and faecal bile acid (FBA) excretion in rats fed a high-fat diet (HFD). Male Sprague–Dawley rats were evenly divided into five groups according to diets as follows: regular diet (control), HFD, HFD enriched with 2 % of SOS (SOS), HFD enriched with 3 % of SOP (SOP) and HFD enriched with 2 % SOS and 3 % SOP (SOSP). The results showed that SOS and SOP significantly reduced plasma total cholesterol, LDL-cholesterol and TAG, whereas HDL-cholesterol concentration was significantly increased. Furthermore, SOS and SOP reduced plasma apoB, apoE and the apoB:apoAI ratio, whereas apoAI was significantly increased. Moreover, SOS and SOP also reduced plasma thromboxane A2 (TXA2) and the TXA2:prostacyclin (PGI2) ratio, whereas plasma PGI2 and nitric oxide were significantly increased. In addition, SOS and SOP significantly reduced serum and liver malondialdehyde concentrations and increased FBA excretion. However, we did not observe obvious influences of SOS and SOP on superoxide dismutase activities in the liver of HFD-fed rats. The combination of 2 % SOS and 3 % SOP showed a more marked effect than SOS or SOP alone in improving the lipid profile, release of vasoactive substances and increasing FBA excretion (P≤0.05). In summary, SOS and SOP might help prevent atherosclerosis through improving abnormal blood lipid levels, regulating vasoactive substances and protecting against oxidative stress.

Key words: Soya oligosaccharides; Soya oligopeptides; Lipid metabolism; Atherosclerosis

CVD is the leading cause of mortality in the world, accounting for the deaths of seventeen million people each year or approximately one-third (29 %) of global deaths annually1). CVD prevention is a global public health priority. According to the estimation of the WHO, 88 % of the global mortality and disease burden from CVD occurs in low- and middle-income countries2). For example, CVD causes over three million deaths each year in China, and at least 230 million Chinese are suffering from CVD. The origin of CVD is multifactorial and its pathological basis is atherosclerosis. Hypercholesterolaemia is recognised as a major and independent risk factor of atherosclerosis and CVD2). About 60–70 % of plasma cholesterol is present in the LDL fraction3). Plasma LDL-cholesterol (LDL-C) crosses the endothelial barrier and reaches the vascular subendothelial intima, whereby promoting monocyte/macrophage differentiation. Subsequently, monocyte-derived macrophages take up cholesterol through scavenger receptors and become lipid-laden foam cells4). Steinberg et al.5) showed that the cholesterol accumulating in macrophage-derived foam cells comes from circulating lipoproteins, mainly from the atherogenic LDL. Therefore, plasma lipids play a critical role in the initiation and progression of the atherosclerotic lesion4), and it is essential to keep blood cholesterol levels in the appropriate range for

Abbreviations: AI, atherosclerosis index; BW, body weight; FBA, faecal bile acid; HDL-C, HDL-cholesterol; HFD, high-fat diet; LDL-C, LDL-cholesterol; MDA, malondialdehyde; PGI2, prostacyclin; SOD, superoxide dismutase; SOP, soya oligopeptides; SOS, soya oligosaccharides; SOP, HFD enriched with 2 % SOS and 3 % SOP; TG, total cholesterol; TXA2, thromboxane A2.

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the prevention of atherosclerosis and CVD. On the basis of a meta-analysis, a 1% decrease in plasma cholesterol level can reduce the risk of CVD up to 3%.

Hypolipidaemic drugs, particularly statins, are the preferred treatment for reducing blood cholesterol levels because of their efficacy and safety. However, dietary modification is the mainstay of treatment of lipid abnormalities and can have a significant cholesterol-lowering effect.

Soyabeans, as a traditional Chinese food, have been consumed for thousands of years in China due to its beneficial effects on health. Soy contains a variety of phytochemicals with special health functions, such as soya isoflavones, saponins, phytic acid, sterol and so on. Besides these, soya oligosaccharides (SOS) and soya oligopeptides (SOP) are two types of bioactive substances extracted from soyabeans. SOS is the general term for soluble sugars in soyabeans, and it is mainly composed of stachyose, raffinose and sucrose. SOP refers to peptides with molecular weights lower than 1000 Da, and it is derived from soya protein by enzyme hydrolysis. Some reports have suggested that both soya protein and SOP have effects on improving the lipid profile in hypercholesterolaemic subjects, whereas SOP is more effective than soya protein. Many studies found that oligosaccharides have cholesterol-lowering properties. However, little information is known about the effect of SOS on lipoprotein metabolism in high-cholesterol-fed animals, especially the combined effects of SOS and SOP on improving dyslipidaemia. It has been shown that probiotics, such as bifidobacteria and lactobacilli, have beneficial effects on cholesterol metabolism in vitro and in vivo. Lan et al. reported that SOS is a powerful prebiotic that can increase caecal bifidobacterium and lactobacilli populations in animals and human subjects. SOP is the necessary and suitable nutrient for the growth of intestinal bifidobacteria.

In the present study, we aimed to determine whether SOS and/or SOP had significant effects on lipid metabolism in a rat model with hyperlipidaemia induced by a high-fat diet (HFD). Besides plasma lipid parameters, we examined vasoactive substances, antioxidant activity and faecal bile acid (FBA) excretion in order to explore the potential application of SOS and SOP in CVD prevention. The second aim of the study was to determine whether the combination of SOS and SOP had additive or synergistic effects on these parameters.

Materials and methods

Materials

SOS extracted from soya bean whey was provided by Shanghai Far-reaching Food Company Limited (Shanghai, China). The total sugar content was ≥ 70%.

SOP was provided by Shandong Zhongshi Dqing Biotech Company Limited (Jinan, China). The peptide content was ≥ 80%.

Animals, diets and experimental protocol

A total of sixty healthy male Sprague–Dawley rats were obtained from the Laboratory Animal Center of the Third Military Medical University, and the body weight (BW) ranged from 190 to 210 g. The animals were individually housed in metabolic cages in an environment-controlled room (20–23°C and 40–60% of humidity) under a 12 h light–12 h dark cycle with free access to food and water. All animals were fed for 8 weeks, and BW was monitored biweekly throughout the study. Food intake was measured three times per week. The animal protocol was approved by the Third Military Medical University Institutional Animal Care Committee and conducted in accordance with the Third Military Medical University guidelines for the care and use of laboratory animals.

After a week of adaptation, sixty Sprague–Dawley rats were evenly divided into five groups according to diets as follows: regular diet (control), HFD, HFD enriched with 2% of SOS (SOS), HFD enriched with 3% of SOP (SOP) and HFD enriched with 2% SOS and 3% SOP (SOSP). The regular diet was composed of the following ingredients (g/100g diet): bean cake, 20; wheat starch, 19; maize starch, 15; rice starch, 20; bran, 15; fishmeal, 5; bone meal, 3; yeast, 1; salt, 1; and cod liver oil, 1. The total protein/carbohydrate/fat content of the regular diet was approximately 18, 52 and 5%, respectively. The ingredients of the HFD were as follows: 10% lard, 2% cholesterol, 1% bile salt and 87% regular diet. In the SOS group, HFD was supplemented with 2% SOS by replacing 2 g of bran. In the SOP group, HFD was supplemented with 3% SOP by replacing 3 g of fishmeal. In the SOP group, HFD was supplemented with 2% SOS and 3% SOP by replacing 2 g of bran and 3 g of fishmeal, respectively. Diets of the HFD and experimental groups were iso-energetic and iso-nitrogenic. At the end of the experiment, after overnight fasting, rats were anaesthetised using pentobarbital sodium, and blood samples from the femoral vein were collected in tubes containing EDTA (1.4 g/l) and Trasylol (100 kU/l). Then, plasma was separated for subsequent biochemical analysis and stored at −70°C. Immediately after killing the animal, liver tissues were dissected and stored at −70°C for later antioxidant activity assay. Atherosclerosis index (AI) was calculated according to the Fridewald formula:

\[
AI = \text{LDL-C}/\text{HDL-C}.
\]

All faeces were collected for three consecutive days at the end of the experiment. The collected faeces were stored at −20°C for later determination of FBA content by phosphomolybdate colorimetry after 16 h of vacuum freeze-drying.

Biochemical analysis

Serum lipid profile analysis. Serum total cholesterol (TC), TAG, LDL-C and HDL-cholesterol (HDL-C) levels were determined by commercial enzymatic test kits (Biosino Bio-technology and Science Inc., Beijing, China) using an automatic biochemical analyser (Type AU2700; Olympus, Tokyo, Japan). ApoA1, apoB and apoE were determined by immunoturbidimetry using assay kits from Biosino Bio-technology and Science Inc. These procedures were fully automated and have been described in detail elsewhere.
**Evaluation of antioxidant activities.** Superoxide dismutase (SOD) activity in the serum and liver was determined by the xanthine oxidase method using a commercially available kit (SOD Detection Kit; Nanjing Jiancheng Bioengineering Institute, Nanjing, China)(15). Malondialdehyde (MDA) in the serum and liver was measured by the thiobarbituric acid method(16) using a commercially available kit (MDA Detection Kit, Nanjing Jiancheng Bioengineering Institute). The assay was based on the ability of a chromogenic agent to react with MDA, yielding a stable chromophore with maximal absorbance at 532 nm.

**Prostanoid release measurements.** In order to measure the release of thromboxane A2 (TXA2) and prostacyclin (PGI2), direct RIA was carried out to determine the concentrations of thromboxane B2 and 6-ketoPGF1α, the stable degradation products of TXA2 and PGI2, respectively(17). RIA kits were supplied by the General Hospital of PLA, Beijing, China.

**Plasma nitric oxide assay.** Plasma NO concentration was determined by colorimetric technique and nitrate reductase method using a Nitrate/Nitrite Colorimetric Assay kit (Nanjing Jiancheng Bioengineering Institute). This assay was based on the enzymatic conversion of nitrate to nitrite by nitrate reductase, and the concentration of NO was indirectly measured by determining both nitrate and nitrite levels in the plasma.

**Determination of faecal bile acids.** Total FBA was determined by spectrophotometry based on the colour reaction between phosphomolybdate and bile acid(18). Protein and pigments were precipitated from faeces by adding ethanol, and total bile acids were extracted by chloroform and methanol (2:1, v/v). Phosphomolybdate reagent (phosphomolybdate acid (10 ml)) was used to form colour reaction, and then it was quantified by a 721 spectrophotometer (wavelength: 690 nm) (Shanghai Phenix Optical Scientific Instrument Company Limited, Shanghai, China).

**Statistical analysis**

All statistical calculations were carried out with Statistical Package of SPSS 13.0 (Release 13.01S China: Beijing Stats Data Mining Company Limited, Beijing, China; permanent license). Data were expressed as mean values and standard deviations. For data with normal distribution and homogeneity of variance, significance of difference \((P<0.05)\) between mean values was determined by ANOVA coupled with the least significant differences (LSD) test, whereas it was determined by the Dunnett-T3 test for data with heterogeneity of variance.

**Results**

**Body weight and food intake**

Table 1 shows that neither the pre-treatment BW of rats nor weight gains after the 8-week treatment were significantly different among the five groups. The mean daily food intake of rats in the HFD and experimental groups was higher than that of the control group during the 8-week experiment; however, the difference was not statistically significant.

**Lipid profile changes**

Table 2 shows the effects of SOS and SOP on serum TC, TAG, LDL-C and HDL-C levels. Serum TC, TAG and LDL-C levels in the HFD group were significantly higher than those of the control group, whereas HDL-C level was significantly lower than that of the control group \((P<0.05)\). The addition of SOS and SOP alone or together with HFD resulted in significantly decreased serum TC, TAG and LDL-C levels, whereas HDL-C level was significantly increased compared with the HFD group \((P<0.05)\). In particular, the combination of SOS

<table>
<thead>
<tr>
<th>Group</th>
<th>TC (mmol/l)</th>
<th>TAG (mmol/l)</th>
<th>LDL-C (mmol/l)</th>
<th>HDL-C (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.59</td>
<td>0.25</td>
<td>0.29</td>
<td>1.03</td>
</tr>
<tr>
<td>HFD</td>
<td>6.26*</td>
<td>0.52</td>
<td>2.76*</td>
<td>4.68*</td>
</tr>
<tr>
<td>SOS</td>
<td>3.90†</td>
<td>0.34</td>
<td>1.37†</td>
<td>1.90†</td>
</tr>
<tr>
<td>SOP</td>
<td>4.09†</td>
<td>0.23</td>
<td>1.65†</td>
<td>1.55†</td>
</tr>
<tr>
<td>SOSP</td>
<td>3.50†‡§</td>
<td>0.19</td>
<td>1.31†‡§</td>
<td>1.07†‡§</td>
</tr>
</tbody>
</table>

TC, total cholesterol; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; HFD, high-fat diet; SOSP, HFD enriched with 2% SOS and 3% SOP.
* Mean values were significantly different from those of the control group \((P<0.05)\).
† Mean values were significantly different from those of the HFD group \((P<0.05)\).
‡ Mean values were significantly different from those of the SOS group \((P<0.05)\).
§ Mean values were significantly different from those of the SOP group \((P<0.05)\).

Table 2. Effects of soya oligosaccharides (SOS) and soya oligopeptides (SOP) on lipid profile of rats (Mean values and standard deviations, n 12 per group)
Table 3 shows the effects of SOS and SOP on plasma apo levels in rats. Plasma apoAI level in the HFD group was significantly lower than those of the control group ($P<0.05$), whereas the apoB level of the SOS group was significantly decreased compared with that of the HFD group ($P<0.05$). SOP was significantly more effective than SOS and SOP alone in altering TC, LDL-C and HDL-C levels ($P<0.05$).

Fig. 1 shows the change of AI value of each group. AI value of the HFD group was significantly higher than that of the control group, whereas the value of the three experimental groups was significantly lower than that of the HFD group ($P<0.05$). There was no statistically significant difference among the control and experimental groups.

**Effects of soya oligosaccharides and soya oligopeptides on plasma apo levels**

Table 3 shows the effects of SOS and SOP on plasma apo levels in rats. Plasma apoAI level in the HFD group was significantly lower, whereas apoB and apoE levels were significantly higher than those of the control group ($P<0.05$). ApoAI levels of the three experimental groups were significantly increased compared with the HFD and control groups ($P<0.05$), and the most significant effect was observed in the SOSP group ($P<0.05$). ApoB levels of the SOP and SOSP groups were significantly increased compared with those of the other three groups, and apoB level of the SOS group was significantly decreased compared with that of the HFD group ($P<0.05$).

**Effects of soya oligosaccharides and soya oligopeptides on vasoactive substances**

Table 4 shows the effects of SOS and SOP on plasma vasoactive substances. Plasma PGI$_2$ and NO levels of the HFD group were significantly lower than that of the control group, whereas TXA$_2$ and the TXA$_2$:PGI$_2$ ratio of the HFD group were significantly higher than those of the control group ($P<0.05$). Plasma PGI$_2$ and NO levels of the three experimental groups were significantly higher than those of the HFD group, whereas TXA$_2$ and the TXA$_2$:PGI$_2$ ratio of the three experimental groups were significantly lower than those of the HFD group, but higher than those of the control group ($P<0.05$). Compared within the three experimental groups, the SOSP group was more effective in reducing TXA$_2$ and the TXA$_2$:PGI$_2$ ratio, as well as increasing NO than the SOS or SOP group ($P<0.05$).

**Superoxide dismutase activity and malondialdehyde content in livers and serum**

Table 5 shows that SOD activity in the serum, as well as MDA contents in the liver and serum of the HFD group were significantly higher than those of the control group ($P<0.05$), whereas SOD activity in the liver of the HFD group was not statistically different from that of the control group. SOD activity in the liver of the three experimental groups was significantly lower than that of the control group ($P<0.05$). SOD activity in serum and the MDA content in the liver and serum of the
Effects of soya oligosaccharides and oligopeptides

Discussion

In the present study, we did not observe significant differences of the mean daily food intake and BW gains among the three experimental groups with a significantly higher than those of the control group, but lower than those of the HFD group (P < 0.05), except for the liver MDA content of the HFD group, which was not significantly different from that of the control group. Among the three experimental groups, the MDAs from liver and serum group were significantly lower than that of the SOSP group (P < 0.05), whereas no significance was obtained from the other experimental groups.

Dry faecal weight and bile acid excretion

Table 6 shows the FBA levels of rats. Dry faecal weight of the HFD group and the three experimental groups were higher than that of the control group. However, there were no significant differences among any groups (P > 0.05). FBA of the three experimental groups was significantly higher than that of the HFD group (P < 0.05). Compared with the three experimental groups, FBA content of the SOSP group was significantly higher than that of the other two experimental groups (P < 0.05).

Table 4. Effects of soya oligosaccharides (SOS) and soya oligopeptides (SOP) on vasoactive substances of rats (Mean values and standard deviations, n 12 per group)

<table>
<thead>
<tr>
<th>Group</th>
<th>TXA2 (ng/l)</th>
<th>PGI2 (ng/l)</th>
<th>NO (μmol/l)</th>
<th>TXA2:PGI2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Control</td>
<td>97</td>
<td>8</td>
<td>136</td>
<td>5</td>
</tr>
<tr>
<td>HFD</td>
<td>511*</td>
<td>27</td>
<td>86*</td>
<td>9</td>
</tr>
<tr>
<td>SOS</td>
<td>209†</td>
<td>18</td>
<td>158†</td>
<td>11</td>
</tr>
<tr>
<td>SOP</td>
<td>237**</td>
<td>23</td>
<td>144**</td>
<td>9</td>
</tr>
<tr>
<td>SOSP</td>
<td>186††§</td>
<td>10</td>
<td>146††§</td>
<td>10</td>
</tr>
</tbody>
</table>

TXA2, thromboxane A2; PGI2, prostacyclin; NO, nitric oxide; HFD, high-fat diet; SOSP, HFD enriched with 2% SOS and 3% SOP.

* Mean values were significantly different from those of the control group (P < 0.05).
† Mean values were significantly different from those of the HFD group (P < 0.05).
‡ Mean values were significantly different from those of the SOS group (P < 0.05).
§ Mean values were significantly different from those of the SOP group (P < 0.05).

Table 5. Effects of soya oligosaccharides (SOS) and soya oligopeptides (SOP) on superoxide dismutase (SOD) activity and malondialdehyde (MDA) content in liver and serum of rats (Mean values and standard deviations, n 12 per group)

<table>
<thead>
<tr>
<th>Group</th>
<th>Livers (U/mg protein)</th>
<th>Serum (U/ml)</th>
<th>MDA (nmol/g liver)</th>
<th>Serum (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Control</td>
<td>662</td>
<td>26</td>
<td>346</td>
<td>16</td>
</tr>
<tr>
<td>HFD</td>
<td>598</td>
<td>60</td>
<td>738*</td>
<td>69</td>
</tr>
<tr>
<td>SOS</td>
<td>581*</td>
<td>41</td>
<td>424**</td>
<td>41</td>
</tr>
<tr>
<td>SOP</td>
<td>565*</td>
<td>100</td>
<td>428*†</td>
<td>53</td>
</tr>
<tr>
<td>SOSP</td>
<td>572*</td>
<td>35</td>
<td>423†‡</td>
<td>51</td>
</tr>
</tbody>
</table>

HFD, high-fat diet; SOSP, HFD enriched with 2% SOS and 3% SOP.

* Mean values were significantly different from those of the control group (P < 0.05).
† Mean values were significantly different from those of the SOSP group (P < 0.05).
‡ Mean values were significantly different from those of the SOP group (P < 0.05).

Discussion

In the present study, we did not observe significant differences of the mean daily food intake and BW gains among the five groups, indicating that the consumption of diet by rats was not altered by high fat, SOS and SOP, and thus the BW gains were not significantly different among the five groups.

The reduction of TC or LDL-C in plasma has been reported to reduce the risk of CVD. Numerous studies in human subjects and animals have shown the hypocholesterolaemic effect of soya protein. Some studies reported that the effect of soya protein on lipid metabolism is attributed to its component of peptides. In the present study, we observed that serum TC and TAG of the SOP group were decreased by 34.7 and 40.2%, respectively, with a powerful prebiotic ability, SOS is fermented in the colon by bifidobacterium, yielding large amounts of SCFA, such as butyrate and...
propionate\(^{25}\). It has been hypothesised that SCFA, in particular propionate and butyrate, are able to influence lipid metabolism\(^{26}\). Butyrate can inhibit hepatic cholesterol synthesis, while propionate may inhibit the synthesis of fatty acids in the liver, thereby reducing the rates of TAG secretion\(^{27}\). Therefore, the present data suggested that the improved lipid metabolism observed in the SOS group was partly caused by an alteration in the absorption and/or synthesis of cholesterol through SCFA. In the present study, we observed that the combined treatment of SOS and SOP reduced TC and TAG levels by 10·3, 14·4 and 4·4 %, 20·6 % compared with SOS and SOP alone, respectively, suggesting that the combination of SOS and SOP was more effective in improving dyslipidaemia, thus reducing the risk of atherosclerotic CVD.

There is increasing evidence that both reducing LDL-C and raising HDL-C can result in significant cardiovascular benefits\(^{28}\). In the present study, LDL-C was reduced by 66·9 % in the SOP group and by 59·4 % in the SOS group, whereas HDL-C was increased by 112·5 and 119·2 %, respectively. These results suggested that these effects of SOS and SOP on LDL-C and HDL-C were beneficial for atherosclerotic CVD. Some studies confirmed the reduction in plasma TC and LDL-C without significant effect on HDL-C by soya protein\(^{19,29}\). Zhao et al.\(^{50}\) observed that SOP reduces serum TC and TAG levels of hyperlipidaemic rats, whereas HDL-C is not significantly altered. However, we observed a significant increase of plasma HDL-C in the SOP group. The precise reason for this inconsistency with other reports remains unclear. It may result from the SOP used in the present study, which was different from other studies, especially the molecular weight of SOP. It has been proposed that the regulation of cholesterol homeostasis by SOP depends on the activation of LDL receptors and LDL degradation in the liver\(^{31}\). Lovati et al.\(^{32}\) demonstrated that peptides derived from soya 7S globulin-\(\alpha\)-subunit are able to up-regulate LDL receptors’ activity, thus resulting in receptor-mediated LDL catabolism\(^{33}\). However, 7S lacking the \(\alpha\)-subunit has no effect on the up-regulation of LDL receptors, indicating that soya peptides with hypocholesterolaemic activity are involved in the regulation of LDL receptors. The observed decrease in AI values suggested that SOS and SOP, especially their combination, were auxiliary in decreasing the risk of atherosclerosis and CVD.

High apoB, the structural protein of LDL, and a high apoB:apoAI ratio are strongly related to the increased atherosclerotic cardiovascular risk. In contrast, high apoAI, mainly representing HDL and being crucial in transferring cholesterol from tissues to the liver, is inversely related to atherogenic risk\(^{34}\). In the present study, we observed a significantly higher apoB and apoB:apoAI ratio, exhibiting an atherogenic lipid profile as well as a significantly lower apoAI in HFD-fed rats. The present data also showed a significant reduction of apoB by 43·3 % and an increase of apoAI by 197·8 % in SOS-fed rats. In a clinical study, Teixeira et al.\(^{50}\) observed that soya protein effectively reduces plasma apoB, whereas the change in apoAI is not significant. However, in the present study, SOP not only reduced apoB but also increased apoAI effectively. Moreover, the combination of SOP and SOS showed a more marked effect on reducing the apoB:apoAI ratio and increasing apoAI than SOP and SOS alone. These data indicated that the combination of SOP and SOS could be more effective in reducing serum lipid risk factors for CVD than SOP or SOS alone.

Endothelial dysfunction plays an important role in the early development of atherosclerosis\(^{35}\). PGI\(2\) and NO are antiatherogenic because of their vasodilating effects, whereas TXA\(2\) is a potent vasoconstrictor\(^{36–38}\). In the present study, we observed that HFD reduced the release of PGI\(2\) and NO, and increased the TXA\(2\) level, suggesting that endothelium-dependent vasorelaxation was attenuated by hyperlipidaemia\(^{39}\), and the effect of hypercholesterolaemia on endothelial cells leads to the inhibition of NO synthesis\(^{45}\). In vitro study has shown that SOP increases the release of PGI\(2\) and NO, but it does not affect TXA\(2\)\(^{40}\). The present study showed that SOP not only increased the release of PGI\(2\) and NO by 67·9 and 48·9 %, respectively, but also decreased TXA\(2\) by 53·6 % compared with the HFD group. SOS exerted similar effects. Furthermore, the combination of SOS and SOP was more effective in reducing TXA\(2\) and the TXA\(2\):PGI\(2\) ratio, as well as increasing NO than SOS or SOP alone, thus improving endothelial dysfunction and cardiovascular health.

It has been shown that oxidative stress contributes to endothelial dysfunction and atherosclerosis\(^{22}\). However, dietary antioxidants may protect against oxidative events in the body, and the antioxidant activity reduces CVD by preventing LDL oxidation\(^{35,41}\). In the present study, oxidative stress occurred in HFD-fed rats with elevated liver and serum levels of MDA, the ultimate product of lipid peroxidation. The antioxidant defence system, such as SOS, is important to protect the body from oxidative damage\(^{42}\). Many experimental results showed that HFD or hyperlipidaemia can reduce SOD activity in rats\(^{43}\). Surprisingly, in the present study, the serum SOD activity of the HFD group was not reduced, and it was higher than that of the control and experimental groups. The present findings are consistent with those of Arafa et al.\(^{44}\), who showed that a HFD does not decrease the liver SOD activity, which is higher than that of normal diet-fed rats and curcumin-treated rats. We supposed that the result was due to the high level of MDA, which resulted in the
increase of SOD activity for maintaining the balance between oxidative stress and antioxidant defences.

Gibbs et al.\(^{(45)}\) reported that the antioxidant activity of soya protein is associated with the fractions 11S or glycinin as the precursor of antioxidant peptides. In another study, it has been shown that the ingestion of soya peptide in rats reduces paraquat-induced oxidative stress by preventing the elevation of the concentration of serum thiobarbituric acid-reactive substances, thus effectively preventing LDL oxidation\(^{(46)}\). The present data show that SOP decreases MDA by 32.8 and 51.6 % in the liver and serum, respectively, thus reducing oxidative stress. Chen et al.\(^{(47)}\) found that peptides with a Pro-His-His sequence demonstrate the greatest antioxidant activity among all tested peptides, and their activity is decreased by removing a His residue from the C-terminus. Chen et al.\(^{(48)}\) reported that the activities of antioxidant enzymes in the liver and serum, such as catalase and SOD, are significantly elevated when SOS is orally administered to rats for 45 d. The present data show that SOS could reduce oxidative stress by increasing the serum SOD activity and reducing the MDA content in SOS-treated rats, suggesting that SOS protected blood vessels and endothelial cells from oxidative damage by inhibiting LDL oxidation, thus protecting against atherosclerosis.

Bile acids are critical in maintaining cholesterol homeostasis. Binding of bile acids and increasing their faecal excretion have been hypothesised as an effective way to reduce cholesterol by dietary factors.\(^{(49)}\) Iwami et al.\(^{(49)}\) reported that SOP binds bile acids, thereby enhancing bile acid and faecal steroid excretion, which may contribute to the hypcholesterolaemic activity. In the present study, SOP-fed rats demonstrated significantly greater FBA excretion than those fed with normal diets or HFD. Greater FBA excretion leads to the increased bile acid production from cholesterol, thus reducing serum cholesterol concentrations. Choi et al.\(^{(50)}\) identified the bile acid-binding region in SOP and found that the glycinin-derived peptide VAWWMY is responsible for this bile acid-binding ability. Therefore, the present data support the hypothesis that the absorption of cholesterol and bile acids could be further decreased by SOP, and the increased bile acid excretion is an important contributor to the hypcholesterolaemic effect of SOP. SOS-fed rats also showed significantly higher excretion of FBA compared with the control and HFD groups. A 30 d study in human subjects\(^{(51)}\) showed that SOS intake of 3 g/d significantly increases faecal bifidobacteria. Therefore, the increasing FBA excretion by the combination of SOS and SOP could be ascribed to an additive or synergistic effect.

In summary, the present study shows that ingestion of 2 % SOS or 3 % SOP influence lipid metabolism, release of vasoactive substances as well as antioxidant activity and increase FBA excretion. In particular, the combination of 2 % SOS and 3 % SOP is more effective than SOS or SOP alone, especially in improving the lipid profile and increasing FBA excretion. These results indicate that SOS and SOP have potential value in CVD prevention. Further studies should focus on clarifying the mechanisms of action of the lipid effects of SOS and SOP.

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