LUPIN PROTEIN ATTENUATES THE DEVELOPMENT OF HYPERTENSION AND NORMALISES THE VASCULAR FUNCTION OF NaCl-LOADED GOTO-KAKIZAKI RATS

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The beneficial cardiovascular effects of soy protein have been studied intensively in recent years. Another protein-rich legume is lupin, which has been shown to have similar effects to those of soy in lowering serum cholesterol levels. In this study we compared the effects of lupin and soy protein on hypertension and vascular functions in spontaneously diabetic Goto-Kakizaki (GK) rat, which develop hypertension when fed a high-salt diet. The rats were fed with a 6% NaCl diet containing either lupin or soy protein isolate (20% weight/weight) for two weeks. In the end of the study the SBP was 18.6 mmHg lower (p<0.001) in the lupin group, and 12.0 mmHg lower (p<0.01) in the soy group than in the control group. Lupin and soy treatments normalised the decreased vasocontraction observed in the NaCl-fed control group, but only lupin treatment improved the impaired endothelium-dependent vasorelaxation. The attenuation of hypertension is likely to be mediated by the corrected vascular dysfunction, whose precise mechanism and the possible clinical relevance remains to be studied further.

Key Words: hypertension, vascular function, lupin protein, soy protein, Goto-Kakizaki rat
In 1999, the US Food and Drug Administration (FDA) approved a health claim for the relationship between consumption of soy protein and reduced risk of coronary heart disease (1). The health claim is largely based on data included in a meta-analysis by Anderson et al. (2). The data from 38 clinical studies indicated that consumption of soy protein rather than animal protein significantly reduced serum concentrations of total cholesterol, LDL cholesterol and triglycerides. Even though the most active research concerning soy has focused on its cholesterol-lowering effect, the mechanisms and active components responsible for the improvement of lipid profiles are still not fully understood. It was initially suggested that the soy estrogens, the isoflavones genistein and daidzein, were likely to be responsible for the hypocholesterolemic effects of soy (2). On the other hand, the role of isoflavones as the main component responsible for the cholesterol-lowering effect of soy has recently been questioned, and it has been suggested that if isoflavones have any health-protecting effect in humans, it is small in comparison with the effect of the protein itself (3). One mechanism by which soy protein has been shown to reduce hypercholesterolemia is the up-regulation of liver β-VLDL-receptors by the α'-constituent subunit of soybean 7S globulin (4-6).

Another protein-rich legume besides soy is lupin (7), which has been cultivated for centuries, mainly for domestic animal feed. At present Australia is the most important lupin-producing country in the world. In Europe lupin is cultivated mostly in France, Russia, Spain, Poland and Italy (8). The protein and amino acid content of lupin seeds and soy are very similar, but the arginine content of lupin protein, in particular, is higher than that of soy protein (9-12). Conversely, the content of sulphur amino acids, lysine and tryptophan is clearly smaller in lupin protein than in soy. The amount of antinutritional compounds, such as alkaloids, saponins, tannins and trypsin inhibitors, is minimal in lupin (11), as is the amount of isoflavones (7, 13). In addition the lupin protein is highly bioavailable and there is no difference in the bioavailability when compared to soy protein (10).

The protein isolate of white lupin (Lupinus albus) has been shown to have a similar hypocholesterolemic effect in rats to that of soy protein (7). Also the lupin kernel fibre as well as whole lupin seeds have been demonstrated to have cholesterol-lowering effect in pigs and in man (14). It has been shown, that a minor lupin protein component, conglutin γ, has a remarkable increasing effect on LDL-uptake and degradation, and this has been suggested as one of the mechanisms behind the hypocholesterolemic effect (7). This finding, however, is based on a study of cultured HepG2 cells and is not as such applicable to proteins that undergo digestion.

Hypertension is a well-known risk factor for various cardiovascular complications and is also closely related to the pathophysiology of type II diabetes. The Goto-Kakizaki (GK) rat is a novel experimental model of type II diabetes. In addition to diabetes, GK rats have also been shown to develop
hypertension when fed a high NaCl diet (15). Apart from the hypocholesterolemic effect of soy, it has been demonstrated that a soy-protein-based diet attenuates the development of hypertension compared to a casein-based diet in spontaneously hypertensive rats (SHRs) (16). The aim of the present study was to compare the effects of lupin and soy protein on blood pressure and vascular functions in GK rats fed on a high-NaCl diet.

MATERIALS AND METHODS

Animals and diets

Twenty-four male GK rats (137 ± 3 g) were purchased from M&B (Lejby, Denmark) and ten male Wistar rats (213 ± 3 g) from Harlan Nederland (Horst, The Netherlands). The study protocol was approved by the Animal Experimentation Committee of the Institute of Biomedicine, Helsinki, Finland. Body weight-matched GK rats were randomised into three treatment groups (n=8/group): (1) a control group, receiving standard rat chow (Harlan Tekland 2018, Harlan Holding, Inc, Wilmington, DE, USA), with high NaCl content (6%), (2) a lupin group (200 g/kg lupin protein isolate mixed with the standard chow, NaCl content 6%) and (3) a soy group (200 g/kg soy protein isolate mixed with the standard chow, NaCl content 6%). (4) The Wistar rats (receiving standard rat chow, NaCl content 0.6%) served as a normotensive control group. Lupin protein isolate (type E, L181) of Lupinus albus, was provided by Fraunhofer IVV (Freising, Germany) and soy protein isolate (Supro 670-IP) by The Solae Company (St Louis, MO, USA). The isoflavone content of the soy protein isolate was 2.2 µmol/g (0.4 µmol/g daidzein, 1.7 µmol/g genistein and 0.09 µmol/g glycitein) as measured by HPLC (17). The isoflavone content of lupin protein isolate has been reported to be below the detection limit 0.1 nmol/g (7, 13).

The rats were housed four to a cage (the Wistar rats, five to a cage) in a standard experimental animal laboratory (illuminated from 6.30 a.m. to 6.30 p.m., temperature +22±1°C) and had free access to tap water and chow during the experiment. The consumption of feed was monitored daily and the consumption of drinking water every other day.

Weight and blood pressure measurement

The rats were weighed weekly using a table scale, and systolic blood pressure was monitored weekly using a tail cuff blood pressure analyser (IITC Life Science, model 179, Woodland Hills, CA, USA). The rats were prewarmed for 15-20 minutes at +32°C to improve the detection of the pulsation of the tail artery. The arithmetic mean of three successive measurements without disturbances of the signal indicated the systolic blood pressure. To minimise stress-induced variations in blood pressure, the same person, in the same peaceful environment, carried out all the measurements.

Collection of blood samples

After the two-week treatment period, the rats were rendered unconscious with CO₂/O₂ (95%/5%) (AGA, Riihimäki, Finland), and decapitated. Blood samples were taken in glass tubes and the serum was separated by centrifugation at +4°C for 15 minutes. The serum samples were stored at -79°C until the lipid and blood glucose determinations. Total cholesterol, HDL cholesterol and triglycerides were measured enzymatically, LDL was calculated by the Friedewald equation (18) and blood glucose determined enzymatically by the hexokinase method (19).
Arterial preparations

The mesenteric artery was cleaned of adherent connective tissue and cut into 3 mm long sections, beginning 5 mm distally from the mesenteric artery-aorta junction. The mesenteric artery rings were carefully stretched between stainless steel hooks and suspended in an organ bath chamber in Krebs-Ringer buffer (pH 7.4) of the following composition (mM): NaCl 119.0, NaHCO$_3$ 25.0, glucose 11.1, CaCl$_2$ 1.6, KCl 4.7, KH$_2$PO$_4$ 1.2, MgSO$_4$ 1.2, and aerated with 96% O$_2$ and 4% CO$_2$. The rings were equilibrated for 45 min at +37°C with a resting tension of 1.0 g. The presence of a functional endothelium in the preparations was confirmed by observing a clear relaxation response to 1 µM acetylcholine in 1 µM noradrenaline precontracted arterial rings. The cumulative relaxation responses to acetylcholine (1nM-10µM) (endothelium-dependent) and sodium nitroprusside (1nM-10µM) (endothelium-independent) were determined after noradrenaline precontraction.

Statistical analysis

Data are presented as means ± SEM. Statistically significant differences between the mean values were tested by ANOVA followed by the Tukey's test for pair wise comparisons. The differences were considered significant when p<0.05. The data were analysed using SPSS (Version 10.1, SPSS Inc. Chicago, IL, USA).

RESULTS

Body weight gain and food and drink consumption

There were no differences in body weight gain between the GK groups during the treatment period (p=0.563) (Table 1). There were no differences in the food and drink consumption between the GK groups during the study.

Blood pressure

Sodium loading increased systolic blood pressure (SBP), which was lowered by the lupin and soy treatments (Fig. 1). At the end of the treatment period, the highest SBP (138.7 ± 1.3 mmHg) was found in the GK control group. The differences in blood pressure levels were, in comparison with the GK control group: lupin group -18.6 mmHg (p<0.001), soy group -12.0 mmHg (p<0.01) and Wistar control group -20.2 mmHg (p<0.001). There was no significant difference in the systolic blood pressure between the Wistar control group and the lupin (p=0.961) and soy (p=0.066) treatment groups.

Arterial responses

The contractile response of the mesenteric arterial rings to 1 µM noradrenaline in the NaCl-fed GK rats was about half that of the normotensive control group (Fig. 2). Lupin and soy treatments normalised the decreased vasoconstriction. Lupin treatment also normalised the endothelium-dependent vasorelaxation to acetylcholine, which was severely impaired in the NaCl-fed GK rats (Fig. 3A). The arteries of the normotensive Wistar rats were more sensitive in endothelium-
independent relaxation to sodium nitroprusside (SNP) than those of the NaCl-fed GK rats (Fig. 3B), and there were no differences between the treatment groups in the relaxation response to SNP.

Table 1 Body weight gain and food and water intake during the two-week treatment period in NaCl-diet fed GK control group (GK), GK rats fed with NaCl and lupin protein diet (Lupin) and GK rats fed with NaCl and soy protein diet (Soy).

<table>
<thead>
<tr>
<th></th>
<th>GK</th>
<th>Lupin</th>
<th>Soy</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, baseline (g)</td>
<td>136.9±6.0</td>
<td>135.3±5.3</td>
<td>138.0±4.3</td>
<td>0.933</td>
</tr>
<tr>
<td>Weight, final (g)</td>
<td>221.5±8.3</td>
<td>203.8±9.3</td>
<td>207.3±6.9</td>
<td>0.433</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>38.4±3.4</td>
<td>38.5±3.0</td>
<td>31.0±2.0</td>
<td>0.251</td>
</tr>
<tr>
<td>Water intake (ml/d)</td>
<td>65.0±0.6</td>
<td>62.9±1.2</td>
<td>64.3±0.6</td>
<td>0.335</td>
</tr>
</tbody>
</table>

1Values are mean ± SEM, n=8 in each group

Fig. 1. The difference in systolic blood pressure (mean ± SEM) in comparison with the Goto-Kakizaki (GK) rats on 6% NaCl diet (GK control group), n=8 in each group. The x-axis indicates GK control group; Lupin, GK rats treated with lupin protein; Soy, GK rats treated with soy protein for two weeks, and W, normotensive Wistar rats. The asterisk (*) denotes the significant difference in comparison with the GK control group (p<0.05)

Fig. 2. The difference in vascular contractile response of rat mesenteric artery rings to noradrenaline (1 µM) (mean ± SEM) in comparison with the Goto-Kakizaki (GK) rats on 6% NaCl diet (GK control group), n=8 in each group. The x-axis indicates GK control group. For abbreviations, see legend for Figure 1. The asterisk (*) denotes the significant difference in comparison with the GK control group (p<0.05)
At the end of the treatment period, total and LDL cholesterol levels were 9% and 10% lower in the lupin group (p=0.06 and p=0.375) and 7% and 18% lower in the soy group (p=0.05 and p<0.05) compared to the control group. HDL cholesterol levels were significantly higher in both the lupin and soy groups (p<0.05). The HDL/LDL ratio was also significantly higher in both the lupin and soy groups (p<0.05). Glucose levels were significantly higher in the soy group (p<0.05).

**Table 2** Plasma lipid and glucose concentrations at the end of the two-week treatment period in NaCl-diet fed GK control group (GK), GK rats fed with NaCl and lupin protein diet (Lupin) and GK rats fed with NaCl and soy protein diet (Soy).

<table>
<thead>
<tr>
<th>Test</th>
<th>GK</th>
<th>Lupin</th>
<th>Soy</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL cholesterol</td>
<td>0.46±0.03</td>
<td>0.41±0.03</td>
<td>0.38±0.04</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>2.00±0.07</td>
<td>1.80±0.05*</td>
<td>1.78±0.07*</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>2.84±0.11</td>
<td>2.59±0.05</td>
<td>2.64±0.10</td>
</tr>
<tr>
<td>HDL/LDL ratio</td>
<td>4.51±0.37</td>
<td>4.58±0.45</td>
<td>5.10±0.50</td>
</tr>
<tr>
<td>Glucose</td>
<td>11.67±0.90</td>
<td>13.38±0.79</td>
<td>15.54±1.34*</td>
</tr>
</tbody>
</table>

*Values are mean ± SEM, n=8 in each group. *p<0.05 vs. GK

**Plasma lipid and glucose levels**

At the end of the treatment period, total and LDL cholesterol levels were 9% and 10% lower in the lupin group (p=0.06 and p=0.375) and 7% and 18% lower in the soy group (p=0.05 and p<0.05) compared to the control group. HDL cholesterol levels were significantly higher in both the lupin and soy groups (p<0.05). The HDL/LDL ratio was also significantly higher in both the lupin and soy groups (p<0.05). Glucose levels were significantly higher in the soy group (p<0.05).
soy group \( (p=0.125 \) and \( p=0.111 \) \) compared to the GK control group (Tab. 2). HDL cholesterol was highest in GK control group. The difference was -0.20 mM \( (p=0.039) \) when compared to the lupin group and -0.22 mM when compared soy group \( (p=0.022) \). There were no significant differences between the groups in the HDL/LDL ratio. Glucose level was higher in the soy group than in the GK control group \( (p=0.018) \), while the glucose level of the lupin group did not differ from that of the GK controls \( (p=0.271) \).

DISCUSSION

In the present study, we compared the effects of lupin and soy protein diets on blood pressure and vascular function in an animal model of type II diabetes, where the development of hypertension was accelerated by sodium loading. Both protein treatments normalised the elevated blood pressure to the level of normotensive controls. Both of the protein treatments also normalised the impaired vasocontraction but only the lupin treatment improved the severely diminished endothelium dependent vascular relaxation.

The attenuation of the hypertension can be explained by the improved vascular function observed in the lupin and soy groups. In the lupin group the effect on blood pressure was greater than in the soy group, and also the improvement of the impaired vasodilatation was more marked in this group. One possible explanation for the positive effect in the lupin group could be the relatively high arginine content of lupin protein; 99.3 mg/g protein in lupin and 57.8 mg/g in soy \( (6,10,11) \). Arginine is a physiological substrate for endothelial nitric oxide synthase (eNOS) and supplementation with L-arginine has been shown to improve vascular function and attenuate blood pressure in other animal models of salt-induced hypertension, the Dahl salt-sensitive rat \( (20,21) \) and DOCA-salt hypertensive rat \( (22) \), as well as in diabetic OLETF-rat \( (23) \). This is in agreement with our observations in Dahl salt-sensitive rats on a six-week dietary arginine supplementation with the amount of arginine comparable to that of the lupin group \( (Pilvi et al. unpublished data) \). The tenability of arginine-hypothesis, should, however be further confirmed in Goto-Kakizaki rats.

Another possible mechanism behind the improved endothelial function could theoretically be through the hypocholesterolemic effect of the proteins. Both proteins studied have been shown to have a positive effect on lipid profiles, and their ability to upregulate LDL-receptors in hepatocytes \textit{in vitro} has been established \( (4,7) \). In the present study there was no statistical difference between the groups in the serum lipid levels, but since this was a short-term study of normocholesterolemic animals, the changes in these variables can only be minimal. To evaluate the effects on serum lipid levels the study should be updated in animals on a lipid diet.
Although it has been suggested that the isoflavones in soy may be the main compounds responsible for the beneficial cardiovascular effects (2, 16, 24), the results of this study indicate otherwise. If only the results obtained from the soy group are considered on their own, the effect of isoflavones cannot be ruled out on the basis of the results of this study. On the other hand, neither in the levels of SBP, nor in the vascular responses, were there any statistical differences between the lupin and the soy treatment groups. Since the amount of isoflavones in lupin protein is minimal (7), it is more likely that the protein itself, rather than the phytoestrogens, is responsible for the beneficial cardiovascular effects. This is in accordance with epidemiological data, which show that an increased protein, and especially plant protein intake is associated with lower blood pressure and an attenuated increase in blood pressure over time, at least in humans (25). The mean intake of lupin protein in the lupin group in this study was 24.1±1.9 g/kg/day. Interestingly, results from a study in Polish subjects indicate beneficial cardiovascular effects of a 30-day treatment of only 36.6 g of lupin protein/day (28).

Like many other dietary protein sources, soy is also known to contain angiotensin converting enzyme (ACE) inhibiting peptides, which have blood pressure lowering effect (26, 27). The soy protein derived ACE-inhibiting peptides have been demonstrated to have an effect on sodium excretion in addition to their blood pressure lowering effect (26). This type of bioactive peptides have so far not been described in lupin protein, but this possibility should be evaluated.

Both plant protein treatments attenuated the development of hypertension, which is most likely due to the improved vascular function. Both the lupin and soy protein treatment normalised the impaired vasocontraction observed in the NaCl-treated GK rats, but only the lupin treatment improved the severely diminished vascular relaxation. The positive effect of the plant protein treatments on blood pressure is independent of lipid profiles or blood glucose levels. The possible beneficial cardiovascular effects of lupin protein in other animal models as well as in humans and the mechanisms involved remain to be clarified.

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