

Non-Hydrolysed and Hydrolysed Soy Protein

A Human Immunological Study

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Daily consumption of soy protein has increased during recent years. Twelve healthy subjects and 12 atopic patients were given 30 g non-hydrolysed soy protein (NHS) or 30 g hydrolysed soy protein (HS) daily during a 3-month period. There were no changes in the following immunological parameters: IgE with specificity for NHS or HS, IgG with specificity for NHS, crossed radioimmuno-electrophoresis with NHS and skin prick test using NHS or HS. Hypertrophy of the pancreas in rats exposed to excessive peroral intake of soy flour has been reported. In our study the size of the pancreas was measured before dosing began and at the end of the study by means of ultrasound. No change in size was shown. Daily intake of 30 g NHS and 30 g HS gave rise to gastrointestinal discomfort, while 15 g of both soy protein preparations were usually accepted.

Key words: crossed radioimmuno-electrophoresis; food intolerance; hydrolysed soy protein; nutrition; pancreas scanning; soy protein.

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CLINICAL ASPECTS

In the food industry it is usual to add soy protein as a cheap protein enrichment. Furthermore, many cow milk intolerant infants are often given cow milk substitutes containing soy protein (NHS). The question is, will this create more soy allergic patients in the future?

Hydrolysed soy protein (HS) has a small molecular weight. Smaller molecules are often not very allergenic. It might be of great importance if it could be shown that hydrolysed soy protein is hypoallergenic.

Twenty-four persons, 12 atopics and 12 non-atopics, were given a diet of 30 g non-hydrolysed soy protein (NHS) or 30 g hydrolysed soy protein (HS). During a 3-month period there were no changes in the specific or non-specific immunological parameters investigated. 15 g of both soy protein preparations was tolerated while 30 g of NHS caused gastrointestinal discomfort in 15% of the atopics and in almost all of the non-atopics. Thus, HS was not shown to be better tolerated than NHS. Further investigations are needed before the allergenic properties of NHS and HS can be evaluated.

The daily consumption of soy protein has increased during recent years, as the food industry is adding soy protein to prepared foods as a cheap protein enrichment. Furthermore, many cow milk intolerant infants are given cow milk substitute containing soy protein. Soy protein taken orally can provoke anaphylactic shock, urticaria and gastrointestinal symptoms, and immediate and late type asthma (2, 4, 6, 7, 9, 10, 11, 12). Hydrolysis has enabled the

production of soy proteins with low molecular weight (2000–8000 dalton). Most allergens have a molecular weight above 10,000–50,000 dalton. Smaller molecules are not very allergenic, and therefore the soy protein hydrolysate might be hypoallergenic.

The main purpose of this study has been to compare the immunological response to hydrolysed soy protein (HS) and non-hydrolysed soy protein (NHS) in 12 healthy subjects and 12

atopic patients given 30 g NHS or 30 g HS daily during a 3-month period. In addition, the influence on the size of the pancreas has been evaluated by means of ultrasound, as nutrition experiments in rats given soy protein have shown enlargement of the pancreas.

MATERIAL AND METHODS

Participants

Twelve healthy volunteers and 12 allergics took part in the study. The non-allergic participants all had normal total IgE examined by PRIST (Pharmacia) and negative prick test for NHS. The allergics had asthma and/or rhinitis and a total IgE between 200–1000 U/ml (normal range 3–263 U/ml). The skin prick test for NHS was negative. Persons suspected of food allergy were not included in the study. The age of the participants was between 20–60 years. The mean age of the atopics and healthy volunteers was 27 years, and 32 years respectively.

Study design

The study was performed during 3 months in the summer of 1980. Six of the allergics and six of the healthy volunteers consumed 30 g NHS daily and the remaining 30 g HS daily. Table 1 shows the type and content, either NHS or HS, of the food offered to the participants. Starting 2 weeks before changing to one of the two soy protein diets, the participants kept a daily record of their symptoms from lungs, eyes, nose and intestinal tract, using the following score system: 1 = light, 2 = moderate, 3 = severe. During this study the participants' daily diets were unchanged apart from a reduced consumption of ordinary foods, as the food containing soy protein corresponded to approximately 500–900 kcal. The body weight of the participants was recorded before and after the trial.

Blood chemistry

The participants came to the out-patient clinic before commencement of the study, and after 7,

30, 60 and 90 days. At each of these visits the following was examined: haemoglobin, leucocyte, and differential counts, thrombocytes, eosinophils, plasma-protein, -albumin, -orosomucoid, -glucose, -sodium, -potassium, -cortisol (morning), -thyroxin, T_3 -RIA, T_3 -resin test, serum-calcium, -cholesterol, -triglyceride.

Immunological test

At the same intervals as mentioned above the following was examined: Immunoglobulin G, A, M and E, allergen-specific IgE by A1-RAST (against NHS and HS) and IgG with specificity for NHS. Before and after the trial crossed radioimmuno-electrophoresis (CRIE) with NHS was performed. For technical reasons CRIE and specific IgE determinations were only carried out for NHS.

Skin prick testing

Before and after the trial all participants had a skin prick test with NHS, HS and soy antigens (from Allergologisk Lab., Copenhagen). The soy protein extract was used in concentration 1:20 w/v. Skin prick test was performed on the volar side of the forearm. As a positive reference histamine chloride 1 mg/ml was used and

Table 1
The composition of the individual soy protein-containing foods used in the study

Product	Quantity	Soy protein content
Strawberry canned	250 g	15 g non-hydrolysed soy protein
Flyte I	1 piece	30 g non-hydrolysed soy protein
Flyte II	1 piece	15 g non-hydrolysed soy protein
Strawberry jelly	500 g	15 g non-hydrolysed soy protein
Cherry drink	1 liter	30 g hydrolysed protein
Orange drink	1 liter	30 g hydrolysed protein
Grape drink	1 liter	30 g hydrolysed protein

Table 2

The amino acid content in hydrolysed (Rynkeby) and non-hydrolysed soy protein

Amino acids	%	g/16 g N
Aspartic acid	7.6	12
Threonine	2.2	3.4
Serine	2.9	4.5
Glutamic acid	13.0	20.0
Proline	3.8	5.9
Glycine	2.6	3.9
Alanine	3.8	5.8
Valine (98 %)	3.3	5.0
Isoleucine	3.2	5.0
Leucine	5.2	8.0
Tyrosine	2.3	3.5
Phenylalanine	3.4	5.2
Lysine	4.3	6.6
Histidine	1.8	2.8
Arginine	4.7	7.2
Cystine (80 %)	0.80	1.2
Methionine (85 %)	1.0	1.6
Tryptophane	0.95	1.5
Raw protein (N $\times$ 6.25)	65.0	

the methods according to the Nordic Guidelines were followed (3).

Description of soy protein

Non-hydrolysed soy protein is washed white soy flakes with a 70% protein content, free from fats and water soluble carbohydrates. They are native proteins with a mol wt of 8,000–600,000 dalton. 50–60% of the proteins have a mol wt of 180,000–350,000 dalton (7S–11S fraction). Hydrolysed soy protein (Rynkeby) consists of HS in which 10% of the peptide bonds are cleaved. The hydrolysate consists mostly of peptides with an average of 7–10 amino acids per strand, which corresponds to a mol wt of 800–2,000 dalton. The amino acid composition of the two soy products is shown in Table 2. The percentage composition is the same for the two products.

NHS was baked in bread or mixed in the fruit preserves in yoghurt. HS was added to liquids with fruit taste. These drinks were made of natural products without additives.

Crossed radioimmuno-electrophoresis (CRIE) analysis

Equipment, reagents and general procedure for crossed immunoelectrophoresis (CIE) were as described by Axelsen et al. (1). Agarose 1% (w/v) was suspended in buffer as described for soy antigen, but the ionic strength was 0.1 for electrophoresis. The first dimension electrophoresis was performed at 10 V cm⁻¹ for 60 min and the second dimension at 3 V cm⁻¹ for 18 h, both at 18°C.

The antigen used in CRIE was 1% (w/v) white soy flakes suspended in Tris/diameal buffer, pH 8.6, Tris 0.073 M, barbital 0.023 M, calcium lactate 0.006 M, ionic strength 0.5, adjusted with NaCl 29.3 g/l, 0.02 M CaCl₂, 3% (w/v) polyethylene glycol 6000 and 0.2 M B-mercaptoethanol were added, mixed for 1 h at room temperature and centrifuged for 15 min at 1000 g. This solution is stable for 4–8 h. Fig. 1 shows a CIE of soy protein (soy white flakes) and CRIE from a soy allergic patient.

Soy protein specific IgG

Radioimmunoprecipitation technique. The assay used was a double-antibody assay. The procedure was as follows: 100 μ l of serum diluted 1:15 in test buffer (sodium borate 55 mmol/l, sodium chloride 100 mmol/l, human serum albumin 14.5×10^{-6} mol/l, sodium acid 0.015 mol/l, pH 8.0) and 100 μ l ¹²⁵I-labelled antigen were allowed to react for 3 h at room temperature, followed by incubation overnight at 4°C with 100 μ l rabbit anti-human IgG (Fc-specific, DAKO, Copenhagen, Denmark) diluted 1:1 with test buffer. To remove non-bound activity the precipitates were washed four times with 2.5 ml test buffer. Centrifugation was performed for 15 min at 3000 rpm. The bound activity was measured in a gammacounter. Non-specific binding in the assay was evaluated individually by inclusion of replicates of each serum sample containing excess of non-labelled soy protein (5).

The optimal amount of anti-IgG for precipitation was ensured by examining the supernatants from a patient with a high total IgG level and from a 1000 donor pool according to

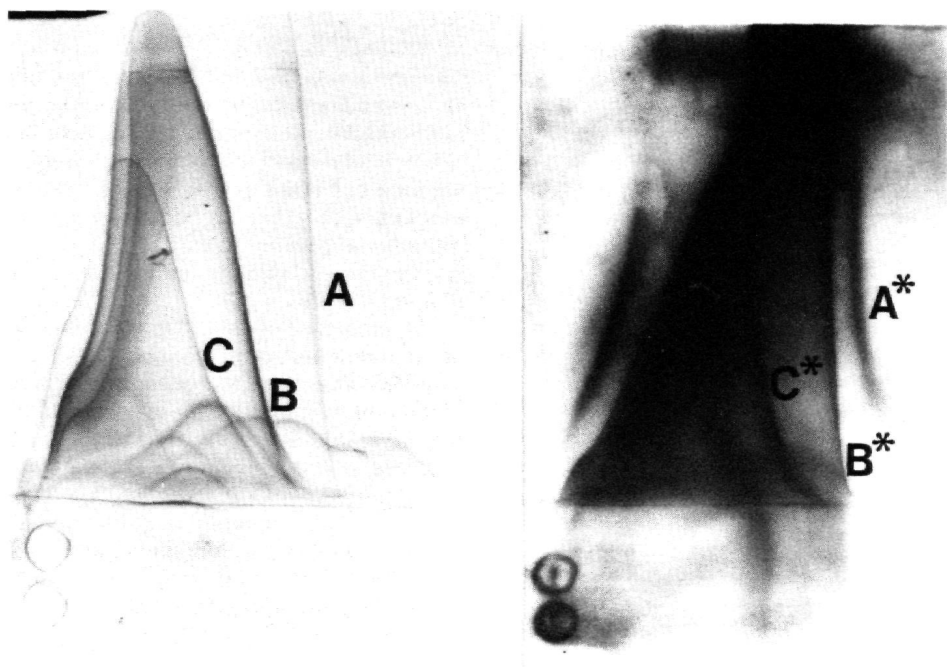


Fig. 1. Left: Soy protein crossed immunoelectrophoresis. Three of the precipitates corresponding to the CRIE picture on the right are marked A, B and C. Right: Autoradiogram (crossed radioimmuno-electrophoresis) from a soy protein allergic patient. Note the three clearly marked precipitates: A*, B* and C*. These are three of the four major allergen precipitates of importance in most soy allergic patients.

Sewel (13). The non-specific binding was subtracted from all assay results and the net cpm used for calculations.

The assay was performed in duplicate.

Preparation of ^{125}I labelled soy proteins. The substance concentration of the soy protein used for iodination was determined by the optical density at 215 and 225 nm on a Unicam SP 1800-spectrophotometer (14).

Soy protein was labelled by the chloramine-T technique according to Hunter (5). To 5 μg protein was added 100 μl 0.1 mol/l phosphate buffer pH 7.5 and 10×10^{-9} mol/l chloramine-T in the presence of 1 mCi sodium ^{125}I in a total volume of 120 μl . After 40 sec of agitation 52×10 mol of sodium metabisulphite in 20 μl was added to stop the reaction and 15 sec later the reaction mixture was diluted with 100 μl 0.1

mol/l sodium phosphate buffer, pH 7.5, containing 14.5×10^{-6} mol/l human serum albumin as carrier protein and potassium iodine 0.03 mol/l. Non-protein bound iodine was removed by gelfiltration on a Sephadex G-50 column (Pharmacia, Uppsala, Sweden). Bed volume was 50 ml and flow rate 40 ml/h. The elution was performed with a phosphate buffer (pH 7.5), containing human serum albumin 14.5×10^{-6} mol/l and sodium chloride 0.1 mol/l. Fractions of 600 μl were collected. The pooled ^{125}I -labelled antigens were 80% precipitable by trichloroacetic acid 0.6 mol/l. Specific activity achieved was $1\text{--}3 \times 10^{-4}$ cpm/ng.

Ultrasonic scanning of the pancreas

The size of the pancreas was measured using an ultrasound test before the dosing began and

after the end of the study to show any changes.

For the ultra-sound test a mechanical, dynamic sector scanner (Aloka type 115) was used with a single transducer of 2.25 MHz.

The scannings were made on persons fasting 6–8 h, and the examinations were carried out by an experienced ultrasound physician. The research subjects were scanned in random order before and after the dose-test and the examiner had no knowledge of whether the persons were atopic or non-atopic. Transverse/oblique scans were carried out corresponding to the longitudinal axis of the pancreas and longitudinal scanning through the aorta. Only those research subjects who offered optimal scanning pictures with regard to production of the morphology of the pancreas and reproducibility after the dose-test were included in the series.

On the transverse/oblique scanning pictures the antero-posterior diameter of the pancreas was measured in front of the middle of the portal vein (head of pancreas) immediately in front of the superior mesenteric artery (body of pancreas), and by a reproducible number of mm left of the superior mesenteric artery (between 10 and 20 mm) (body/tail of pancreas). In addition, the distance from the duodenal arch to the superior mesenteric artery was measured (head of pancreas). On the longitudinal scanning pictures the antero-posterior diameter of the pancreas in front of the splenic vein and the cranio-caudal diameter in front of the superior mesenteric artery were measured.

Participants were weighed before and after the trial.

RESULTS

Subjective clinical symptoms

Most of the non-allergics had subjective discomfort, evenly distributed between the NHS and HS groups. Two patients from each soy group had to retire after 10 days due to severe swelling of the abdomen, diarrhoea and stomach pain. The symptoms disappeared without treatment after 2 weeks. These two persons were working at the same place and might have

suffered from severe infectious gastroenteritis. The remaining 10 had slighter symptoms with swelling and tendency to diarrhoea for 1–3 weeks, a single case for 6 weeks. This resulted in dose reduction to 1/2 or 3/4 dose for the whole period for two participants and for 2–7 weeks for five participants. Only two of the allergics had discomfort in the form of swelling and nausea for 3 and 6 weeks respectively. Both NHS. One of the patients reduced the intake of soy protein to 1/2 dose for 1 month and 3/4 dose the following month. Neither participants were taking HS. One had diarrhoea for 1 day. One participant could not take more than 3/4 dose NHS due to lack of appetite.

Para clinical data

Non-immunological blood assays showed no significant changes.

Immunological blood assays

IgE. Total IgE was increased in some grass pollen allergics at the beginning of the study; this could have been due to grass pollen exposure in June/July, which stimulated the production of specific IgE directed against grass pollen. During the experiment none had increased RAST-values for NHS or HS.

CRIE tests gave a positive precipitation line for one individual prior to the test and an unchanged arc after. By means of an inhibition technique it was demonstrated that the radio-staining was allergen specific.

Soy protein-specific IgG. Four sera from patients with allergic diseases contained measurable amounts of soy protein-specific IgG before the trial. However, the amount was very small, ranging from 2.2 to 2.6 times the non-specific binding in the assay. In one of the patients specific IgG increased from 2.5 to 3.8 times non-specific binding during the trial (the coefficient of variation for the IgG as assay was 20%, whereas the mean coefficient of variation within experiments was 5%). The amount of specific IgG showed no correlation with clinical

side effects. None of the normal controls contained measurable amounts of specific IgG before or at the end of the trial.

Weight. Seventeen participants had a weight increase of between 0.7 kg and 3 kg and four patients remained unchanged.

Skin prick test. Skin prick test using NHS and HS remained negative (less than half the area of positive histamine reference 1 mg/ml of histamine chloride) during the trial.

Ultrasound scanning of pancreas. Twenty-two subjects were scanned by ultrasound, of which five had to be excluded due to unsatisfactory picture quality. Seven of the 17 persons included in the material were atopics and 10 were non-atopics.

The average antero-posterior diameter of the pancreas in front of the portal vein was 7.1 mm (5–16 mm) before, and 6.9 mm (5–16 mm) after the dose test. The corresponding diameters in front of the splenic vein were 8.0 mm (5–16 mm)/7.9 mm (6–16 mm), 12.2 mm (9–20 mm)/12.3 mm (10–20 mm) and 7.8 mm (5–16 mm)/8.1 mm (5–16 mm). The average distance from the duodenum to arteria mesenterica superior was 37.9 mm (30–50 mm) before and 39.1 mm (30–50 mm) after the dose test.

The average cranio-caudal diameter of the pancreas in front of the superior mesenteric artery was 25.6 mm (20–34 mm) before and 25.8 mm (11–34 mm) after the experiment.

The average difference in the antero-posterior diameter (thickness of pancreas) before and after the dose test was an increase of 0.1 mm, and the average difference in the cranio-caudal diameter in front of the superior mesenteric artery (width of pancreas) was an increase of 0.2 mm. The average difference in the distance from the duodenum to the superior mesenteric artery before and after the dose test was 1.2 mm.

In 17 of 22 of the participants ultrasound scanning was of such good picture quality that it was possible to make an accurate measurement of the size of the pancreas.

Measurement before and after the study

showed no statistically significant change in the parameters mentioned.

DISCUSSION

In this study of atopic and non-atopic subjects on a fixed daily intake of either NHS or HS it is possible to show a change in the specific immunological response to soy antigens, apart from a single immunological parameter. Thus there was a significant, but still slight increase in soy-specific IgG in one atopic, who otherwise showed no other signs in laboratory tests or clinically, which could be described as soy allergy. Surprisingly enough there were a considerable number of participants who reacted with subjective discomfort in the form of swelling of the stomach, nausea and tendency to diarrhoea. This tendency was much greater in the non-atopics than in the atopics.

Because of these side effects, eight non-atopics had to reduce their daily soy intake in varying periods, two were taken out of the study. In the atopic group only three had gastrointestinal discomfort, but in only one of them did this lead to a dose reduction for a period. This discrepancy between the two groups is inexplicable. There has been no difference in frequency of side effects between the two soy preparations. NHS was washed white soy flakes and HS was made from washed white soy flakes. It is possible that a small amount of oligosaccharides was left in the soy protein preparations even after washing. If this was the case it could explain the stomach ache, as during digestion in the intestine oligosaccharides will produce a great amount of air. In 1980 McGuinness et al. (8) found hypertrophy and hyperplasia of the pancreas in rats exposed to excessive peroral intake of soy flour. Therefore, in this study we have attempted by means of ultrasound scanning to measure the pancreas immediately before and in the last week of the dose test in order to show any changes.

With the ultrasound scanning technique described it was not possible to show any enlargement of the pancreas during the 3 months of daily soy protein intake.

From our study it can thus be concluded that

a daily intake of both 30 g NHS and 30 g HS gave rise to subjective gastrointestinal discomfort unacceptable to 15% of the atopics and nearly all of the non-atopics. In most of these cases 15 g of soy protein was acceptable. We have not revealed the pathogenetic mechanisms responsible for this intolerance, but an IgE-mediated allergy seems excluded and a pancreatic dysfunction is not substantiated.

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