Effects of Consumption of Processed Soy Proteins on Minerals and Digestion in Man

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ABSTRACT

Most of our knowledge of digestion and utilization of minerals and other components of foods is derived from model experiments with animals. Results indicate that digestion and utilization depend not only on type of foodstuff, but also on, e.g., animal species, nutritional status and overall diet composition. Despite these limitations, such model experiments help us in delineating problem areas and in finding solutions. To verify the conclusions drawn, tests in human beings, preferably under normal living conditions, are needed. Results of animal experiments with soy protein materials indicate that no major effects from the consumption of heat-treated soy proteins are to be expected. As systematic human experiments are scarce, we carried out a large scale experiment to assess acceptation and the possible physiological effects of substituting soy proteins for conventional proteins in the usual diet. We compared two diets in a 4 + 4 week crossover design with 92 healthy volunteers. One diet contained a wide variety of soy protein foods, mainly made with concentrates (test diet), about 25% of the protein intake being from soy. The other diet (control) contained products made from conventional protein sources. Although most of the 100 parameters investigated showed no difference, statistically significant reactions to soy were observed in mineral metabolism and digestion. All these changes are well within normal physiological ranges, do not indicate unfavorable trends, and can be explained by adaptation to shifts in dietary composition due to substitution of soy protein materials for conventional protein sources. In a separate experiment with 12 subjects, the effect of processing soy proteins on flatulence was investigated. It was found that refining can remove up to two-thirds of the oligosaccharides stachyose and raffinose, with a parallel decrease in volume of intestinal gas production. We conclude that these results confirm the prevailing view that soy protein materials are acceptable for our daily food.

INTRODUCTION

In the Far East, soybeans have been widely accepted as a common protein source for thousands of years. In contrast, soy protein materials acceptable for the Western palate have been available only recently. Defatted meals, concentrates, and isolates are the result of new technological processes, and have to be considered as novel proteins. As a consequence, their effects on health and well-being have to be assessed.

Most of our knowledge of nutritional properties is derived from model experiments with animals under standardized conditions. Such models help us in delineating problem areas, and in optimizing processing. The advantages of the animal model are: factors can be studied in isolation or in combination, and extreme conditions are possible. The disadvantages of the animal model are that the human body may react differently, and that the conditions used are far from the everyday human experience, especially in Western societies: we are used to a wide variety of mixed foods, and not to a standard laboratory chow.

On the basis of numerous chemical analyses and animal experiments (1), one may conclude that properly processed soy protein materials are unlikely to cause health problems. Experience with human soy consumption, as e.g. in the US school lunch program, seems to warrant this conclusion. Because model findings are based on a wide variety of procedures, products, diets and animals, we felt the need to supplement these data by appropriate human studies, as recommended by the former Protein Calorie Advisory Group (PAG) of the United Nations (2).

This paper discusses aspects of mineral metabolism and digestion as investigated in our experiments with volunteers under everyday conditions.

EXPERIMENTAL

Our first study was designed to detect possible physiological and behavioral effects of replacing conventional protein sources by soy protein sources in the diet, under the following conditions: a continuously high soy protein intake for a long period; normal patterns of eating and living; an experimental model that can detect small changes.

We designed two experimental diets in the form of identical, isonitrogenous, four-week menu cycles. About one quarter of the protein in the soy diet was from soy, mostly refined concentrates prepared by alcohol extraction. The control diet had the same food items, made with only conventional proteins. Diets consisted of normal meals and were organoleptically similar. Table I shows examples of the 52 products that were made both in soy and control

TABLE I

Examples of Products Specially Prepared for the Experiment. A Total of 52 Products Was Developed in Both a Soy and a Control Composition. The Source of Soy Protein Was Refined Soy Concentrate.

Examples		Soy protein % of total protein
Entrees	soup	56
	ragout	73
Main dish	hamburgers	55
	goulash	68
	chili con carne	50
	fishburgers	53
Dessert	pudding	100
	pancakes	54
Cold cuts	paté	51
Bread and cake	assorted breads	60
	biscuits	60
Snacks	sausage rolls	39
	nut chocolate	92

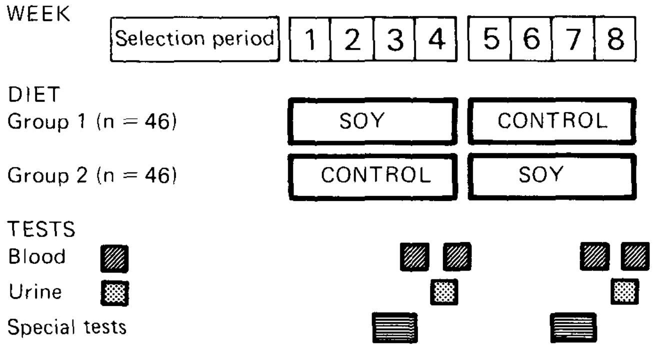

FIG. 1. Design of the experiment for physiological effects of a high soy intake. The diets were identical four week menu cycles. In the soy diet, 25% of the protein was from soy protein concentrate.

varieties. Despite the requirement of organoleptical similarity, the average soy protein intake could be maintained at 23 g a day.

The diets were compared with 92 volunteers in a 4 + 4 week crossover design (Fig. 1). This design gives a maximum sensitivity to detect differences due to the two diets. The experiment was successfully finished after 8 weeks with 89 participants. Both diets were equally well accepted, food and nutrient intakes were similar, guaranteeing that the reactions could be ascribed to the different protein sources and not to differences in food habits.

The criteria we investigated are listed in Table II. Those changes that were statistically significant ($P_2 \leqslant 0.05$) are in italic type. All these changes were relatively small, and well within normal clinical limits. This paper discusses the findings on mineral metabolism and digestion. For a discussion of other aspects, see (3).

TABLE II

Analyses Performed during Comparison of Soy and Control Diets over Two Four-Week Periods with 92 Subjects

Food intake	Acceptance-Protein-Carbohydrate-Fat-Alcohol-Energy
Haematology	RBC-Hb-PCV-MCV-MCH-MCHC-WBC-Monocytes *Lymphocytes* - Banded Neutrophils - *Segmented Neutrophils* - Eosinophils - Basophils - ESR Platelet Aggregation
Serum enzymes	γGT - ICHD - GPT - GOT - *AP* - LDH - αHBDH - CPK
Serum immunology	*IgA(♀♀)* - Ige - IgG - IgM - *Soya Specific IgE(♀♀)* Immunoconglutinin
Serum, other	Albumin - Protein - Urea - Uric Acid - Glucose Bilirubin - Glycerol - *Cholesterol* - T_3 Ratio Thyroxin - *Inorg. Phosphate (Fasting,* Fed) Na - K - Ca - *Mg (Fasting, Fed)* - Cl - Fe - Fe Binding Fe Saturation
Feces	Weight - Dry Matter - Triglycerides - Bile Acids *Fatty Acids* - Nitrogen - Ca - *P* - *Mg* - *Zn*
Urine	Volume - pH - Urea - Uric Acid - Creatinine Nitrogen - Ca - Mg
Miscellaneous	*Defecations* - Intestinal Transit - *Body Weight* G.I. Reactions - Flatulence - Blood Pressure

[a]Analyses showing significant differences between diets ($P_2 \leqslant 0.05$) are in italic type.

MINERAL METABOLISM

Recently, both Rackis and Anderson (4) and O'Dell (5) have reviewed the factors that affect mineral bioavailability in soy protein products. Soy protein materials can be classified as having low or high bioavailabilities, depending on conditions of manufacture. The use in animal experimentation of industrial grade soy isolates manufactured for technical uses like glueing and paper sizing has contributed to misunderstandings in this area.

Under practical dietary conditions, availability studies can give completely different results, owing to several interfering factors, such as the presence of other minerals; the source(s) of these minerals; the presence of chelating compounds like phytate, fiber and carbohydrates; the binding of these chelating agents to proteins.

Results of this study throw some light on the question whether high levels of soy protein concentrate in a normal Western diet significantly alter mineral metabolism (see

TABLE III

Effect of a High-Soy Protein Diet on Mineral Metabolism: Mean Values in Blood Serum, Urine and Feces after Four Weeks Consumption of Experimental Diets[a]

Criterion		n	Diet: Soy	Diet: Control	Diff.	P_2 Value
Calcium - serum	mmol/l	20	2.64	2.66	-0.02	0.61
- urine	mmol/14h	20	4.9	5.2	-0.3	0.42
- faeces	mmol/24h	20	23.4	23.5	-0.1	0.92
Inorganic phosphate						
- serum (fasting)	*mmol/l*	*85*	*1.14*	*1.17*	*-0.03*	*0.04*
- serum (postalimentary)	mmol/l	20	1.10	1.14	-0.04	0.07
Total phosphorus-faeces	*mmol/24h*	*20*	*23.0*	*20.6*	*2.4*	*0.05*
Magnesium						
- serum (postalimentary)	*mmol/l*	*20*	*0.87*	*0.82*	*0.05*	*0.01*
- serum (fasting)	*mmol/l*	*85*	*0.75*	*0.76*	*-0.01*	*0.04*
- urine	mmol/24h	88	5.24	5.27	-0.03	0.86
- faeces	*mmol/24h*	*20*	*11.7*	*10.1*	*1.6*	*0.02*
Sodium-serum	mmol/l	20	135.1	135.8	-0.7	0.37
Potassium-serum	mmol/l	20	5.0	5.1	-0.1	0.13
Chloride-serum	mmol/l	20	102.6	101.7	0.9	0.16
Zinc-serum	μmol/l	89	17	16	1	0.16
Zinc-faeces	*μmol/24h*	*20*	*192*	*216*	*-24*	*0.02*
Iron-serum	μmol/l	89	19.9	20.0	-0.1	0.92
Iron-binding capacity	μmol/l	89	62.3	61.8	0.5	0.29
Iron saturation	%	89	32.6	33.2	-0.6	0.67

[a]Statistically significant differences ($P_2^2 \leqslant 0.05$) are given in italic type.

TABLE IV

Trypsin Inhibitor Activity of Finished Products, Analyzed According to a Modification of (8), in mg of Trypsin Inhibited by 1 g Product (Mean ± SEM)

Product	n	Soy	Control
Bread	12	0.17 ± 0.014	0.17 ± 0.018
Sausages	6	0.21 ± 0.035	0.21 ± 0.027
Minced meat	7	0.26 ± 0.021	0.27 ± 0.015
Meat stews	6	0.15 ± 0.034	0.17 ± 0.025
Rice and noodle dishes	6	0.08 ± 0.017	0.08 ± 0.011

Table III). The isonitrogenous exchange of proteins (mainly soy for meat) caused the soy diet to provide more calcium, phosphorus, magnesium and potassium, but less zinc than the control diet. In addition soy products contributed to the phytate content of the diet, but much less than the level found to induce reduction of mineral absorption (4). Serum calcium remained unchanged upon switching from one diet to another, as were urine and feces' values.

Phosphorus in soy is primarily bound to phytate. The serum inorganic phosphate levels were somewhat lowered after soy feeding. Most likely, this small change has no physiological significance, but it occurred so systematically that the difference is statistically significant, thus underlining the sensitivity of the experimental design. The higher phosphorus excretion with the soy diet may be ascribed to its higher intake.

The magnesium levels clearly reflect the higher intakes from the soy diet. An increase after a meal is followed by a reaction in the fasting state in the form of a very small but systematic lowering. The increased intake is excreted via the feces. Literature (5) suggests that this usually occurs via the urine. Sodium, potassium, and chloride in serum remained unchanged. The lower fecal excretion of zinc in the soy diet period reflects the lower intake. The serum zinc levels remained unchanged. Recent findings (6) with adolescent girls indicate that zinc availabilities in meat and soy are similar, and that soy protein does not affect zinc utilization under practical conditions.

Iron bioavailability in properly processed soy protein materials approaches that of iron in animal foods (7), and iron levels of our control and soy diets were comparable. It therefore is understandable that serum iron, iron-binding capacity, and iron saturation showed no change upon consumption of soy proteins. All reactions that we found in mineral metabolism were small, they did not indicate long term risks, and can in general be described as adaptations to changes in the dietary composition.

DIGESTION

Raw soy proteins contain components that inhibit the protein-splitting enzymes of the intestinal tract. Such trypsin inhibitor activity is a common property of many foods (1) and is reduced to acceptable levels by heat treatment. We analyzed samples of both soy and control products after preparation, using a modification of Kakade's method (8). As the results in Table IV show, no differences between the diets were observed. This means that introduction of properly heated soy proteins does not lead to appreciable changes in overall trypsin inhibitor activity of the diet. Results of fecal analyses are shown in Table V. The higher fatty acid excretion in the feces during the control period equals ca. 1% of the total fat intake. Although statistically significant, this observation has no practical meaning. One might speculate that a small part of the fatty acids in animal tissue is structurally bound to cell membrane material and hence more difficult to digest.

The higher defecation frequency on the soy diet comes down to one defecation every fortnight. This is a very small increase. Together with a lack of change in other criteria such as excreted fecal mass, dry matter content and intestinal transit time, it suggests no increase of dietary fiber activity by the soy protein products. Our diets, however, contained a fair level of dietary fiber. Moreover, comparison of the fecal characteristics with those observed in other investigations (9,10) indicates that our values are typical for consumers of high fiber Western diets. Consequently, any extra contribution from soy products in the diet may have had little effect. It would be interesting to investigate whether the indigestible carbohydrate in soy concentrate will have an effect in subjects with a low fiber food pattern, the group that is most in need of some intestinal stimulation.

FLATULENCE

In the previous investigation, the subjects consistently reported a higher incidence of intestinal gas production during the soy diet period. We wanted to express these findings in an objective way, and made standardized sound recordings on 20 men in sound-proof rooms after test meals. The ear clearly discriminates the different characteristics of the sound tracks, and the recordings on paper are visibly different, but efforts to express these differences in numbers for further evaluation failed. Although we do not consider flatulence a health problem, it is socially unacceptable to many people.

Therefore, we devised a separated experiment to answer two questions: 1. What is the intestinal gas production after a soy protein meal in comparison with a conventional mean? 2. To what extent can flatulence be reduced by refining of soy concentrates?

Flatulence in soy and other legumes is mainly ascribed to the microbial degradation in the intestine of the undigested oligosaccharides raffinose and stachyose (11). During the refining process, these compounds are partially removed by the alcohol-water extraction. We compared

TABLE V

Effect of a High-Soy Protein Diet on Digestion, Mean Fecal Values: Feces were Collected for 5 Days towards the End of Each Period

Criterion		n	Diet: Soy	Diet: Control	Diff.	P_2 Value
Excretion	g/24h	20	185	174	11	0.17
Dry matter excretion	g/24h	20	40	39	1	0.64
Dry matter content	%	20	22.3	23.0	-0.7	0.19
Total Fatty Acids	*mmol/24h*	*20*	*9.8*	*12.2*	*-2.4*	*0.009*
Triglycerides	mmol/24h	20	0.66	0.64	0.02	0.83
Bile acids	mmol/24h	20	0.61	0.55	0.06	0.38
Nitrogen	mmol/24h	20	148	150	-2	0.85
Defecations	*no/24h*	*89*	*1.17*	*1.11*	*0.06*	*0.001*
Intestinal transit time	h	20	40	37	3	0.47

TABLE VI

Soy Protein Materials for the Flatulence Study: Twelve Subjects Were Given Test Meals Containing 75 g of the Materials on Four Consecutive Days; Meat and Fish Were Used for the Control Meals

Soy protein material		Raffinose %	Stachyose %	Sucrose %
Defatted meal	(50% protein)	1.14	5.54	7.39
Refined concentrate	(60% protein)	0.48	3.28	0.41
Refined concentrate	(70% protein)	0.19	1.78	0.42

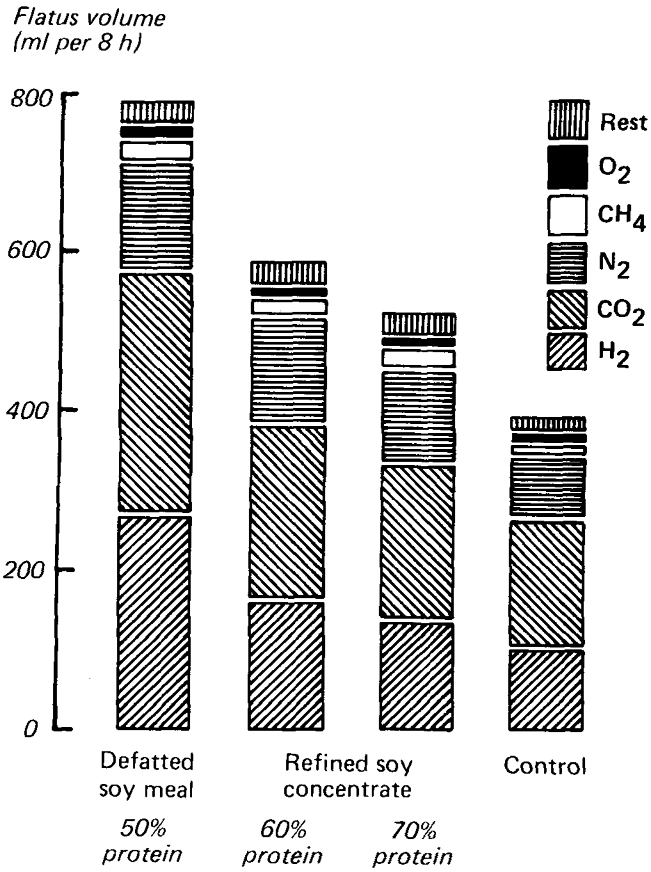

FIG. 2. Effect of several soy protein materials in test meals on flatus production in man. Meals contained 75 g (dry basis) of the test materials.

four test meals, differing only in the protein source, viz: defatty soy meal; a 60% protein refined soy concentrate, as used in the previous experiment; a 70% protein concentrate; meat and fish as control. Oligosaccharide contents are shown in Table VI.

The test meals were offered to twelve students, members of a rowing team, after their daily training. The meals were eaten on four consecutive days at 11:00 a.m., in a double-blind setting. From 2 to 10 hr after the meal, egested flatus was collected, using Stomahesive® (E.R. Squibb & Sons, Inc.), a skin adhesive material, via polyethylene tubing connected with multilayer laminated bags. The volume of gas produced was measured every two hr, and the composition determined by gas chromatography.

As could be expected, the subjects showed great individual variability, but the composite results (see Fig. 2) show a clear trend. The control meal (right column) caused a mean flatus egestion of 400 ml. Most subjects hardly noticed this low level flatulence, perhaps because they had learned to accept it as an unavoidable minor problem.

Defatted soy in the test meal caused a high awareness of intestinal movements, and a doubling of gas production as compared to the bland control meal. The refined concentrates caused intermediate reactions: the 60% protein type halved the flatulence, while in the 70% protein concentrate the flatulence was reduced to about one-third of the original potency.

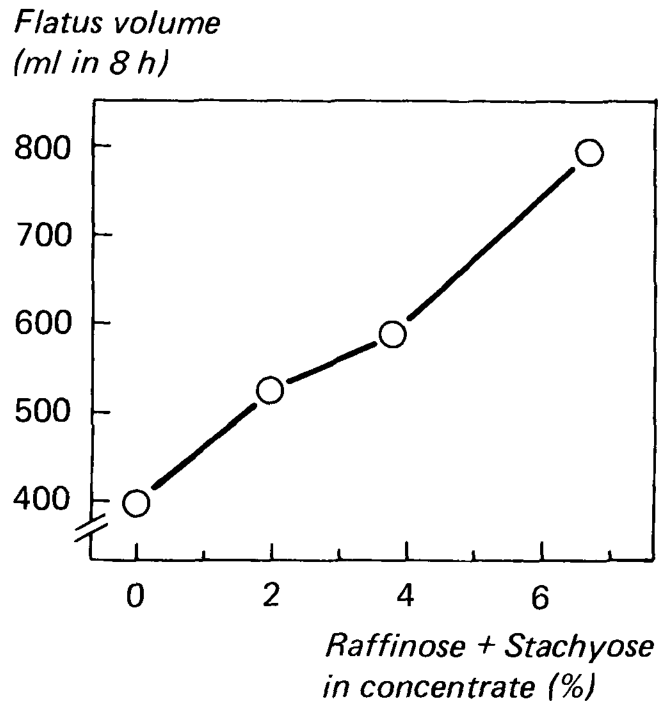

FIG. 3. Relationship between oligosaccharides in soy protein concentrates and flatulence in man.

All these differences are statistically significant ($P \leqslant 0.05$) with the exception of that between the refined concentrates. The same relationships are found in the quantities of carbon dioxide and hydrogen. Both gases are typical for anerobic microbial fermentation of undigested carbohydrates.

Figure 3 shows a linear relationship between raffinose plus stachyose content in the soy protein materials and the extra flatus volume. On the basis of these findings, one can use the content of these two sugars for predicting the flatulence properties of soy protein materials.

OBSERVATIONS

From the first experiment, we conclude that high levels of soy protein concentrate in the diet under practical conditions do not induce physiologically significant changes. Those changes that were found statistically significant after four weeks of continuous high consumption were small and within normal limits, and did not indicate any long term hazard. Most changes can be explained on the basis of small shifts in dietary composition as a result of substituting soy protein concentrate for conventional protein sources. The results of the second trial demonstrate that flatulence due to concentrate consumption is largely eliminated by proper refining. These human experiments confirm the prevailing view that soy protein materials are a new and welcome tool in nutrition. This tool enables us to provide the consumer with foods that are not only wholesome and acceptable but also are in line with modern concepts of good nutrition.

ACKNOWLEDGMENT

The authors express their gratitude to the volunteers, whose loyal cooperation made these studies successful. They also thank the many colleagues for their collaboration, especially those of Unilever Research Colworth House, England, and the Central Institute for Nutrition and Food Research TNO, Zeist, The Netherlands.

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