

Pancreatic digestive hydrolase activities in growing rats fed alternately on raw and heated soya-bean flour

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The time-course effects of two diets containing raw (RSF) or heated (HSF) soya-bean flour on the digestive enzyme levels in the pancreas and in pancreatic juice were investigated in growing rats fed, alternately, on RSF or HSF diets for two 4-week periods. These values were compared with those obtained in a control group fed on a casein diet. RSF and HSF diets lowered N balance (84.8 (SE 0.9), 82.6 (SE 0.8) and 79.9 (SE 0.8) % with control, HSF and RSF diets respectively, at the third week). However, they increased protease activities compared with the control diet (3-fold for trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) activities in pancreas contents and outputs with the RSF diet; 2-fold for trypsin in pancreas contents and outputs and by 60% for chymotrypsin contents with the HSF diet). The poorer nutritional N utilization might be attributable to soya-bean flour heat-stable (lectins) and heat-labile components (trypsin inhibitors). The decrease in lipid apparent digestibilities in RSF and HSF diets (97.0 (SE 0.8), 91.1 (SE 0.9) and 90.4 (SE 0.7) % with control, RSF and HSF diets at the seventh week) were correlated with a diminution in apparent lipase (EC 3.1.1.3; measured without addition of exogenous colipase), potential lipase (measured with addition of saturated amounts of exogenous colipase) and colipase activities. Compared with control values, gains in potential and apparent lipase outputs were diminished by nearly 40% and gain in colipase outputs by 60% with RSF and HSF diets. These results show clearly that heated or raw soya-bean flours have a significant inhibitory effect on lipase digestive enzyme activities in the pancreas and in its secretion, which might explain impaired lipid digestibility.

Soya-bean flour: Pancreas: Lipase: Phospholipase A₂

Soya-bean flour contains antinutritional factors. Protease inhibitors found in raw soya-bean flour (RSF) have been extensively studied. It is well established that giving RSF to rats causes growth depression, pancreatic enlargement and stimulation of its secretory activity (Liener & Kakade, 1980). Most of the deleterious effects of RSF have been generally attributable to trypsin inhibitor (TI; MacGuinness *et al.* 1984). However, rats fed on diets supplemented with soya-bean flours devoid of TI activity also developed pancreatic hypertrophy (Naim *et al.* 1982). This effect can be partly attributed to the low digestibility of RSF proteins which favours the formation of highly stable complexes between proteases and proteins and disturbs the negative feedback regulation of cholecystokinin (CCK) secretion (Liener, 1981).

In vitro studies have shown that RSF contains compounds that inhibit pancreatic lipase

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Table 1. *Composition of the diets (g/kg)**

	Ingredients	Control diet	Raw or heated soya-bean flour diets
Protein	Milk casein†	200	—
	Soya-bean protein‡	—	200
Carbohydrates	Maize starch§	562.2	437.5
	Soya-bean carbohydrates‡	—	125
Lipids	Soya-bean triacylglycerols	102	102‡
	Soya-bean phospholipids	8	8
Fibres	(Agar-agar)	40	22.5
	Soya-bean fibre‡	—	17.5
	Cellulose	26	26
Others	Salt mix¶	28.3	28.3
	Vitamin mix**	5.7	5.7
	Moisture	40	40‡

* Diets were isoenergetic (16.9 MJ/kg) and were given in powdered form.

† Prolabo (Paris, France).

‡ Ingredients originating from soya-bean flour (Soyapan, Edelseja GMBH, 2000 Hamburg, Germany).

§ Etablissements Louis François (Saint Maur les Fossés, France).

|| Cellulose originating from vitamin and salt mixtures.

¶ UAR, 205B (Villemoisson, Epinay sur Orge, France). The salt mix provides the following amounts (mg/kg diet): CaHPO₄ 17200, KCl 4100, NaCl 4000, MgO 420, MgSO₄ 2000, Fe₂O₃ 120, Fe₂SO₄·7H₂O 200, trace elements 400 (MnSO₄·H₂O 98, CuSO₄·5H₂O 20, ZnSO₄·7H₂O 80, CoSO₄·7H₂O 0.16, KI 0.3).

** UAR 200 vitamin mixture, containing (mg/kg diet): retinol 12, cholecalciferol 0.125, thiamin 40, riboflavin 30, pantothenic acid 140, pyridoxine 20, inositol 300, cyanocobalamin 0.1, ascorbic acid 1600, DL- α -tocopherol 340, menadione 80, nicotinic acid 200, choline 2720, pteroylmonoglutamic acid 10, *p*-aminobenzoic acid 100, biotin 0.6.

(EC 3.1.1.3) and which are heat labile proteins (Satouchi & Matsushita, 1976). Inhibitors of pancreatic lipase have been partially purified in protein fractions isolated from RSF (Widmer, 1977).

Gargouri *et al.* (1984) have shown that lipase inhibition by proteins, including soya-bean flour proteins, does not result from the formation of a soluble complex between enzyme and inhibitor, but from the fact that proteins bound to the oil-water interface prevent enzyme interaction with their substrate. The extent to which this inhibitory effect of proteins might affect enzymic digestion of dietary fat has not been studied *in vivo*. However, in a previous short-term study we have shown that soya-bean-flour components, which are partially thermolabile, decrease lipid digestion and absorption (Khalifa *et al.* 1992).

The effects of lipase inhibitors, previously characterized in soya-bean flour, on the synthesis and secretion of pancreatic lipolytic enzymes and on fat digestion in rats fed on RSF or heated soya-bean flour (HSF) diets were investigated. In relation to this, apparent and potential lipase, colipase and phospholipase A₂ (EC 3.1.1.4) activities, in parallel with protease activities (trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1)), were measured in the pancreas and in its secretion. Experiments were carried out in growing animals for two 4-week periods with RSF and HSF diets being fed alternately. Comparisons were performed using a reference diet containing 200 g casein/kg.

EXPERIMENTAL

Animal and diets

Male Wistar rats (n 180; Iffa-Credo, L'Arbresle, France), weighing 80 (SE 5) g at the beginning of the experiment, were given a balanced control diet (200 g casein/kg) for 1 week. After this adaptation period their weight was 100 (SE 8) g. They were then divided into three equal groups and fed on different diets as follows: the S1 group were fed on a HSF diet for one 4-week period (phase 1) followed by a RSF diet for a second 4-week period (phase 2); the S2 group were fed on the RSF diet during phase 1 and the HSF diet during phase 2; a control group (C) was fed on the original reference diet throughout the experiment (8 weeks). Diets were isoenergetic (16.9 MJ/kg) and were given in powdered form. The composition of the diets is shown in Table 1. Rats were kept in wire-bottomed cages at constant temperature (25°) and humidity (65%) with light provided for 12 h daily (07.00–19.00 hours). They had *ad lib.* access to food and tap water. The general guidelines for the care and use of laboratory animals were followed (Council of European Communities, 1986).

Commercially available soya-bean flour (Soyapan) was purchased from Edelseja (Hamburg, Germany). HSF was obtained by autoclaving RSF at 130° for 9 min, under pressure (1.5 bar). Under these experimental conditions, only 21% of the trypsin inhibitor activity originally present in RSF remained in HSF (Khalifa *et al.* 1992) and this residual activity had no antinutritional effect (Rackis, 1981). HSF contained 30% of the pancreatic lipase inhibitor activity found in RSF (Gargouri *et al.* 1984). The fatty acid composition (%) of total lipids in soya-bean flour was: palmitic 12.4, stearic 2.8, oleic 18.9, linoleic 57.7, and linoleic 8.1. After heating, the fatty acid composition remained unchanged.

Food intake and body weight were measured daily on six rats from each group. To determine the nutritional efficiency of diets, faeces and urine were collected and pooled for several 1-week periods. N balance is the ratio between N retained and N ingested, and lipid digestibility is the ratio between lipid ingested and lipid excreted in faeces, as follows:

$$\text{N balance} = \frac{\text{N ingested} - \text{N excreted (urinary + faecal)}}{\text{N ingested}} \times 100,$$

$$\text{lipid digestibility} = \frac{\text{lipid ingested} - \text{lipid excreted (faecal)}}{\text{lipid ingested}} \times 100.$$

The food and protein conversion efficiency ratios were determined as follows:

$$\text{food conversion efficiency ratio} = \frac{\text{gain in body weight (g)}}{\text{food intake (g)}},$$

$$\text{protein conversion efficiency ratio} = \frac{\text{gain in body weight (g)}}{\text{protein intake (g)}}.$$

Pancreas and pancreatic juice samples

At days 2, 7, 14, 21 and 28 of each phase (1 and 2), six rats from each group were anaesthetized with Imalgene (Iffa-Mérieux, Lyon, France; 100 mg/kg body weight injected intraperitoneally) and fitted with a double pancreatic and biliary cannula, according to the technique of Colwell (1951), to obtain pure pancreatic juice. After 6 h fasting, pancreatic juice was collected on ice for 24 h. During the sampling period, rats were fasted but had access *ad lib.* to tap water containing NaCl (120 mM) and CaCl₂ (27 mM). No bile or pancreatic juice was re-introduced into the intestinal lumen. Then the pancreas was

removed, washed with NaCl (150 mM), trimmed free of fat, weighed, diluted with ice-cold water (1 ml/100 mg tissue) and homogenized using a Potter-Elvehjem homogenizer (Braun, Helsingen AG, Germany) to obtain pancreatic extracts. Portions of pancreatic juice and pancreas extracts were kept at -30° until assayed.

Chemical analysis and enzyme activity determination

Protein levels were determined according to the technique of Lowry *et al.* (1951) using bovine serum albumin as standard. Total lipids of faeces were extracted for analysis by the method of Folch *et al.* (1957).

Enzyme activities were assayed on specific substrates by titrimetry, using a pH-stat Radiometer (Radiometer Analytical Instruments Division, Copenhagen, Denmark).

Trypsin activity was determined by measuring the hydrolysis of *N*-benzoyl-L-arginine ethyl ester hydrochloride and that of chymotrypsin by degradation of *N*-acetyl-L tyrosine ethyl ester monohydrate, after activation of the proenzyme forms by exogenous trypsin, using the method of Figarella (1966). The production of free amino acids was automatically neutralized by 20 mM-NaOH solution.

Triacylglycerol lipase (apparent and potential) and colipase activities were assayed according to the technique of Rathelot *et al.* (1975), using a triolein substrate. Apparent lipase activity was measured without addition of colipase to the incubation medium, and potential lipase activity with a saturating amount of colipase, i.e. 5 μ g crude exogenous porcine colipase, because there is evidence that colipase from one species activates the lipase of other species (Canioni *et al.* 1977).

Determination of colipase requires previous lipase inactivation of pancreatic juice and pancreas extracts. This was achieved by lowering the pH of samples to 2.0 at 0° for 10 min, then restoring it to pH 9.0 with NaOH (100 mM). Portions of acid-treated samples of pancreatic juice (100 μ l) or pancreas (500 μ l) were added to an assay system mixture containing bile salts and a purified preparation of rat lipase (40 U) devoid of colipase (Ouagued & Girard-Globa, 1980), then lipase activity was measured. Colipase was determined in terms of its ability to restore lipase activity.

Phospholipase A₂ was determined according to the method of Belleville & Clement (1971), using egg yolk phospholipids as substrate. Triolein and phospholipid hydrolyses were measured for 10 and 20 min respectively. The production of free fatty acids was automatically neutralized by a 20 mM-NaOH solution.

One enzyme unit corresponds to 1 mmol substrate hydrolysed/min at the appropriate pH and temperature.

Results were expressed as their arithmetical means with their standard errors. Values are means of gain in activity per pancreas per day (for contents and outputs) during phases 1 and 2 (Δ (day 2–day 7), Δ (day 7–day 14), Δ (day 14–day 21), Δ (day 21–day 28)).

Statistical analysis

Statistical analysis was performed on control, S1 and S2 groups during phases 1 and 2, using a simple crossover design (Armitage & Berry, 1987) e.g. Δ 1 (day 7–day 14 phase 1)– Δ 2 (day 7–day 14 phase 2); Student's *t* test was carried out on the Δ 1– Δ 2 values. The significance of the difference is given by *t* and *P* values.

RESULTS

Body weights and food intake

Compared with control values, at the end of phase 1 the weight of rats fed on the HSF (S1) or RSF (S2) diets dropped to 82 and 74% respectively (Fig. 1). These values remained lowered during phase 2, and at the end of this phase the weights of rats fed on both soya-

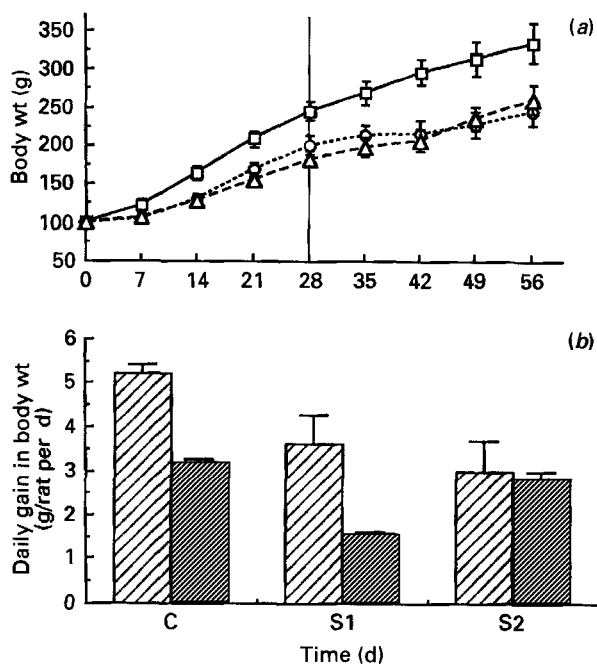


Fig. 1. Effect of alternate feeding of diets containing raw (RSF) or heated (HSF) soya-bean flour on (a) body weight and (b) daily gain in body weight in rats. Rats were given one of the diets for 28 d (phase 1) then changed onto the alternate diet for a further 28 d (phase 2); the point of change is indicated in panel (a) by a vertical line. Values are means with their standard errors indicated by vertical bars. (—□—), control (C) group fed on a diet containing 200 g casein/kg for 56 d; (—○—), S1 group, fed on the HSF diet during phase 1 and the RSF diet during phase 2; (—△—), S2 group, fed on the RSF diet during phase 1 and the HSF diet during phase 2; (▨), phase 1; (■), phase 2. Statistical analysis (Student's *t* test): C v. S1, *t* 12.56, *P* < 0.001; C v. S2, *t* 11.12, *P* < 0.001; S1 v. S2, *t* 12.09, *P* < 0.001. For details of diets and procedures, see Table 1 and pp. 535–536.

bean flours were only 70% of control values. After the diet change the body weight curves of rats fed on the raw and heated soya-bean flours intersected between day 42 and day 49 of the experiment. Gains in body weight were significantly different between all three dietary groups (Fig. 1).

During each phase, the lowest food intakes/d per rat (Table 2) were obtained with the RSF diet (S2 during phase 1 and S1 during phase 2).

N balance and apparent lipid digestibility (Table 2)

In the control group, N balance values were unchanged with age. N balances were higher in rats fed on the HSF diet than in those fed on the RSF diet, but these N balance values were lower than those of the control group.

The highest values of lipid digestibility were obtained in the control group and remained steady throughout the experiment (97 (SE 0.5) %). Values were higher with the RSF than with the HSF diet.

Food and protein conversion efficiency ratios (Table 3)

Compared with control values, during phase 1 both food and protein conversion efficiency ratios (Table 3) were lowered by soya-bean flours (–25 and –30%, with HSF and RSF diets respectively), whereas during phase 2 these values were diminished with RSF (–26%), but increased with HSF (+17%).

Table 2. Food intake, N balance and lipid digestibility in rats fed on a control diet (C) for 56 d, or fed alternately on diets containing raw soya-bean flour (RSF) or heated soya-bean flour (HSF) for 28 d each in cross-over design*

(Mean values with their standard errors for six rats)

Stage of experiment	Group	Food intake (g/d per rat)		N balance (%)		Lipid digestibility (%)	
		Mean	SE	Mean	SE	Mean	SE
Week 3 (phase 1)	C	23.4	1.50	86.0	0.7	97.5	0.8
	S1† HSF	21.1	0.31	79.8	0.6	79.7	0.5
	S2† RSF	13.4	0.25	65.3	0.3	86.4	0.7
Week 7 (phase 2)	C	31.4	2.59	84.8	0.9	97.0	0.8
	S1 RSF	18.9	1.16	79.9	0.7	91.1	0.9
	S2 HSF	23.6	1.89	82.6	0.8	90.4	0.7
C v. S1		<i>t</i> 5.339 <i>P</i> < 0.001		<i>t</i> 2.95 0.001 < <i>P</i> < 0.01		<i>t</i> 2.34 0.02 < <i>P</i> < 0.05	
C v. S2		<i>t</i> 4.989 <i>P</i> < 0.001		<i>t</i> 9.72 <i>P</i> < 0.001		<i>t</i> 2.43 0.02 < <i>P</i> < 0.05	
S1 v. S2		<i>t</i> 5.952 <i>P</i> < 0.001		<i>t</i> 5.659 <i>P</i> < 0.001		<i>t</i> 9.73 <i>P</i> < 0.001	

* For details of diets and procedures, see Table 1 and pp. 535-536.

† Group S1 received the HSF diet during phase 1 and the RSF diet during phase 2; group S2 received the RSF diet during phase 1 and the HSF diet during phase 2.

Table 3. Food and protein conversion efficiency ratios in rats fed on a control diet (C) for 56 d, or fed alternately on diets containing raw soya-bean flour (RSF) or heated soya-bean flour (HSF) for 28 d each in a cross-over design*

(Mean values with their standard errors for six rats per group for 28 d)

Group	Food conversion efficiency ratio		Protein conversion efficiency ratio		Statistical significance of diet effects
	Mean	SE	Mean	SE	
Phase 1					
C	0.287	0.0144	1.435	0.0715	C v. S1 0.01 > <i>P</i> > 0.001
S1† HSF	0.219	0.0131	1.094	0.0656	C v. S2 <i>P</i> < 0.001
S2† RSF	0.200	0.0090	1.001	0.0450	S1 v. S2 0.02 > <i>P</i> > 0.01
Phase 2					
C	0.107	0.0043	0.534	0.0214	C v. S1 <i>P</i> < 0.001
S1 RSF	0.079	0.0039	0.395	0.0198	C v. S2 0.01 > <i>P</i> > 0.001
S2 HSF	0.125	0.0082	0.625	0.0406	S1 v. S2 <i>P</i> < 0.001
Phase 1 v. phase 2					C <i>P</i> < 0.001 S1 <i>P</i> < 0.001 S2 <i>P</i> < 0.001

* For details of diets and procedures, see Table 1 and pp. 535-536.

† Group S1 received the HSF diet during phase 1 and the RSF diet during phase 2; group S2 received the RSF diet during phase 1 and the HSF diet during phase 2.

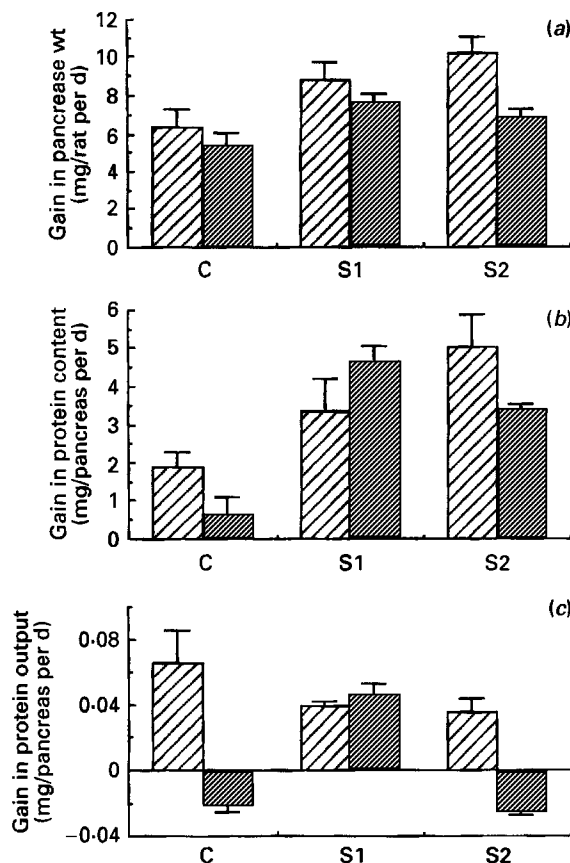


Fig. 2. Effect of alternate feeding of diets containing raw (RSF) or heated (HSF) soya-bean flour on daily gains in (a) pancreas weight, (b) pancreas protein content and (c) pancreatic protein output in rats. Rats were given one of the diets for 28 d (phase 1, ▨) then changed onto the alternate diet for a further 28 d (phase 2, ▩). Values are means with their standard errors indicated by vertical bars. C, control group, fed on a diet containing 200 g casein/kg for 56 d; S1 group, fed on the HSF diet during phase 1 and the RSF diet during phase 2; S2 group, fed on the RSF diet during phase 1 and the HSF diet during phase 2. For details of diets and procedures see Table 1 and pp. 534–536. Statistical analysis: panel (a), C v. S1 t 3.835, 0.01 > P > 0.001; C v. S2 t 2.166, 0.05 > P > 0.02; S1 v. S2 t 4.621, P < 0.001; panel (b), C v. S1 t 2.404, 0.05 > P > 0.02; C v. S2 t 2.939, 0.01 > P > 0.001; S1 v. S2 t 3.561, 0.01 > P > 0.001; panel (c), C v. S1 t 6.443, P < 0.001; C v. S2 t 1.778, not significant; S1 v. S2 t 2.68, 0.02 > P > 0.01.

Pancreas weights and protein contents and protein outputs in pancreatic juice (Fig. 2)

At the end of phases 1 and 2 the relative pancreas weights (pancreas weight/body weight $\times 10^3$) were 1.8, 2.0; 2.8, 3.1; 3.5, 2.5, in groups C, S1 and S2 respectively (results not shown). The values were correlated with daily gain in pancreas weight per rat (6.35, 5.35; 8.77, 7.58; 10.23, 6.85 mg, during phases 1 and 2, in groups C, S1 and S2 respectively) and the daily gain in protein contents (1.87, 0.63; 3.33, 4.62; 5.03, 3.38 in groups C, S1 and S2 respectively).

In the control group the daily gain in protein outputs was positive during phase 1 (+0.065 mg/d) and negative during phase 2 (−0.021 mg/d). During phase 1 the daily gain was similar in groups fed on soya-bean flours (0.038 and 0.035 mg/d in groups S1 and S2, respectively) but lower than that observed in the control group. During phase 2 the highest values were obtained in group S1 (0.045 mg/d), while values in group S2 (−0.025 mg/d) were decreased and similar to those of the control group (−0.03 mg/d).

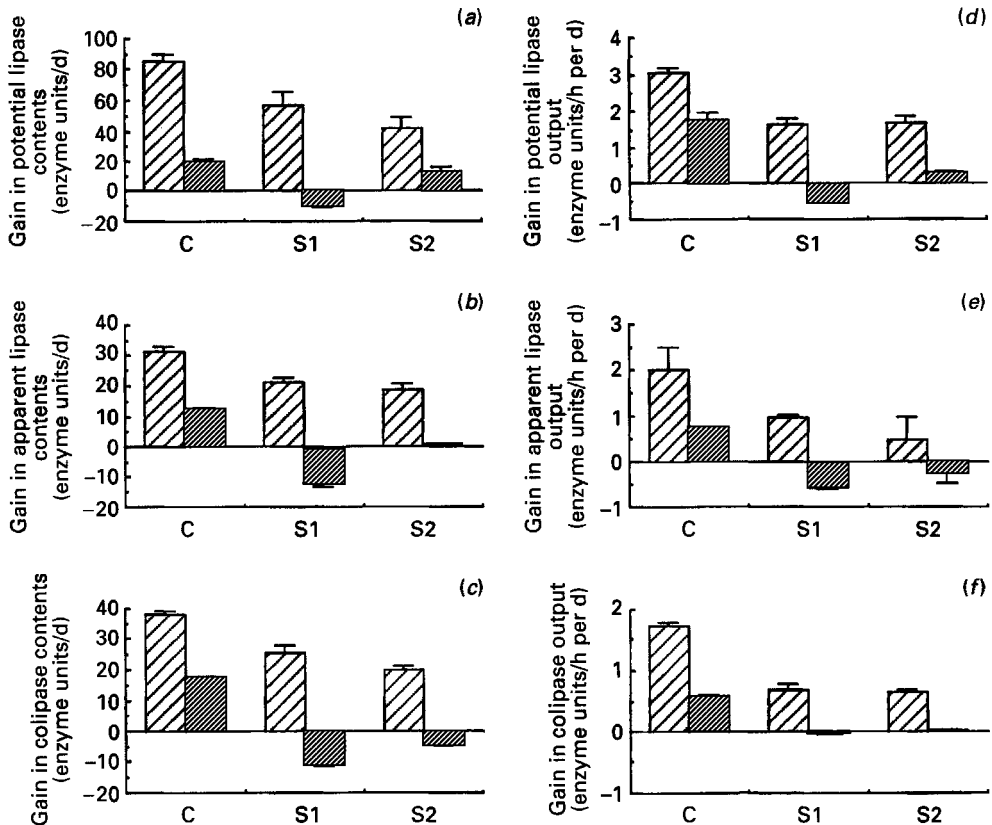


Fig. 3. Effect of alternate feeding of diets containing raw (RSF) or heated (HSF) soya-bean flour on (a) potential and (b) apparent lipase (*EC* 3.1.1.3), and (c) colipase activities in the pancreas (enzyme units/pancreas) and (d, e, f) in pancreatic juice outputs (enzyme units/h per pancreas) of rats. Rats were given one of the diets for 28 d (phase 1, ▨) then changed onto the alternate diet for a further 28 d (phase 2, ▩). Values are means with their standard errors indicated by vertical bars. C, control group, fed on a diet containing 200 g casein/kg for 56 d; S1 group, fed on the HSF diet during phase 1 and the RSF diet during phase 2; S2 group, fed on the RSF diet during phase 1 and the HSF diet during phase 2. For details of diets and procedures, see Table 1 and pp. 535–536. Statistical analysis: panel (a), C v. S1 t 5.39, $P < 0.001$; C v. S2 t 3.205, $0.01 > P > 0.001$; S1 v. S2 t 12.26, $P < 0.001$; panel (b), C v. S1 t 7.974, $P < 0.001$; C v. S2 t 4.090, $P < 0.001$; S1 v. S2 t 1.370, not significant; panel (c), C v. S1 t 2.614, $0.05 > P > 0.02$; C v. S2 t 2.959, $0.01 > P > 0.001$; S1 v. S2 t 2.882, $0.02 > P > 0.01$; panel (d), C v. S1 t 3.27, $0.01 > P > 0.001$; C v. S2 t 2.52, $0.05 > P > 0.02$; S1 v. S2 t 1.854, not significant; panel (e), C v. S1 t 5.862, $P < 0.001$; C v. S2 t 6.181, $P < 0.001$; S1 v. S2 t 1.558, not significant; panel (f), C v. S1 t 5.65, $P < 0.001$; C v. S2 t 5.887, $P < 0.001$; S1 v. S2 t 1.75, not significant.

Digestive enzyme activities

The Armitage & Berry (1987) simple crossover design allows comparison of two groups during both phases of the experiment. In the control group the daily gain in all enzyme activities (contents and outputs) was higher during phase 1 than during phase 2.

The daily gains in lipase (potential and apparent) and colipase activities in pancreas and in pancreatic juice were decreased in groups S1 and S2 compared with control values, but higher in groups S1 and S2 fed on the HSF diet compared with the RSF diet (Fig. 3).

In pancreatic juice during phases 1 and 2, colipase activity: potential lipase activity ratios were 0.54 (SE 0.08), 0.35 (SE 0.12); 0.42 (SE 0.03), 0.26 (SE 0.04) and 0.45 (SE 0.09), 0.27 (SE 0.02) in groups C, S1 and S2 respectively (results not shown).

The highest daily gain in phospholipase A_2 activity in the pancreas was obtained with the HSF diet, but pancreatic juice outputs were not correlated with pancreas contents (Fig.

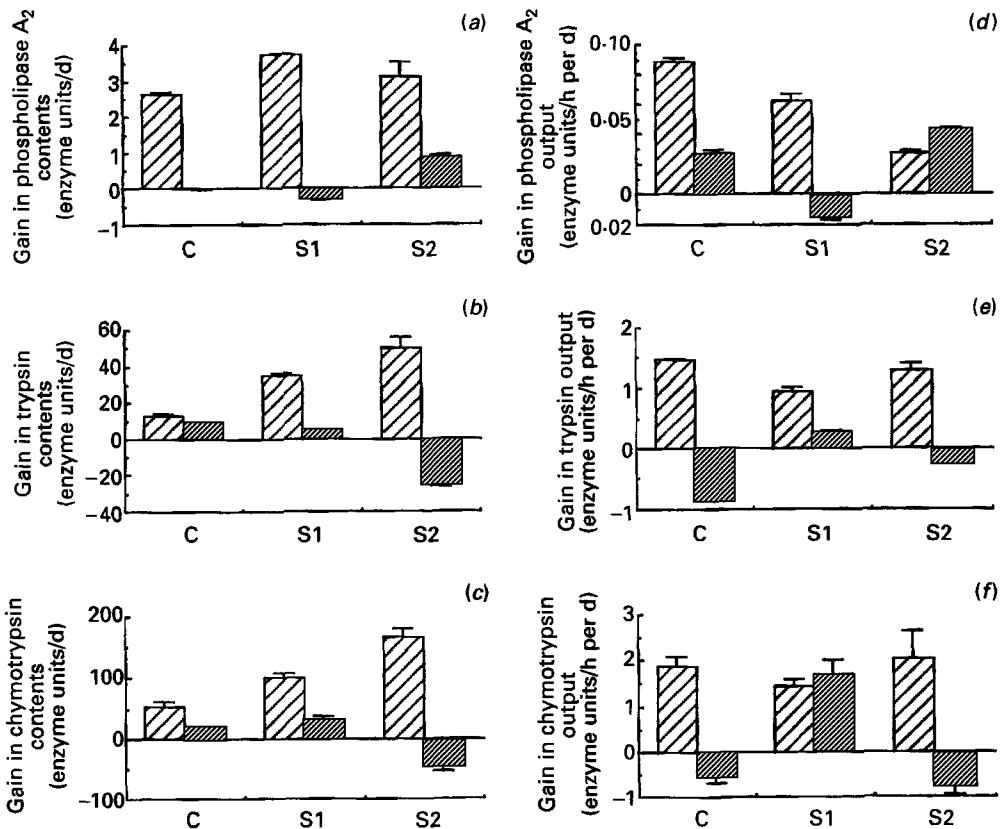


Fig. 4. Effect of alternate feeding of diets containing raw (RSF) or heated (HSF) soya-bean flour on (a) phospholipase A_2 ($EC\ 3.1.1.4$), (b) trypsin ($EC\ 3.4.21.4$) and (c) chymotrypsin ($EC\ 3.4.21.1$) activities in the pancreas (enzyme units/pancreas) and (d, e, f) in pancreatic juice outputs (enzyme units/h per pancreas) in rats. Rats were given one of the diets for 28 d (phase 1, \square) then changed onto the alternate diet for a further 28 d (phase 2, \blacksquare). Values are means with their standard errors indicated by vertical bars. C, control group, fed on a diet containing 200 g casein/kg for 56 d; S1 group, fed on the HSF diet during phase 1 and the RSF diet during phase 2; S2 group, fed on the RSF diet during phase 1 and the HSF diet during phase 2. For details of diets and procedures, see Table 1 and pp. 535–536. Statistical analysis: panel (a), C v. S1 $t\ 2.22$, $0.05 > P > 0.02$; C v. S2 $t\ 1.22$, not significant; S1 v. S2 $t\ 2.967$, $0.01 > P > 0.001$; panel (b), C v. S1 $t\ 3.443$, $0.01 > P > 0.001$; C v. S2 $t\ 3.249$, $0.01 > P > 0.001$; S1 v. S2 $t\ 13.649$, $P < 0.001$; panel (c), C v. S1 $t\ 6.152$, $P < 0.001$; C v. S2 $t\ 9.299$, $P < 0.001$; S1 v. S2 $t\ 3.076$, $0.01 > P > 0.001$; panel (d), C v. S1 $t\ 3.495$, $0.01 > P > 0.001$; C v. S2 $t\ 2.529$, $0.05 > P > 0.02$; S1 v. S2 $t\ 1.75$, not significant; panel (e), C v. S1 $t\ 2.419$, $0.05 > P > 0.02$; C v. S2 $t\ 2.293$, $0.05 > P > 0.02$; S1 v. S2 $t\ 2.545$, $0.05 > P > 0.02$; panel (f), C v. S1 $t\ 2.186$, $0.05 > P > 0.02$; C v. S2 $t\ 2.333$, $0.05 > P > 0.02$; S1 v. S2 $t\ 3.21$, $0.01 > P > 0.001$.

4). The greatest gain in outputs was obtained in the control group during phase 1 and group S2 fed on the HSF diet during phase 2.

The higher daily gain in trypsin and chymotrypsin activities in the pancreas was observed in group S1 during phase 1, and in the control group for trypsin, and group S1 for chymotrypsin activity during phase 2 (Fig. 4). Trypsin and chymotrypsin activities in pancreatic juice outputs were not correlated with those of pancreas contents.

DISCUSSION

A previous study has shown that the highest contents and outputs of hydrolase/g pancreas are observed when control rats are 8–9 weeks old (Prost *et al.* 1988). In the present experiment, rat pancreas was subjected to maturation during phase 1, correlated with larger

body and pancreas growth. Rats were adult during phase 2 with lower body and pancreas growth (Figs. 1, 2).

The Armitage & Berry (1987) simple crossover design test was used to compare the effects of alternate RSF and HSF diets on S1 and S2 rats at the same age, during the same growth period. Control values were also compared with S1 and S2 values with this test. Feeding rats alternately with RSF or HSF diets allowed differentiation between the effects of heat-stable and heat-labile factors in soya-bean flour on pancreatic enzyme synthesis and secretion. The original aspect of this study is the comparison of activities for each enzyme in pancreatic tissue and juice in the same rat.

S1 and S2 groups fed on both soya-bean diets (raw and heated) showed slow growth which was correlated with reduction in food and N intakes and N balance, particularly with the RSF diet (Table 2). The reduction in food intake was dependent on different factors. The RSF diet contains a trypsin inhibitor which slows down the gastric transit of ingested food (De Mulanaere, 1964) and stimulates CCK release (Green *et al.* 1986). Brand & Morgan (1981) have shown that oral administration of trypsin inhibitor to rats is followed by a great CCK peak in plasma. The increase in plasma CCK may strongly inhibit stomach emptying (Green *et al.* 1986). Moreover, soya-bean proteins generally, and protease inhibitors particularly, are not easily digested by the gastrointestinal tract of rats (Kakade *et al.* 1973).

The relative pancreas weight of rats fed on both soya-bean diets, particularly those subjected to the RSF diet, was higher than that of control rats. This increase and its consequences have been reviewed by Gumbmann *et al.* (1986). Prolonged feeding with soya-bean flour containing trypsin inhibitor induces chronic CCK release in excessive amounts and pancreatic hypertrophy. Temler & Mettraux (1986) have shown that the increase in gastrin activity of rats fed on soya-bean trypsin inhibitor also contributes to pancreatic hypertrophy. Moreover, soya-bean flour contains various antinutritional factors such as lectins which are thermostable and resistant to proteolytic breakdown, and induce cellular hyperplasia and pancreatic hypertrophy in spite of heat treatment (Grant *et al.* 1987).

Hypersecretion of pancreatic proteases, namely cysteine-rich trypsin and chymotrypsin, enhances the requirement for S-containing amino acids in the synthesis of pancreatic hydrolases. Growth retardation could be attributable to the fact that cysteine and methionine would be less available for protein synthesis (Crass & Morgan, 1982). In the present study, soya-bean proteins were less suitable than casein for protein synthesis as shown by the nutritional efficiency of soya-bean proteins (Table 3) which was lower than that of casein.

Determination of apparent lipid digestibility indicated that rats fed on soya-bean diets (raw or heated) increased their lipid faecal excretion. This diminution in lipid digestibility could be attributable to an intraluminal lipolytic activity decrease, on account of the presence of amphiphilic soya-bean proteins (Gargouri *et al.* 1984) and a diminished colipase activity: potential lipase activity ratio observed in the pancreatic juice of rats fed on HSF and RSF. A positive correlation between values of apparent lipid digestibility and colipase:lipase ratios was observed. The highest correlation values observed during phases 1 and 2 of the experiment were obtained with the control diet, followed by RSF and HSF.

The RSF and HSF diets depressed lipase and colipase activities, and to a lesser extent phospholipase A₂ activity in the pancreas and its secretion. Adaptation of pancreatic enzyme synthesis to diet composition was well demonstrated. Lipase and phospholipase A₂ activities are enhanced when the diet is enriched with triacylglycerols (Belleville *et al.* 1978). The decrease in lipid digestibility with raw or heated soya-bean diets at least partly accounts for the depression in lipolytic activity. It could be hypothesized from *in vitro* data reported by Gargouri *et al.* (1984) and from the present observations that amphiphilic soya-

bean proteins cause partial inhibition of *in vivo* dietary fat hydrolysis by pancreatic lipase and colipase. Moreover, previous studies have shown that HSF causes a partial inhibition of lipase and colipase activities (Khalifa *et al.* 1992). In the gut, bile salts and proteins lead to inactivation of lipase by physical separation of the enzyme from its substrate. This inhibition is reversed by colipase which forms a specific complex with lipase and brings the enzyme into close contact with the interface, thus indirectly allowing interfacial activation (Van Tilbeurgh *et al.* 1992). Under these conditions it could be hypothesized that colipase is the limiting factor of lipase activity in the intestinal tract. This, in turn, might cause a decrease in the plasma concentration of metabolites resulting from the cellular degradation of fatty acids. Metabolites such as α -acetoacetate and β -hydroxybutyrate are considered by Lavau *et al.* (1974) to be mediators of pancreatic lipase synthesis. However, our results indicated that the decrease in lipid digestibility was lower (-18 and -11% for S2 and S1 respectively, during phase 1 and -6% during phase 2) than that observed in lipase and colipase activities (nearly -40% for potential and apparent lipase, and -60% for colipase outputs for RSF and HSF during phases 1 and 2). This could account for the secretion of excess lipolytic enzymes in pancreatic juice under normal conditions.

Only a few studies have been carried out on the changes in lipase, colipase and phospholipase A₂ activities in pancreas and pancreatic juice, brought about by alternate feeding of a diet containing raw and heated soya-bean flours. Konijn *et al.* (1970) have shown that the pancreatic lipolytic activity is higher in rats adapted to a HSF diet than in rats fed on the RSF diet. Raw proteins have greater solubility than heated proteins (De Muelenaere, 1964) in the intestinal tract and this could modify the interaction between the enzyme and its substrate. However, the decrease in lipase and colipase activities was induced by heat-labile as well as heat-stable factors since it was observed in rats fed on both RSF and HSF diets. These factors cannot be the same as those inhibiting *in vitro* lipase activity (Satouchi & Matsushita, 1976; Gargouri *et al.* 1984), since they affect lipase and colipase biosynthesis and secretion.

In conclusion, alternate feeding of RSF and HSF diets in the rat allowed study of the effects of heat-stable and heat-labile factors in soya-bean flour respectively, on the synthesis and secretion of pancreatic digestive enzymes. The most striking effect of feeding soya-bean flour diets was the net decrease in lipase and colipase activities in pancreatic tissue and juice. Phospholipase A₂ activities were less affected. In contrast, protease levels were increased.

So far, the mechanism by which factors in soya-bean flour influence the synthesis of enzymes in the pancreas has been unknown. Control of the pancreatic secretion composition by diet may not only be attributable to CCK but to other intestinal hormones together with metabolites resulting from the transformation of nutrients. Moreover, our results indicate non-parallelism in the enzyme activities of pancreas and its secretion, suggesting changes in enzyme retention thresholds in acinar cells which vary with RSF or HSF diets.

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