Short term effect of feeding raw or heated soya flour and casein meals on lipid intestinal digestion and absorption in rat

Fatma Khalifa, Véronique Bertrand, Jacques Belleville, Louis Sarda and Josiane Prost

Unité de Recherches de Nutrition Cellulaire et Métabolique, Dijon Cedex, and Laboratoire de Biochimie, Faculté des Sciences St-Charles, Marseille, France

In vivo and in vitro studies were carried out to investigate the effects of raw or heated soya flour consumption on lipid digestion and absorption. Rats were fed mixed [^{14}C]-triolein and raw or heated soya flours by gastric intubation and thoracic lymph was collected (experiment A). Raw or heated soya flours mixed with [^{3}H]-oleic acid and [^{14}C]-triolein were used to study lipid digestion and absorption (experiment B). Triolein digestion and absorption were studied by using the technique of the intestinal loop isolated in situ (experiment C). In vitro studies were performed to measure lipase and colipase activities on triolein substrate. The results obtained with raw or heated soya flours were compared with those obtained with casein meals. Raw soya flour significantly decreased triolein absorption. The emptying of the stomach was highly retarded. The absorption of oleic acid was higher than that of triolein with raw and heated soy flours but they were increased with heated soya flour. These absorptions were lower with raw and heated soya flours compared with casein meals. The apparent lipase activity was higher with both soya meals, and it was increased by heating. Activity of potential lipase was higher with heated soya flour than with casein meals. Colipase activity was reduced with both soya flours. Our data show that lipid digestion is not inhibited by soya flour consumption, but that only lipid absorption is diminished. The soya flour components which decrease this absorption are partially thermolabile.

Keywords: soya flour; lipid digestion; lipid absorption; casein; rat

Introduction

Soya flour contains proteins that are inhibitors of digestive enzymes. Inactivation of the pancreatic proteases, trypsin and chymotrypsin, by protein inhibitors from soya beans is well documented.[1,2] Inhibitors form specific molecular complexes with the enzymes. Feeding rats the diet containing soya flour decreases body growth, causes pancreas hypertrophy, and stimulates synthesis of the proteolytic enzymes in pancreas. In vitro studies by Satouchi et al.[3,4] and by Gargouri et al.[5,6] have provided evidence that amphiphilic proteins from soya flour inhibit the hydrolysis of triacylglycerols by pancreatic lipase. Inhibition results from the binding of the proteins to the lipid-water interface. However, hydrolysis is partly restored by adding colipase in large excess to lipase and bile salts to the reaction system. Little is known on the effects of these proteins on fat digestion and absorption under in vivo conditions. Gargouri et al.[7] have further shown that pure human gastric lipase, which acts in vivo at the stomach level under acidic conditions, is irreversibly inhibited by amphiphiles, including proteins from soya, that decrease the surface tension at the lipid-water interface below 8 dynes per cm. It therefore appeared that dietary soya protein might decrease the activity of lipases of the gastrointestinal tract (preduo-

Address reprint requests to Professor Jacques Belleville at the Unité de Recherches de Nutrition Cellulaire et Métabolique, Faculté des Sciences Mirande, B.P. 138, 21004 Dijon Cedex, France.
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denal and pancreatic lipases) in vivo and, thereby, affect hydrolysis and absorption of ingested fat. Our previous results (unpublished data) have shown that lipid digestibility and potential lipase and colipase activities were decreased as early as 48 hr of feeding raw or heated soya flour meals. To answer the question whether one meal containing raw or heated soya flour can inhibit digestion or absorption of lipids, experiments have been performed with [^{14}C]-triolein or [^{3}H]-oleic acid + [^{14}C]-triolein mixed with raw or heated soya flour, or with reference meal of the same composition, but containing casein instead of soya proteins. Rats were fed their meals either by intragastric intubation or by using the technique of the intestinal loop isolated in situ. In vitro, apparent and potential lipase and colipase activities have been measured on triolein substrate to investigate if the inhibition could be attributable to digestive or absorption processes.

Materials and methods

Animals

Male Wistar rats (Iffa-Credo, L'Arbresle, France) weighing 250 ± 31g were used. Before experiment, they were fed ad libitum a commercial diet (U.A.R, Epinay sur Orge, France) containing 17% protein, 3% lipid, 58.7% carbohydrate, 4.3% fiber, 5% minerals, and 2% vitamins. They had free access to tap water. Animals were housed individually in stainless-steel cages at constant temperature (25°C) and humidity (65%). Light was provided for 12 hours (07.00–19.00).

Materials

[9-10(n)^{3}H]-oleic acid (185.10^{9} Bq/mmol^{-1}) was purchased from Amersham (Buckingham shire, UK) and [^{14}C]-triolein (2.556.^{6} Bq/mmol^{-1}) from C.E.A. (Saclay, France), pure triolein from Nu-Chek-Prep (Elysian, MN, USA) and pure oleic acid from Prolabo (Paris, France). Commercially available soya flour (Soyapan) was obtained from Lecithos-France SA (St-Maur les Fossés, France) and casein from Prolabo (Paris, France). Composition of soyapan is given in *Table 1*.

Table 1 Ponderal composition of soya flour and casein meals (g/100g)

	Soya meals with Soyapan[a] raw (RSF) and heated (HSF) meals	Casein[b] meals raw (RC) and heated (HC) meals
Protein	32.4	32.4
Lipid	21.0	0.6 + 20.4 soya oil[c]
Carbohydrate	37.1	5.0 + 32.1 corn starch[d]
Fibers	3.5	0.0 + 3.5 Agar agar[b]
Moisture	6.0	3.4 + 2.6 water

Both diets were intubated as raw or heated gruels. The casein meal gives the same quantity of nutrients as soya meal.

The lipid composition of soyapan (by weight) was: 78% triacylglycerol; 5% diacylglycerol; 2% monoacylglycerol; 9% free fatty acid; and 6% phopholipid. The lipid composition of the phospholipid fraction was: 41% phosphatidylcholine; 31% phosphatidylethanolamine; 11% phosphatidylinositol; 6% phosphatidylserine, and 11% lyso derivatives. The fatty acid composition of the total lipids of soya flour was as follows (by weight): 1% lauric acid; 0.5% myristic acid; 12.5% palmitic acid; 3% stearic acid; 18% oleic acid; 57% linoleic acid; 8% linolenic acid.

[a] Lecithos-France SA, St Maur les Fossés, France.
[b] Prolabo, Paris, France.
[c] Commercially available product.
[d] Etbs Louis François SA, Saint Maur les Fossés, France.

Test meals

Test meals were prepared with soyapan flour or reference meal containing casein as protein source. The composition of these meals is given in *Table 1*. Heated soya flour (HSF) and heated casein meals (HC) were obtained after autoclaved at 130°C for 9 min under the pressure of 1.5 bar. After heating, the lipid composition of HSF and HC were not changed. Residual trypsin inhibitor activity and pancreatic lipase inhibitor activity of HSF represented 21% and 30%, respectively, of those of raw soya flour meal (RSF). According to Rackis[2] this residual trypsin inhibitor activity had no nutritional effect. Trypsin inhibition was measured in a pH stat, using the decreased activity of trypsin (0.1 mEq/min^{-1}) by trypsin inhibitor. Trypsin assays were performed using Figarella's technique[8].)

In vivo experiments

Experiment A. The rats were fasted 6 hours before thoracic duct cannulation to collect lymph according to the Bollman et al. technique.[9] A polyethylene catheter was inserted under anesthesia with Imalgene (Iffa Mérieux, Lyon, France) (100 mg/Kg body weight^{-1}). Only rats with normal lymph flow (2–3 mL/h^{-1}) were kept for further lipid absorption studies. One day after surgery, for each meal, five fasted rats having free access to NaCl 12 mmol/L and KCl 2.7 mmol/L solution, received either RSF, HSF, or casein meals by gastric intubation. Each rat was fed a meal prepared by mixing 100 mg of pure triolein and [^{14}C]-triolein (176 Bq/mmol^{-1}) with 450 mg of RSF, HSF, or raw casein meal (RC). Before intubation, meals were homogenized by ultrasonication in 2 mL of distilled water. Samples of lymph were collected every hour for 6 hours following meal intubation. Then lymph collected during the next 18 hours was pooled. At that time, after a light anesthesia, rats were killed by a blow on the neck. The contents of the stomach was scraped and those of the small and large intestines were rinsed out, and contents of stomach and intestines were separately collected. Feces were collected for the 24-hour period following meal intubation and mixed with the content of large intestine. Organ tissues were finally homogenized in 100 mL of chloroform/methanol (L/L) (vol/vol) by means of an Ultraturax homogenizer. Lipids were extracted from lymph, organ contents, and tissue homogenates and further analyzed for their composition and radioactivity.

Experiment B. To avoid laparotomy stress that can slow down gastric emptying, another experiment was performed without lymph collection. Five rats were fed each test meal by gastric intubation. Each test meal consisted of mixed pure triolein (192 mg), oleic acid (96 mg), [^{14}C]-triolein (176 Bq/mmol^{-1}) and [^{3}H]-oleic acid (119 Bq/mmol^{-1}) with 450 mg of RSF, HSF, RC, or HC. Six hours after intubation of the test meal, rats were killed. Stomach, small, and large intestines were removed. Then stomach and its contents, small intestine and its contents, intestinal mucosa, residual small intestine tissue, and large intestine and its contents were homogenized as described above and their lipid contents were analyzed for their composition and radioactivity.

Experiment C. A polyethylene catheter was first inserted into the pancreatic duct of anesthetized rats, at the level of its junc-

tion with duodenum, in order to divert bile and pancreatic juice secretions. Rats were placed in modified Bollman-type restraint cages and were fasted for 24 hours, but were allowed free access to tap water. On the next day, a segment of the small intestine was isolated in situ. The first ligature was effected about 2 cm after the junction to the pancreatic duct and the second ligature was effected 20 cm below. A cannula was then inserted at both ends of the isolated intestinal loop. Before meal injection, the intestinal loop was carefully rinsed with 20 mL 15 mmol/L NaCl to remove potential remaining bile and pancreatic enzymes. Fifteen animals were divided into three equal groups. Each group received a particular test meal. Each meal contained 100 mg of pure triolein and [^{14}C]-triolein (176 Bq/mmol^{-1}) with 100 mg of RSF, HSF, or RC. Mixtures were homogenized with 2 mL distilled water by ultrasonication. To simulate the chemical effects of gastric transit on ingested food, 0.75 mL 0.1 M HCl were added to homogenized meal. The pH of homogenized meal was about 3.00. After 30 min at 37°C under constant stirring, rat bile (120 µL) was added to each meal and pH was raised to 7 with 0.1 M NaOH. Finally, rat pancreatic juice (50 µL) was added to homogenized mixture, just before injection into the intestinal loop. Bile and pancreatic juice were derived from the same pools. Volumes of bile and juice added to meals represented the average amounts that are normally secreted in rat during the time of experiment (20 min). After 20 min, the intestinal loop was rinsed by injection of 20 mL 15 mmol/L NaCl and the intraluminal content with unabsorbed lipids was collected. Lipids were immediately extracted. The intestinal loop was removed by cutting small intestine at ligature levels. The intestinal segment was opened by a longitudinal incision and spread on a cooled glass plate. Mucosa cells were scraped off using a glass spatula. Residual intestinal tissue was homogenized as described in experiment A. Lipids were extracted from each fraction (intraluminal content, mucosa cells, and residual intestine tissue). Lipids were analyzed for their composition. Radioactivity was determined on each lipid fraction.

Lipid analysis. On each sample originating from experiments A, B, and C, the total lipids were extracted according to Folch et al..[10] Separation of lipids was performed in duplicate by thin layer chromatography according to the Stahl technique.[11] Fatty acid compositions from the different lipid fractions separated by thin layer chromatography were analyzed as butyl esters by gas liquid chromatography according to Clement and Bezard[12] using pentadecanoic acid as internal standard for quantitative analysis. The gas liquid chromatography using Becker Packard chromatograph model 417 (Packard Instrument, Rockville, MD) was performed using a capillary column (length: 30 M; internal diameter: 0.35 mm; stationary phase: carbowax 20 M; flow of N_2: 3 mL/min^{-1}).

Determination of radioactivity. After separation of the lipid fractions by thin layer chromatography, plates were scraped and gel was introduced into a scintillation vial that contained 1 mL methanol. Two hours later, 14 mL of scintillation liquid, xylene/permafluor 1/10 (vol/vol) (Packard) were added. Radioactivity was counted in a Packard Tricarb model 300 CD scintillation spectrometer. Quenching was automatically corrected.

In vitro experiment

The in vitro experiment was carried out to separate the meal effect on the triolein digestion from intestinal absorption. The apparent and potential lipase and colipase activities were measured. In this experiment, the same meals as those of experiment C were used. In vitro incubations have been achieved with a pH stat Radiometer (Copenhagen, Denmark) with continuous stirring at 25°C and pH 9.0. After a stable baseline the same pancreatic juice sample (50 µL) originating from the same pool, has been added to each incubation. Protein contents of pancreatic juice have been estimated according to the Lowry et al. technique.[13] In vitro activities were measured on triolein substrate alone, then with 100 mg HSF, RSF, HC, or RC.

Enzyme assays. The entry of dietary fats into the villus cells requires the conversion of water insoluble long-chain triacylglycerols into fatty acids and 2-monoacyglycerols by the pancreatic lipolytic system. It also requires the participation of bile salts that form a micellar phase with the fatty acids and 2-monoacylglycerols. Bile salts at physiologic concentration have been shown to strongly inhibit the action of pure pancreatic lipase.[14,15] This inhibition is reversed by colipase, a protein cofactor of lipase also found in pancreatic secretion.

Determination of apparent and potential lipase and colipase activities were performed according to the Rathelot et al. technique.[16] Triolein substrate was constituted of 1 vol olive oil and 9 vol 10% arabic gum (Prolabo) sonicated 10 min in a generator Alcatel (20–200) at maximum power. The final assay mixture contained 7 mL substrate, 1 mL 22 mmol/L tauroglycocholate (Prolabo), 1 mL 125 mmol/L NaCl + 20 mmol/L $CaCl_2$. Apparent lipase activity was measured without addition of colipase in incubation medium and potential lipase activity with addition of saturating amounts of colipase, ie, 5 µg of crude exogenous porcine colipase[17] because there is evidence that colipase from one species activates lipase of other species. Determination of colipase activity requires previous lipase inactivation of pancreatic juice. This was achieved by acidification of incubation medium to pH 2.0 for 2 min to destroy lipase activity. After returning to pH 9.0 with 0.1 M NaOH, a purified preparation of rat lipase (40 U) devoid of colipase, was added and lipase activity was measured. Colipase was determined as its possibility to restore lipase activity. Triolein hydrolysis was measured during 10 min. The production of free fatty acids in medium was automatically neutralized by a 20 mmol/L NaOH solution. The results are expressed as µEq of fatty acid released/min^{-1}/mg protein^{-1}.

Statistical analysis

Data were expressed as their means ± SEM. Statistical evaluation of the data was carried out by analysis of variance and by the classification of the means using Duncan's new multiple range test.[18]

Results

In vivo experiments

Experiment A. Effects of raw and heated soya flours on the absorption of triolein given by gastric intubation.

The total radioactivity recovered in lymph, organ contents, and tissue homogenates accounted for 70%–80% of that given as meal containing triolein and RSF, HSF, or RC. The relative distribution of the radioactivity in the lipid fractions extracted from lymph, small, and large intestines is reported in *Table 2*. In rats fed meals containing HSF or RC, most of the radioactivity recovered in lipid extracts (74% and 82%, respectively) was found in lymph. Only a small proportion of the radioactivity (5.0% and 3.5%, respectively) was obtained in lipids extracted from the stomach. Conversely, only 42% of the radioactivity

Table 2 Experiment A: Relative radioactivity distribution recovered in the lipids extracted from lymph, small, and large intestine of rats fed [^{14}C]-triolein mixed with raw or heated soya flour or raw casein meal

Meal	Radioactivity			
	Lymph	Stomach and its content	Small intestine and its content	Large intestine and its content and feces
Triolein + RSF	42.2 ± 14.6^{b}	51.4 ± 12.6^{b}	6.5 ± 1.1^{b}	0.5 ± 0.4^{c}
Triolein + HSF	73.7 ± 20.2^{a}	5.0 ± 2.6^{a}	19.0 ± 2.1^{a}	3.5 ± 0.8^{b}
Triolein + RC	81.9 ± 19.1^{a}	3.5 ± 1.6^{a}	13.0 ± 5.1^{a}	1.1 ± 0.5^{a}

Meals were given by gastric intubation.
RSF and HSF, raw and heated soya flours. RC, raw casein meal.
Lymph was collected over a 24-hour period.
Data are given as percents of the total recovered radioactivity. Values are given as means ± SEM for five rats by group. After analysis of variance, classification of means was performed using Duncan's new multiple range test. Means with different superscript letters along the same vertical line are significantly different ($P < 0.05$).

appeared in lymph, and the major part of radioactive lipids (51%) was present in the fraction isolated from the stomach of rats fed RSF. Direct observations have pointed out that the RSF meal still adhered to the stomach wall at the end of experiment, and the label adhered to the soya flour.

The rate of radioactivity appearance in lymph during the 24-hour period following meal intubation is shown in *Figure 1*. From the curves of *Figure 1* it can be observed that, with the three meals, radioactivity was maximal in lymph two hours after triolein intubation. This observation indicates that the relative rate of triolein absorption was not significantly affected by the presence of RSF or HSF, although the net amount of radioactive lipids in lymph was significantly lower (−50%) in rats fed RSF, compared with HSF and RC values.

Experiment B. Effects of raw and heated soya flours on the distribution of [^{3}H]-oleic acid and [^{14}C]-triolein along the gastrointestinal tract after meal intubation.

This experiment was carried out with RSF, HSF, RC, or HC with [^{3}H]-oleic acid and [^{14}C]-triolein. Results are given in *Table 3*. A large and significant decrease in triolein and oleic acid absorptions were observed with RSF (−62% and −54%, respectively) compared with RC values. These absorptions were markedly enhanced when soya flour was heated. However, the highest values were obtained with HC. With both casein meals, the triolein and oleic acid absorptions were not significantly different, but triolein absorption was decreased to a larger extent than that of oleic acid with both soya meals. Most of the radioactivity recovered from triolein and oleic acid was observed in stomach. However, stomach radioactivity contents were significantly higher with RSFand HSF compared with RC and HC values. In the content of small intestine, the proportions of triolein and oleic acid with RSF were significantly decreased compared with HSF, RC, and HC values. In mucosa, these proportions were diminished with both soya flour meals compared with both casein meals. In small intestine tissue and large intestine + feces, very low proportions of triolein and oleic acid were found with RSF. Triolein absorption was particularly reduced with RSF.

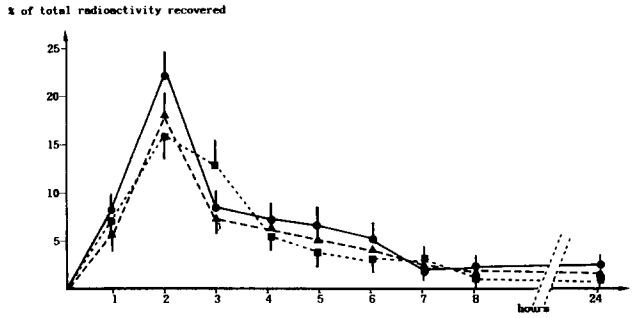

Figure 1 Relative rate of radioactivity appearance in lymph of rats fed [^{14}C]-triolein mixed with raw, heated soya flour, or casein meals. Meals were given by gastric intubation. Radioactivity in each lymph fraction is expressed as percent of total radioactivity recovered in lymph over 24-hour period. ■-----■ Raw soya flour meal, ▲- - - -▲ heated soya flour meal, ●———● casein meal.

Experiment C. Effects of raw and heated soya flours on triolein absorption investigated in isolated intestinal loop in situ.

Absorbed triolein was estimated as the difference between radioactivity introduced into the intestinal loop and that recovered in the lipids extracted from intestinal luminal content. Data reported in *Table 4* show that the net uptake of radioactive triolein was significantly decreased with RSF compared to HSF or RC values. The distribution of radioactivity in lipid fraction obtained from intraluminal contents is presented in *Table 5*. HSF induced a lower significant proportion of radioactivity in the triacylglycerol fraction and a higher significant proportion of radioactivity in the free fatty acid, monoacylglycerol, diacylglycerol, and cholesterol ester fractions. These findings might be indicative of increased lipolysis in the presence of HSF as compared with RC or RSF meals.

Table 3 Experiment B: [^{3}H]-oleic acid and [^{14}C]-triolein recovered at the different steps of gastrointestinal tract 6 hours after intubation of meals

Meal	Stomach	Small intestine content*	Small intestine mucosa*	Small intestine tissue*	Large intestine + feces*	Total radioactivity recovered†	Total radioactivity absorbed†
[^{3}H]-oleic acid RC	88.74 ± 3.71^a	1.58 ± 0.37^b	5.26 ± 1.74^a	3.47 ± 1.20^a	0.95 ± 0.39^a	30.9 ± 2.44^a	69.1 ± 2.44^a
[^{14}C]-triolein	87.93 ± 4.18^a	1.45 ± 0.34^b	5.90 ± 2.09^a	3.52 ± 2.25^a	1.20 ± 0.5^a	36.2 ± 2.63^a	63.8 ± 2.63^a
[^{3}H]-oleic acid HC	88.50 ± 0.72^a	1.84 ± 0.16^b	5.07 ± 0.70^a	3.95 ± 1.40^a	0.69 ± 0.17^a	17.8 ± 3.18^d	82.2 ± 3.18^d
[^{14}C]-triolein	88.60 ± 0.30^a	1.10 ± 0.15^{ab}	5.30 ± 0.80^a	3.80 ± 1.10^a	1.21 ± 0.24^a	20.1 ± 2.55^d	79.9 ± 2.55^d
[^{3}H]-oleic acid RSF	97.18 ± 0.34^b	0.73 ± 0.27^a	1.35 ± 0.43^b	0.74 ± 0.19^b	0.00 ± 0.00^c	67.8 ± 0.12^b	32.2 ± 0.12^b
[^{14}C]-triolein	97.02 ± 0.42^b	0.75 ± 0.23^a	1.37 ± 0.44^b	0.76 ± 0.19^b	0.10 ± 0.01^c	76.0 ± 1.51^b	24.0 ± 1.51^b
[^{3}H]-oleic acid HSF	92.61 ± 0.01^b	1.51 ± 0.00^b	2.23 ± 0.02^b	3.29 ± 0.01^a	0.36 ± 0.01^b	34.0 ± 1.11^a	66.0 ± 1.11^a
[^{14}C]-triolein	95.66 ± 0.65^b	1.66 ± 0.05^b	1.56 ± 0.44^b	0.73 ± 0.21^b	0.40 ± 0.05^b	42.4 ± 1.80^c	57.6 ± 1.80^c

RSF and HSF, raw and heated soya flour meals. RC and HC, raw and heated casein meals.
* Data are given in percents of total recovered radioactivity in gastrointestinal tract.
† Data are given in percents of intubated radioactivity.
Values are given as means ± SEM for five rats by group. After analysis of variance, classification of means was performed using Duncan's new multiple range test. Means with different superscript letters along the same vertical line are significantly different ($P < 0.05$).

Table 4 Experiment C: Absorption of triolein with raw or heated soya flour meals or raw casein meals

Meal	Recovered radioactivity: Intestinal content	Intestinal mucosa	Intestinal tissue	Absorbed radioactivity
Triolein + RSF	77.7 ± 0.4^b	0.9 ± 0.6^a	0.5 ± 0.3^a	21.0 ± 0.3^b
Triolein + HSF	71.2 ± 0.6^a	0.9 ± 0.4^a	0.3 ± 0.1^a	27.6 ± 0.8^a
Triolein + RC	70.1 ± 0.7^a	0.9 ± 0.1^a	0.3 ± 0.15^a	28.4 ± 0.6^a

RSF and HSF, raw and heated soya flour meals. RC, raw casein meal.
Data are expressed as percents of the total radioactivity intubated into the intestinal loop.
Experiments were carried out using the technique of the intestinal loop isolated in situ.
Values are given as means ± SEM for seven rats by group. After analysis of variance, classification of means was performed using Duncan's new multiple range test. Means with different superscript letters along the same vertical line are significantly different ($P < 0.05$).

Table 5 Experiment C: Distribution of radioactivity among lipids extracted from the intraluminal content of intestinal loop isolated in situ

Meal	Radioactivity in lipid fractions: Triacylglycerols	Diacylglycerols	Monoacylglycerols + Phospholipids	Cholesterol esters	Free fatty acids
Triolein + RSF	79.2 ± 4.3^a	8.1 ± 1.8^a	4.0 ± 0.5^a	0.3 ± 0.1^a	8.3 ± 1.7^a
Triolein + HSF	36.8 ± 4.3^b	20.8 ± 0.9^b	10.8 ± 2.6^b	3.8 ± 2.7^a	27.7 ± 2.0^b
Triolein + RC	78.6 ± 1.9^a	7.2 ± 0.1^a	4.0 ± 0.9^a	1.6 ± 0.3^a	8.5 ± 0.6^a

RSF and HSF, raw and heated soya flour meals. RC, raw casein meal.
[^{14}C]-triolein was mixed with raw or heated soya flour and casein meals.
Data are expressed as percents of total radioactivity intubated into the intestinal loop.
Values are given as means ± SEM for seven rats by group. After analysis of variance, classification of means was performed using Duncan's new multiple range test. Means with different superscript letters along the same vertical line are significantly different ($P < 0.05$).

Table 6 In vitro activities of apparent lipase, potential lipase, and colipase in presence of 100 mg of various meals

Meal	Apparent lipase	Potential lipase	Colipase	Colipase / Potential lipase
Without	64.8 ± 0.1[b]	86.5 ± 0.1[a]	48.6 ± 0.1[d]	0.56 ± 0.01[d]
RSF	61.1 ± 1.6[b]	73.0 ± 1.0[b]	8.8 ± 0.7[a]	0.12 ± 0.02[c]
HSF	92.4 ± 5.9[c]	94.6 ± 10.8[c]	13.5 ± 1.4[b]	0.14 ± 0.04[bc]
RC	51.6 ± 5.7[a]	80.5 ± 1.1[a]	18.9 ± 4.1[c]	0.23 ± 0.05[ab]
HC	68.1 ± 7.6[b]	71.1 ± 6.2[b]	18.2 ± 1.1[c]	0.26 ± 0.04[a]

RSF and HSF, raw and heated soya flour meals. RC and HC, raw and heated casein meals. Results are expressed as means ± SEM for four incubations by meal. Enzyme activities were expressed in specific activity (μEq of hydrolysed substrate/min^{-1}/mg protein^{-1}). After analysis of variance, classification of means was performed using Duncan's new multiple range test. Means with different superscript letters along the same vertical line are significantly different ($P < 0.05$).

In vitro experiments

Effects of raw and heated soya flours on triolein hydrolysis as studied with in vitro incubations.

The results are given in *Table 6*. Apparent lipase activity was not impaired with RSF and HC; enhanced by HSF; but depressed by RC, compared with the activity obtained in the absence of meal. Potential lipase activity was diminished with RSF and HC and increased with HSF. Colipase activity was strongly decreased by the four meals, particularly with both soya meals and especially with RSF. These results infer a marked reduction in colipase/potential lipase ratio values, particularly with both soya meals.

Discussion

The aim of this study was to investigate the short-term effect of raw and heated soya meals compared with casein meals, on triolein digestion and oleic acid absorption in rat.

Our data indicated that with RSF, 52% of radioactive lipid was found in the gastric content 24 hr after intubation in experiment A, instead of 3.5% and 5% with HSF and casein meals, respectively. This result was confirmed in experiment B when gut tract was removed after 6 hours of experiment. Direct observation of the gastric bowl at the end of these experiments confirmed that RSF meal was firmly sticking to the stomach wall, probably because RSF contains trypsin inhibitor and highly active surface proteins. This is in accordance with the observations by De Mulanaere,[19] Lyman et al.,[20] and Lyman and Wilcox,[21] that addition of RSF or soybean trypsin inhibitors (SBTI) to meals slows down the gastric transit of ingested food. Moreover, gastric motility is known to be markedly impaired by laparotomy. Perhaps this accounts for the particular inability of stomach to eject RSF in experiment A. Perhaps also the study design in which no further meals were given has created a highly artificial situation because with normal patterns of eating, meal residue would surely be diluted and washed out much more effectively than in this study. Another hypothesis was that RSF proteins and protease inhibitors stimulate CCK release more than HSF proteins and casein.[22,23] Green et al.[24] have shown that increased plasma CCK may strongly inhibit stomach emptying. Moreover, soya proteins in general, and protease inhibitors in particular, are not easily digested by gastrointestinal tract of rats.[25]

Pancreatic lipase and colipase operate when the triacylglycerol substrate is present at the oil-water interface of micelles. Bile salts, added to such system, bind to the interface, decrease the surface pressure, and displace lipase and most other proteins from the interface. This leads to inactivation of lipase by physical separation of the enzyme from its substrate.[26] This inhibition is reversed by colipase. Colipase binds to the lipase substrate interface in the presence of bile salts, and anchors lipase, making possible a lipase-substrate complex.[27]

The lower lipase activity (*Table 6*) and oleic acid and triolein absorptions (*Table 4*) observed with RSF, compared with HSF values, might be partly attributable to a partial inactivation of lipase and colipase by the highly active surface proteins of RSF. As shown by Gargouri et al.,[6] RSF actually contains proteins that can act as inhibitors of pancreatic lipase. In presence of bile salts, inhibition is counteracted by colipase in excess of lipase (potential lipase activity). However, under physiologic conditions, the colipase:lipase ratio is approximately one, which might not be sufficient to involve maximal lipolysis in the presence of inhibitory proteins. Moreover, colipase is secreted as procolipase.[25] If SBTI inhibits trypsin activity, procolipase-colipase conversion could be diminished, involving lipase activity decrease.

Results in *Table 6* indicate that soya meals impaired in vitro triolein digestion to a lesser extent than casein meals, because apparent lipase activities were higher with soya meals. The values obtained with HSF were highest, which was in agreement with the results shown in *Table 5*. Heating soya flour increased lipase activity. Therefore, in soya flour and casein, inhibiting thermolabile factors were effective, particularly in soya flour. Indeed HSF involved an enhanced activity to 51.3% compared with RSF, when HC values were only raised to 32% compared with RC values. A possible explanation might result from various solubility rates in dietary proteins. The higher solubility in the

more proteins could inhibit the enzyme-substrate contact. Studies have shown that raw SBTI and soya proteins have greater solubility than their respective heated products.[19]

The gain of activity between potential and apparent lipase was more substantial with raw meals and the highest increase was obtained with RC. These results may indicate that colipase action was reduced with RSF and RC and that a greater quantity of this cofactor was required to obtain adequate lipase activity with both these meals.

In vitro, the inhibiting effects on apparent lipase activity were greater with both casein meals than those obtained with soya meals. In each incubation, as lipase and colipase supply was the same, our data indicate that meal components, especially in casein meals, inhibited the enzyme-substrate contact and that part of this inhibition was suppressed by heating. We can hypothesize that proteins played the main role in this inhibitor effect. However, the fiber action cannot be omitted. Indeed, agar-agar contains mostly soluble fibers that have a greater capacity to form gel than insoluble fibers. The large proportion of soluble fibers involved reduced digestion and absorption of lipids. This effect could partly lead to the decrease in apparent lipase activity with casein meals.

Triolein absorption was studied using the technique of the intestinal loop isolated in situ. Data on *Table 3* indicate that after 20 min, [^{14}C]-triolein absorption was reduced with RSF to 23.9% and 28.4%, respectively, compared with HSF and RC values. These were in agreement with the results in *Table 4*, obtained by intubation of the meals, but the difference was enhanced with time, because after 6 hours, triolein absorption was decreased with RSF to 41.2% and 54.1%, respectively, compared with HSF and RC values. Triolein digestion was enhanced with HSF (*Table 5*) in comparison with RSF and RC, because in intestinal loop, this meal led to the presence of markedly less triacylglycerols and higher diacylglycerols, monoacylglycerols, and free fatty acids than RSF and RC values. Triolein absorption requires two steps: digestion followed by actual absorption. Absorption can be studied by oleic acid disappearance from digestive tract. The comparison between oleic acid and triolein absorptions showed only a slight difference with both soya meals, −8.4% for triolein absorption compared with oleic acid absorption.

In the short term (20 min) HSF involved a higher triolein digestion than those of RSF and RC (*Table 4*), but after 6 hours and a gastric stage, this advantage was reduced (*Table 3*).

Our data (*Tables 5 and 6*) show that lower digestion and absorption of triolein obtained with RSF (*Table 4*) were essentially attributable to the absorption step. The heating of casein meal raised triolein and oleic acid absorptions, to 8.2% and 19%, respectively, whereas, the heat treatment of soya flour enhanced these values to 70% and 56.4%, respectively. Therefore, in casein meals, active thermolabile components that inhibit lipid digestion and absorption were present, but components with similar effect were in larger quantities and/or were more efficient in soya flour. Triolein and oleic acid absorptions were lower with HSF and RSF than with HC or RC, in spite of a higher degree of in vitro apparent lipase activity with both soya meals. This result could also be accounted for by the presence of thermostable lectins in HSF and RSF.[28,29] Lectins might interact with glycoproteins of the brush border and impair lipid absorption from mixed micelles. But, other effects such as impaired diffusion or increased thickness of the unstirred layer could account for the changes observed between soya meals and casein meals.

In conclusion, in vivo experiments on the effects of soya flour on triolein digestion and absorption in rats have shown that highly tensioactive proteins of raw soya flour may be responsible for impaired emptying of the stomach and therefore for the observed reduced absorption of ingested lipid. This effect was markedly increased by laparotomy. Thermolabile proteins might also decrease intragastric and intestinal intraluminal lipolysis. Our data indicate that soya flour contained more inhibiting lipid absorption components than casein meal and the latter contained more inhibiting in vitro lipase activity components. As heating improved lipid absorption and lipase activity, a part of these inhibiting compounds was thermolabile. However, in spite of normal lipolysis activity, heated soya flour contained an inhibitory activity on lipid absorption because differences prevailed between the effects of both heated meals. A part of these components was thermostable and might interfere with lipid absorption at the brush border level. These inhibiting effects of soya flour and casein meals can play a significant physiologic role on the appearance rate of dietary lipids in lymph.

Abbreviations

RSF raw soya flour meal
HSF heated soya flour meal
RC raw casein meal
HC heated casein meal
SBTI soybean trypsin inhibitor

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