Adaptation of exocrine pancreas to dietary proteins: Effect of the nature of protein and rat strain on enzyme activities and messenger RNA levels

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We compared the effect of high protein diets enriched with casein, fish, or soybean on enzyme content and mRNA levels in pancreata of postweaning and adult rats of two different strains. In the first experiment, 72 male Fischer rats (age 5 weeks) were divided into six groups and fed with one of six diets containing 20% or 50% protein as fish meal, casein, or soybean for 1 or 3 weeks. In a second experiment, 36 Fischer and 36 Wistar rats were divided into six groups and fed with the same diets for 1 week. In both experiments, rats were sacrificed at the end of the experimental feeding period and pancreata were excised and prepared for biochemical assay and mRNA extraction. Activities and mRNA levels were determined for each enzyme (amylase, lipase, chymotrypsin, trypsin, and elastases). Pancreas weight and its total protein content were modulated by the amount of dietary protein and duration of diet. These parameters were significantly different in Fischer and Wistar rats. In the latter strain, the nature of dietary protein also influenced pancreas weight. In Fischer rats, amylase specific activity was decreased after feeding 50% casein diet for 1 or 3 weeks and 50% fish or soybean diets for 3 weeks. The decrease of specific mRNA was more pronounced after a 3-week than after a 1-week feeding, suggesting that transcriptional regulation replaced progressively a translational one. In Wistar rats, amylase specific activity was not modified, but mRNAs were decreased after feeding high-protein diets. Lipase specific activity and mRNAs were not modified by any diet in any group. Chymotrypsin specific activity was increased after feeding 50% casein and soybean diets for 1 week and any 50% protein diet for 3 weeks. This effect was more pronounced in adult rats fed high protein diets for 1 week. In young Fischer rats, chymotrypsinogen mRNAs were increased after feeding 50% casein diets; in adult rats of both strains this parameter was increased by all diets except when the 50% soybean diet was provided to Wistar. Trypsin specific activity was increased after feeding 50% casein diet for 1 week and 50% fish or soybean diet for 3 weeks in Fischer rats but not altered in Wistar. The expression of trypsinogen mRNA was only increased after feeding 50% casein diet to both strains and 50% soybean diet to Wistar rats, suggesting that the regulation of its expression is different with the nature of protein. Elastase specific activities were increased by high-protein diets in both strains, but this effect was more pronounced in Wistar rats; these enzyme mRNAs were not altered, suggesting that the regulation was translational. In conclusion, it appears that the kinetics of adaptation of enzymes is different depending on the nature of dietary protein and the strain of rats used in the experiment. Amylase biosynthesis is regulated at the transcriptional level. Chymotrypsinogen and trypsinogen mRNA level showed that casein-induced adaptation was modulated via transcription, while other diets induced adaptation via posttranscriptional events. Elastases adapt differently depending on the nature of the protein and the regulation of their expression is mostly posttranscriptional. (J. Nutr. Biochem. 5:84–94, 1994.)

Keywords: rat pancreas; dietary adaptation; gene expression

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Introduction

Since the report of Pavlov[1] in the late 1800s, the adaptation of the exocrine pancreas to quantitative changes in

dietary substrate has been described in various species.[2,3] A consensus fact is that protease, lipase, and amylase secretion and contents change proportionally in response to the amount of their respective dietary substrates, i.e., protein, lipids, and carbohydrate. Aside from effect of dietary amount, the incidence of the nature of dietary protein on pancreatic adaptation has rarely been studied. Authors reported that the effect of dietary proteins on pancreatic enzymes is inversely proportional to their digestibility and depends on an adequate proportion of amino acids.[4–6] For example, diets containing 64% high-quality proteins such as casein or fish concentrate increased chymotrypsin as opposed to low-quality proteins such as zein or gluten, which did not.[4] Moreover, administration of a diet containing casein versus soybean or egg protein led to higher chymotrypsin (soybean and egg diets) and trypsin (egg diet only) specific activities.[5,6] Also, in the weanling and adult pig, a diet containing the standard level of mixed wheat-fish protein had a similar effect on protease secretion as a casein semi-synthetic diet, but amylase and lipase were more sensitive to the mixed diet than to the semi-synthetic.[7,8] In our laboratory we demonstrated that protease secretion was different in pigs fed rapeseed or casein protein.[9] In the first experiment reported here, we attempted to analyze the effect of feeding diets containing 20 or 50% protein either as casein, fish concentrate, or soybean meal, on pancreatic enzyme content and mRNA levels for 1 and 3 weeks to postweaning male Fischer rats (5-weeks-old).

The comparison of data from several authors may also be impaired by the rat strain used. As an example, it has been demonstrated previously that Fischer rats are less sensible to pancreatic cancer than Wistar.[10] In pig species, it has been reported that intestinal carbohydrase activities differ with breed.[11] Feeding Sprague-Dawley rats with 8.5 to 40% casein diet for 6 days[12] or with 15 to 70% casein diet for 24 hours[13] proportionally increased trypsin activities, but no difference was observed when the same 15 and 70% casein diets were fed to Wistar rats for 2 months.[14] However, these data are difficult to compare because the protocol used are rather different. Therefore, in a second experiment, we compared a 1-week adaptation in Fischer and Wistar adult rats fed with diets containing 20 or 50% protein as casein, fish, or soybean.

Methods and materials

Experimental protocols

In both experiments, rats were housed in pairs and had free access to acidified water and food. The composition of the six experimental diets (20 or 50% protein as casein, fishmeal concentrate, or soybean meal and isolate) is illustrated in *Table 1*.

In the first experiment, 72 male Fischer rats weighing 105 ± 1 g (age 5 weeks) were divided into six groups and received one of the experimental diets. After a 1-week treatment, 36 rats (six per group) were sacrificed by decapitation under ether anesthesia and the pancreas was removed, weighed, and divided into two parts. The first part (left lobe) was immediately used for mRNA extraction. The second part (right lobe) was frozen until protein, DNA, RNA, and enzyme activities were assayed. The remaining 36 rats were sacrificed after 3-week treatment, and the same analyses were performed on the collected and frozen pancreatic tissue.

Table 1 Composition of diets in percent of dry weight and crude energy (J/ 100 g dry weight). Carbohydrates and lipids provided by soybean and fish meal were taken in account for calculation of the total amount of fat and carbohydrate in the diets.

	Casein		Fish		Soybean	
Casein	22.2	55.5	—	—	—	—
Methionine	0.3	0.3	—	—	—	—
Fish concentrate[a]	—	—	24	60	—	—
Soybean meal[b]	—	—	—	—	44	44
Soybean isolate[c]	—	—	—	—	—	33
Corn oil	4	4	3.6	—	3	3
Corn starch	63	29.7	65.4	33	45.5	12.5
Cellulose	5	5	5	5	2	2
Minerals[d]	4.5	4.5	0.8	0.8	4.5	4.5
Vitamins[e]	1	1	1.2	1.2	1	1
Crude energy[f]	408	399	420	413	397	402
% protein	20	50	20	50	20	50
% lipid	4	4	6	6	4	4

[a]Fish concentrate (CPSP 80) contained approximately 85% proteins, 10% lipids, and 5% minerals.
[b]Soybean meal contained approximately 44.4% protein, 2.3% lipid, 6.4% cellulose, and 3.8% carbohydrates. No trypsin inhibitor activity was detected.
[c]Soybean isolate contained approximately 88.5% protein, 1.5% lipid, and 1.5% cellulose.
[d]In mg/g of mineral complex: calcium phosphate, $2H_2O$ 380, potassium phosphate 240, calcium carbonate 180, sodium chloride 69, magnesium oxyde 20, magnesium sulfate 90, iron sulfate 8.6, zinc sulfate 5, manganese sulfate 5, copper sulfate 1, cobalt carbonate 0.02, potassium iodine 0.04, ammonium molybdate 0.02, sodium selenate 0.02, sodium fluoride 0.8, cromium and potassium sulfate, 12 H_2O 0.5.
[e]In mg/ kg diet: choline concentrate (50%) 1500, vitamin E 100, vitamine A acetate 10, vitamine D3 25, niacine 45, calcium pantothenate 30, thiamine chlorhydrate 10, riboflavine 10, pyridoxine chlorhydrate 10, ascorbic acid 100, paraaminobenzoic acid 50, folic acid 2, cyanocobalamine 13.5, rovimix H2 (biotin) 10, menadione 1, meso-inositol 50, saccharose 8033.5.
[f]Obtained by calculation.

In the second experiment, 36 Fischer and 36 Wistar rats weighing 207 ± 3 and 197 ± 3 g, respectively, were divided into six groups (n = 6 per group and per rat strain) and received one of the six experimental diets. After a 1-week treatment, they were sacrificed by decapitation and the pancreas was collected and processed as described above.

Protein and enzyme assays

Pancreata were homogenized in ice-cold distilled water (1 g/ 7 mL). Protein and enzyme contents (amylase, lipase, chymotrypsin, trypsin, and elastase) were determined as published previously.[15]

mRNA extraction and dot-blot hybridization

Total RNA was prepared by the procedure of Chirgwin et al.[16] with slight modifications. Immediately after excision, pancreata were homogenized on ice with a Polytron (15 seconds, medium speed) in 8 mL of a buffer containing 5 M guanidinium thiocyanate (Fluka, AG, Basel, Switzerland), 50

mM (Tris)-Cl (pH 7.5), 25 mM EDTA, and 0.1 M mercaptoethanol. This solution was then precipitated in 0.75 volume ethanol and 25 mM acetic acid at −70° C for at least one night. After a 10-minute centrifugation (−10° C, 5,000 g in a Sorval SS-34 rotor), the pellet was resuspended in 0.5 volume 7.5 M guanidinium hydrochloride (Fluka, AG), 200 mM EDTA, pH 7.5, 5 mM dithiothreitol. The RNA was reprecipitated in ethanol as described above. This dissolution-precipitation step was repeated once, reducing the volume by half. The resulting pellet was washed with ethanol to remove any remaining guanidinium hydrochloride, and finally dissolved in water (1 mL). Total RNA was quantified spectrophotometrically at 260 nm, reprecipitated in 66% ethanol, 0.2 M potassium acetate, pH 5 and stored at −70° C until assays. Qualitative analysis of total RNA was routinely performed on 1% agarose gel electrophoresis.[17] Gene expression was determined by dot blot hybridization using six rats per group unless otherwise indicated in the Results section. Undegraded RNA samples were denatured in 7.4% formaldehyde, 10 × standard sodium phosphate EDTA (SSPE) by heating at 65° C for 15 minutes. Exact concentration was measured by spectrophotometry at 260 nm, and RNA were dotted onto nitrocellulose in sequential dilutions using a manifold apparatus (Minifold I, Schleicher und Schüll, Dassel, Germany). Filters were air dried and baked at 80° C for 30 minutes.

cDNA probes were labeled with (α^{32}P)-dCTP (Amersham, Les Ulis, France) using a random primer kit (Boehringer, Mannheim, Germany). The specific activity of the probes was routinely 10^9 cpm/μg after an overnight incubation at room temperature. Rat amylase (1400 bp), lipase (930 bp), chymotrypsinogen (600 bp), and trypsinogen (745 bp) probes were kindly provided by C. Wicker (CNRS, Marseille, France).[18,19] Rat elastase I (pcXp 13; 900 bp) and elastase II (pcXp 30; 600 bp) were a gift from R. McDonald (Texas University, Dallas, TX, USA).[20] Filters were prehybridized for 4 hours at 42° C in 10 mL hybridization buffer (5 × SSPE, 50% formamide, 5 × Denhardts' reagent, 0.1% sodium dodecylsulfate (SDS), 100 μg/mL denatured salmon sperm DNA).[17] Hybridization was carried out at 42° C for 20 hours in the above buffer containing the denatured random primed probe (5 × 10^6 cpm/mL). After hybridization, filters were washed in 2 × standard sodium citrate (SSC), 0.5% SDS at 55° C for 3 × 45 minutes and then in 0.1 × SSC, 0.1% SDS at 55° C for 45 minutes. Filters were then blotted dry and exposed to X-ray film. After film development, filter-bound radioactivity was determined by Cerenkov counting in an LKB scintillation spectrometer. Slopes of the dot intensity of each dilution were calculated for the linear portion of the curves by linear regression.

On each nitrocellulose filter, mRNAs from one rat per group were spotted. Hybridization was performed in the same conditions, and specific binding was expressed as percentage of corresponding control on each filter (20% protein diet). By normalizing the pancreatic levels of each specific mRNA to those of the control diet, it was therefore possible to evaluate the relative levels of specific mRNA in total cellular extracts from the pancreas of individual animals in each group.

Statistical analysis

The SAS software package was used for statistical analysis (SAS Institute Inc., Cary NC, USA). For weights and all biochemical parameters, a three-way analysis of variance (ANOVA) was performed. In both experiments, amount of dietary protein and its nature were considered as classes. In addition, duration of treatment in the first experiment and rat strain in the second experiment were also considered. In each analysis, the interaction of all classes were also taken into account. After general linear model procedure, groups were compared by a Duncan test (multiple groups). mRNAs were compared with a Wilcoxon T test.

Results

Effect of the nature of dietary protein and duration of treatment on the rat pancreas (1st experiment)

Body weight gain, pancreatic weight, and total pancreatic protein content are represented in *Table 2*. The growth of young Fischer rats was not impaired by any diet. The data of three-way ANOVA are represented in *Table 3*. The amount of protein and duration of treatment significantly affected pancreatic weight and its total protein content ($P < 0.0001$); the nature of protein did not influence these parameters and there was no interaction between any parameter.

Pancreatic weights (mg/100 g body weight) were increased after feeding 50% fish and soybean diets for 1 week ($P < 0.01$ and 0.05, respectively, as compared with

Table 2 Effect on body weight gain, pancreatic weight, and total protein content of feeding experimental diets for 1 or 3 weeks to post-weaning Fischer rats.

Diet	C20	C50	F20	F50	S20	S50
1-week feeding						
Body wgt gain	27 ± 4	23 ± 2	32 ± 5	32 ± 3	26 ± 4	26 ± 5
Panc wgt (mg/100 g)	517 ± 37	515 ± 55	373 ± 45	534 ± 15**	430 ± 24	587 ± 34*
Protein (mg)	178 ± 8	191 ± 33	123 ± 18	215 ± 9*	154 ± 23	216 ± 18
3-week feeding						
Body wgt gain	141 ± 21	134 ± 18	170 ± 17	149 ± 16	131 ± 9	152 ± 20
Panc wgt (mg/100 g)	387 ± 21	465 ± 28	348 ± 36	420 ± 38	427 ± 36	441 ± 38
Protein (mg)	244 ± 15	293 ± 14	215 ± 29	354 ± 64*	205 ± 8	261 ± 17

C20 and C50, 20 and 50% casein diets; F20 and F50, 20 and 50% fish diets; S20 and S50, 20 and 50% soybean diets.
Results are means ± sem.
*$P < 0.05$ and **$P < 0.01$ when compared with respective 20% protein diet.
Panc. wgt, pancreatic weight (mg/100 g final body weight).
Body weight gain is the ratio: (BWF-BW0) × 100/BW0. BW0, body weight on day 0, BWF, body weight at the end of experiment.

Table 3 Incidence of amount and nature of dietary protein and duration of experiment in young Fischer rats on body weight gain and pancreatic adaptation (pancreatic weight, total protein content, and enzyme specific activities) (first experiment)

Class	BW gain	Panc. wt	Protein	Amylase	Lipase	Chtrypsin	Trypsin	Elastase
Amount	0.5820	0.0001	0.0001	0.0001	0.2145	0.0001	0.0001	0.0001
Protein	0.2876	0.6864	0.4503	0.6548	0.0341	0.2452	0.2482	0.8046
Duration	0.0001	0.0001	0.0001	0.1064	0.0017	0.1177	0.3247	0.0075
Am × Du	0.9183	0.8224	0.5576	0.0384	0.4406	0.0565	0.1190	0.1930
Am × Pr	0.4972	0.1852	0.0700	0.1237	0.6710	0.8194	0.8120	0.1248
Pr × Du	0.6883	0.9399	0.1375	0.0581	0.2720	0.0084	0.0029	0.0665

P values were calculated by three-way analysis of variance for each parameter and each class.
BW gain, body weight gain; Panc. wt, pancreatic weight; Protein = total protein content; Chtrypsin, chymotrypsin; Amount (Am), 20 or 50% of protein in diet; Protein (Pr) = casein, fish, or soybean; Duration (Du), 1 or 3 weeks.

their 20% counterparts) but this effect was not significant after 3 weeks. If total protein content is considered, we observed a significant increase after feeding 50% fish concentrate diet (1.6-fold when feeding for 1 or 3 weeks, $P < 0.05$ when compared with 20% fish diet).

Specific enzyme activities (U/mg protein) and mRNA levels (expressed as percentage of control) are represented in *Figure 1*, while the results of the three-way ANOVA are illustrated in *Table 3*.

Amylase specific activity was affected by the level of protein in the diet ($P < 0.0001$) regardless of the nature of protein. However, the nature of dietary protein significantly interacted with duration of experiment ($P < 0.0384$). After feeding the 50% casein diet for 1 week, amylase specific activity was decreased by 30% (as compared with 20% casein diet, $P < 0.01$); this effect was enhanced when the same diet was fed for 3 weeks (50% of the 20% casein level; $P < 0.01$). Feeding other 50% protein diets significantly decreased amylase specific activity after 3 weeks only. mRNAs were significantly decreased after feeding the 50% casein or soybean diets for 3 weeks ($P < 0.05$).

Three-way ANOVA showed that nature of diet influenced lipase specific activity ($P < 0.0341$). However, only the group of rats fed the 50% casein diet was statistically different from the one fed the 20% fish diet ($P < 0.05$). mRNA levels were not modified except when feeding 50% soybean diet for 3 weeks, which increased this parameter ($P < 0.05$).

The three-way ANOVA pointed out a significant incidence of amount of protein on all serine protease ($P < 0.0001$) specific activities. Moreover, this analysis exhibited a significant interaction between the nature of the protein and duration of experiment on chymotrypsin ($P < 0.0084$) and trypsin ($P < 0.0029$) specific activities. The effect of 50% casein diet on chymotrypsin specific activity was maximum after 1 week of experiment (two-fold increase, $P < 0.01$), but this activity seemed to be less affected after feeding the casein-rich diet for 3 weeks (1.5-fold increase, $P < 0.05$). Feeding 50% fish diet for 1 week did not modify chymotrypsin specific activity, but this parameter reached more than a two-fold increase after 3 weeks ($P < 0.01$), while feeding soybean-rich diet for 1 week induced a 1.5-fold increase of this parameter ($P < 0.01$); prolonging this diet for 3 weeks induced a two-fold increase of chymotrypsin specific activity ($P < 0.01$). Chymotrypsinogen mRNA levels were significantly increased after feeding the 50% casein diet for 1 and 3 weeks but not after feeding the 50% fish or soybean diets.

The same pattern was observed for trypsin specific activities, but the increase was more modest. mRNAs were only increased after feeding 50% casein diet for 3 weeks.

Elastase specific activities were significantly increased after feeding fish-enriched diets for 1 and 3 weeks (1.2-fold, $P < 0.05$). Other 50% protein diets did not affect this parameter. Elastase mRNAs were not modified.

Effect of the nature of dietary protein, and rat strain on the rat pancreas (2nd experiment)

In adult Fischer rats, body weight gain was not impaired by feeding any 20% diet for 1 week, while feeding 50% casein diet decreased this parameter ($P < 0.05$). No significant effect was observed in Wistar rats, although the same trend was observed (*Table 4*) as alleged by the multiple-way ANOVA ($P < 0.0005$).

Table 5 summarizes the results of a three-way ANOVA performed on this experiment. The amount of dietary protein altered pancreatic weight and its total protein content ($P < 0.0001$ and $P < 0.0035$, respectively), while the nature of dietary protein only affected pancreatic weight ($P < 0.0147$). Rat strain influenced pancreatic weight and its total protein content ($P < 0.0059$ and $P < 0.0092$, respectively). In Fischer rats, total protein content was only increased after feeding 50% fish diet (1.4-fold, $P < 0.01$ when compared with the respective 20% diet). In Wistar rats, feeding 20% fish diet altered pancreatic weight, but no 20% diet significantly modified pancreatic protein content. In the same rat strain, feeding 50% protein diets increased pancreatic weights (1.3-fold, $P < 0.05$ for 50% casein; 1.4-fold, $P < 0.01$ for 50% fish, and 1.5-fold, $P < 0.01$ for 50% soybean as compared with their respective control), while only 50% soybean increased its total protein content (1.5-fold, $P < 0.01$ when compared with the respective 20% diet).

Specific enzyme activities (U/mg protein) and mRNA levels (percentage of control) are represented in *Figure 2*.

Amylase specific activity was significantly altered by

Table 4 Effect on body weight gain, pancreatic weight, and total protein content of feeding experimental diets for 1 week to Wistar and Fischer rats

Diet	C20	C50	F20	F50	S20	S50
Fischer rats						
Body wgt gain	13 ± 1	5 ± 1*	19 ± 1	17 ± 1	16 ± 3	13 ± 1
Panc. wgt (mg/100g)	421 ± 26	454 ± 34	363 ± 24	417 ± 27	410 ± 27	462 ± 39
Protein (mg)	213 ± 13	241 ± 8	185 ± 11	246 ± 12**	224 ± 14	262 ± 21
Wistar rats						
Body wgt gain	29 ± 6	17 ± 7	32 ± 7	20 ± 3	30 ± 6	12 ± 9
Panc. wgt (mg/100g)	363 ± 10	484 ± 47*	323 ± 40	468 ± 18**	422 ± 35	640 ± 27**
Protein (mg)	225 ± 5	269 ± 22	256 ± 24	271 ± 69	234 ± 17	352 ± 23*

C20 and C50, 20 and 50% casein diets; F20 and F50, 20 and 50% fish diets; S20 and S50, 20 and 50% soybean diets.
Results are means ± sem.
*$P < 0.05$ and **$P < 0.01$ when compared with respective 20% diet.
Panc. wgt, pancreatic weight (mg/100g final body weight).
Body weight gain is the ratio: (BWF-BW0) × 100/BW0. BW0, body weight on day 0, BWF, body weight at the end of experiment.

Table 5 Incidence of amount and nature of dietary protein, and rat strain on body weight gain and pancreatic adaptation (pancreatic weight, total protein content and enzyme specific activities) (second experiment)

Class	BW gain	Panc. wt	Protein	Amylase	Chtrypsin	Trypsin	Elastase
Amount	0.0005	0.0001	0.0035	0.0001	0.2145	0.0001	0.0001
Protein	0.0897	0.0147	0.2487	0.3786	0.3934	0.9527	0.4347
Strain	0.0001	0.0059	0.0092	0.0001	0.0001	0.0001	0.0405
Am × St	0.0552	0.0317	0.8279	0.5522	0.0244	0.0005	0.0111
Am × Pr	0.8781	0.3953	0.4263	0.1754	0.9193	0.2140	0.0588
Pr × St	0.4476	0.0855	0.6827	0.9734	0.4963	0.5079	0.7391

P values were calculated by three-way analysis of variance for each parameter and each class.
The ratio of lipase specific activity is not represented because no class had a significant effect on these parameters.
BW gain, body weight gain; Panc. wt, pancreatic weight; Protein, total protein content; Chtrypsin, chymotrypsin; Amount (Am), 20 or 50% of protein in diet; Protein (Pr), casein, fish, or soybean; Rat strain (St), Fischer or Wistar.

the amount of dietary protein and rat strain (three-way ANOVA, *Table 5*, $P < 0.0001$ for both variables) but not by the nature of dietary protein. Feeding Fischer rats with 50% soybean decreased amylase specific activity and mRNA by 50% ($P < 0.01$ for specific activity and $P < 0.05$ for mRNA when compared with 20% soybean). In Wistar rats we did not observe any effect of diets on amylase specific activity but mRNA levels were significantly decreased by 50% after feeding any protein-rich diets ($P < 0.05$).

Lipase specific activity and mRNAs were not modified by any diet at any time.

The three-way ANOVA pointed out a significant influence of amount of protein on protease specific activities ($P < 0.0001$). Rat strain was responsible for differences among serine proteases. Moreover, this analysis exhibited a significant interaction of the amount of protein with strain on chymotrypsin ($P < 0.0244$), trypsin ($P < 0.0005$), and elastase ($P < 0.0111$) specific activities.

Chymotrypsin specific activity was not different in all 20% protein diets. This enzyme specific activity was increased by all the protein-rich diets. In Fischer rats, all 50% diets induced approximately a 1.7-fold increase of specific activity and 1.2- to 1.5-fold increase in mRNA levels ($P < 0.05$). In Wistar rats, specific activities were significantly increased after feeding all diets (two-fold, $P < 0.01$), and mRNAs were only increased after feeding 50% casein and fish diets (1.5-fold, $P < 0.05$).

In Fischer rats, trypsin specific activity was not modified whatever the nature of protein in 20% protein diets, and was increased after feeding 50% casein and fish diets (respectively, 1.5- and 1.7 fold, $P < 0.01$ and $P < 0.05$). Trypsinogen mRNA levels were only increased after feeding 50% casein diet ($P < 0.05$). In all groups of Wistar rats, trypsin specific activity was much lower than in Fischer rats. Moreover, after feeding 50% casein or soybean diets to Wistars, we did not measure any increase in trypsin specific activity, although mRNAs were increased.

Elastase specific activities were not different in all 20% protein diet groups and they were significantly increased after feeding 50% fish diet to Fischer rats (1.3 fold, $P < 0.05$). However, mRNAs were not modified. In Wistar rats, all three protein-rich diets increased this parameter ($P < 0.01$), but mRNAs were not modified either.

Discussion

In this work, we investigated the effect of various dietary proteins on pancreatic adaptation to high protein dietary

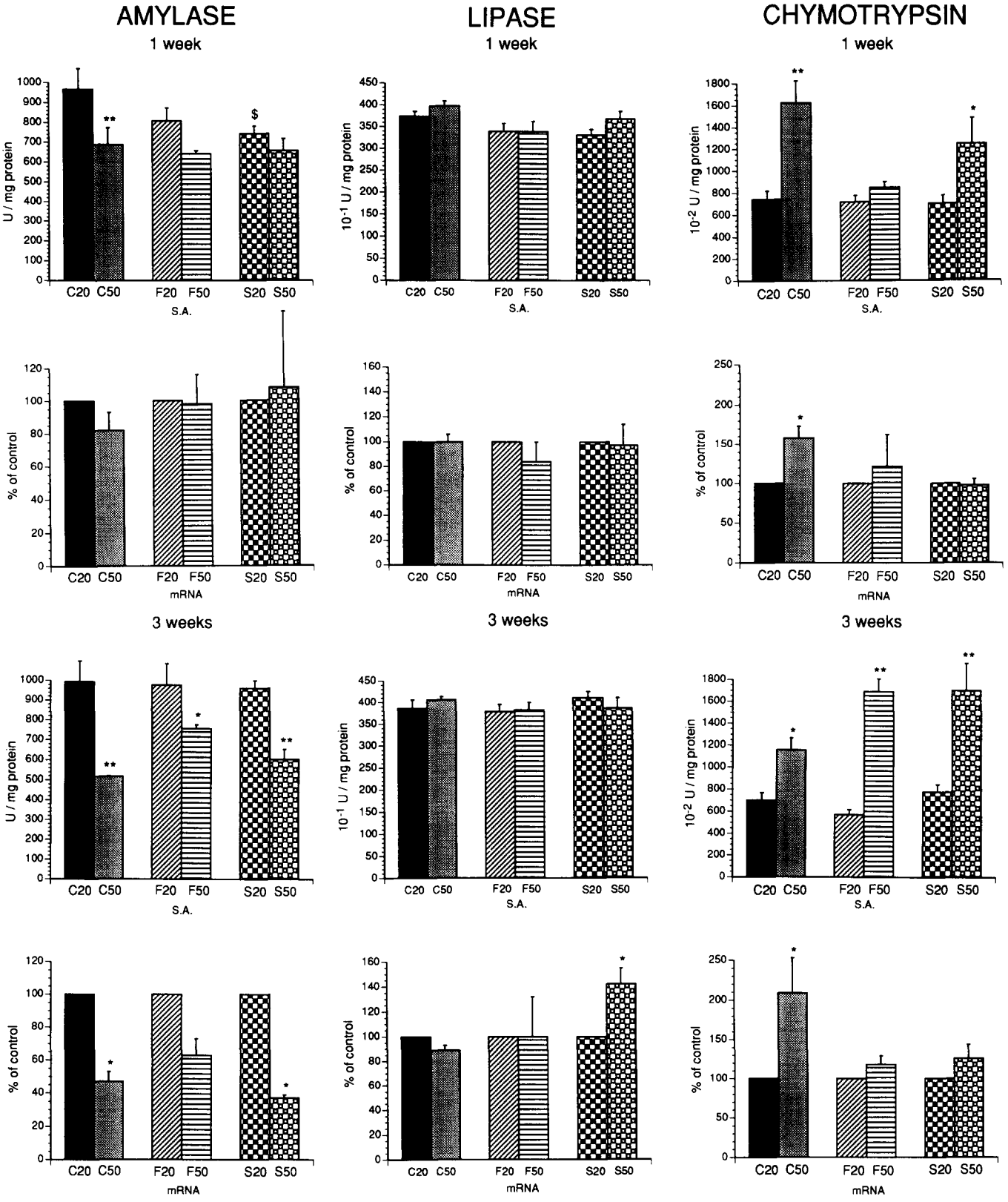

Figure 1 Effect of feeding experimental diets for 1 or 3 weeks to post-weaning Fischer rats on enzyme specific activities (U/mg protein) and mRNA levels (expressed as percentage of respective 20%).
S.A., specific activity.

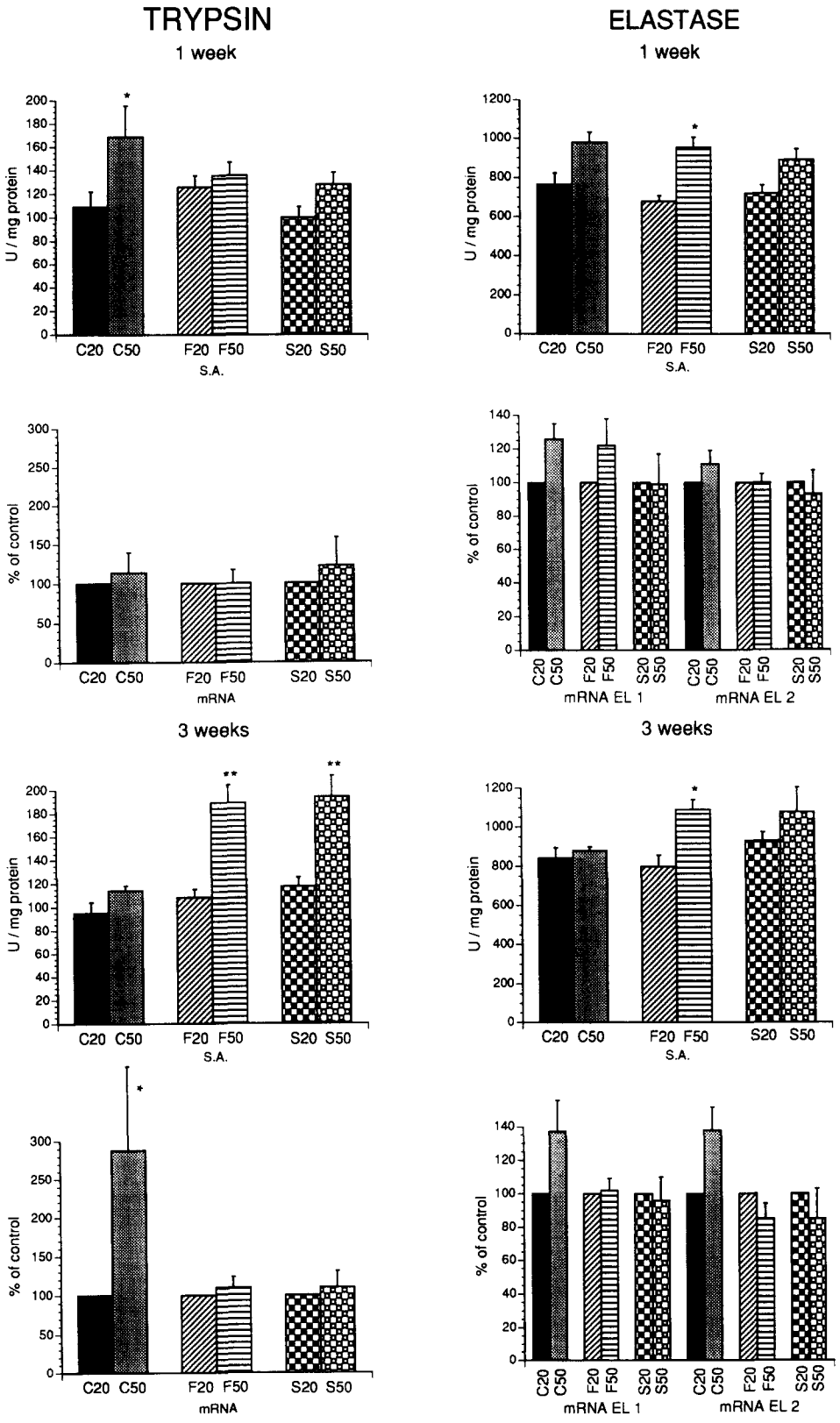

Figure 1 Continued
*$P < 0.05$ and **$P < 0.01$ when compared with the corresponding 20% protein diet.
$ $P < 0.01$ when compared with 20% casein diet.
EL1 and EL2, (pro)elastase 1 and 2;
The number of animals per group was five for enzyme activities and six for mRNA except for lipase (3 weeks casein, $n = 5$) and elastase (all groups, $n = 5$).

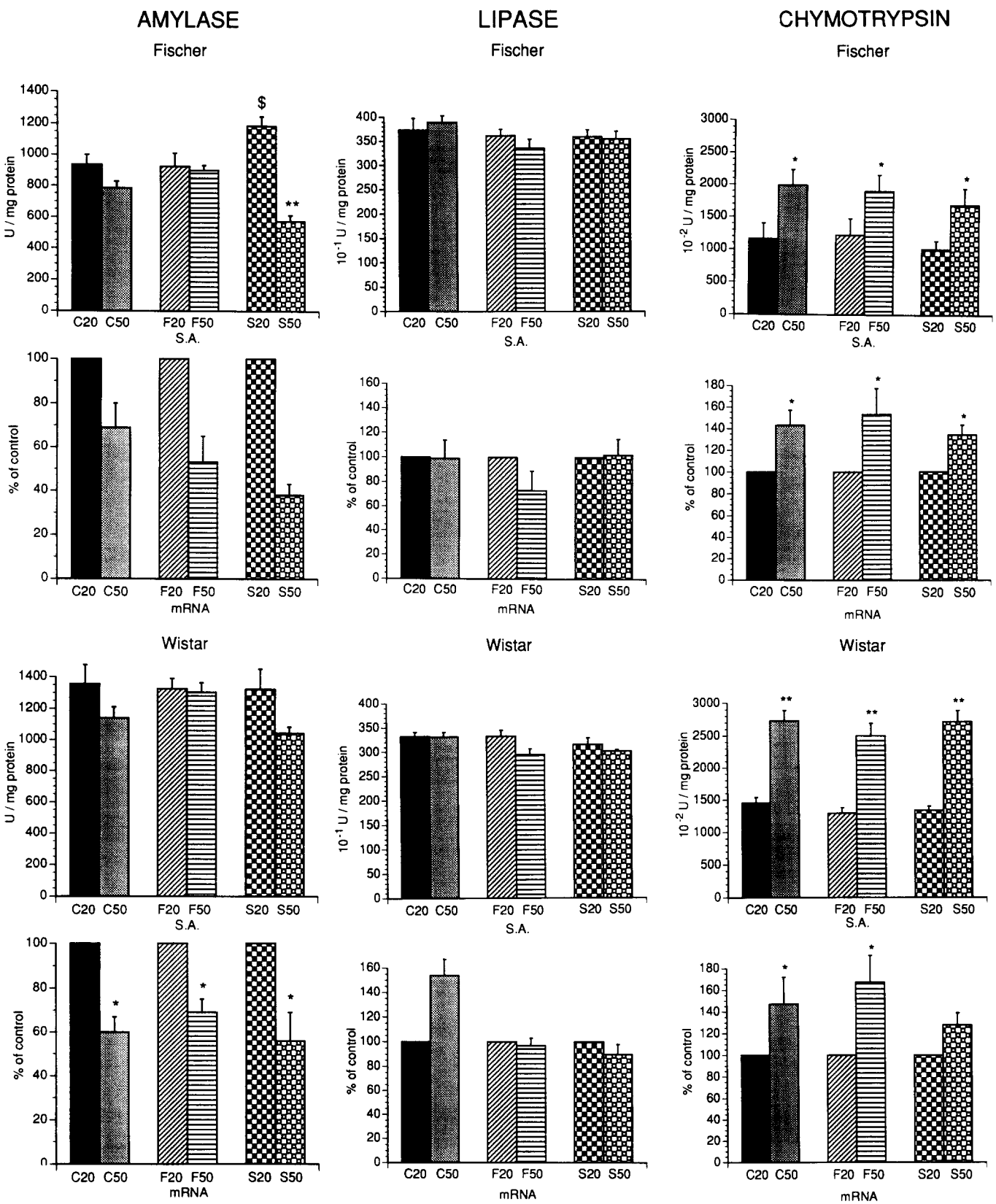

Figure 2 Effect of feeding experimental diets for 1 week to adult Wistar and Fischer rats on enzyme specific activities (U/mg protein) and mRNA levels (expressed as percentage of respective 20%). C20 and C50, 20 and 50% casein diets; F20 and F50, 20 and 50% fish diets; S20 and S50, 20 and 50% soybean diets.
S.A., specific activity.
Results are mean ± sem.

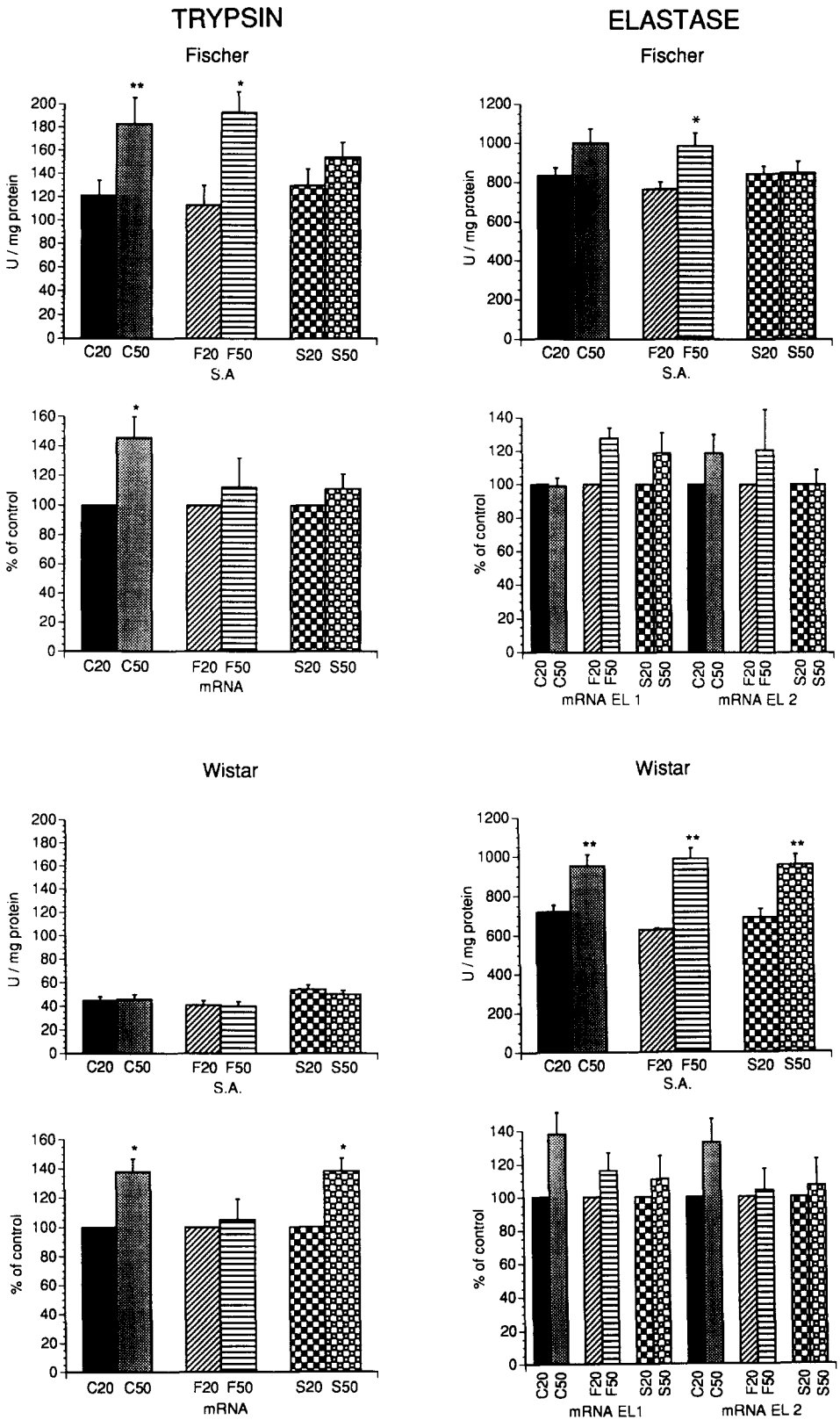

Figure 2 Continued
*$P < 0.05$ and **$P < 0.01$ when compared with the corresponding 20% protein diet.
\$ $P < 0.05$ when compared with 20% casein diet.
EL1 and EL2, (pro)elastase 1 and 2;
The number of animals per group was five for enzyme activities and six for mRNA except for lipase (all groups, $n = 5$) and elastase 2 (Fischer casein and fish, $n = 5$).

levels in rats of different ages and strains. The administration of high protein diets slightly impaired the growth of adult rats. In previous studies in which dietary proteins were supplied as casein to Sprague-Dawley rats[12,13] or adult Wistar rats[5] there was no effect on body weight or it was slightly decreased.[21]

Pancreatic growth was altered by the amount of dietary protein in both experiments and by nature of protein and rat strain in the second experiment. In Wistar rats only, pancreatic size was significantly increased by feeding high dietary proteins. These data are in agreement with previous studies.[22,23] When casein was used, this increase in pancreatic weight has been shown to reflect both hyperplasia and hypertrophy.[23]

When elaborating on the experimental diets for further comparison of their respective effect, it is important to keep them isocaloric so that the daily consumption is equivalent for all diets. Therefore, the increase in dietary protein is usually obtained by decreasing carbohydrate content. It has been demonstrated previously that high casein diets increased protease specific activities at the expense of amylase.[2,3] We report here that the alteration of dietary carbohydrate induced by the change in protein source did not modify the adaptation of amylase to the amount of these carbohydrates. However, the decrease in amylase was modulated by duration of the experiment as well as rat strain. As a matter of fact, amylase tissue content and mRNA levels were more affected in young rats than in adult rats. In young Fischer rats, amylase specific activity and mRNA were progressively decreased over the 3 weeks of the experiment. After feeding 50% casein or soybean diets for 3 weeks, amylase mRNA levels were also significantly decreased parallel to specific enzyme activities, suggesting that the regulation of amylase biosynthesis was mostly transcriptional. In adult Wistar rats, amylase mRNA levels were significantly decreased after feeding low carbohydrate diets, although specific activity was not affected. These data confirm findings previously described by other authors using Wistar and Sprague-Dawley rats fed high casein diets.[24,25] We may suggest that specific activity was not modified because enzyme secretion was inhibited to the extent of synthesis. As a matter of fact, Lee at al.[21] observed an increase in amylase pancreatic contents and a slight decrease of its intestinal contents after feeding rats with decreasing levels of carbohydrate.

The amount and nature of dietary fat remained constant in the casein and soybean diets. In the fish diets, the fat level was slightly higher (6% instead of 4%) than in the four other diets to match the level of fat contained in the 20% fish diet with that of the 50% fish diet. However, this discrepancy did not influence lipase activity. Moreover, the nature of dietary lipids was very different between the 20% and 50% fish diets. As a matter of fact, corn oil contains mostly 18:2(n-6) fatty acids, while fish oil mostly contains 20:5 and 20:6(n-3) fatty acids. Although no data have been reported in the pancreas, it is likely that alteration of plasma membranes due to the inversion of the n-6:n-3 ratio may affect pancreatic function. It has been shown previously that long chain versus medium chain[26] and highly unsaturated versus satured fatty acids[27,28] enhanced the synthesis of pancreatic lipase in adult rats. However, no study compares the effect of corn oil with fish oil on lipase biosynthesis.

Although the effect of the nature of dietary protein on pancreas has been assessed by several authors,[5-9] this paper is the first one to compare pancreatic adaptation to various protein-rich diets. The two- and three-way ANOVA did not highlight a specific interaction of the nature of protein on pancreatic enzyme adaptation to high protein diets. Yet, we observed that in young Fischer rats adaptation of chymotrypsin and trypsin specific activities to casein-rich diets was faster than in rats fed other protein-rich diets, and that the gene expression (as measured by mRNA levels) was more affected by casein-enriched diets. As previously described, the biosynthesis of chymotrypsinogen and trypsinogen is increased after feeding casein-rich diets via transcription.[24,29] Trypsin specific activity was increased after feeding 50% fish or soybean diets, but mRNAs were not modified, suggesting that adaptation to these diets involved mostly posttranscriptional events. As opposed to these two enzymes, elastase activities were preferentially increased by the 50% fish diet, while elastase I and II mRNAs were not modified, suggesting that biosynthesis of elastase was regulated at the posttranscriptional level in young rats. The mechanisms involved in these enzyme biosyntheses may vary with the nature of protein.

This paper suggests that rat strain is a major factor in the discrepancies between studies. As a matter of fact, multiple-way ANOVA showed that this class influences all parameters. In Wistar rats, chymotrypsin and elastase specific activities were increased after feeding 50% protein diets with no regard to the nature of proteins. Trypsin was not affected. These data are in accordance with previous works in which authors could not show a significant difference in trypsin specific activity between 24% and 40% or 15% and 70% casein diets fed for 2 to 4 weeks.[12,14] In Fischer rats, we report that 50% casein diet increased chymotrypsin, trypsin, and elastase activities while feeding them with 50% fish diets increased only trypsin. Moreover, we did not detect any modulation of mRNA expression. As a matter of fact, Giorgi et al.[29] showed that in Sprague-Dawley rats elastase I mRNA was increased after feeding a 70% casein diet but not a 50% casein diet as compared with a 15% casein diet. It appears therefore, that the increase in mRNA is more modest but also not concomitant with the increase in enzyme activity.

In conclusion, this paper indicated that pancreatic adaptation to high protein diets seems to be widespread independent of the nature of protein or the age and strain of rats. Important observations suggest that the factors involved in pancreatic adaptation are different according to the nature of protein: (1) the course of induction of the adaptive response varies as a function of diets, suggesting that the route of regulation involved may be more or less direct (whether the first messenger acts directly on pancreas or stimulates the secretion of another peptide active on pancreas), (2) some dietary proteins act more at the transcriptional level, others at the

posttranscriptional level, and (3) Wistar rats are more sensitive to dietary adaptation than Fischer rats.

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