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EXPRESSION OF RAT PANCREATIC LIPASE GENE IS MODULATED BY A LIPID-RICH DIET AT A TRANSCRIPTIONAL LEVEL

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The amount of cytoplasmic mRNAs specific for pancreatic lipase and amylase as well as transcription of the corresponding genes were investigated in rats fed a diet containing 25 % sunflower oil. Concentration of lipase mRNA was actually increased by the lipid-rich carbohydrate-low diet and reached a maximum level after 2 days, but further remained constant for at least 10 days. In contrast, about a two-fold decrease in the concentration of pancreatic amylase mRNA was only observed after rats were fed the high-lipid diet for 10 days. Transcription rate measurements on isolated nuclei from pancreatic tissue indicated that the concentration of nascent lipase and amylase transcripts was consistent with a transcriptional regulation of expression of these two genes. © 1990 Academic Press, Inc.

Pancreatic lipase (EC 3.1.1.3) is known for a long time to be a key enzyme in the digestion of dietary lipids. The effect of type and amount of lipid substrates on the enzyme activity level in pancreatic tissue has been extensively studied during the past two decades (1-4). Surprisingly enough, very few studies concerning lipase biosynthesis have nevertheless been reported (4-5). We have recently shown that lipase activity closely paralleled the rate of enzyme synthesis in the pancreas of rats fed isocaloric diets containing increasing amounts (0-30 %) of lipids for a 10-day period (4) as well as in the early days of consumption of a 25 % lipid diet (5). We have also shown that ingestion of dietary lipids resulted in lipase mRNA accumulation (6). In the following, in order to determine the respective part of transcriptional and post-transcriptional events in dietary regulation of lipase mRNA levels, we compare changes in the rate of gene transcription in isolated nuclei to changes in accumulation of lipase specific mRNA in the pancreas of control and experimental rats. Since the carbohydrate content of the fat-rich diet was much lower than that of control diet, to maintain isocaloricity, both the level and synthesis of pancreatic amylase mRNA (EC 3.2.1.1) were examined as well. The overall results indicate that the control of expression of lipase and amylase genes is essentially transcriptional.

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Abbreviations used in this paper :DTT, dithiothreitol ; PMSF, phenylmethylsulfonyl fluoride ; SDS, sodium dodecyl sulfate ; TCA, trichloroacetic acid.

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EXPERIMENTAL PROCEDURES

Chemicals

[α-^{32}P] dCTP (110 TBq/mmol), [α-^{32}P] UTP (30 TBq/mmol) and L-[^{35}S] Methionine (37 TBq/mmol) were obtained from Amersham Corp. (Les Ulis, France). Human placental ribonuclease inhibitor, creatine phosphate, creatine phosphokinase, tRNA from baker's yeast and ribonucleotide triphosphates were from Boehringer (Mannheim, FRG). All other reagents and dietary components used throughout this study have been previously indicated (4, 6).

Animals

Male Wistar rats (180-200 g, body weight) were purchased from Iffa-Credo (L'Arbresle, France) and fed either a standard diet (22 % casein, 62 % starch and 3 % sunflower oil) or a lipid-rich diet (22 % casein, 12.5 % starch and 25 % sunflower oil) for various periods of time (0-10 days). Composition of both isocaloric standard diet and lipid-rich diet (Table I) essentially differed from that previously used (4, 6) by the fact that sodium bicarbonate was replaced by cellulose to avoid the possible effect of bicarbonate on the level of plasma cholecystokinin. Preparation of pancreatic lobules, synthesis of radiolabeled secretory proteins, analysis of radioactive proteins, RNA purification and dot-blot analysis were as previously reported (4-6).

Recombinant DNA

The cDNA clone for rat pancreatic lipase was that previously described (6) while amylase cDNA clone was different from that we already used (5, 6). The latter is nearly a full-length insert (1400 bp) which was characterized by its nucleotidic sequence determined by Sanger's dideoxy procedure (7). The DNA of pUC 9 plasmid as well as that of a recombinant plasmid containing sequences complementary to actin mRNA (8) were included as internal standards in the hybridization experiments.

Run-on transcription on isolated nuclei

Nuclei were prepared from the pancreatic glands of control and experimental animals essentially as described by Schibler et al. (9) and stored at - 70° C. Transcription experiments were carried out according to Gariglio et al. (10) in a final volume of 110 µl containing 10-20 µg DNA (about 4 x 10^6 nuclei). After a 20-min incubation period at 25°C and addition of 900 µl of solution K (10 mM Tris-HCl pH 7.4, 100 mM NaCl, 2 mM KCl, 1 mM EDTA, 0.5 % SDS and 100 µg/ml proteinase K), the resulting mixture was incubated at 37°C for 30 min. Digestion of DNA was then performed by RNase-free DNase (final concentration, 10 µg/ml) at 37°C for 10 min. Following another

Table I: Diet composition (%)

Component	Standard diet	Lipid-rich diet
Sunflower oil	3	25
Casein	22	22
Starch	62	12.5
Mineral mix	6	6
Vitamin mix	1	1
Cellulose	6	33.5
Energy provided as fat (%)	7.4	62

addition of solution K (400 µl) and subsequent incubation for 30 min at 37°C, the medium was twice-extracted with 1 vol. of phenol-chloroform-isoamyl alcohol (25:24:1, v/v/v), and RNA was precipitated at O°C for 10 min. from the aqueous phase with 50 µl of ice-cold 100 % TCA (w/v). The resulting pellet was resuspended in 200 µl buffer (20 mM Hepes pH 7.5, 5 mM Mg Cl_2, 1 mM $CaCl_2$, 1 mM $MnCl_2$) and treated with RNase-free DNase (20 µg/ml) in the presence of ribonuclease inhibitor (25 units) at 37°C for 30 min. After a final extraction with the phenol-chloroform-isoamyl alcohol mixture, as mentioned above, and subsequent precipitation with ethanol, the pellet was dissolved in an appropriate volume of 0.1 % SDS for hybridization

Filter hybridization

Plasmid DNA (5µg) was first boiled in 0.1 N NaOH for 2 min, quickly chilled and adjusted to 2 M ammonium acetate, and finally spotted onto nitrocellulose filters wich were previously soaked in 2 M ammonium acetate and mounted on a slot-blot apparatus (from BRL). Filters were then vacuum-baked at 80°C for 2 h, hybridized in glass tubes in a final volume of 200 µl containing 50 mM Pipes, pH 7.0, 0.5 M NaCl, 33 % formamide, 0.1 % SDS, 2 mM EDTA and 20 µg/ml yeast tRNA, in vitro transcribed and ^{32}P-RNA assayed at 45°C for 48 h. After hybridization, the filters were washed 4 times in 1 x SSC (0.15 M NaCl, 0.015 M sodium citrate) containing 0.1 % SDS at 45°C for 1h each time. Radioactive slots were visualized by autoradiography and resulting autoradiograms were scanned for densitometry using a GS 300 equipement (Hoefer Scientific Instruments).

RESULTS

Relative amounts of biosynthesized lipase and amylase, and relative mRNA levels

In order to determine the time-course response of synthesis of pancreatic lipase and amylase to high-lipid diet ingestion, we measured the incorporation of ^{35}S-labeled methionine into the enzymes relative to that into the pool of secretory proteins by isolated lobules. The relative synthesis of lipase and amylase were expressed as the amount of radioactivity incorporated by each protein divided by that incorporated by total secretory proteins. Table II shows that the observed effect on biosynthesis of each enzyme was time-dependent since diet consumption resulted in a 1.57-fold and 1.88-fold increase in the biosynthesis of lipase, and in a 1.35-fold and 1.75-fold decrease in that of amylase after 5 and 10 days, respectively.

The levels of pancreatic mRNAs specific for lipase and amylase were determined by dot-blot hybridization with the corresponding complementary cDNA probes. Figure 1a and Table II show that lipase mRNA level was significantly increased even after a single day (1.4-fold) of ingestion of the lipid-rich diet. Maximum induction of lipase messenger (about 1.8-fold) was reached from the second day onwards. In contrast, a slight decrease (10-20 %) in amylase mRNA was observed from day 0 to day 5 followed by a sharp decrease (about 2-fold) on day 10 (Fig. 1b and Table II). This rather long lag period might indicate that the level of amylase mRNA decreased as a secondary consequence of a lower amount of carbohydrate in the diet, but not as a direct consequence. Parallel variations in lipase and amylase synthesis as well as in the corresponding messenger levels were observed throughout the experiments.

Transcription assays

To determine whether the accumulation of lipase mRNA following high-lipid diet consumption was due to some mRNA stabilization or resulted from enhanced gene transcription, we measured

Table II : Changes in lipase and amylase relative synthesis and mRNA levels induced by a lipid-rich diet

Diet Administration (day)	Lipase		Amylase	
	Relative Synthesis	mRNA level	Relative Synthesis	mRNA level
0	6.1 ± 0.5	1	50.5 ± 2.2	1
1	6.7 ± 0.6	1.41 ± 0.08	46.9 ± 2.2	1.04 ± 0.16
2	7.8 ± 0.8	1.79 ± 0.28	44.0 ± 4.2	0.83 + 0.13
3	8.9 ± 0.5	1.83 ± 0.16	36.9 ± 2.4	0.99 ± 0.17
5	9.6 ± 0.7	1.99 ± 0.33	37.2 ± 4.0	0.79 ± 0.14
10	11.5 ± 1.1	1.83 ± 0.15	28.8 ± 5.8	0.48 ± 0.10

The relative rates of biosynthesis of pancreatic enzymes were determined by incorporation of (^{35}S)methionine into pancreatic secretory proteins, and expressed as percentage of radioactivity incorporated into individual proteins compared to radioactivity incorporated into total pancreatic proteins.
mRNA content specific for lipase and amylase was estimated by cutting out radioactive spots from figure 1 and counting them. Typical hybridization lines were obtained by linear regression. From the slope of each line, it was possible to calculate the amount of a specific mRNA in the mixture of total RNA. Contents of cytoplasmic mRNA were normalized to those in the pancreas of control rats.

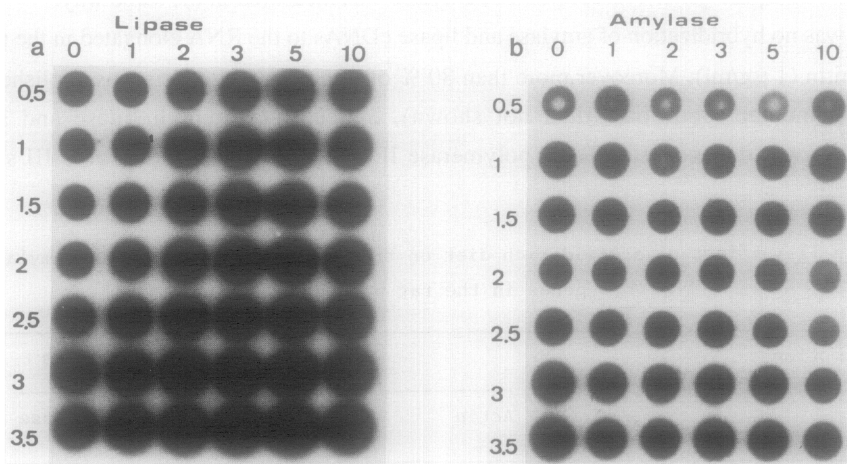

Fig. 1. Autoradiography of a single dot-blot hybridization experiment with lipase and amylase cDNA probes. Total pancreatic RNA (0.5-3.5 µg) isolated from rats fed the lipidic diet for 0, 1, 2, 3, 5, and 10 days was denatured, spotted onto nitrocellulose and hybridized with ^{32}P-labeled nick-translated specific cDNA probes Numbers on top indicate the time (day) of ingestion of hyperlipidic diet, those to the left refer to amounts (µg) of total pancreatic RNA dotted on nitrocellulose filters.

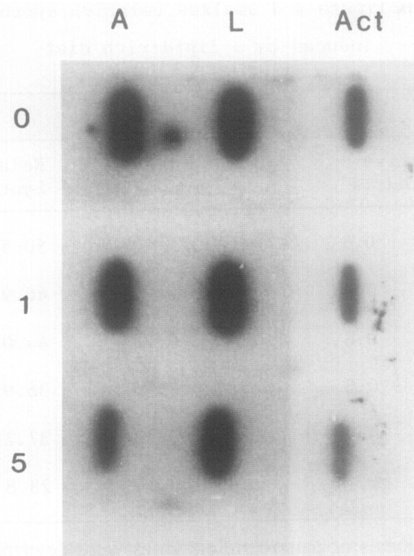

Fig. 2. Lipase and amylase gene transcription in isolated nuclei from rat pancreatic tissue, as influenced by ingestion of a lipid-rich diet for 0, 1 and 5 days. Nascent RNA chains produced by isolated nuclei from control and lipid-stimulated animals were labeled as described under Materials and Methods. Labeled RNA transcripts were hybridized to excess amounts (10 μg) of amylase (A), lipase (L) and cellular actin (Act).cDNAs immobilized on nitrocellulose filters.Actin cDNA was included as an internal standard Autoradiography was performed with an intensifying screen for 1 day at -80°C.

transcription activities in isolated nuclei. This assay which predominantly estimates the elongation of *in vivo* initiated RNA chains is therefore a direct indication of the relative number of polymerases on lipase gene. Transcription of amylase was measured in the same way.

There was no hybridization of amylase and lipase cDNAs to the RNA elongated in the presence of α-aminitin (1 μg/ml). Moreover,more than 80 % of total RNA synthesis was abolished under such experimental conditions (data not shown), indicating that both lipase and amylase transcription was dependent on RNA polymerase II activity. Figure 2 and Table III show the

Table III: Effect of a lipid-rich diet on transcription of lipase and amylase genes in the rat pancreas

Consumption period	Relative transcription rates		
	Actin	Lipase	Amylase
Control	1	3.3	3.1
1 day	1	4.7	3.4
5 days	1	5.7	1.5

Transcription rates were normalized to that of actin. The pancreatic glands of 5 animals per group were pooled before nuclei extraction.

relative rates of transcription of lipase and amylase genes in normal and lipid-fed rats for 1 and 5 days. The DNA of a recombinant plasmid containing sequences complementary to actin mRNA was included as an internal standard in the hybridization experiment. Since lipase transcription was progressively induced in the nuclei of adaptated animals and closely paralleled mRNA stimulation, it was concluded that dietary lipids regulated lipase mRNA concentration at a transcriptional level. In a similar way, the decrease in amylase mRNA levels after 5 and 10 days of consumption of the lipid-rich diet is probably the result of an altered rate of amylase gene transcription.

DISCUSSION

In the present study, dietary lipids were found to progressively increase the biosynthesis of pancreatic lipase which was directly proportional to that of the specific messenger. In fact, the about 2-fold enhancement of lipase mRNA was already obtained after 2 days of diet intake and did not further increase up to 10 days. Consequently, these results are different from those previously reported by us (5) according to which a biphasic induction pattern of lipase mRNA was observed probably as a result of the presence of sodium bicarbonate in experimental diets. The latter has been reported to have side effects on plasma cholecystokinin levels as well as on enzyme secretion and biosynthesis (11-13). As opposite with lipase, the level of amylase mRNA was belatedly decreased as low as 2-fold on the 10th day of high-lipid diet ingestion.

In order to understand the underlying mechanism(s) of lipase messenger accumulation, and that of the decay of amylase mRNA, gene transcription measurements in isolated nuclei from control and adaptated animals were carried out. This was achieved by "run-on" assays wich predominantly measured the elongation of in vivo initiated RNA chains. A progressive stimulation of lipase gene transcription was found to occur as a function of time of adaptation to the lipid-rich diet. Thus, the stimulation of lipase transcription may entirely explain messenger accumulation during dietary manipulation. Similarly, amylase transcription rate was inhibited by the lipid diet, to an extent comparable to that of the messenger decay, implying once more the existence of a transcriptional regulation of the expression of amylase gene.

In conclusion,although transcription of pancreatic amylase gene has been recently shown to be induced by glucocorticoids (14) we demonstrate here for the first time that enhanced transcription of another pancreatic gene, namely that of lipase, is responsible for the increase in both enzyme activity and specific messenger level in the pancreatic tissue of rats adapted to a lipid-rich diet

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