Dietary regulation of cholesterol esterase mRNA level in rat pancreas

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Abstract This study investigates the effect of a high fat/high cholesterol diet on cholesterol esterase biosynthesis in rat pancreas. Results showed that rats fed a high fat/high cholesterol diet, with or without bile salt supplementation, exhibited increased levels of pancreatic cholesterol esterase mRNA. An average of a 2-fold increase in cholesterol esterase mRNA was observed after 1 day of feeding the atherogenic diet. A maximal 3- to 4-fold induction was observed after 4 days on the special diet. The level of pancreatic cholesterol esterase mRNA declined subsequently, resulting in a new steady state level that remained significantly higher than cholesterol esterase mRNA level in control rat pancreas. The feeding of high fat diet without cholesterol, or high cholesterol diet without high fat content, did not result in significant increase in pancreatic cholesterol esterase mRNA when compared to that observed in control chow-fed animals. The increase in cholesterol esterase mRNA after high fat/high cholesterol feeding paralleled the increased in pancreatic lipase mRNA. The high fat/high cholesterol-induced increase in cholesterol esterase mRNA was due to increase rate of transcription, as demonstrated by nuclear run-on assays. Additionally, in vitro incubation experiments of pancreatic lobules with [^{35}S]methionine showed higher rates of ^{35}S-labeled cholesterol esterase synthesis with lobules from the high fat/high cholesterol-fed animals. Taken together, these results demonstrated that high fat/high cholesterol diets increased cholesterol esterase mRNA level and enzyme biosynthesis in rat pancreas. The coordinated regulation of cholesterol esterase with another lipid digestive enzyme, the pancreatic lipase, suggested an important role for these proteins in dietary lipid absorption through the gastrointestinal tract.—**Brodt-Eppley, J., and D. Y. Hui.** Dietary regulation of cholesterol esterase mRNA level in rat pancreas. *J. Lipid Res.* 1994. **35:** 27–35.

Supplementary key words serum cholesterol • bile salt • pancreatic lipase

Epidemiological studies have documented a direct relationship between high plasma cholesterol level and the premature development of atherosclerosis (1). Both dietary cholesterol and cholesterol synthesized endogenously, primarily by the liver and the intestine, contribute to the level of cholesterol in the circulation. Kesäniemi and Miettinen (2) have shown that cholesterol absorption efficiency is a contributing factor for plasma cholesterol level and a risk factor for premature development of atherosclerosis. Currently, very little information is available on the mechanisms and the regulation of the dietary cholesterol absorption process. The available literature suggests that at least two proteins, the intestinal acyl-CoA:cholesterol acyltransferase (ACAT) (3) and the pancreatic cholesterol esterase (4, 5), are involved with cholesterol transport through the gastrointestinal tract.

Research in the role of the intestinal ACAT on cholesterol absorption has been limited to studies with ACAT inhibitors due to difficulties in purification of the enzyme and cloning of its cDNA (6, 7). In contrast, numerous studies have been performed in characterization of the pancreatic cholesterol esterase. The role of the pancreatic cholesterol esterase in dietary cholesterol absorption was demonstrated by observations that the removal of the pancreas dramatically reduced cholesterol absorption through the intestine (4). The reinfusion of pancreatic juice containing the cholesterol esterase, but not cholesterol esterase-depleted pancreatic juice, restored both the mucosal esterase activity and the intestinal cholesterol absorption process (5). Cholesterol malabsorption observed in human subjects with exocrine pancreatic insufficiency could also be restored to nearly normal levels by exogenous enzyme substitution with preparations containing the pancreatic cholesterol esterase (8). A direct role of pancreatic cholesterol esterase in mediating intestinal absorption of free and esterified cholesterol was demonstrated by Bhat and Brockman (9). The latter study showed that incubation of rat intestinal sacs with cholesterol-containing micelles in the presence of cholesterol esterase resulted in a 3- to 5-fold enhancement of intracellular cholesterol and cholesteryl ester accumulation compared to intestinal sacs incubated in the absence of the enzyme. More recently, specific inhibitors of cholesterol esterase, such as the phenoxylphenyl carbamates WAY-121,751 and WAY-121,898, were shown to be effective inhibitors of cholesterol absorption (10) and prevented

Abbreviation: ACAT, acyl-CoA:cholesterol acyltransferase.

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hypercholesterolemia in cholesterol-fed animals (11). In addition to its postulated role in dietary cholesterol absorption, the pancreatic cholesterol esterase also acts in concert with pancreatic lipase to hydrolyze emulsified triglycerides and facilitates dietary fat absorption. Therefore, understanding the physiological functions and mechanisms controlling the biosynthesis of the pancreatic cholesterol esterase will be useful for future studies aimed at limiting dietary fat and cholesterol absorption and reducing the risk of cardiovascular disease.

In a previous study with the rat pancreatoma cell line AR42J, we have reported that cholesterol esterase biosynthesis can be induced by gastric hormones such as cholecystokinin and secretin (12). The mechanism of hormone-induced cholesterol esterase biosynthesis appeared to be a post-transcriptional event without alteration in cellular cholesterol esterase mRNA. The purpose of the current investigation was to determine whether alteration in specific nutrients in the diet can also regulate cholesterol esterase gene expression. The results will show that prolonged feeding of rats with a diet enriched with fat and cholesterol leads to increased levels of cholesterol esterase mRNA in the pancreas.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague-Dawley rats, weighing approximately 300 g, were purchased from Harlan Laboratories (Madison, WI) and housed individually in a room maintained at 23–26°C under 12-h light and 12-h dark cycles. All animals were fed ad libitum with Purina 5001 chow diet and had free access to water. Experimental animals were given either the Purina chow diet 5001 as control or one of the following five experimental diets, made by Harlan Tecklad (Madison, WI). Experimental diet 1 contained low fat (4.5%) and high cholesterol (1.25%). Diet 2 contained low fat, high cholesterol, and 0.5% sodium cholate. Diet 3 contained high fat (11.5%) without cholesterol or cholate, and diets 4 and 5 contained high fat with high cholesterol, with or without cholate supplementation, respectively. To minimize possible variations due to time of feeding and/or diurnal variations in lipid metabolism, food was removed from the animals at 9:30 AM in the morning. Rats were fasted for 4 h prior to their killing at 1:30 PM.

Preparation and quantitation of rat pancreatic RNA

The rats were anesthetized with approximately 80 mg of sodium pentobarbital per kg of body weight. The abdominal cavity was opened and a portion of the pancreas was removed and immediately homogenized in 5 ml of RNAzol (Cinna/Biotecx Laboratories, Houston, TX) as described (13). The RNA was further purified by extraction with chloroform and then precipitated with isopropanol. The RNA was washed with 70% ice-cold ethanol and then resuspended in diethyl pyrocarbonate-treated water. The quality and quantity of RNA samples were assessed spectrophotometrically at OD_{260} and OD_{280}. The absorbance ratio of 1.88 to 2.02 was consistently observed.

Slot blot analysis of pancreatic RNA

The RNA was denatured with formaldehyde before hybridization. For a typical RNA sample, 10 μg of RNA was incubated for 15 min at 68°C with 50% formamide and 7% formaldehyde in SSC buffer (150 mM NaCl, 15 mM sodium citrate). The samples were cooled on ice, two volumes of cold 20 × SSC were added, and then applied to a nitrocellulose paper mounted on a Bio-Rad slot blot apparatus (Richmond, CA). The sample in each well was washed three times with 10 × SSC and then vacuum-dried for 2 h at >80°C. The nitrocellulose paper containing the RNA samples was stored in a vacuum-sealed bag at −20°C until use.

A 2.0 kB cDNA fragment containing the sequence for the full length rat cholesterol esterase cDNA was obtained by digestion of the recombinant plasmid pUC-13 with *Eco*RI (14). A universal actin cDNA probe, encoding amino acid residues 202–374 of α-actin, was obtained as a gift from Dr. James Lessard (Children's Hospital, Cincinnati, OH). A plasmid containing the human pancreatic lipase cDNA (15) was obtained as a gift from Hoffmann-La Roche Ltd. (Basel, Switzerland). The pancreatic lipase cDNA was obtained by *Hin*dIII digestion. The cDNAs were radiolabeled with ^{32}P by the random priming method to a specific activity of approximately 0.5–1 × 10^8 cpm/μg. Hybridization of the cDNA to the RNA samples on nitrocellulose paper was carried out for 18 h at 37°C in buffer containing 50% formamide, 4 × SSC, 50 mM NaH_2PO_4, 5 × Denhardt's solution, and 10% dextran sulfate. The nitrocellulose was washed in 1 × SSC and 0.1% sodium dodecyl sulfate initially at room temperature, and then 4 times at 50°C for 15 min each. Previous studies from our laboratory have shown that these hybridization and washing conditions were specific, yielding single bands of expected sizes on Northern blot analysis with either cholesterol esterase or actin cDNA probes (12, 14). Similar specificity was also observed with the pancreatic lipase cDNA probe. The radiolabeled bands on the nitrocellulose filter were excised from the paper using the autoradiograph as a template and then quantitated by counting in a liquid scintillation counter.

Serum cholesterol determination

Blood samples were collected from the rats by cardiac puncture immediately following pancreatectomy. The blood was allowed to clot for 1 h at 4°C and then cen-

trifuged at 5,000 *g* for 30 min. The serum was removed and used for cholesterol determination as described previously (16).

Pancreatic cholesterol esterase biosynthesis

Cholesterol esterase protein biosynthesis by rat pancreas was performed according to previously described procedure (17). The rats were anesthetized and the abdominal cavities were opened. The pancreas was inflated by direct injection of Krebs Ringer buffer. Individual lobules were excised and placed in Krebs Ringer buffer containing the entire complement of amino acids, except methionine. The incubation medium also contained the following protease inhibitors: 2 mM benzamidine, 2 mM hydrocinnamic acid, and 0.5 mM sodium benzoyl-D,L-arginine. The pancreatic lobules were pulsed with the same medium containing 9.2 μM of [^{35}S]methionine (1083 Ci/mmol) for 1 h at 37°C. At the end of the incubation period, 2-ml aliquots of media were withdrawn for immunoprecipitation with cholesterol esterase antibodies (12). Immunoprecipitated radioactivity was quantitated by liquid scintillation counting. The amount of cholesterol esterase synthesized by each pancreatic lobule was normalized based on the amount of cellular DNA, as determined by the fluorescent Hoechst dye method (18).

Nuclear run-on transcription assays

Pancreatic nuclei were isolated using similar procedure described previously by Schibler et al. (19). Briefly, a portion of control or experimental pancreas was suspended in 10 ml of buffer A (10 mM HEPES-KOH, pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM EGTA, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 2 mM benzamidine, 0.3 M sucrose) and then homogenized with 8–12 strokes in a Potter-Elvehjem homogenizer. The samples were made 0.3% Nonidet P-40 and then rehomogenized with an additional 1–2 strokes. The homogenates were centrifuged at 1200 *g* for 10 min and washed twice with buffer A. Crude nuclei were resuspended in 3 ml of buffer B (15 mM HEPES, pH 7.5, 60 mM KCl, 15 mM NaCl, 0.15 mM spermine, 0.5 mM spermidine, 14 mM β-mercaptoethanol, 0.1 mM EGTA, 0.1 mM EDTA) and then centrifuged over 9.5 ml of buffer B at 160,000 *g* for 2 h in an SW-40 rotor at 4°C. Nuclei were resuspended in storage buffer (20 mM Tris-HCl, pH 7.9, 75 mM NaCl, 0.5 mM EDTA, 8.5 mM dithiothreitol, 0.125 mM phenylmethylsulfonyl fluoride, and 50% glycerol) and kept at −70°C. The nuclei were quantitated via a Giemsa stain and counted on a hemocytometer. Transcription assays were conducted based on the method of Gariglio, Bellard, and Chambon (20). The transcription assay was performed with 5×10^6 nuclei, 1 mM each of [^{32}P]CTP (3000 Ci/mmol), ATP, GTP, and UTP, in buffer containing 95 mM Tris-HCl, pH 7.8, 54 mM NaCl, 50 mM ammonium sulfate, 4 mM $MgCl_2$, 1.2 mM dithiothreitol, 0.4 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, and 29% glycerol. After 30 min of incubation, 900 μl of solution K (10 mM Tris-HCl, pH 7.4, 100 mM NaCl, 2 mM KCl, 1 mM EDTA, 0.5% SDS, 100 μg/ml proteinase K) was added and then incubation was continued for an additional 30 min at 37°C. At the end of the incubation period, the reaction mixture was added to RNAzol for RNA isolation. The final pellet was dissolved in diethyl pyrocarbonate-treated water.

A solution containing plasmid DNA (1, 2.5, or 5 μg) was boiled in 0.1 N NaOH for 2 min, chilled quickly on ice, and then adjusted to 2 M ammonium acetate. The DNA was applied to nitrocellulose paper mounted in a slot blot apparatus (Bio-Rad). The filters were baked at 80°C in a vacuum oven for 2 h. The nitrocellulose paper containing the DNA samples was then used for hybridization with radiolabeled RNA obtained from nuclear run-on transcription assays. The hybridization took place in 2 ml of buffer (50 mM PIPES, 0.5 M NaCl, 33% formamide, 0.1% SDS, 2 mM EDTA, 5 mg/ml salmon sperm DNA) at 45°C for 52 h. Filters were rinsed once in 1 × SSC with 0.1% SDS at room temperature, then washed twice at 50°C for 30 min each. Dried filters were exposed to X-ray films and the autoradiograms were scanned by a densitometer (Fisher Biotech FB910).

Statistical analysis

Data were analyzed using Student's *t*-test, ANOVA and the Duncan's ad hoc test with the program Number Cruncher Statistical System Version 5.03 (Kaysville, UT).

RESULTS

Initial experiments were conducted to determine the optimal amount of sample to be used for measuring cholesterol esterase mRNA level in rat pancreas. Results showed that a linear response of cholesterol esterase cDNA hybridization signal ($r = 0.99$) was obtained using 1–20 μg of total pancreatic RNA (**Fig. 1**). Therefore, 10 μg of RNA from each sample was used in subsequent experiments to measure the level of cholesterol esterase mRNA under various metabolic conditions.

Feeding rats with a diet containing 11.5% fat and 1.25% cholesterol resulted in increased plasma cholesterol levels (**Fig. 2**). The increased level of plasma cholesterol level was small, but significant, after 1 day feeding of the special diet (Fig. 2). Continuous feeding of the animals with the high fat/high cholesterol diet without cholate did not result in further increase in plasma cholesterol (Fig. 2). In contrast, supplementation of the high fat/high cholesterol diet with 0.5% cholate resulted in continuous increase of plasma cholesterol, up to a level 5–6 times that in control rats (Fig. 2). Results of these studies are consistent with a previous report that bile salt supplementation is required to produce hypercholesterolemia in rats (21).

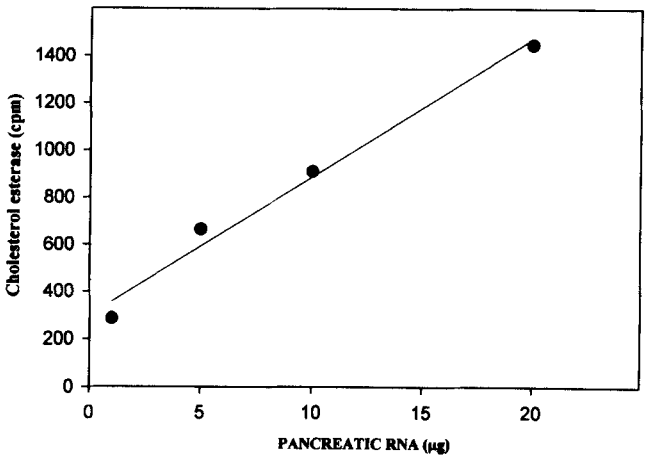

Fig. 1. Cholesterol esterase cDNA hybridization to rat pancreatic RNA. Total RNA was isolated from freshly isolated pancreas by RNAsol extraction. Various concentrations of total pancreatic RNA were applied to nitrocellulose paper through a slot blot apparatus and hybridized with ^{32}P-labeled cholesterol esterase cDNA. The hybridization was carried out for 18 h at 37°C in buffer containing 50% formamide, 4 × SSC, 50 mM NaH_2PO_4, 5 × Denhardt's solution, and 10% dextran sulfate. The filters were washed once with 1 × SSC and 0.1% SDS at room temperature and then 4 times at 50°C. The radiolabeled bands on the nitrocellulose filter were excised and subjected to scintillation counting. The results were reported as the amount of cholesterol esterase binding to the RNA.

To determine the influence of a high fat/high cholesterol diet, with or without cholate, on pancreatic cholesterol esterase gene expression, total RNA was extracted from the pancreas of rats fed the different diets for various times and used for hybridization with cholesterol esterase cDNA. An actin cDNA probe was used as control in these studies to normalize the amount of RNA in each sample. The ratio of hybridized cholesterol esterase cDNA to actin cDNA hybridization was determined by direct counting of the filters as described in Experimental Procedures.

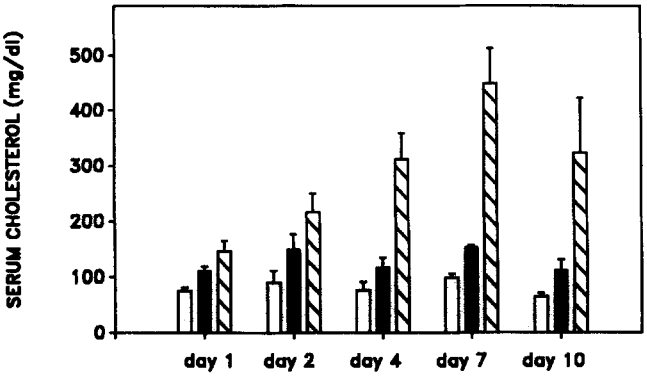

Fig. 2. Serum cholesterol results from rats fed either the control Purina 5001 diet (open bars), diet containing 11.5% fat and 1.25% cholesterol (solid bars), or the high fat/high cholesterol diet with 0.5% cholate (hatched bars), for the duration as indicated. The results were averaged from duplicate determinations with six animals from each group in days 1, 4, 7, and 10. Three animals were used per group for the determination in day 2. The error bars represented standard deviation of the means.

The ratio obtained from rats fed the control diet was assigned an arbitrary value of 1.0. Results showed that the cholesterol esterase mRNA level increased significantly ($P < 0.05$) after 1 day of consuming the high fat/high cholesterol diets (**Fig. 3**). The level of cholesterol esterase mRNA continued to increase, reaching a maximal level of 3- to 4-fold induction after 4 days on these special diets. The cholesterol esterase mRNA level decreased after 4 days of fat and cholesterol feeding, resulting in a level after 10 days that was 1.5-fold higher ($P < 0.05$ compared with control) than that observed with chow-fed animals (Fig. 3). The inclusion of bile salt in the diet resulted in slightly higher pancreatic cholesterol esterase mRNA level (Fig. 3). However, the differences observed between the two high fat/high cholesterol groups were not statistically significant.

Another set of experiments was performed to determine the effects of prolonged feeding of fat and cholesterol on cholesterol esterase mRNA level in rat pancreas. These experiments were prompted by observations of a gradual decrease in cholesterol esterase mRNA level after 4 days. In these experiments, rats were fed the high fat/high cholesterol diets for 28 days and results were compared with chow-fed animals housed under exact conditions. The results revealed that pancreatic cholesterol esterase mRNA level after 28 days of the high fat/high cholesterol diet was very similar to that observed after 10 days and averaged 1.5-fold higher than those in chow-fed animals (data not shown). Therefore, prolonged feeding of rats with a diet enriched with fat and cholesterol

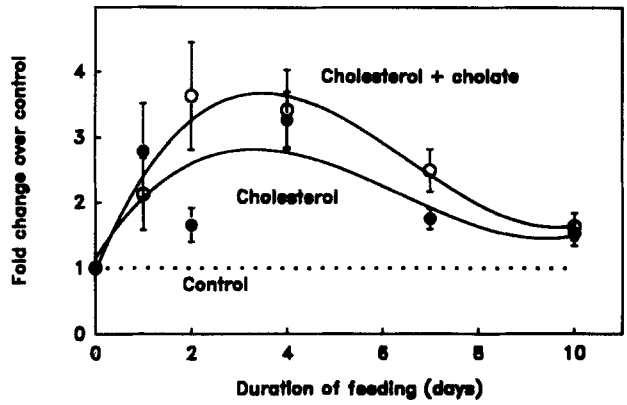

Fig. 3. Dietary effects upon rat pancreatic cholesterol esterase mRNA. Rats were fed either the control diet (dotted line), high fat/high cholesterol diet (filled circles), or high fat/high cholesterol diet with cholate (open circles) for the period indicated. The rats were killed 4 h after food removal. Total pancreatic RNA was extracted from each animal immediately after killing. Duplicate samples of 10 μg RNA were applied to each slot on the slot blot apparatus. The samples were hybridized with either cholesterol esterase cDNA or actin cDNA. The ratio of hybridized cDNA was determined by scintillation counting. The hybridization ratio observed for RNA obtained from rats fed the control diet was set at 1.0. Each symbol represents six animals from each group with the exception of day 2 (three rats per point) and day 4 (eight animals per point). The error bars represent standard error of the mean.

resulted in a new steady state level of pancreatic cholesterol esterase mRNA that was significantly higher than that observed in control animals.

Experiments were also conducted to determine whether increased pancreatic cholesterol esterase mRNA level in response to the high fat/high cholesterol diet results in increased cholesterol esterase protein biosynthesis. In these experiments, pancreatic lobules obtained from rats fed the various diets were incubated in vitro with [^{35}S]methionine for 1 h at 37°C. At the end of the incubation period, ^{35}S-labeled cholesterol esterase secreted into the medium and in the cell lysate was subjected to immunoprecipitation with anti-rat cholesterol esterase antibodies (22) as described in Experimental Procedures. Results showed that the pancreas from rats consuming the high fat/high cholesterol diet synthesized and secreted 1.5-fold more ^{35}S-labeled cholesterol esterase than pancreas obtained from control rats (**Fig. 4**). Cholesterol esterase biosynthesis was also higher (1.25-fold) from pancreas of rats fed this special diet for 7 days in comparison with control rats. The increase in cholesterol esterase biosynthesis was statistically significant for the high fat/high cholesterol effects ($P < 0.05$). However, cholesterol esterase synthesis from pancreas of rats fed the high fat/high cholesterol diet for 4 days was not significantly different from rats fed the same diet for 7 days ($P = 0.45$).

The mechanism for dietary-induced increase in pancreatic cholesterol esterase mRNA was investigated by nuclear run-on transcription assays. The nuclei from rats fed the control and experimental diets were isolated and used for transcription assays in the presence of [^{32}P]CTP, as described in Experimental Procedures. The in vitro

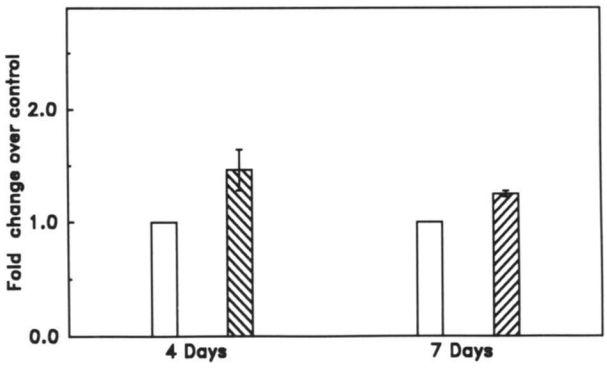

Fig. 4. Cholesterol esterase biosynthesis in pancreatic lobules excised from rats fed either the control diet (open bars) or diet containing high fat/high cholesterol and cholate (diagonally lined bars) for 4 or 7 days. Pancreatic lobules were obtained from the animals after a 4-h fast. The lobules were incubated with medium containing [^{35}S]methionine for 1 h at 37°C. The amount of ^{35}S-labeled cholesterol esterase synthesized by each lobule was determined by immunoprecipitation with antibodies against rat cholesterol esterase and was normalized to the amount of DNA in each lobule. The ^{35}S-labeled cholesterol esterase synthesized by pancreatic lobules from rats fed the control diet was set to equal 1.0. Each bar represents four rats with error bars representing the standard error of the mean.

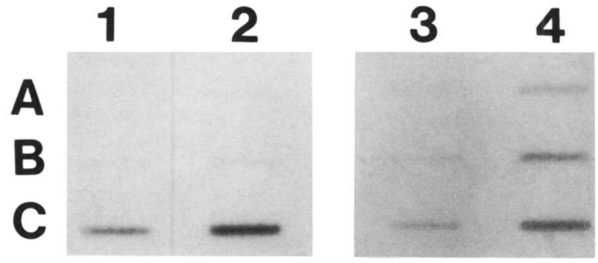

Fig. 5. Autoradiography of cholesterol esterase gene transcription in isolated nuclei of rat pancreas. Nascent RNA chains produced in vitro in the presence of [^{32}P]CTP with isolated nuclei from rats fed the 5001 control chow diet (lanes 1 and 3), or those from rats fed the high fat/high cholesterol/cholate diet (lanes 2 and 4), were hybridized to 1, 2.5, and 5 μg of cholesterol esterase cDNA (A, B, and C, respectively) immobilized on a slot blotted nitrocellulose paper. The hybridization condition was 45°C for 52 h. The filters were washed with 1 × SSC buffer containing 0.1% SDS once at room temperature and then twice at 50°C before exposure to X-ray films.

transcribed products were then analyzed by hybridization to denatured cholesterol esterase cDNA immobilized on nitrocellulose filters. The results showed more intense hybridization signals using transcription products from nuclei of fat and cholesterol-fed animals, compared with those from nuclei of animals fed the control diet (**Fig. 5**). The signals observed by hybridization with 5 μg of denatured cholesterol esterase cDNA on the blot were then scanned with a densitometer for quantitation. The results obtained from three separate scans were averaged for comparison. The results showed that cholesterol esterase transcriptional activity was 3-fold higher in pancreas from animals fed the experimental diet ($P < 0.001$).

The effects of high fat/high cholesterol diet on pancreatic gene expression may not be restricted to the cholesterol esterase. Another pancreatic enzyme that has been shown to be important for fat absorption is the pancreatic lipase (23). Although previous studies have shown that lipid-rich diet induced pancreatic lipase mRNA level and transcriptional activity (24, 25), these studies were performed with a cDNA corresponding to the pancreatic lipase-related protein and not with a pancreatic lipase-specific probe (26). Therefore, these experiments were repeated herein by probing the pancreatic RNA obtained in our studies with a cDNA corresponding to authentic pancreatic lipase (15). The results showed that feeding animals a high fat/high cholesterol diet also increased the level of pancreatic lipase mRNA (**Fig. 6**). The increase in pancreatic lipase mRNA paralleled the increase in cholesterol esterase mRNA with maximal response observed after 4 days. The level of pancreatic lipase mRNA also decreased with prolonged feeding of the high fat/high cholesterol diet (Fig. 6).

Previous studies have reported that increase in pancreatic lipase mRNA was responsive to a high fat diet and

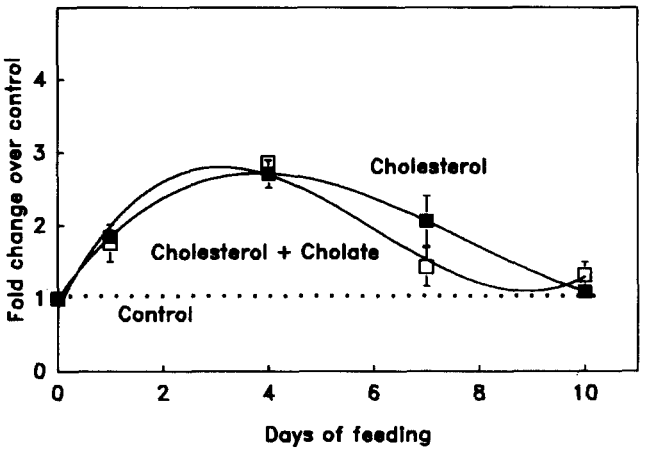

Fig. 6. Dietary effects on rat pancreatic lipase mRNA. Rats were fed the control diet (dotted line), or a high fat/high cholesterol without cholate (filled squares) or with cholate supplementation (open squares). Pancreatic RNA were isolated and used for hybridization with a pancreatic lipase cDNA probe. Each symbol represents duplicate determination from three rats per group. The amount of pancreatic lipase cDNA hybridization was normalized to the hybridization signal with the actin cDNA probe. The ratio of hybridization observed with RNA isolated from rats fed the control chow diet was set to equal 1.0.

that dietary cholesterol was not required for stimulation (24, 25). To determine whether increase in pancreatic cholesterol esterase mRNA requires the presence of both high fat and high cholesterol, rats were fed special chows containing either low fat plus cholesterol, low fat plus cholate, or high fat alone. After feeding these diets for 4 days, the rats were killed to obtain pancreatic RNA samples for analysis. Results showed that feeding the diet containing low fat and high cholesterol evoked a slightly higher level of pancreatic cholesterol esterase mRNA compared with that observed in the control group (**Fig. 7**). However, regression analysis of the data revealed that the difference was not statistically significant. Furthermore, animals fed diets with high fat alone, or with 0.5% cholate, also showed no difference in pancreatic cholesterol esterase mRNA level when compared with animals fed the normal chow diet (Fig. 7).

DISCUSSION

Previous studies conducted in our laboratory using the rat pancreatoma AR42J cells as an in vitro model have demonstrated translational regulation of cholesterol esterase biosynthesis by gastric hormones. These studies showed that cholesterol esterase mRNA isolated from gastric hormone-stimulated AR42J cells was translated more efficiently than those isolated from control cells (12). The results presented in the current study demonstrated that feeding animals with a high fat/high cholesterol diet resulted in increased cholesterol esterase biosynthesis via increased level of cholesterol esterase mRNA. The increased cholesterol esterase biosynthesis in response to secretin, a fat-induced gastric hormone, together with the ability of high fat/high cholesterol diet to increase cholesterol esterase mRNA, suggested an important role of this enzyme in the lipid absorption process. These studies also revealed that pancreatic cholesterol esterase biosynthesis is regulated at both transcriptional and translational levels by nutritional and/or hormonal manipulations.

The dual mode of cholesterol esterase regulation is reminiscent of the regulation of other pancreatic digestive enzymes. In the early 1900s, Pavlov proposed that the digestive process is regulated by the variable presence of different digestive enzymes in the gut (27). According to his hypothesis, the particular needs for digesting a meal are met by appropriately varying the amount of different digestive enzymes in the intestine. The presence of these digestive enzymes is primarily due to their synthesis and secretion by the pancreas. The first substantial experimental evidence in support of this theory was provided by studies showing that varying the dietary composition led to variations in the relative enzyme content in rat pancreas (28). The observations, corroborated by many subsequent studies (reviewed in ref. 29), indicated that high carbohydrate diets produced a substantial increase in amylase and a concomitant decrease in serine proteases.

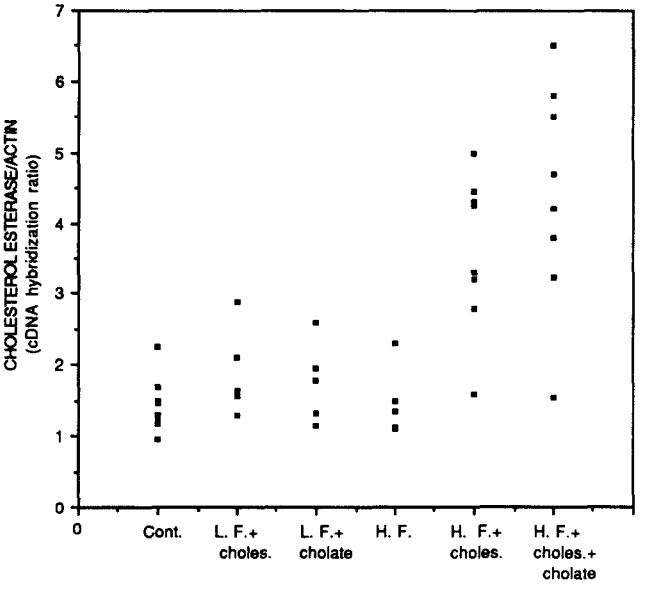

Fig. 7. Cholesterol esterase mRNA levels in pancreas of rats fed different diets. The rats were fed either a control diet (cont.) or diets containing various combinations of either 4.5% fat (L.F.), 1.25% cholesterol (choles.), 11.5% fat (H.F.), and/or 0.5% cholate (cholate) for 4 days. Total pancreatic RNA was extracted from each animal and duplicate samples of 10 μg RNA were applied to nitrocellulose paper mounted in a slot blot apparatus. The samples were hybridized with either cholesterol esterase or actin cDNA probes. The hybridization ratio of the probes was determined as described in the legend to Fig. 3. Each point on the graph denotes data obtained from an individual rat.

In contrast, high protein diets led to increased protease activity and no change in amylase. The level of pancreatic lipase was unchanged by these dietary manipulations but could be induced by a high fat diet (30). The high fat diet had no effect (24) or decreased (25) the expression of the carbohydrate digestive enzyme amylase.

Subsequent studies revealed that pancreatic digestive enzyme biosynthesis could be regulated by both transcriptional and translational mechanisms. At the acute level, a protein-rich diet was shown to increase biosynthesis of proteolytic enzymes, such as trypsinogen and chymotrypsinogen, by increasing the release of the gastric hormone cholecystokinin (31). The change in trypsinogen biosynthesis occurred in two phases. During the first 6 h of hormone stimulation, increase in trypsinogen biosynthesis was due to activation of mRNA translation (32–34). Feeding animals a high protein diet for several days resulted in a 3- to 4-fold increase in trypsinogen and chymotrypsinogen mRNA (35). Likewise, feeding animals a high carbohydrate diet for several days was shown to result in a 9-fold increase of amylase mRNA in the pancreas (36).

The data presented in this manuscript revealed the coordinate regulation of cholesterol esterase and pancreatic lipase mRNA levels in response to high fat/high cholesterol diets. The data showed that inclusion of bile salt in the diet, which was required to elicit a hypercholesterolemic response, was not necessary to induce pancreatic cholesterol esterase and pancreatic lipase gene expression. These results further suggest that bile salt affects plasma cholesterol by suppression of hepatic lipoprotein uptake, and possibly independent of dietary lipid absorption through the intestine. This study also showed that although cholate supplementation augmented the dietary cholesterol effects on serum cholesterol level, little or no effect of cholate supplementation was observed for dietary stimulation of cholesterol esterase mRNA. These observations suggested that the induction of these pancreatic enzymes was not due to increased bile flux and was probably independent of plasma cholesterol level. The increase in cholesterol esterase mRNA was most likely due to increased influx of dietary fat and cholesterol into the intestinal lumen.

Previous studies have shown that pancreatic lipase biosynthesis could be increased by feeding a high fat diet without cholesterol. In contrast, our results demonstrated that induction of cholesterol esterase gene expression required the presence of both high fat and high cholesterol in the diet. The different mode of regulation for the two genes may be related to the functional properties of the two different pancreatic digestive enzymes. While the pancreatic lipase is specific for digestion of triglycerides in the absorptive process, the pancreatic cholesterol esterase is involved with dietary cholesterol absorption (4, 5, 9, 10), and also acts in concert with pancreatic lipase for the complete digestion of dietary fats. The parallel regulation of pancreatic lipase and cholesterol esterase in response to high fat and high cholesterol diet suggests possible common fat-responsive element(s) in the pancreatic lipase and cholesterol esterase genes. The cholesterol esterase gene may contain additional cholesterol-responsive elements not present in the pancreatic lipase gene that are required to act in concert with the fat-responsive elements in regulating cholesterol esterase gene expression. This hypothesis may be tested by examining promoter sequences in the respective genes.

An interesting observation of the current study is the immediate effect of diet-induced cholesterol esterase and pancreatic lipase mRNA level in rat pancreas. The results showed significant increase after 1 day and that maximal level was reached after 4 days of fat and cholesterol feeding. The level of cholesterol esterase and pancreatic lipase mRNA actually decreased with prolonged feeding of the special diets, reaching a new steady state level that was higher than that in control rat pancreas. Although the apparent decrease in cholesterol esterase mRNA with prolonged feeding of the high fat/high cholesterol diet remains unexplained, it is interesting to note that the amount of ^{35}S-labeled cholesterol esterase synthesized in vitro by pancreas obtained from the 4- and 7-day groups was statistically similar. Based on these observations, it is possible to speculate that the cholesterol esterase mRNA measured after 4 days of cholesterol feeding may contain cholesterol esterase populations with different translational efficiency. Some of the cholesterol esterase mRNA may be inactive or sequestered in a compartment inaccessible to the translational machinery. Prolonged feeding of high fat/high cholesterol diet may be required to convert the inactive cholesterol esterase mRNA to an actively translated mRNA. Alternatively, prolonged feeding of high fat/high cholesterol diet may result in the replacement of inactive cholesterol esterase mRNA by newly synthesized active-form of the mRNA. Metabolic regulation of cholesterol esterase mRNA translation has been reported previously (12). Possible translational differences of the cholesterol esterase mRNA in control and diet-induced rat pancreas await additional detailed characterization on the structure and translation activity of the isolated mRNA.

The initial increase, followed by decrease to a new steady state level, of pancreatic lipase mRNA in response to the experimental diet was different from results reported by other investigators (24, 25). Wicker, Scheele, and Puigserver (24) demonstrated a 2.2-fold increase in pancreatic lipase after feeding rats a 10% lipid diet for 10 days. A 3.9-fold increase was observed when dietary lipid content was increased to 25% (24). In a later study, Wicker and Puigserver (25) reported a maximum increase (1.4-fold) of pancreatic lipase after 2 days of feeding a diet with 25% sunflower oil. The latter study also reported

that the pancreatic lipase mRNA level remained constant for at least 10 days. Although it is difficult to reconcile the differences observed between these results and our experiments, several key differences in experimental methodology were noticeable. First, the type of lipids used in the diet was different between the studies. While Wicker and her colleagues used sunflower oil in both studies, our experiments utilized cocoa butter, in addition to cholesterol, as the source of lipids. Second, the cDNA probes used in the two studies were also different. The earlier studies used a cDNA with sequence corresponding to the lipase related protein-1 (26). Our studies used a probe identified as pancreatic lipase cDNA (26). Accordingly, it is possible that different pancreatic lipase genes may respond differently to a lipid-rich diet. Alternatively, different sources of lipids in the diet may result in differential regulation of the pancreatic lipase genes. Toward this end, it is noteworthy that differential effects of specific fat type on pancreatic lipase biosynthesis, especially at low threshold levels, have been reported previously (30). However, the ultimate resolution of the observed differences will require detailed experiments to determine the effects of specific dietary fat type on expression of the various pancreatic lipase genes.

The authors acknowledge Dr. Yan Huang for many helpful discussions, and Mr. James Kissel and Mr. Chris Carter for technical assistance. This research was supported by Grant DK40917 from the National Institutes of Health. Julia Brodt-Eppley was supported by NIH Training Grant HL07527.

Manuscript received 4 March 1993 and in revised form 7 June 1993.

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