Response of Rat Exocrine Pancreas to High-Fat and High-Carbohydrate Diets (44496)

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Abstract. **Intake of diets with high fat content is a risk factor for acute pancreatitis and pancreatic cancer. The underlying mechanisms leading to the development of these diseases due to high fat intake are currently unknown. The current study was designed in rats to determine the physiologic and pathological consequences of a high-fat diet that contained excess amounts of cottonseed oil or a high-carbohydrate diet that contained high amounts of sucrose on the exocrine pancreas. Rats were maintained on the diets for 4 weeks, and a cannula was inserted into the right jugular vein and one into the pancreatic duct for collection of pancreatic juice. Volume of the pancreatic juice and concentrations of amylase, lipase, and trypsinogen in the pancreatic juice were measured before and after infusions of CCK-8. Results showed that basal and CCK-stimulated pancreatic outputs of volume, amylase and lipase but not trypsinogen, were significantly elevated in intact rats given a high-fat diet when compared with rats given a high-carbohydrate diet. Forty-eight hours later, rats were sacrificed, and parts of the pancreas were removed for isolation of pancreatic acinar cells and for histopathologic studies. Pancreatic acini isolated from rats on a high-fat diet showed significantly lower basal and CCK-stimulated amylase release when compared with those on a high-carbohydrate diet. Histology of the pancreas of rats on a high-carbohydrate diet appeared normal; however, the pancreas of rats on high-fat diet showed significant alterations in exocrine pancreas. These results showed abnormalities in the exocrine pancreas of rats on a high-fat diet, that were not found in rats on a high-carbohydrate diet; further, they support the contention that a high-fat diet has a deleterious effect on the pancreas.** [P.S.E.B.M. 2000, Vol 223]

Pavlov in early 1900 described the adaptation of the exocrine pancreas to dietary contents (1). Subsequent studies showed that the content of pancreatic proteases, amylase and lipase, change in proportion to the dietary content of their respective substrates, namely protein, carbohydrate, and fat (2–9). Studies reported by Brannon have provided evidence for a pretranslational mechanism of the adaptation of proteases, amylase, and lipase to their respective substrates (10). In subsequent studies, Ricketts and Brannon have shown that the amount of fat independent of its type regulates pancreatic lipase at translational or post-translational levels (11). In studies conducted in dogs, rats, and humans, it has been shown that fat given orally and *via* intraduodenal route induces the release of CCK indicating that CCK is one of the mediators by which the pancreas adapts to dietary fat (12–18). Diets rich in high carbohydrate or high fat also affect carbohydrate metabolism and insulin secretion in rats as shown by Ramiraez *et al.* (19). Other studies in conscious dogs showed that fat and carbohydrate had little or no effect on basal exocrine pancreatic secretion but did have some effect on postprandial exocrine pancreatic output (20, 21).

Chronic intake of excessive amounts of dietary fat are known to be associated with hyperlipidemia, a condition whose pathological consequence is acute pancreatitis (22, 23). In addition, diets rich in fat may also be a significant risk factor in the etiology of pancreatic cancer (24, 25). The underlying mechanisms by which fat induces deleterious effects on pancreatic physiology and pathology are unclear. The current study was designed in rats to provide a better understanding of the physiological and pathological consequences of those diets on the pancreas. The chronic effects

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of high-fat (in the form of cottonseed oil) and high-carbohydrate (in the form of sucrose) diets on both *in vivo* and *in vitro* pancreatic responses were studied in the same animal.

Materials and Methods

Materials. The following substances were purchased: soybean trypsin inhibitor (type I-S), bovine serum albumin (BSA), HEPES from Sigma Chemicals (St. Louis, MO); COOH-terminal octapeptide of cholecystokinin (CCK-8) from Peninsula Laboratories (Belmont, CA); purified collagenase (type CLSPA) from Worthington Biochemical (Freehold, NJ); and Eagle's minimal essential medium from GIBCO (Grand Island, NY).

Animals and Diets. Twenty-one Male Sprague-Dawley rats, body weights ranging from 250 g initially to 400 g at sacrifice, were maintained in a screen-bottomed cage at 22°C on a 12:12-hr light:dark cycle with free access to water. The rats were divided randomly into two groups, and rats in each group were fed *ad libitum* with diets containing either high fat ($n = 11$) or high carbohydrate ($n = 10$) that were mixed with water (0.7 g/ml of water). The diets were prepared through a commercial source and satisfied AIN-76 standard (R-M-H 3000; Agway Inc., Syracuse, NY). The high-fat diet contained 45% fat (excess amounts of vegetable cottonseed oil including mono, poly, and saturated fats with no cholesterol) and 29% carbohydrates whereas the high-carbohydrate diet contained 58% carbohydrates (mainly sucrose) and no fat. Each diet contained ≈ 20% protein in the form of vitamin-free casein. The diets were mixed with USB total vitamins and vitamin supplements, celufil, non-nutritive bulk, and salts (salt mix USP XXII). The fat content in the high-fat diet included mono, poly, and saturated fats with no cholesterol. The carbohydrate source in the high-carbohydrate diet included sucrose. Each of these diets had equal calories as determined by a nutrient analysis tool and was therefore isocaloric. Identical diet preparation was used as in our studies reported earlier (26).

Surgical Preparation. Each rat was kept on a high-fat or high-carbohydrate diet for 4 weeks. For surgery, rats were fasted for 24 hr and anesthetized with a mixture of ketamine-HCl and acepromazine maleate (1:10). A cannula (PE 90, I.D. 0.86 mm, O.D. 1.27 mm) was inserted into the right jugular vein to collect blood samples and for infusion of CCK for studies as described below. After a midline abdominal incision was made, a PE-50 cannula was placed and secured in the pancreatic duct for collection of pancreatic juice. One other external PE-50 cannula was placed in the duodenum to reperfuse the pancreatic juice into the duodenum. A third PE-50 cannula was placed in the common bile duct to route bile into the duodenum.

All cannulas were supported and secured by purse-string nylon sutures with 6.0 Prolene (Ethicon, Somerville, NJ). They were exteriorized through the subcutaneous tissue. The incision was then closed, and each rat was transferred to a Bollman type restraining cage (Fisher Scientific, Fair Lawn, NJ) for recovery. During the 24-hr recovery period, the animals received water but no food. All rats received intraduodenal saline, 1.58 ml/hr, and intravenous saline, 0.4 ml/hr.

***In Vivo* Studies.** Twenty-four hours after surgery, rats were lightly anesthetized, and pancreatic juice was collected continuously for two 30-min periods prior to CCK infusion. CCK-8, dissolved in saline containing 0.5% bovine serum albumin, was administered intravenously at a rate of 0.15 μg/kg for 30 min. After CCK infusions, pancreatic juice was collected for 90 min. The volume of pancreatic juice was measured with a Hamilton syringe, and an aliquot was immediately frozen at 20°C for assay of pancreatic enzymes. The remaining pancreatic juice was returned into the duodenum.

***In Vitro* Studies.** Forty-eight hours after *in vivo* studies, blood samples from rats were collected, and rats were then sacrificed. Each pancreas was rapidly removed, freed from fat and lymph nodes, and weighed. Portions were used for histology or for isolation of pancreatic acini. Dispersed pancreatic acini were isolated by enzymatic digestion according to the method reported previously (27, 28). After isolation, acini were suspended in incubation buffer at a density of 0.6 mg of protein/ml and preincubated at 37°C for 60 min; 2-ml aliquots of cell suspension (0.3 mg of acinar protein/ml) were then distributed into 25-ml polycarbonate Erlenmeyer flasks. CCK-8 was added in graded doses, and the flasks were incubated for 30 min at 37°C with shaking at 60 cycles/min. Each incubation was performed in duplicate flasks. Incubation buffer contained 128 m*M* NaCl, 4.7 m*M* KCl, 1 m*M* Na_3PO_4, 1.28 m*M* $CaCl_2$, 0.56 m*M* $MgCl_2$, 10 m*M* HEPES, 11.1 m*M* glucose, 0.5% BSA, 0.1 g/l soybean trypsin inhibitor, and Eagle's minimal essential medium. The buffer was routinely equilibrated with 100% oxygen and titrated to pH 7.4.

Assays. Amylase activity was measured by the method of Jung (29), using procion yellow starch as substrate. The trypsinogen level was determined after activation with enterokinase, following the method of Erlanger (30). Lipase activity was measured titrimetrically using emulsion oil as substrate (31). Protein content of acinar cells was measured by the method of Bradford (32). Concentrations of CCK in plasma were measured by a specific radioimmunoassay that had been reported previously and validated (33, 34). The integrated responses of enzymes in the pancreatic juice were calculated by the method described previously (35).

Histopathology. Histological studies of the pancreas were conducted by methods as described earlier (36). Briefly, the pancreas from rats on high-fat and high-carbohydrate diets were carefully isolated, trimmed of fat, and fixed in 10% buffered formalin. The formalin-fixed pancreases were then dehydrated with ethanol and embedded in paraplast. Sections of 5–6 μm were cut and stained with hematoxylin and eosin.

Statistics. The data were expressed as the mean ± SEM. Data were subjected to analysis by Student's *t* test followed by analysis of variance (ANOVA). A *P*-value ≤ 0.05 was considered significant.

Results

***In Vivo* Studies.** Mean body weights of rats prior to being placed on high-fat or high-carbohydrate diets were close to 250 g for both groups. After 4 weeks on high-fat and high-carbohydrate diets, body weights increased to 392 ± 17 g for the high-carbohydrate group and 379 ± 12 g for the high-fat group. In addition, after 4 weeks, pancreatic weights were 2.67 ± 0.24 g and 2.05 ± 0.1 g, and plasma levels of CCK were 20.2 ± 4.9 p*M* and 20.9 ± 4.2 p*M* for rats on high-carbohydrate and high-fat diets, respectively; however, these values were not significantly different from each other.

Basal volumes of pancreatic juice from rats on high-fat diets were significantly greater than those of rats on high-carbohydrate diets (Fig. 1). In addition, basal outputs (U/30 min) of amylase (50 ± 6), lipase (78 ± 14), and trypsinogen (150 ± 21) in pancreatic juice of rats on high-fat diets were higher than those of rats on high-carbohydrate diets (25 ± 6, 48 ± 16, and 50 ± 15), respectively.

CCK infusion induced increases of volume, amylase, lipase, and trypsinogen outputs in each dietary group. The effectiveness of CCK response to these two dietary groups was further evaluated by calculating the integrated enzyme outputs in the pancreatic juice that was collected for a 90-min period Table I. Outputs (U/90 min) of amylase, lipase, and trypsinogen were significantly higher for rats on high-fat diets when the same were compared with outputs of rats on high-carbohydrate diets, respectively (Table I). Normalization of the enzyme outputs with respect to volume resulted in final outputs (U/ml/90 min) of amylase (32 ± 4.2 vs 18.6 ± 10.0), lipase (56.9 ± 19.6 vs 29.8 ± 19.4), and trypsinogen (136.0 ± 26.9 vs 147.0 ± 94.7) for high-fat diets, and for high-carbohydrate diets, respectively. Integrated outputs in amylase and lipase, but not trypsinogen, of rats on high-carbohydrate diets were significantly higher compared with rats on high-fat diets (Table I).

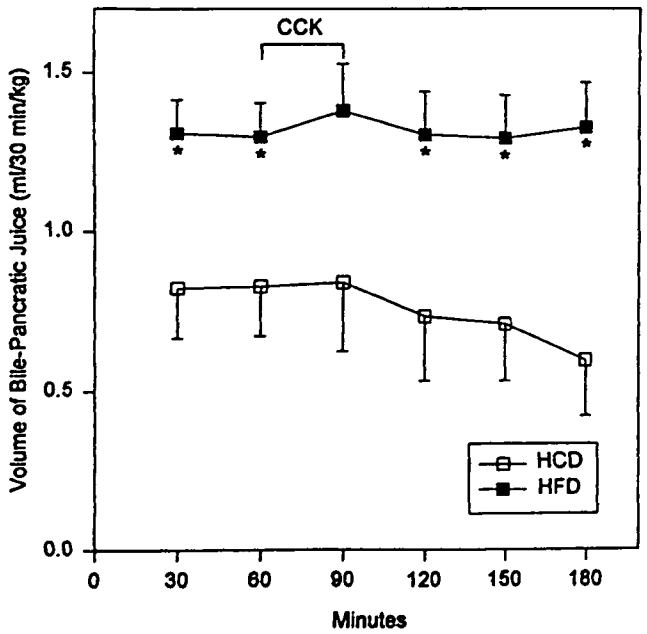

Figure 1. Effects of dietary fat and carbohydrate on pancreatic juice volume. Pancreatic juice was collected continuously in 30-min samples for 1 hr under basal conditions. CCK-8 was administered intravenously at a rate of 0.15 µg/kg for 30 min as indicated. After administration of CCK-8, sampling of pancreatic juice was conducted every 30 min for 2 hr. HFD, rats fed a high-fat diet; HCD, rats fed a high-carbohydrate diet. Data are the mean ± SEM; **P* < 0.05 when compared with HCD.

***In Vitro* Studies.** Amylase release in response to graded doses of CCK was measured in pancreatic acini isolated from the same rats at basal level and with graded doses of CCK (Fig. 2). The release of amylase by CCK in acini from rats on high-fat diets was significantly lower than that measured from rats on high-carbohydrate diets. The response by the group on high-carbohydrate diets was identical to those on normal carbohydrate and normal fat diets (data not shown).

Pathological Findings. No histologic alterations were detected in the pancreas of rats on the high-carbohydrate diets (Fig. 3, top panel). These findings were not different from normal carbohydrate and normal fat diets. However, the pancreas of rats on high-fat diets showed increased numbers of pyknotic cells when compared with high-carbohydrate diets (Fig. 3, bottom panel shown by arrowhead → (P), edematous swelling (Fig. 3, bottom panel shown by arrowhead → (S), and cytoplasmic vacuolization (Fig. 3, bottom panel shown by arrowhead → (V)).

Discussion

The current study was conducted with rats that were fed either a high-fat–normal-carbohydrate diet or a high-carbohydrate–no-fat diet to determine if these dietary components had different effects on the exocrine pancreas. Rats were maintained on these diets for 4 weeks. Body weights, pancreatic weights, and plasma levels of CCK were not significantly different in rats that were fed either types of diet. After surgery, in *in vivo* studies, pancreatic secretions were measured in pancreatic juice. Subsequently, rats were sacrificed, and pancreatic acini were isolated to measure CCK-stimulated amylase response for *in vitro* studies. Histological examination of the pancreas was also conducted.

In *in vivo* studies, volumes of bile pancreatic juice measured at basal and in response to CCK-8 were significantly greater in rats that were fed a high-fat diet when compared with those measured in rats on a high-carbohydrate diet. These results suggest that feeding rats a high-fat diet for 4 weeks affects their pancreatic volume outputs in a manner that is different from those fed high-carbohydrate diets. This increase in volume outputs may be due to the release of secretin, reported to be released by fat (12, 18) and whose

Table I. Effect of Dietary Fat and Carbohydrate on Integrated Pancreatic Enzyme Outputs

	HCD (U/90 min)	HFD (U/90 min)	HCD (U/ml/90 min)	HFD (U/ml/90 min)
Amylase	41.8 ± 22.7	125.0 ± 16.4[a]	18.6 ± 10.0	32 ± 4.2[a]
Lipase	67.0 ± 43.7	221.9 ± 76.6[a]	29.8 ± 19.4	56.9 ± 19.6[a]
Trypsinogen	330.8 ± 213.1	531.8 ± 105.1	147 ± 94.7	136.3 ± 26.9

Note. Pancreatic juice was collected continuously in 30-min samples for 1 hr under basal conditions. CCK-8 was administered intravenously at a rate of 0.15 μg/kg for 30 min as indicated. After administration of CCK-8, samples of bile-pancreatic juice were collected every 90 min. Integrated responses were calculated as described in Materials and Methods (33). Data are the mean ± SE; [a]$P < 0.05$ when compared with HCD. HCD, high carbohydrate diet; HFD, high fat diet.

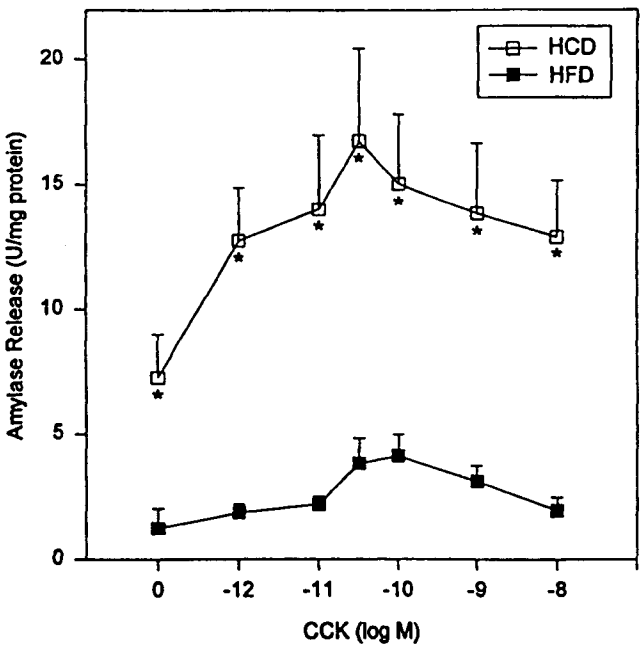

Figure 2. Effects of dietary fat and carbohydrate on amylase release from isolated rat pancreatic acini. CCK-8–stimulated amylase release was determined in isolated dispersed pancreatic acini and expressed as U/mg of acinar protein. Data are the mean ± SEM; *$P < 0.05$ when compared with HFD.

biologic action is to increase pancreatic volume output (18). Plasma levels of secretin were not measured in the current study.

Changes in dietary components produced significantly greater outputs of amylase and lipase, but not trypsinogen, in rats fed a high-fat diet when compared with those measured in rats fed high-carbohydrate diets (Table I). The effects of a high-fat diet appeared to have more dramatic effect on lipase output when compared with amylase. These differences in lipase and amylase outputs were also noted when the results were expressed as U/ml/90 min, but the effects of these diets on trypsinogen outputs were almost equal between the two groups. The data suggest that pancreatic enzyme outputs are specific for dietary components and vary in proportion to the dietary content of their respective substrates. This reasoning is further supported by the fact that trypsinogen outputs were not different in rats that were fed equal amounts of protein.

Recently, it was shown that daily ingestion of fat or ethanol in rats for 12 days did not alter plasma CCK levels (37). The radioimmunoassay for CCK used in this study measures mostly larger forms of CCK (i.e., CCK-33 and CCK-39) and does not effectively detect smaller forms of this peptide, especially CCK-8. Therefore, the results of this study do not rule out the possibility that the diet could affect endogenous levels of CCK-8. Thus, there is a possibility that active forms of CCK and secretin as well as other regulatory peptides could be released differentially in response to these two diets, and the action of these released hormones may affect the receptor-mediated pathways leading to exocrine pancreatic secretions.

In *in vitro* studies, CCK-induced amylase release from pancreatic acinar cells expressed as U/mg protein was significantly greater in rats on a high-carbohydrate diet when compared with the same rats on a high-fat diet. These differences were significant before and after infusion of CCK and were expected because of the larger amounts of amylase needed to digest the large amount of carbohydrate present in the high-carbohydrate diet. The decreased response of CCK in acinar cells isolated from high-fat diet rats may be due to the exhaustion and downregulation of CCK receptors.

Results from the pathological findings showed that feeding a high-fat diet, but not a high-carbohydrate diet, induced significant histological changes in the exocrine pancreas as evidenced by the appearence of pyknotic nuclei, cellular edema, and vacuolization (Fig. 3, bottom panel). The appearance of cytoplasmic vacuoles has been recognized as an indicative criterion for early pancreatitis development in rats and mice (36, 38–41). Because the exocrine pancreas of rats on a high-carbohydrate diet appeared to be normal in contrast to that of rats on a high-fat diet, the results indicated that feeding rats with different amounts of fats and carbohydrates may have different pathologic effects on the pancreas.

In summary, in *in vivo* studies, pancreatic volume, amylase and lipase, but not trypsinogen, outputs were greater in the pancreatic juice of rats fed a diet containing high amounts of cottonseed oil (high fat) when compared with those found in rats on a diet containing high amounts of sucrose (high carbohydrate). In contrast, in *in vitro* studies, amylase release from isolated acini, taken from rats on a high-carbohydrate diet was greater than that found in acini from rats on a high-fat diet. The most important and novel findings in these studies were the abnormalities in the pan-

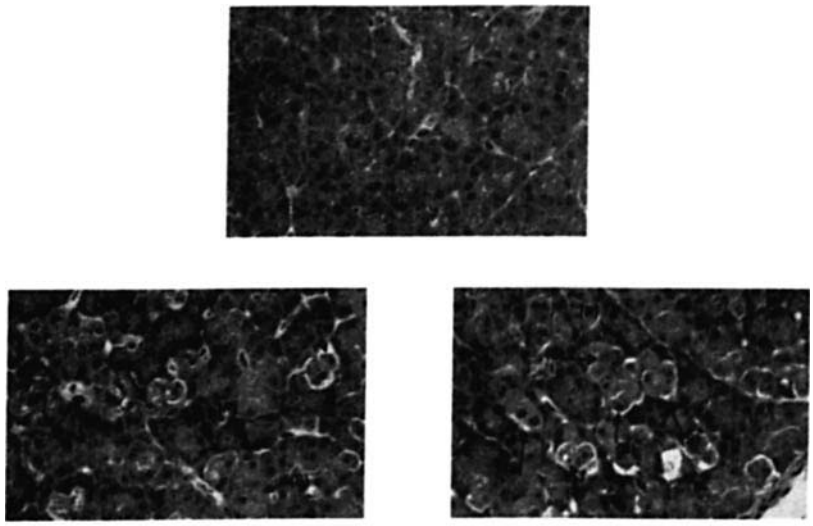

Figure 3. (Top panel) Pancreas of male rats on high-carbohydrate diet. No histopathological changes noted. (Bottom left panel) Pancreas of male rats on high-fat diet for 28 days. Increasing number of pyknotic cells and vacuolization were seen as shown by arrow heads; (P) pyknotic cells, (V) vacuoles. (H & E, 250x). (Bottom right panel) Pancreas of male rats on high-fat diet for 28 days. Extensive edematous swelling is seen in many acinar cells as shown by arrowheads (S). Some pyknotic cells are also present (P). (H & E, 250x).

creas of rats on a high-fat diet that were not found in the pancreas of rats on a high-carbohydrate diet. The results support the contention that a high-fat diet may have deleterious effects on pancreatic pathophysiology.

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