Pancreatic Exocrine Response to Long-Term High-Fat Diets in Rats

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ABSTRACT

Context Although the synthesis and secretion of pancreatic enzymes are dependent on the composition of diet, little is known about the long-term adaptation of the exocrine pancreas to the chronic intake of high-fat, low-carbohydrate diets.

Objective The effects of long-term (48-weeks) high-fat, low-carbohydrate feeding on the intracellular activity as well as on the secretory activities of the pancreas regarding the secretion of amylase and lipase in isolated pancreatic lobules of rats were investigated.

Animals Twenty-six male Sprague-Dawley rats were randomly divided into two groups.

Design The high-fat diet group was fed a diet containing 12.3% Kcal of carbohydrates, 76.0% Kcal of fats, and 11.7% Kcal of proteins for 48 weeks. In the control group, the rats were fed a standard diet (50.3% Kcal of carbohydrates, 33.2% Kcal of fats, and 16.5% Kcal of proteins) for the same period of time.

Main outcome measures The intracellular activity of alpha-amylase and lipase were defined as enzyme activity per 1 mg protein in the pancreatic homogenate. The secretory activity of the pancreatic enzymes was defined as enzyme activity per 1 mg protein or as the mean percentage of released enzyme activity in the medium to that initially retained in the lobules over the entire 90 minute incubation period.

Results The intracellular activity as well as the secretory activities of pancreatic amylase in the basal and in the cholecystokinin (10 pmol/L)-stimulated states of high-fat, low-carbohydrate diet fed rats were significantly lower than that in the control rats. Nevertheless, those of lipase were not significantly different between the two groups. However, when the secretory enzyme activity was illustrated as the percentage released, basal as well as CCK-stimulated amylase and lipase activity were not significantly different between the two groups.

Conclusions The reduction of the intracellular amylase activity and the secretory amylase activity may be a long-term adaptive response of the pancreas to the reduced carbohydrate composition of the diets. Moreover, the reduction of the secretory amylase activity may be due to reduced intracellular amylase activity rather than to the altered sensitivity of the acinar cells to cholecystokinin. However, we failed to observe a long-term adaptive response of pancreatic lipase to increased fat composition of diets.

INTRODUCTION

It has been well-documented that the chronic intake of a high-fat diet is associated with
hyperlipidemia, whose pathologic consequences are acute pancreatitis, atherosclerosis, obesity, non insulin-dependent diabetes mellitus and other disorders [1, 2, 3]. In addition, a high-fat diet may also be a significant risk factor in pancreatic cancer [4]. The elevation of blood lipid levels due to the ingestion of excess dietary fat can lead to the development of several disorders. The majority of fat digestion occurs in the upper intestine by lipase secreted from the exocrine pancreas. It has been reported that the contents of pancreatic amylase, lipase and protease change in proportion to the dietary contents of their respective substrates, namely carbohydrates, fats and proteins [5, 6]. In addition, the synthesis, mRNA content and transcription of pancreatic lipase are regulated by dietary fat [7, 8]. The secretion of lipase from the exocrine pancreas is also dependent on the fat composition of the diet [9, 10]. However, considerable controversy exists concerning dietary fat and a consequent adaptation of the pancreatic enzymes. It has been demonstrated that dietary fat increases the lipase activity of the exocrine pancreas in mice, rats and pigs [7, 8, 9, 10]. Nevertheless, in other studies of conscious dogs, dietary fat failed to change the expression and secretion of pancreatic lipase [11, 12]. Although it has been well-documented that short-term fat given orally or intraduodenally induces the release of cholecystokinin (CCK) which is one of the potent mediators in pancreatic adaptation to dietary fat [13, 14, 15, 16], little is known about the adaptation of the exocrine pancreas to the chronic intake of dietary fat. Since increasing the fat component in diets results in the decrease of the carbohydrate component [5], pancreatic amylase could also be influenced by a reduced carbohydrate component. Thus, the present study was undertaken to investigate the effects of very long-term high-fat, low-carbohydrate diet (HF-LCD) fed rats. Isolated pancreatic lobules of rats were utilized to test the enzyme secretion in this study in order to eliminate the possible influence of the extrinsic nerves and hormones on exocrine secretion.

MATERIALS AND METHODS

Animals and Diets

Prior to sacrifice, twenty-six male Sprague-Dawley rats (4 weeks of age, weighing 100-150 g) were maintained for a period of 52 weeks in an environmentally-controlled room with a temperature of 23±2°C, relative humidity of 55±5% and a 12-h light:dark cycle with free access to water. The rats were randomly divided into two groups of 13 rats each. As shown in Table 1, the HF-LCD group was fed a diet containing 12.3% Kcal of carbohydrates, 76.0% Kcal of fat, and 11.7% Kcal of protein over a 48-week period. In the control group, the rats were fed a standard diet (containing 50.3% Kcal of carbohydrates, 33.2% Kcal of fat, and 16.5% Kcal of protein) for the same 48 weeks. The standard diets were prepared using a commercial source of AIN-93G purified rodent diet (Dyets, Bethlehem, PA, USA). The diets were mixed with cellulose (10 g/kg),

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>HF-LCD</th>
<th>Control diet</th>
</tr>
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<tbody>
<tr>
<td>Carbohydrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Corn starch</td>
<td>100</td>
<td>480</td>
</tr>
<tr>
<td>- Sucrose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Fats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Soybean oil</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>- Lard</td>
<td>500</td>
<td>120</td>
</tr>
<tr>
<td>Protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Casein</td>
<td>190</td>
<td>190</td>
</tr>
<tr>
<td>Energy (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>12.3%</td>
<td>50.3%</td>
</tr>
<tr>
<td>Fats</td>
<td>76.0%</td>
<td>33.2%</td>
</tr>
<tr>
<td>Protein</td>
<td>11.7%</td>
<td>16.5%</td>
</tr>
<tr>
<td>Metabolizable energy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Kcal/kg)</td>
<td>6,510</td>
<td>4,610</td>
</tr>
</tbody>
</table>

Table 1. The composition of experimental diets.
L-cystine (3 g/kg), choline bitartrate (2.5 g/kg), AIG-93G vitamin mixture (10 g/kg), AIG-93G mineral mixture (35 g/kg), and t-butylhydroquinone (0.014 g/kg).

**Preparation of the Pancreatic Lobules**

After an overnight fast with free access to water, the animals were killed by cervical dislocation and the pancreatic lobules were obtained. The pancreatic lobules were prepared according to a method previously described [17]. In brief, the pancreas was quickly removed and immersed in modified calcium-free HEPES (25 mmol/L)-buffered Ringer solution with the following composition (Sigma-Aldrich, St. Louis, MO, USA): NaCl 113 mmol/L, HEPES 25 mmol/L, KCl 4.7 mmol/L, MgCl₂ 1.2 mmol/L, D-glucose 5.6 mmol/L, sodium pyruvate 5 mmol/L, sodium fumarate 5 mmol/L, sodium glutamate 5 mmol/L, and L-glutamine 2 mmol/L. The medium also contained 2% (v/v) essential amino acids, 1% (w/v) BSA, and 0.1% (w/v) soybean trypsin inhibitor. The solution was maintained at pH 7.4, while being continuously gassed with 100% O₂ at 37°C. Whole pancreatic lobules were spread out by injecting calcium-free HEPES-Ringer solution gassed with 100% O₂ into the loose connective tissue. The separated lobules, about 6 mg wet weight, were then obtained by excision. Four lobules were incubated in 4 mL of calcium-contained (2.5 mmol/L) HEPES-buffered Ringer solution gassed with 100% O₂ for an equilibration period of 30 min.

**Effect of Diets on the Intracellular Activities of Pancreatic Amylase and Lipase**

The intracellular activities of alpha-amylase and lipase were measured in pancreatic lobules isolated from the control rats and the HF-LCD fed rats. The lobules were mechanically homogenized in 1% Triton X-100 buffer (pH 8.0) containing (mmol/L) CaCl₂ 2.5 mmol/L, HEPES 25 mmol/L, and KCl 0.1 mmol/L. Alpha-amylase activity in the homogenate was determined according to a method previously described, using starch as a substrate [18, 19]. Lipase activity was measured titrimetrically using lipase assay reagent (Sigma-Aldrich, St. Louis, MO, USA). Protein content in pancreatic tissue was measured using BCA protein assay reagent (Sigma-Aldrich, St. Louis, MO, USA).

**Effects of Diets on the Secretory Activities of Pancreatic Amylase and Lipase in the Basal and in the CCK-stimulated States**

To determine the secretory enzyme activity, 2 mL of the incubation medium was continuously collected every 30 min over the entire 90-min of the experimental period in an ice-chilled test tube and was immediately replaced with the same volume of fresh medium. The secretory activities of amylase and lipase in the basal state were measured in the pancreatic lobules isolated from the control rats and the HF-LCD fed rats without any secretagogues. To stimulate pancreatic enzyme secretion, synthetic sulfated CCK-8 (Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 10 pmol/L was introduced into the culture medium, and the concentration was then maintained by replacement with 2 mL of fresh medium containing 10 pmol/L of CCK after the sample collection every 30 min. To calculate the enzyme activity initially present within the lobules, alpha-amylase and lipase activity not only in the incubation medium but also in the tissue were determined.

**ETHICS**

All the animals involved in this experiment received humane care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animal (1996)” prepared by the National Academy of Sciences.

**STATISTICS**

All results are presented as means±SE. The intracellular activities of amylase and lipase were defined as amylase and lipase activity per 1 mg of protein in the pancreatic
homogenate. The secretory activities of amylase and lipase were defined as U/90 min/mg protein, which is the enzyme activity cumulatively secreted over the entire 90-min period in the medium per 1 mg of protein initially present in the lobules, and as a % total, which is enzyme activity cumulatively secreted over the entire 90-min period in the medium per enzyme activity initially present within lobules. The protein amount and the enzyme activity initially present in the lobules were calculated from the sum of the secreted value and the retained value. The data were analyzed using the Student’s t test by means of the SPSS version 9.0 for Windows. Differences were considered significant when the two-tailed P value was less than 0.05.

RESULTS

Intracellular Activities of Pancreatic Amylase and Lipase

The mean body weights of the rats prior to being fed the control diet and the HF-LCD were 120±6 g and 121±7 g, respectively. After 48 weeks, their body weights increased to 408±22 g and 499±28 g in the control group and in the HF-LCD group, respectively. Significantly greater weight gain (P=0.016) was observed in the HF-LCD group. As shown in Figure 1a, the intracellular alpha-amylase activity in the pancreatic lobules isolated from HF-LCD fed rats was 71.6±5.0 U/mg protein, which was significantly lower (P=0.007) than that in control rats (95.0±5.8 U/mg protein). However, the intracellular lipase activity (Figure 1b) in the HF-LCD fed rats (329±21 U/mg protein) was not significantly different (P=0.362).

Secretory Pancreatic Enzyme Activities in the Basal and in the CCK-stimulated States

The pancreatic lobules of the control rats spontaneously secreted amylase and lipase in the basal state at a rate of 11.1±1.5 U/90 min/mg protein and 25.4±2.7 U/90 min/mg protein, respectively. As shown in Figure 2, the lobules of the HF-LCD fed rats spontaneously secreted amylase at a rate of 6.85±1.47 U/90 min/mg protein, which was notably lower (P=0.030) than that in control rats while they secreted lipase at a rate of 27.7±2.9 U/90 min/mg protein, which was similar to that in control rats (P=0.342). However, when secretory enzyme activity was illustrated as the percentage released, the
secretory activities of amylase and lipase in basal state of HF-LCD group were not significantly different from the control group (Table 2).

As shown in Figure 3, the CCK-stimulated pancreatic amylase secretion was dose-dependent. Significance (P<0.001) was observed beginning with 10 pmol/L of CCK, and maximal effect was observed in 1 nmol/L of CCK. CCK at a concentration of 10 pmol/L significantly increased the secretory amylase activity from the concentration of 10 pmol/L, and maximal effect was observed at 1 nmol/L of CCK.

DISCUSSION

In the present study, we first report that the intracellular and the secretory activity of amylase were reduced by very long-term (48 weeks) high-fat, low-carbohydrate feeding. In our study, we used a control diet containing 50.3% Kcal of carbohydrates and a HF-LCD containing 12.3% Kcal of carbohydrates. In general, increasing the fat component in the diets results in a decrease in the carbohydrate component [5]. It has been well-established that high-fat, low-carbohydrate feeding results in a decrease of the mRNA expression of pancreatic amylase [20, 21] and high-carbohydrate feeding results in an increase of the mRNA expression of pancreatic amylase in rats [22]. These results strongly indicate that high-fat feeding may reduce pancreatic amylase synthesis due to a low-carbohydrate component present in the duodenum for an extended period of time, namely a long-term adaptation.

In in vitro studies, a high-fat diet decreased the basal or the CCK-stimulated secretion of amylase, and a high-carbohydrate diet increased them in rats [10, 21, 23, 24]. In our study, the secretory amylase activities in the basal and in the CCK-stimulated states were lower in HF-LCD fed rats than in control diet

Table 2. The secretory amylase and lipase activities in the basal and in the CCK-stimulated states illustrated as a % total in pancreatic lobules isolated from control rats and HF-LCD fed rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Secretry alpha-amylase activity (% total)</th>
<th>Secretry lipase activity (% total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>CCK-stimulated</td>
</tr>
<tr>
<td>HF-LCD</td>
<td>9.5±1.1</td>
<td>14.5±1.6</td>
</tr>
<tr>
<td>Control</td>
<td>11.4±1.1</td>
<td>16.1±0.9</td>
</tr>
<tr>
<td>P value</td>
<td>0.107</td>
<td>0.340</td>
</tr>
</tbody>
</table>

Each data represents the mean±SE from 13 experiments.
fed rats. However, when the secretory amylase activity was illustrated as the percentage released, the secretory amylase activities in the basal and in the CCK-stimulated states of high-fat feeding rats were not significantly different from those of control diet fed rats. Therefore, the reduced amylase activities in the basal and in the CCK-stimulated states, illustrated as U/90 min/mg protein, may be due to the reduced intracellular amylase activity rather than to altered sensitivity of the pancreatic lobules to CCK. These results suggest that the synthesis of pancreatic amylase may adapt to the reduced load of carbohydrates for digestion in the small intestine.

Generally, increasing dietary fat stimulates the synthesis [7, 25], mRNA content [8, 26] and transcription [27] of pancreatic lipase. However, this adaptation of lipase seems to be time-dependent since controversial results have been observed in relation to the length of time of dietary fat feeding. Some studies in dogs showed that dietary fat feeding for only 8 days failed to change the expression of pancreatic lipase [11, 12]. However, other studies showed that lipase was sensitive to high-fat feeding for 4 weeks [8, 10]. It has recently been reported that elevated lipase mRNA expression is sustained for 8 weeks but returned to normal levels by 16 weeks in high-fat feeding mice [7]. Similarly, intracellular lipase activity was also unchanged by long-term HF-LCD feeding despite a reduction in the intracellular amylase activity in the present study. Although the mechanism of unchanged intracellular lipase activity is not known, it seems that an adaptation of intracellular lipase activity to a HF-LCD diet may need appropriate feeding duration, and may not be sustained for a long time.

It is known that dietary fat enhances the pancreatic secretion of lipase [9, 10, 16]. CCK is thought to be one of the potent mediators of an adaptation of pancreatic lipase, since short-term fat given orally or intraduodenally has been known to induce the release of CCK which is one of the potent stimulators of enzyme secretion of the pancreas [13, 14, 27, 28, 29]. Interestingly, it was reported that there were no differences in plasma CCK secretion between lean and obese people in a fasting state as well as in response to the ingestion of a mixed meal [30, 31]. Moreover, CCK-deficient mice clearly demonstrated that CCK was not required for the long-term protein-induced adaptation of pancreatic protease [32], despite the fact that CCK has been considered the primary mediator of dietary protein-induced changes in the pancreas [5]. Although there were conflicting reports regarding the role of CCK on the adaptation of the production of pancreatic enzymes, we failed to modify the secretory lipase activities in the basal and in the CCK-stimulated states by long-term HF-LCD feeding as compared to control diet feeding. We used CCK at a concentration of 10 pmol/L which is a relatively lower, but physiological [27, 28, 29], concentration as compared to other studies; thus, the secretory lipase activity may be unchanged in this study. This result may also be an age-related phenomenon because there is a reduction in lipase secretion in older humans [33, 34]. Therefore, further study about pancreatic lipase secretion with a higher dose of CCK or other secretagogues is needed to elucidate the adaptive response of pancreatic lipase to HF-LCD feeding.

From the above results, we conclude that reduction of intracellular and secretory amylase activity may be a long-term adaptive response of the pancreas to a reduced carbohydrate component, and this long-term adaptation of the pancreatic secretion of amylase may be due to the changes in the intracellular amylase activity rather than to the altered sensitivity of the acinar to CCK.

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Keywords alpha-Amylase; Cholecystokinin; Dietary Fats; Lipase; Time

Abbreviations CCK: cholecystokinin; HF-LCD: high-fat, low-carbohydrate diet
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