Feeding activates protein synthesis in mouse pancreas at the translational level without increase in mRNA

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Sans, Maria Dolors, Sae-Hong Lee, Louis G. D’Aleyc, and John A. Williams. Feeding activates protein synthesis in mouse pancreas at the translational level without increase in mRNA. Am J Physiol Gastrointest Liver Physiol 287: G667–G675, 2004. First published April 29, 2004; 10.1152/ajpgi.00505.2003.—To determine the mechanism of meal-regulated synthesis of pancreatic digestive enzymes, we studied the effect of fasting and refeeding on pancreatic protein synthesis, relative mRNA levels of digestive enzymes, and activation of the translational machinery. With the use of the flooding dose technique with [3H]phenylalanine, morning protein synthesis in the pancreas of Institute for Cancer Research mice fed ad libitum was 7.9 ± 0.3 nmol phenylalanine·10 min⁻¹·mg protein⁻¹. Prior fasting for 18 h reduced total protein synthesis to 70 ± 14% of this value. Refeeding for 2 h, during which the mice consumed 29% of their daily food intake, increased protein synthesis to 117.3 ± 4.9% of the control level. Pancreatic mRNA levels of amylase, lipases, trypsins, chymotrypsin, elastases, as well as those for several housekeeping genes tested were not significantly changed after refeeding compared with fasted mice. By contrast, the major translational control pathway involving Akt, mTOR, and S6K was strongly regulated by fasting and refeeding. Fasting for 18 h decreased phosphorylation of ribosomal protein S6 to almost undetectable levels, and refeeding highly increased it. The most highly phosphorylated form of the eIF4E binding protein (4E-BP1) made up the 14.6% of total 4E-BP1 in normally fed animals, was only 2.8% after fasting, and was increased to 21.4% after refeeding. This was correlated with an increase in the formation of the eIF4E-eIF4G complex after refeeding. By contrast, feeding did not affect eIF2B activity. Thus food intake stimulates pancreatic protein synthesis and translational effectors without increasing digestive enzyme mRNA levels.

Digestive enzymes; protein translation; mice

Pancreatic protein synthesis is required for production of secreted digestive enzymes, growth of the pancreas, and the replacement of normal cellular components. In the mature pancreas, ~90% of protein synthesis has been estimated to be devoted to a mixture of about 20 digestive enzymes (34, 39). Digestive enzymes are secreted after food intake, with secretion mediated largely by neural and gastrointestinal hormone stimulation. To match digestive enzyme synthesis to dietary need, synthesis of digestive enzymes also needs to be controlled. Early studies (8, 46) showed that prolonged fasting decreased both pancreatic protein content and protein synthesis and that these were restored by refeeding. Whereas long-term dietary changes are known to affect mRNA expression (46), short-term meal-to-meal control needs to be immediate, reversible, and flexible. Such control of protein synthesis in other cell types is mainly at the translational level (25, 26).

Early studies on the neural and hormonal regulation of pancreatic protein synthesis were conflicting as reviewed by Case (8). With the advent of isolated acini and pancreatic lobules, gastrointestinal hormones, cholinergic stimulation, and insulin were all shown to stimulate protein synthesis (19–21, 40). Rausch et al. (33) showed that in vivo infusion of CCK and secretin increased the rate of total protein synthesis and that of specific digestive enzymes measured subsequently in vitro in isolated lobules with little or no change in mRNA during the first 24 h of stimulation. The studies with isolated acini showed that the stimulatory effect was directly on acini and also did not require synthesis of new mRNA (19–21, 31).

Recent studies (4, 5) have shown that pancreatic secretagogues can activate the translational machinery both in vitro and in vivo (6). The primary pathway being activated in acinar cells is the PI3K-PKB-mTOR pathway (38). In the rat pancreas, CCK stimulation leads to phosphorylation of eIF4E-BP1 (also known as PHAS-I), which leads to the release of eIF4E, the mRNA cap binding protein, and its subsequent incorporation into the eIF4F complex. In addition, CCK stimulation activates S6K, leading to the phosphorylation of ribosomal protein S6, which is believed to enhance translation of mRNA species with a terminal oligopyrimidine tract (26, 27). These effects are all blocked in vitro by rapamycin, an inhibitor of mTOR (5). The other major regulatory site in many cells, eIF2B, was not activated by CCK in vitro, although its inhibition may reduce acinar protein synthesis (35).

The present study was designed to evaluate whether meal-stimulated pancreatic protein synthesis is accompanied by translational activation, changes in mRNA levels, or both. We studied the effect of fasting and refeeding in mice to model meal-stimulated pancreatic secretion and digestive enzyme synthesis. After it was established that pancreatic protein synthesis was stimulated by normal refeeding, we analyzed the possible changes in the mRNA of several digestive enzymes and confirmed that the increase in protein synthesis was not due to the increase of mRNA but rather was accompanied by the activation of the translational machinery, specifically eIF4F complex formation and S6K activation. No change was seen in the activity of eIF2B.

MATERIAL AND METHODS

Materials. GDP, DT, phenylalanine, and SYBR Green were from Sigma (St. Louis, MO). TRIZol reagent and all oligonucleotides were purchased from Invitrogen (Carlsbad, CA). All other reagents were of analytical grade.

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from Invitrogen (Carlsbad, CA); RNeasy and the RNase-Free DNase set were from Qiagen (Valencia, CA); TaqMan (reverse transcription reagents), dNTPs and the Primer Express Primer software were from Applied Biosystems (Foster City, CA). Goat anti-rabbit and antimouse IgG conjugated to horseradish peroxidase and enhanced chemiluminescence (ECL) reagent were from Amersham Pharmacia Biotech (Piscataway, NJ); 10, 15, and 4–20% Tris-HCl precast gels, broad-range prestained SDS-PAGE standard markers, and fluoroscein and the iCycler IQ real-time PCR detection system software were from Bio-Rad (Hercules, CA); nitrocellulose membranes were from Schleicher & Schuell (Keene, NH). [3H]GDP (11.3 Ci/mmol) was from New England Nuclear Life Science Products (Boston, MA); L-[2,3,4,5,6-3H]phenylalanine (L-[3H]Phe) was from Amersham Bio-reagents, dNTPs and the Primer Express Primer software were from Qiagen (Valencia, CA); TaqMan (reverse transcription reagents) and Waters AccQ-Fluor Reagent Kit were from Millipore (Millford, MA). Protein A–linked antibody was a gift from Dr. Scot R. Kimball (Pennsylvania State University, Hershey, PA); and rabbit anti-eIF4E antibody was a gift from Dr. R. E. Rhoads (Louisiana State University, Shreveport, LA).

Experimental design. All studies were carried out on male ICR mice (Harlan, Indianapolis, IN) weighing 26–32 g fed Purina 5001 chow (LabDiet, St, Louis, MO) and placed in a 12:12-h light-dark cycle (9:00 PM but with a second peak between 3:00 and 6:00 AM). When mice were provided water ad libitum but were fasted 18 h and then refed (at 9:00 AM), they consumed 1.6 ± 0.3 g or 29% of their normal daily intake in 2 h, with most in the first hour. For physiological studies, mice were fed pelleted chow and separated into different groups: fed (ad libitum), fasted (for 18 h, starting at 3:00 PM), refed 1, 2, or 3 h after the 18-h fast, and refasted 6 h (fasted for 18 h, then refed for 2 h followed by refasting for 6 h).

Measurement of pancreatic protein synthesis. Pancreatic protein synthesis was determined using the flooding dose technique, originally described by Garlick et al. (12). With the use of the protocol of Lundholm et al. (24) used to measure liver protein synthesis in mice, we injected 0.4 Ci/g of L-[3H]Phe together with unlabeled L-Phe (1.5 μmol/g) by the intraperitoneal route in a volume of 300 μL. Ten minutes later, pancreases were rapidly removed and frozen in liquid nitrogen. Frozen pancreas was subsequently homogenized in 10 vol of 0.6 N perchloric acid (PCA) and processed as described previously (36). L-Phe was measured by HPLC on a C18 reverse-phase column after precolumn derivatization with Waters AccQ-Fluor Reagent kit to produce a stable fluorescent derivative. Protein synthesis was calculated from the rate of radioactive L-Phe incorporation into pancreatic protein using the specific radioactivity of pancreatic PCA-soluble L-Phe as representative of the precursor pool and expressed as nanomoles of L-Phe per milligram of protein.

**Table 1. Primers used for quantitative RT-PCR of mouse pancreas RNA**

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<thead>
<tr>
<th>Name</th>
<th>Accession Number</th>
<th>Orientation</th>
<th>Primer Sequence (5’ → 3’)</th>
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<tr>
<td>β2-Microglobulin</td>
<td>XM 123906</td>
<td>sense</td>
<td>GAC CCC GAC TGA GAC TGA TAC A</td>
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<tr>
<td></td>
<td></td>
<td>antisense</td>
<td>CGA TCC CAG TAG AGC TGG TGG</td>
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<td>X 52803</td>
<td>sense</td>
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<td></td>
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<td>M32599</td>
<td>sense</td>
<td>GAA GAC AGT AGT AGA CTC GAC A</td>
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<tr>
<td></td>
<td></td>
<td>antisense</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>NM 009885</td>
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<tr>
<td></td>
<td></td>
<td>antisense</td>
<td>GCA CAG CGG GCA CAT CA</td>
</tr>
<tr>
<td>Triglyceride lipase</td>
<td>AY387690</td>
<td>sense</td>
<td>TCT GGC TCA TTC GAA ATC TCA AG</td>
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<td></td>
<td></td>
<td>antisense</td>
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<td>sense</td>
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<td>NM 011646</td>
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60°C for 1 min (repeated 40 times), and 55°C for 1 min. To distinguish between products and primer-dimers, we monitored the melt curve, obtained by increments of 0.5°C every 10-s interval from 55°C until it reached 96°C. Finally, all primer pairs were also validated with standard dilution curve (1:2, 1:4, 1:8, 1:16, 1:32). Data were analyzed using I-Cycler IQ real-time PCR detection system software to analyze the melt curve, the standard curve, and the quantitative amplification. The fluorescence resulting from the incorporation of SYBR Green 1 dye into the double-stranded DNA produced during the PCR reaction was quantitated to obtain the threshold cycle (Ct) value for each sample. Because Ct readings represent measurements on a log scale, a mean Ct value was calculated for each sample as 2Ct, where ΔCt is the mean control Ct minus the individual Ct. This converts the log scale Ct values to a relative number in linear form that can be used to calculate a mean ± SE for each group.

Gene sequences for housekeeping genes and digestive enzymes were primarily obtained from the GenBank NCBI Sequence Viewer (http://www.ncbi.nlm.nih.gov). The mouse pancreatic triglyceride lipase sequence was obtained by PCR amplification from reverse-transcribed mouse pancreatic RNA, using primers based on an EST sequence (AK002353) and the rat lipase sequence (M58369) and sequenced in both directions with multiple overlapping primers. The resulting full-length sequence is now present in GenBank as AY387690. Primers for quantitative RT-PCR (Table 1) were designed using the Primer Express software from ABI (Foster City, CA).

Measurement of PKB/Akt activity. Activity of the Akt kinase was measured using the nonradioactive Akt kinase assay kit from Cell Signaling, as described previously (36). Briefly, pancreas samples were homogenized in 2 ml lysis buffer, homogenates were centrifuged for 15 min at 10,000 g, and Akt was immunoprecipitated from 500 μg protein. The resulting immunoprecipitate was then incubated with GSK-3 fusion protein as substrate at 30°C for 30 min in the presence of ATP and kinase buffer. Phosphorylation of GSK-3 was measured by Western blot analysis using an anti-phospho-GSK-3α/β (Ser21/9) antibody. Quantitation in this case, and in all Western blot analysis, was performed using Multi-Analyt software (Bio-Rad).

Measurement of eIF2B activity. Determination of eIF2B activity in pancreatic tissue was performed as described previously (17, 35) by measuring the rate of exchange of [3H]GDP present in an exogenous eIF2β-5H[GDP complex for free nonradiolabeled GDP in pancreatic tissue samples. The guanine nucleotide exchange activity was measured as a decrease in eIF2β-5H[GDP complex bound to nitrocellulose filters and expressed as nanomoles of GDP exchanged per minute per milligram of acinar protein or as a percentage of the control group (35).

Evaluation of the phosphorylation state of Akt, the ribosomal protein S6, and eIF4E binding protein 4E-BP1. The phosphorylation state of Akt and ribosomal protein S6 was determined by the relative amount of Akt or S6 in the phosphorylated form, quantitated by protein immunoblot analysis using affinity-purified antibody that specifically recognizes Akt and S6 when phosphorylated at Ser473 and Ser240/244, respectively. Aliquots of pancreas lysates were resolved in a 10% SDS-PAGE gel, transferred to nitrocellulose, followed by Western blot analysis using the anti-phospho Akt and S6 antibodies (1:1,000) and detected by ECL. To evaluate possible changes in the amount of these proteins, the same membranes were stripped and reprobed for total Akt (with an antibody that recognizes all forms) and ribosomal protein S6 using polyclonal antibodies to Akt and S6 at 1:500.

The phosphorylation state of the eIF4E-binding protein (4E-BP1) was determined by protein immunoblot analysis using an antibody that recognizes all forms of 4E-BP1. 4E-BP1 resolves into multiple electrophoretic forms during SDS-PAGE depending on which and how many, sites are phosphorylated (30). Unlike more rapidly migrating forms (α and β), the form exhibiting the slowest migration, referred to as the γ-form, does not bind to eIF4E. For this analysis, aliquots of pancreas lysates were boiled for 10 min, and after they were cooled to room temperature, they were centrifuged at 1,000 g for 30 min at 4°C. Supernatant proteins were resolved in a 15% SDS-PAGE, transferred to nitrocellulose, followed by Western blot analysis using the 4E-BP1 polyclonal antibody (1:3,000), and detected by ECL. The amount in the γ-band was calculated as a percentage of the total.

Coimmunoprecipitation of eIF4G with eIF4E and formation of the eIF4F complex. To quantify the formation of the eIF4F complex, we analyzed the association of eIF4E with eIF4G by coimmunoprecipitation, as previously described (6). Briefly, pancreatic samples were homogenized in 2 ml buffer, centrifuged at 10,000 g for 10 min at 4°C, and the supernatant, containing microsomes and soluble protein, was used to analyze translation factors. The association of eIF4G and eIF4E was assessed by analyzing the amount of eIF4G bound to eIF4E immunoprecipitated using specific anti-eIF4E antibody, as described previously (36). The immunoprecipitates were resolved on a 4–20% gradient SDS-PAGE followed by Western blot analysis using anti-eIF4G antibody (1:2,000).

Statistical analysis. Data are reported as means ± SE, which were obtained from three to five different experiments with three to five animals per group in each experiment. Statistical analysis was carried out by one-way ANOVA and the post hoc Fisher’s protected least significant differences test on the StatView program (SAS Institute, Cary, NC). Differences with P < 0.05 were considered significant.

RESULTS

Feeding stimulates pancreatic protein synthesis. To validate the flooding-dose technique for measuring pancreatic protein synthesis in mice, we measured the uptake of radioactive phenylalanine into plasma and pancreas as a function of time. After the administration of phenylalanine, plasma phenylalanine increased ~3.5-fold (to 252 ± 15 μM). The time course for the specific activity (dpm/nmol of l-Phe) of the pancreatic intracellular nonprotein pool is represented in Fig. 1A, and

![Fig. 1. Time course of specific radioactivity (A) and l-[3H]phenylalanine (Phe) incorporation into pancreatic protein (prot; B) in ICR mice. Values are expressed as disintegrations per minute per nanomole of Phe in the pancreatic acid soluble pool in A and as nanomoles of incorporated Phe per milligram of protein in the acid precipitable pool in B. All data points are means ± SE of 10–12 mice per group.](http://ajpgi.physiology.org/content/287/9/A669/F1)
An effect of fasting to decrease and refeeding to increase back amount of cDNA was used as a template for PCR. Except for quantitative real-time PCR. Total RNA was isolated and after purity three housekeeping proteins, and ribosomal 18 sRNA by quantitative real-time PCR. To determine whether changes changed by the feeding status.

Effects of feeding on mRNA levels and the translational machinery primarily at the 2-h time point. Pancreatic protein synthesis determined at 9:00–10:00 AM in mice fed ad libitum was 7.9 ± 0.3 nmol of 1- Phe·10 min−1·mg protein−1. Fasting for 18 h reduced pancreatic protein synthesis to 70.8 ± 1.4% of control-fed animals (Fig. 2). Refeeding for 1 h increased protein synthesis to levels similar to the fed group, and refeeding for 2 h increased pancreatic protein synthesis to 171.3 ± 4.9% of the fed group or >50% compared with the fasted group (Fig. 2). Protein synthesis measured after 3 h refeeding returned close to fasted levels; 77.4 ± 9.7% of control (Fig. 2). When the animals were fasted again for 6 h after a 2-h refeeding, protein synthesis levels had also returned to the fasted levels, being 68.2 ± 7.7% of control (Fig. 2). These results indicate that stimulating gastrointestinal activity in mice by feeding transiently stimulates pancreatic protein synthesis. Because increased stimulation of protein synthesis was seen after 1 and 2 h refeeding and was maximal after 2 h, we measured the effects of feeding on mRNA levels and the translational machinery primarily at the 2-h time point.

Pancreatic mRNA levels of digestive enzymes were not changed by the feeding status. To determine whether changes in protein synthesis might be due to translational regulation, we evaluated the mRNA levels of nine digestive enzymes, three housekeeping proteins, and ribosomal 18 sRNA by quantitative real-time PCR. Total RNA was isolated and after purity was checked, equal amounts were used for RT, and a standard amount of cDNA was used as a template for PCR. Except for an effect of fasting to decrease and refeeding to increase back to the control for the housekeeping gene cyclophilin A, there were no significant changes between the housekeeping genes analyzed at any of the experimental conditions (Table 2). Only small differences were seen in the different treatments among amylase, chymotrypsin, elastase 1 and 2, triglyceride lipase, carboxyl ester lipase, and the three analyzed trypsin isoforms, with three increasing and five decreasing in response to refeeding. None of these changes was statistically significant (Table 2). Thus changes at the mRNA level cannot explain changes in protein synthesis seen during fasting and feeding (Fig. 2).

The translation initiation factor eIF2B activity is not modified by the feeding status. Because changes seen in pancreatic protein synthesis were not related to changes in mRNA for digestive enzymes, we analyzed the possible translation regulatory mechanisms that could be involved in this effect. Modulation of eIF2B activity is known to be an important regulatory point in translation initiation in some systems, because eIF2 is required for binding of initiator tRNA to the ribosome (16). Extracts of control-fed pancreas exhibited an eIF2B activity of 34.5 ± 3.3 nmol·min−1·mg protein−1 (n = 9). No significant reduction or increase of this activity was seen at any of the different experimental conditions (Fig. 3). We conclude that this regulatory step in translation initiation is active regardless of the feeding status, and thus it is not a limiting factor for normal pancreatic protein synthesis regulation.

Feeding stimulates the Akt/mTOR pathway. Because the Akt/mTOR pathway is known to be activated in the pancreas by physiological doses of CCK (47), we determined whether this stimulatory pathway was also activated by normal feeding in mice. Pancreatic Akt activity decreased after 18 h of fasting to 72.9 ± 9.4% of the fed group levels (Fig. 4A) and increased 1.4% of control-fed animals (Fig. 4 inset). No significant reduction or increase of this activity was seen at any of the different experimental conditions (Fig. 3). We conclude that this regulatory step in translation initiation is active regardless of the feeding status, and thus it is not a limiting factor for normal pancreatic protein synthesis regulation.

Table 2. Relative mRNA quantities of digestive enzymes and housekeeping genes in pancreas of animals fed ad libitum, fasted for 18 h, and refeed for 2 h

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Fed</th>
<th>Fasted</th>
<th>Refed</th>
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<td>Housekeeping genes</td>
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<tr>
<td>β2-Microglobulin</td>
<td>1.07±0.12</td>
<td>0.90±0.06</td>
<td>0.92±0.09</td>
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<tr>
<td>Cyclophilin A</td>
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<td>0.71±0.05*</td>
<td>1.06±0.14†</td>
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<td>GAPDH</td>
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<td>0.86±0.15</td>
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<td>18S RNA</td>
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<td>Digestive enzymes</td>
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<tr>
<td>Amylase 2</td>
<td>1.01±0.10</td>
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<td>1.23±0.11</td>
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<td>1.03±0.10</td>
<td>0.84±0.08</td>
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<td>1.24±0.11</td>
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<td>0.92±0.12</td>
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</table>

Values are means ± SE for 7 animals per group. Groups: Fed refers to animals fed ad libitum, with pancreas removed at 9–10 AM. Fasted refers to animals fasted for 18 h, and refeed refers to animals fasted for 18 h and refeed for 2 h. *P < 0.05 vs. fed group; †P < 0.05 vs. fasted group.
the ribosomal protein S6 and the 4E-BP1. The phosphorylation of the ribosomal protein S6 on Ser240/244 was used as a readout of S6 kinase activity; there was no change in the total amount of S6K in the experimental groups (data not shown). S6 phosphorylation was significantly decreased to 11.8 ± 3.2% of the fed group after 18 h of fasting and highly increased after refeeding to 394 ± 41.6% of fed levels (Fig. 5A). The phosphorylation of the 4E-BP1 was also significantly decreased on fasted animals and enhanced after refeeding for 2 h. Fasting reduced the highest phosphorylated γ-form to 2.8 ± 0.7% of total compared with 14.6 ± 3.6% in the fed group (Fig. 5B). Refeeding for 2 h increased 4E-BP1 phosphorylation with 21.4 ± 3.0% of total in the γ-form (Fig. 5B). Both, S6 and 4E-BP1 phosphorylation partially reversed following a 6-h fast after refeeding (data not shown). Phosphorylation of 4E-BP1 leads to the release of eIF4E, which participates in the formation of the eIF4F complex, another one of the main regulatory points on translation initiation. The association of the mRNA cap binding protein eIF4E with eIF4G by coimmunoprecipitation was measured as an indicator of eIF4F formation. The presence of the eIF4F complex was not significantly changed during fasting (Fig. 6) but was increased to 155 ± 13% (n = 4) of the fed control group after 1 h and to 170 ± 17% after 2 h of refeeding. All of these results together demonstrate that the Akt/mTOR pathway is activated by feeding. Both, the increased formation of eIF4F and the phosphorylation of ribosomal protein S6 stimulate the synthesis of digestive enzymes as well as that of ribosomal proteins and translation factors necessary for maintaining this acutely activated synthesis of digestive enzymes.

DISCUSSION

The present study was designed to evaluate the cellular mechanisms involved in the normal (meal to meal) stimulation of pancreatic digestive enzyme synthesis in mice. Fasting inhibited total pancreatic protein synthesis, and refeeding transiently increased it without changes in the mRNA levels of the digestive enzymes in any of these conditions. However, the increase in protein synthesis was accompanied by the activation of important regulatory points in protein translation (Fig. 7) that have been reviewed in detail elsewhere (38). Although the activity of the first regulatory point in translation initiation (eIF2B) was not modified by the feeding status, the formation of the eIF4F complex and the phosphorylation of 4E-BP1 and the ribosomal protein S6, downstream of the Akt/mTOR pathway, were enhanced after refeeding.

Different methods have been used to measure protein synthesis involving administration of labeled amino acids with subsequent measurement of the incorporation of label into
protein. The flooding-dose technique used in this study is based on the “flooding” of all precursor compartments with unlabeled amino acid (9, 12). Because accurate assessment of the specific radioactivity of the precursor amino acid at the site of protein synthesis is important, the administration of the labeled amino acid together with a large bolus of unlabeled amino acid makes the specific activities in all precursor pool compartments more alike than if the labeled compound is given as a tracer dose. The estimation of the precursor labeling specific activity from a readily accessible compartment pools such as the intracellular or plasma pool should, therefore, reflect that of the aminoacyl-tRNA, the direct precursor of protein synthesis, and remains almost constant for a certain period of time after injection (10).

We used L-[3H]Phe as a radioactive tracer because of its widespread use and because Sweiry and coworkers (45) demonstrated that phenylalanine transport into cells of the perfused rat pancreas was not a rate-limiting factor for protein synthesis. The flooding-dose technique has been well characterized in muscle (9, 11, 12), liver (7, 24), and heart (12), and in the present study, it has been adapted to measure pancreatic protein synthesis in mice. First, the specific activity of the intracellular unincorporated pancreatic compartment was measured at different time points to confirm that it remained almost constant for a certain period of time after injection (Fig. 1A) (12, 24, 45). Second, the actual incorporation of L-[3H]Phe into pancreatic protein at different time points was calculated (Fig. 1B). It was found that this parameter increased nearly linearly from 5 to 20 min after L-[3H]Phe administration, and an intermediate time point (10 min), well placed in the linear range and used elsewhere (12), was chosen to be used in all consecutive studies.

To match digestive enzyme secretion and synthesis to dietary need, synthesis of digestive enzymes needs to be controlled and the short-term meal-to-meal stimulation has to provide an immediate, reversible, and flexible response. Because ~90% of pancreatic protein synthesis has been estimated to be devoted to digestive enzymes (34, 39), all of the results presented in this study implicitly reflect the changes in digestive enzyme synthesis after fasting and refeeding, and consequently, our discussion flows around this premise.

The results for total protein synthesis obtained for a 10-min period after 2 h of refeeding were higher than the rate after 1 and 3 h of refeeding (Fig. 2). One possible explanation for the maximal stimulation 2 h after refeeding is the delay between presentation of food and nutrients entering the small intestine. Another explanation could be that there is a delay between the

Fig. 5. Effect of fasting and feeding on the phosphorylation of S6 (A) and eIF4E binding protein 4E-BP1 (B). In A, S6 phosphorylation is expressed as arbitrary units (A.U.), and each column is the means ± SE of 9–14 animals/group. Representative blots for S6 phosphorylation and total S6 are shown in the insets. In B, the phosphorylation levels of 4E-BP1 are represented as the amount of 4E-BP1 in the γ-phosphorylated form as a percentage of the total 4E-BP1. Inset: representative immunoblot with positions of α-, β-, and γ-forms of 4E-BP1 is noted on left. The most highly phosphorylated γ-form exhibits the slowest electrophoretic mobility and does not bind eIF4E. Data are means ± SE of 9–12 animals/group. *P < 0.05 vs. fed group, #P < 0.05 vs. fasted group.

Fig. 6. Effects of fasting and feeding on the eIF4F complex formation, measured as eIF4E and eIF4G coimmunoprecipitation. Data for coimmunoprecipitated eIF4E and eIF4G are expressed as percentage of fed group levels and are the means ± SE of 8–12 animals/group. Representative blots for eIF4G associated with eIF4E and total eIF4E are shown in the insets. *P < 0.05 vs. fed group, #P < 0.05 vs. fasted group.
stimulation of pancreatic secretion and the synthesis of new digestive enzymes after food intake (41); a decrease in hormonal and cholinergic stimulation after peak eating could correlate with the decreased protein synthesis found after 3 h. Fasting mice again for 6 h, after 2 h of refeeding, brought total protein synthesis levels down to the levels of the 18-h fasted animals, indicating that the pancreas can reach its “basal” synthetic point at this time after being stimulated by a meal.

Fasting is known to reduce protein synthesis in skeletal muscle and liver (1, 7), and refeeding stimulates it in the same organs (11, 18, 43, 44, 51). In the present study, we describe a reduction of total protein synthesis after 18 h of fasting in mouse pancreas, similar to what was found in rat liver and muscle in comparable experimental conditions (1, 7). These results are in agreement with a general effect of the feeding status on total protein synthesis of a variety of different organs whose activity can be more or less dependent on the animal’s feeding status. The comparison of our results with other early pancreas studies (8, 46) is difficult, because those studies were carried out under very different experimental conditions (long fasting times, up to several days), different species of animals, and different methods to measure protein synthesis (i.e., in vitro measurements of total protein synthesis after in vivo treatment). However, the majority of these studies also showed reduced pancreatic protein synthesis with fasting (8).

The partial reduction of total protein synthesis seen in the present study with mice indicates that in the basal or interdigestive state, pancreatic protein synthesis (and consequently, digestive enzyme synthesis) is reduced but not completely blocked; it remains active to maintain certain levels of stored digestive enzymes and replace the ones that are still being secreted (41). Not surprisingly, the fractional rates of pancreatic protein synthesis are among the highest in mammalian tissues (45). Moreover, the lack of effect of fasting on Akt activity (Fig. 4), eIF2B activity (Fig. 2), and eIF4F complex formation (Fig. 6) compared with the control-fed group would be in concordance with keeping the synthetic machinery active under these conditions. Because it is known that CCK and cholinergic analogs stimulate these factors at submaximal doses (38), it could likely be that interdigestive (basal) stimulation of the pancreas by the cholinergic nervous system and continuous (or phasic) secretion of CCK (41) maintained the activity of these factors that could account for the synthesis of digestive enzymes during fasting.

In the same conditions (18 h fasting), mRNA levels of the studied housekeeping genes and digestive enzymes did not change, compared with the control-fed group. These results clearly indicate that the regulation of digestive enzyme synthesis in fasting has to be mainly related to changes in the translation of mRNAs into protein. Along these lines, the
increase of total protein synthesis seen after refeeding for 1 and 2 h, compared with the fasted group, without an increase of the digestive enzymes mRNA levels, indicates that the stimulation of pancreatic protein synthesis after food intake is also regulated at the translational level.

Although the results from early studies were conflicting (8), those from Black and Webster (3) and Morisset and Webster (29) indicated that translational control was involved in the regulation of pancreatic protein synthesis by feeding. Only now, however, a clear involvement of specific translation factors has been described. In mouse pancreas, protein synthesis levels were enhanced after 2 h of refeeding and correlated with activation of the Akt/mTOR pathway, as shown by the increase in Akt activity and phosphorylation, the phosphorylation levels of two downstream effectors of mTOR, 4E-BP1 and the ribosomal proteins S6, and in the formation of the eIF4F complex. The activation of the Akt/mTOR pathway will lead to an increased phosphorylation of S6 and 4E-BP1 and formation of the eIF4F complex that binds the capped mRNA to start translation (13) similar to what has been described in muscle and liver (18). The large phosphorylation of the S6 ribosomal protein in this study indicates that the synthesis of more translational machinery and ribosomal proteins (27) is activated by refeeding. It is known that the phosphorylation of S6 can be regulated by CCK (4, 5, 36), insulin (42), and the branched-chain amino acid leucine (37) in rat pancreas as has been shown in other organs (2, 23, 49). Cholinergic stimulation and insulin have been demonstrated to activate protein synthesis in pancreas (21, 28, 48) and the translational machinery in other organs (15, 32, 48) as well. Thus the stimuli involved in the food intake process that increases vagal neural activity, plasma CCK levels and protein and amino acids from the food itself (41), could each contribute to the stimulation of pancreatic protein synthesis (14, 22) and the activation of translational machinery in the exocrine pancreas after a meal. The lack of effect on pancreatic eIF2B activity by the feeding status may be explained by the already high basal activity of eIF2B in pancreas (35) that was not altered by fasting and/or feeding.

In conclusion, normal feeding in ICR mice stimulates pancreatic protein synthesis by stimulating the translational machinery through the Akt/mTOR pathway, without changes at the mRNA level. Because food intake triggers several gastrointestinal stimuli that are known to both stimulate pancreatic exocrine secretion and affect the translational machinery, it is very likely that all of them, at least to some extent, play a role in the stimulation of pancreatic protein synthesis in this situation. More studies are necessary to elucidate the contribution of each of these factors in the integrated regulation of pancreatic protein synthesis that occurs in response to feeding.

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