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Adaptive changes in translation initiation activities for rat pancreatic protein synthesis with feeding of a high-protein diet

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Abstract

We have previously demonstrated that dietary protein induced pancreatic hypergrowth in pancreaticobiliary diverted (PBD) rats. Dietary protein and dietary amino acids stimulate protein synthesis by regulating translation initiation in the rat skeletal muscle and liver. The aim of the present study was to determine whether feeding a high-protein diet induces activation of translation initiation for protein synthesis in the rat pancreas. In PBD rats in which the bile–pancreatic juice was surgically diverted to the upper ileum for 11–13 days, pancreatic dry weight and protein content were doubled compared with those in sham rats and further increased with feeding of a high-protein diet (60% casein diet) for 2 days. These pancreatic growth parameters were maintained at high levels for the next 5 days and were much higher than those of sham rats fed a high-protein diet. In both sham and PBD rats, feeding of a high-protein diet for 2 days induced phosphorylation of eukaryotic initiation factor 4E-binding protein 1 and 70-kDa ribosomal protein S6 kinase, indicating the activation of the initiation phase of translation for pancreatic protein synthesis. However, this increased phosphorylation returned to normal levels on Day 7 in PBD but not in sham rats. We concluded that feeding a high-protein diet induced pancreatic growth with increases in the translation initiation activities for pancreatic protein synthesis within 2 days and that prolonged feeding of a high-protein diet changed the initiation activities differently in sham and PBD rats.

Keywords: Dietary protein; Pancreatic growth; Bile–pancreatic juice diversion; Rats; Eukaryotic initiation factor 4E-binding protein 1; 70-kDa ribosomal protein S6 kinase

1. Introduction

Dietary protein enhances pancreatic secretion and consumption of a high-protein diet stimulates pancreatic growth and induces pancreatic protease [1,2]. Proteins and amino acids in food may signal information to the mucosal surface of the small intestine and from there are transmitted to the pancreas through hormonal and nervous systems. One factor involved in pancreatic adaptation is luminal protease activity derived from the bile–pancreatic juice (BPJ). Decreasing protease activity in the proximal small intestinal lumen increases pancreatic secretion and pancreatic protease synthesis through the negative feedback mechanism [3,4]. The masking of luminal protease activities by ingested proteins is responsible for the adaptive growth of the pancreas after consumption of dietary protein [5].

The regulatory action of dietary protein occurs in concert with endogenous factors, especially pancreatic proteases and an endogenous cholecystokinin (CCK)-releasing peptide (monitor peptide) [6] in BPJ; however, it is difficult to examine the direct action of the dietary protein itself on the regulation of the pancreatic adaptation in normal rats. Rats with chronic BPJ diversion from the proximal small intestine [pancreaticobiliary diverted (PBD) rats] are suitable models for examining the effects of dietary protein independent of protease activities in BPJ.

We have previously shown that feeding of a high-protein diet for 3 days [7] and for 7 days [8] induced pancreatic growth and protease production in PBD rats and that the induced levels were much higher than those in normal (sham) rats. Dietary protein or dietary amino acids, especially leucine, stimulate protein synthesis by regulating the initiation phase of translation in the rat skeletal muscle and liver [9,10]. Although the translation initiation process is composed of numerous steps, a principal stage in the

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regulation of translation initiation involves the binding of mRNA to 40S ribosome [11]. This step requires a multisubunit complex referred to as eukaryotic initiation factor (eIF) 4F. The formation of the eIF4F complex is prevented by eIF4E-binding protein 1 (4E-BP1), which sequesters the mRNA cap-binding protein, eIF4E, into an inactive complex under hypophosphorylation conditions. The phosphorylation of 4E-BP1 promotes the assembly of the eIF4F complex. The phosphorylation of the 70-kDa ribosomal protein S6 kinase (S6K1) is associated with its kinase activity [12,13], and the increased activity is implicated in stimulating the initiation phase of the translation [14].

The signaling by growth factors and amino acids converges at the mammalian target of rapamycin (mTOR) and acts to control the phosphorylation status of 4E-BP1 and S6K1 [15]. However, it is unclear how dietary protein influences pancreatic growth and how stimulation of dietary proteins influences translation initiation activities for pancreatic protein synthesis in rats.

The aim of the present study was to determine whether feeding of a high-protein diet induces activation of the translation initiation for rat pancreatic protein synthesis regulated via the mTOR pathway. We examined changes in the pancreas after feeding diets with normal and high levels of casein for 2 or 7 days in PBD rats compared with those in sham rats.

2. Methods and materials

2.1. Animals and diets

Male Sprague–Dawley rats (Japan SLC, Hamamatsu, Japan) weighing approximately 200 g were fed a semipurified sucrose–casein-based diet for 4–6 days for acclimation. The acclimated rats were divided into two groups. Rats in one group underwent surgery to establish chronic pancreaticobiliary diversion [16]. After a 24-h fast, surgery was performed to divert the BPJ to the ileum by transposition of a duodenal segment including the ampulla of Vater under anesthesia by intraperitoneal injection of pentobarbital sodium (40 mg/kg body wt, Abbott Laboratories, North Chicago, IL, USA). Briefly, a 2- to 3-cm segment of the duodenum containing the ampulla of Vater was cut off after ligation of the proximal end of the segment. End-to-side anastomosis was carried out between the cut edge of the anal side of the segment and the lateral opening on the upper ileum (45 cm distal to the ligament of Treitz), and both cut edges of the duodenum were end-to-end anastomosed (PBD rats). The rats in the other group were subjected to a sham operation; that is, a 1-cm segment distal to the ampulla of Vater was transected and end-to-end anastomosed (sham rats).

After the operation, the rats were fed a semipurified, sucrose-based, fat-free diet containing a 250 g casein/kg diet [25% casein diet (NC diet)] as described previously [7] for a recovery period of 11–13 days. After recovery from operative damage, sham and PBD rats were divided into four subgroups each. The rats of one subgroup were immediately killed (Day 0). The rats of another subgroup were fed the NC diet for 7 days, and the rats of the other two subgroups were fed a 60% casein diet (HC diet), in which sucrose of the NC diet was replaced by casein up to 600 g casein/kg diet, for 2 or 7 days to evaluate time-dependent pancreatic adaptation to the high-protein diet. We used fat-free diets because dietary fat also stimulates CCK secretion. Rats in all groups had free access to the assigned diet and water. The experiments were performed in a room controlled at 23±2°C, with a 12-h light/12-h dark cycle (8:00 AM–8:00 PM light period).

Rats of each group were killed under pentobarbital anesthesia. Immediately, a segment of pancreatic tissue (approximately 100 mg) was removed from the dorsal area for quantification of 4E-BP1 and S6K1 as described below. The residual pancreatic tissue was removed and frozen in liquid nitrogen for measurement of protein content.

The study was approved by the Hokkaido University Animal Committee, and the animals were maintained in accordance to the guidelines for the care and use of laboratory animals of the Hokkaido University.

2.2. Analysis

A segment of the pancreatic tissue was homogenized in Buffer A [10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid at pH 7.4, 150 mM NaCl, 1 mM EDTA, 5 mM ethylene glycol-bis(α-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, 100 mM NaF, 50 mM α-glycerophosphate, 2 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine and 1 mM sodium vanadate 1% Triton X-100, 10 mM MgCl2, 10 mM KH2PO4, 10 μg/ml aprotinin, 10 μg/ml leupeptin] using a Polytron homogenizer. The homogenate was centrifuged at 10,000×*g* for 10 min at 4°C.

For quantification of 4E-BP1, an aliquot of the 10,000×*g* supernatant was boiled for 10 min then centrifuged at 10,000×*g* for 30 min at 4°C. The resulting supernatant was mixed with an equal volume of buffered 2×SDS, and the diluted sample was electrophoresed on 15% polyacrylamide gel and subjected to protein immunoblot analysis by using rabbit anti-4E-BP1 polyclonal antibodies (Santa Cruz Biotechnology, CA, USA) [17]. For quantification of S6K1, an aliquot of the 10,000×*g* supernatant was combined with an equal volume of buffered 2×SDS, electrophoresed on 7.5% polyacrylamide gel and subjected to a protein immunoblot analysis using rabbit S6K1 polyclonal antibodies (Santa Cruz Biotechnology) [18]. Protein content was measured in the freeze-dried residual pancreas by a modified version of Lowry's method [19,20].

2.3. Calculations

Protein content in the pancreas was expressed as milligrams in the whole pancreas (the sum of the wet weight of the removed pancreas segment used plus that of the residual pancreas was equal to the weight of the whole pancreas).

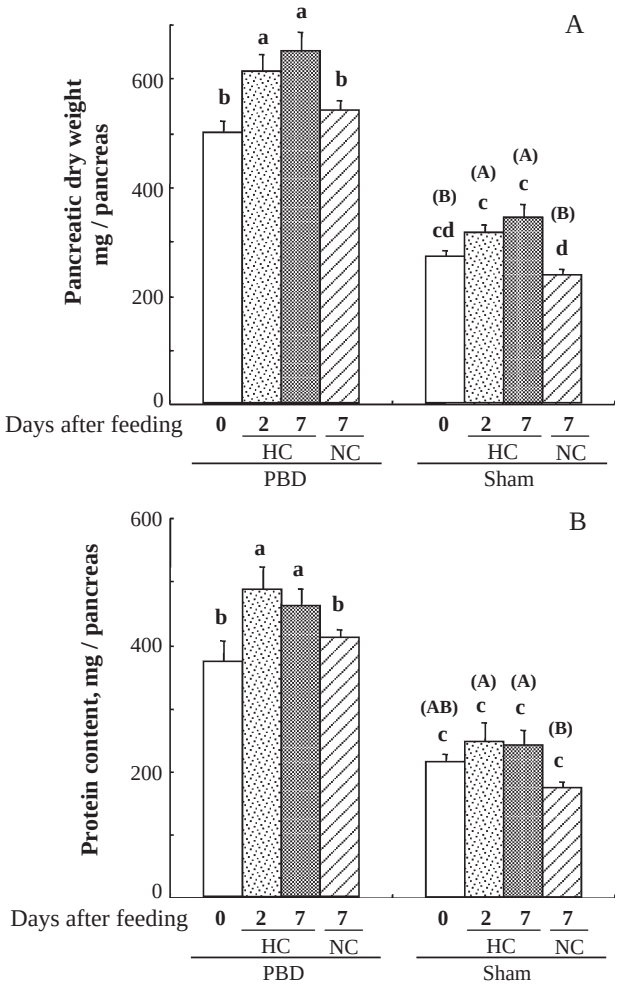

Fig. 1. Changes in pancreatic dry weight (A) and protein content (B) 2 and 7 days after feeding an NC diet or an HC diet in sham and PBD rats. Results are means±S.E.M. ($n=6–9$). The P value in one-way ANOVA for all the groups was <.001 for dry weight and protein content. The P values for the four diet groups on Day 7 evaluated by two-way ANOVA were <.001, .005 and .661 for PBD, diet and PBD × Diet, respectively, for dry weight and <.001, <.001 and .876, respectively, for protein content. [a,b,c,d]Values not sharing a common letter are significantly different among all the groups ($P<.05$). [A,B]Values not sharing a common letter are significantly different within the four groups of sham rats ($P<.05$).

4E-BP1 protein content was calculated as the total density by protein immunoblot analysis per amount of applied proteins. The data were analyzed by one-way analysis of variance (ANOVA) in all the groups (eight groups) or in four groups of sham rats. Also, data on the four diet groups

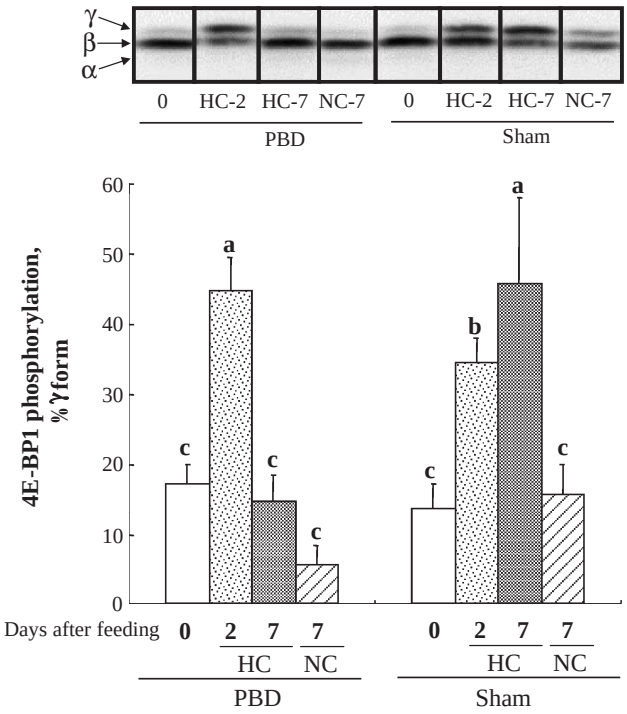

Fig. 2. Changes in the phosphorylation of pancreatic 4E-BP1 2 and 7 days after feeding an NC or an HC diet in sham and PBD rats. Values show degrees of phosphorylation in 4E-BP1 expressed as a percentage of the γ-phosphorylated form to the total 4E-BP1. Each value is the mean±S.E.M. for six to nine rats. The P value evaluated by one-way ANOVA for all the groups was <.001 and the P values evaluated by two-way ANOVA within the four diet groups on Day 7 were .003, .004 and .104 for PBD, diet and PBD × Diet, respectively. [a,b,c]Values not sharing a common letter are significantly different among all the groups ($P<.05$).

on Day 7 were analyzed by two-way ANOVA. The significance of the differences among all eight groups or among the four groups of sham rats (only Fig. 1) was determined by Duncan's multiple-range test ($P<.05$; SAS version 6.07, SAS Institute, Cary, NC, USA).

3. Results

Body weight gain and food intakes are summarized in Table 1. Food intake was lower in the HC group than in the NC group on Day 7 in PBD rats. From the results of two-way ANOVA on Day 7, food intake and body weight gain were influenced by diet but not by PBD.

Pancreatic dry weight and protein content were much higher in the PBD rats than in the sham rats on Day 0

Table1
Food intake and body weight gain 2 and 7 days after the start of feeding the test diets

Groups	Sham-HC	PBD-HC	Sham-HC	PBD-HC	Sham-NC	PBD-NC
	Day 2		Day 7			
Food intake (g/day)	20.4±1.2	18.7±0.6	19.5±1.1^{b,c}	18.7±0.9^{c}	21.8±1.3^{a,b}	22.8±0.5^{a}
Body weight gain (g/day)	4.8±0.8	3.9±0.8	5.1±0.3^{b}	5.5±0.3^{a,b}	5.8±0.4^{a,b}	6.4±0.3^{a}

Values are means±S.E.M. ($n=6–9$). P values in one-way ANOVA for all the groups were .013 for food intake and .032 for body weight gain. P values evaluated by two-way ANOVA within the four diet groups on Day 7 were .926, .003 and .363 for PBD, diet and PBD × Diet, respectively, for food intake and .143, .020 and .750, respectively, for body weight gain. Values not sharing a common superscript letter are significantly different within the same day ($P<.05$).

(Fig. 1). Feeding of the HC diet in PBD rats further increased both pancreatic growth parameters on Day 2. The maximal value in pancreatic protein content was on Day 2. The induced levels of the pancreatic growth parameters by feeding of the HC diet were maintained over the next 5 days in PBD rats. On Day 7, pancreatic dry weight was higher in the HC groups than in the NC groups in PBD and sham rats, respectively. Protein content in PBD rats was also higher in the HC group than in the NC group. By statistical analysis within sham rats, protein content was higher in the HC group than in the NC group on Day 7.

The phosphorylation state of 4E-BP1 can conveniently be examined by resolution of the phosphorylated forms of the protein during SDS–polyacrylamide gel electrophoresis; that is, 4E-BP1 is resolved into multiple electrophoretic forms, termed α, β and γ, representing differentially phosphorylated forms of the protein (Fig. 2). The most highly phosphorylated form, the γ form, exhibits the slowest electrophoretic mobility and is the only form not bound to eIF4E. The percentage of the γ form to the sum of the three form intensities showing initiation activity was similarly increased by feeding of the HC diet for 2 days in both sham and PBD rats. The increased phosphorylation of 4E-BP1 returned to normal levels on Day 7 in PBD rats but not in sham rats. The percentage of the γ form was further increased with prolonged feeding of the HC diet (Day 7) in sham rats. Prolonged feeding of the NC diet did not increase the 4E-BP1 phosphorylation in either the sham rats or the PBD rats. Total 4E-BP1 protein content (sum of α, β and γ form densities per applied protein) did not change with time or diets (data not shown, $P=.498$, $n=60$).

S6K1 is typically resolved into multiple electrophoretic forms after separation by electrophoresis on SDS–polyacrylamide gel with increased phosphorylation being associated with decreased electrophoretic mobility (Fig. 3), termed α, β and γ [21]. We quantified the ratio of the more heavily phosphorylated (more slowly migrating) forms to the total immune reactivity because phosphorylated β and γ forms possess kinase activity. Phosphorylation of S6K1 was higher in the HC group than in the NC group on Day 2 in PBD rats, and the increased phosphorylation was returned to the basal value on Day 7. The percentages of phosphorylated S6K1 in sham rats tended to be changed in a similar manner to those in PBD rats without significant differences. Phosphorylation of S6K1 in NC group did not increase in either the sham rats or the PBD rats.

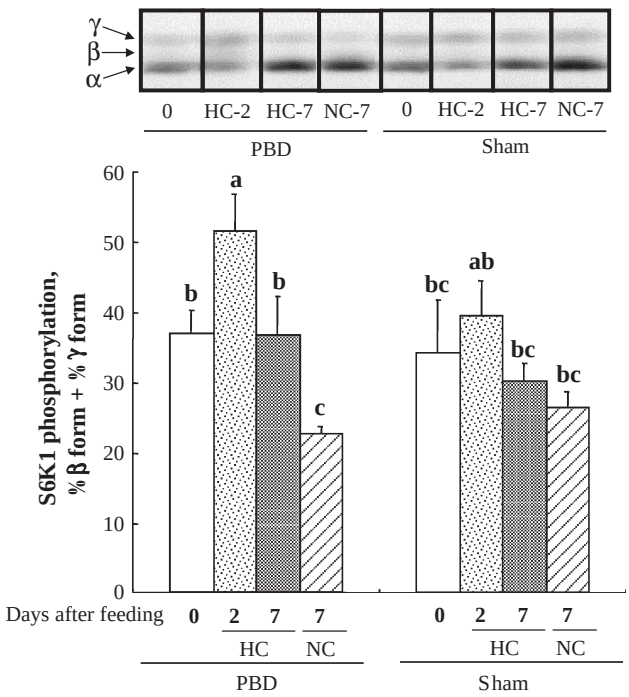

Fig. 3. Changes in the phosphorylation of S6K1 in the pancreas 2 and 7 days after feeding an NC diet or an HC diet in sham and PBD rats. S6K1 is resolved into multiple electrophoretic forms on SDS–polyacrylamide gels. Values display percentages of S6K1 in the β and γ forms to the total S6K1. Each value is the mean±S.E.M. for six to nine rats. The P value evaluated by one-way ANOVA for all the groups was <.001 and the P values evaluated by two-way ANOVA within the four diet groups on Day 7 were .397, .016 and .895 for PBD, diet and PBD × Diet, respectively. [a,b,c]Values not sharing a common letter are significantly different among all the groups ($P<.05$).

4. Discussion

We have previously shown that pancreatic growth and pancreatic protease production were induced by dietary protein independent of luminal BPJ by using PBD rats [7,8]. In this study, we examined changes in the translation initiation activities in chronic PBD rats 2 and 7 days after a change in diet from a normal to a high level of protein. The pancreatic dry weight and protein content were increased by feeding of a high-protein diet for 2 days, and the increments were much larger than those in sham rats on Day 7. These results demonstrate that dietary protein induced pancreatic growth independent of BPJ, which is consistent with the results of our previous studies. CCK [22,23] and dietary protein [9] are known to stimulate protein synthesis in the pancreas. The process of protein synthesis is regulated through several steps including translation initiation. The present study showed that rapid pancreatic growth was associated with phosphorylation of 4E-BP1 and S6K1, which indicates activation of translation initiation of protein synthesis through the mTOR pathway. Regulatory factors of the translation initiation downstream of the mTOR pathway are known to be activated by CCK in the rat pancreas [24]. Recently, it has been reported that the secretion level of CCK, a potent stimulator of the exocrine pancreas, is increased by dietary protein in a manner independent of BPJ [25–28], and our previous study demonstrated that pancreatic growth in PBD rats is dependent on CCK [8]. These previous and present results suggest that CCK is involved in the activation of mTOR-dependent translation initiation by dietary protein both in sham and PBD rats. Direct stimulation with peptides derived from dietary protein on CCK-producing cells and increasing endogenous luminal

CCK-releasing peptides other than the monitor peptide [29,30] are proposed mechanisms to promote CCK secretion in PBD rats. Bioactive peptides such as β-casomorphins are derived from casein [31]. Also, we found CCK-releasing active peptides, β 51–63, derived from soybean β-conglycinin [32]. Some peptides derived from casein may be involved in the promotion of CCK release.

It is well known that PBD induces CCK hypersecretion, and we previously showed that protein feeding further increased blood CCK levels in PBD rats [7]. CCK receptors exist in at least two binding states, high- and low-affinity states [33–35]. CCK stimulates acinar cell growth through both high- and low-affinity receptors [33], and each binding state appears to have a different set of second messengers and different biologic effects [35]. CCK increased by dietary protein in PBD rats possibly induced pancreatic growth through a low-affinity receptor. However, the high-protein diet induced pancreatic growth even in the CCK-deficient mice compared with that in rats fed the high-fat and high-carbohydrate diet [36]. Moreover, we previously showed that a high-amino acid diet also induced pancreatic growth independent of CCK [37]. These previous findings indicate the possibility that the increases in translation initiation activity by dietary protein in PBD rats do not depend on CCK. Further studies are needed to clarify this hypothesis.

There were differences in changes in the translation initiation activity between PBD and sham rats to adapt to the prolonged feeding of a high-protein diet; that is, the large increase in phosphorylation of 4E-BP1 caused by the high-protein diet on Day 2 returned to the basal level in PBD rats on Day 7 but remained at an elevated level in sham rats. In PBD rats fed a high-protein diet, pancreatic protein content was maintained at a high level although the activation level of the initiation returned to the basal level on Day 7. We observed that there were no changes in the total 4E-BP1 level and that phosphorylation of S6K1 similarly changed after feeding a high-protein diet in PBD rats. These findings show that, in PBD rats but not in sham rats, a translation initiation process was inactivated by prolonged feeding of a high-protein diet. Our previous study showed that pancreatic protein synthesis remained at a high level in PBD rats fed an HC diet for 2 weeks [38]. These results suggest that another pathway to the promotion of pancreatic protein synthesis is activated by prolonged feeding of a high-protein diet in PBD rats.

In conclusion, feeding PBD rats with a high-protein diet for 2 days induced pancreatic growth that was associated with the activation of mTOR-dependent translation initiation activities. However, activation of a different pathway for protein synthesis was suggested with prolonged feeding of a high-protein diet.

References

[1] Grossma MI, Greengard H, Ivy AC. The effect of dietary composition of pancreatic enzymes. Am J Physiol 1943;138:676–82.

[2] Schick J, Verspohl R, Kern H, Scheele G. Two distinct adaptive responses in the synthesis of exocrine pancreatic enzymes to inverse changes in protein and carbohydrate in the diet. Am J Physiol 1984; 247:G611–616.

[3] Green GM, Olds BA, Matthews G, Lyman RL. Protein, as a regulator of pancreatic enzyme secretion in the rat. Proc Soc Exp Biol Med 1973;142:1162–7.

[4] Schneeman BO, Lyman RL. Factors involved in the intestinal feedback regulation of pancreatic enzyme secretion in the rat. Proc Soc Exp Biol Med 1975;148:897–903.

[5] Morisset J, Guan D, Jurkowska G, Rivard N, Green GM. Endogenous cholecystokinin, the major factor responsible for dietary protein-induced pancreatic growth. Pancreas 1992;7:522–9.

[6] Iwai K, Fukuoka S, Fushiki T, Tsujikawa M, Hirose M, Tsunasawa S, et al. Purification and sequencing of a trypsin-sensitive cholecystokinin-releasing peptide from rat pancreatic juice. J Biol Chem 1987;262:8956–9.

[7] Hara H, Ochi Y, Kasai T. Bile–pancreatic juice-independent increases in pancreatic proteases and intestinal cholecystokinin by dietary protein in rats. Proc Soc Exp Biol Med 1998;217:173–9.

[8] Hara H, Ohyama S, Hira T. Luminal dietary protein, not amino acids, induces pancreatic protease via CCK in pancreaticobiliary-diverted rats. Am J Physiol 2000;278:G937–945.

[9] Yoshizawa F, Kimball SR, Vary TC, Jefferson LS. Effect of dietary protein on translation initiation in rat skeletal muscle and liver. Am J Physiol 1998;275:E814–820.

[10] Yoshizawa F, Sekizawa H, Hirayama S, Hatakeyama A, Nagasawa T, Sugahara K. Time course of leucine-induced 4E-BP1 and S6K1 phosphorylation in the liver and skeletal muscle of rats. J Nutr Sci Vitaminol 2001;47:311–5.

[11] Pain VM. Initiation of protein synthesis in eukaryotic cells. Eur J Biochem 1996;236:747–71.

[12] Cheatham B, Vlahos CJ, Cheatham L, Wang L, Blenis J, Kahn CR. Phosphatidylinositol 3-kinase activation is required for insulin stimulation of pp70 S6 kinase, DNA synthesis, and glucose transporter translocation. Mol Cell Biol 1994;14:4902–11.

[13] Chung J, Grammer TC, Lemon KP, Kazlauskas A, Blenis J. PDGF- and insulin-dependent pp70S6 activation mediated by phosphatidylinositol-3-OH kinase. Nature 1994;370:71–5.

[14] Sonenberg N. mRNA 5′ cap-binding protein eIF4E and control of cell growth. In: Hershey JWB, Mathews MB, Sonenberg N, editors. Translational control. New York: Cold Spring Harbor Laboratory Press/Plainview; 1996. p. 245–89.

[15] Gingras AC, Raught B, Sonenberg N. Regulation of translation initiation by FRAP/mTOR. Genes Dev 2001;15:807–26.

[16] Newman BM, Lee PC, Tajiri H, Cooney DR, Lebenthal E. Pancreaticobiliary factors in the modulation of small intestinal enterokinase in the rat. Am J Physiol 1986;250:G103–108.

[17] Kimball S.R, Jurasinski CV, Lawrence Jr JC, Jefferson LS. Insulin stimulates protein synthesis in skeletal muscle by enhancing the association of eIF-4E and eIF-4G. Am J Physiol 1997;272:C754–759.

[18] Kimball SR, Horetsky RL, Jefferson LS. Signal transduction pathways involved in the regulation of protein synthesis by insulin in L6 myoblasts. Am J Physiol 1998;274:C221–228.

[19] Lowry OH, Rosebrough HJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193: 265–75.

[20] Sugawara K. Influence of Triton X-100 on protein determination by Lowry procedure. Agric Biol Chem 1975;93:2429–30.

[21] Weng QP, Andrabi K, Kozlowski MT, Grove JR, Avruch J. Multiple independent inputs are required for activation of the p70 S6 kinase. Mol Cell Biol 1995;15:2333–40.

[22] Korc M. Regulation of pancreatic protein synthesis by cholecystokinin and calcium. Am J Physiol 1982;243:G69–75.

[23] Korc M, Bailey AC, Williams JA. Regulation of protein synthesis in normal and diabetic rat pancreas by cholecystokinin. Am J Physiol 1981;241:G116–121.

[24] Bragado MJ, Tashiro M, Williams JA. Regulation of the initiation of pancreatic digestive enzyme protein synthesis by cholecystokinin in rat pancreas in vivo. Gastroenterology 2000;119: 1731–9.

[25] Beucher S, Levenez F, Mireille Y, Corring T. Effects of gastric digestive products from casein on CCK release by intestinal cells in rat. J Nutr Biochem 1994;5:578–84.

[26] Cordier-Bussat M, Bernard C, Haouche S, Roche C, Abello J, Chayvialle JA, et al. Peptones stimulate cholecystokinin secretion and gene transcription in the intestinal cell line STC-1. Endocrinology 1997;138:1137–44.

[27] Nishi T, Hara H, Kasai T. Guanidinated casein hydrolysate stimulates pancreatic secretagogue release by direct action to the intestine in rats. Proc Soc Exp Biol Med 1998;218:357–64.

[28] Nishi T, Hara H, Aoyama Y. Guanidinated casein hydrolysate stimulation of cholecystokinin release via pancreatic enzyme- and cholinergic-independent mechanisms in rats. Biosci Biotechnol Biochem 1999;63:1070–4.

[29] Spannagel AW, Green GM, Guan D, Liddle RA, Faull K, Reeve Jr JR. Purification and characterization of a luminal cholecystokinin-releasing factor from rat intestinal secretion. Proc Natl Acad Sci USA 1996;93:4415–20.

[30] Herzig KH, Schön I, Tatemoto K, Ohe Y, Li Y, Fölsch UR, et al. Diazepam binding inhibitor is a potent cholecystokinin-releasing peptide in the intestine. Proc Natl Acad Sci USA 1996;93:7927–32.

[31] Henschen A, Lottspeich F, Brantl V, Teschemacher H. Novel opioid peptides derived from casein (beta-casomorphins): II. Structure of active components from bovine casein peptone. Hoppe Seyler Z Physiol Chem 1979;360:1217–24.

[32] Nishi T, Hara H, Asano K, Tomita F. The soybean β-conglycinin β 51–63 fragment suppresses appetite by stimulating cholecystokinin release in rats. J Nutr 2003;133:2537–42.

[33] Hoshi H, Logsdon CD. Both low- and high-affinity CCK receptor states mediate trophic effects on rat pancreatic acinar cells. Am J Physiol 1993;265:G1177–1181.

[34] Galas MC, Lignon MF, Rodriguez M, Mendre C, Fulcrand P, Laur J, et al. Structure–activity relationship studies on cholecystokinin: analogues with partial agonist activity. Am J Physiol 1988;254: G176–182.

[35] Matozaki T, Goke B, Tsunoda Y, Rodriguez M, Martinez J, Williams JA. Two functionally distinct cholecystokinin receptors show different modes of action on Ca^{2+} mobilization and phospholipid hydrolysis in isolated rat pancreatic acini. Studies using a new cholecystokinin analog, JMV-180. J Biol Chem 1990;265:6247–54.

[36] Lacourse KA, Swanberg LJ, Gillespie PJ, Rehfeld JF, Saunders TL, Samuelson LC. Pancreatic function in CCK-deficient mice: adaptation to dietary protein does not require CCK. Am J Physiol 1999;276: G1302–1309.

[37] Hara H, Narakino H, Kiriyama S. Induction of pancreatic growth and proteases by feeding a high amino acid diet does not depend on cholecystokinin in rats. J Nutr 1995;125:1143–9.

[38] Hara H, Shiota H. Differential increases in syntheses of newly identified trypsinogen2 isoforms by dietary protein in rat pancreas. Exp Biol Med 2004;229:772–80.