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MOLECULAR MECHANISMS OF PANCREATIC DYSFUNCTION INDUCED BY PROTEIN MALNUTRITION

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Abstract

Background & Aims: Dietary protein deficiency results in diminished capacity of the pancreas to secrete enzymes needed for macronutrient digestion. Previous work has suggested that modulation of the mTOR pathway by the hormone cholecystokinin (CCK) plays an important role in normal digestive enzyme synthesis after feeding. The purpose of this study was to elucidate the role of mTOR in protein deficiency-induced pancreatic dysfunction.

Methods: Wild-type and CCK-null mice were fed protein-deficient chow for four days and then allowed to recover on control chow in the presence or absence of the mTOR inhibitor rapamycin. The size and secretory capacity of the pancreas rapidly decreased after feeding protein-deficient chow. Refeeding protein-replete chow reversed these changes in both wild-type and CCK-null mice. Changes in the size of the pancreas were paralleled by changes in the content and secretion of digestive enzymes, as well as the phosphorylation of downstream targets of mTOR. Administration of the mTOR inhibitor rapamycin decreased regrowth of the pancreas, but did not affect digestive enzyme content or secretory capacity.

Conclusions: These studies demonstrate that dietary protein modulates pancreatic growth, but not digestive enzyme synthesis, via CCK-independent activation of the mTOR pathway.

Background & Aims

The exocrine pancreas synthesizes and secretes the majority of the enzymes responsible for macronutrient digestion. The effects of dietary protein on pancreatic function in experimental animals date from the end of the nineteenth century when Pavlov noted that diet influences pancreatic secretion¹. The first published report on the long-term effects of protein deficiency in humans described a syndrome, referred to as kwashiorkor, associated with vomiting and the

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presence of large amounts of undigested food in the stool of individuals having adequate caloric intake, but who consumed a protein-deficient diet². Additional studies suggested that kwashiorkor may result from pancreatic dysfunction³ and studies employing electron microscopy demonstrated alterations in the size of the acinar cells during kwashiorkor, leading to the hypothesis that the exocrine pancreas is incapable of synthesizing secretory proteins in the absence of dietary protein⁴.

Consumption of large amounts of protein induces growth of the adult rodent pancreas^{5, 6}. Dietary protein stimulates the release of gastrointestinal hormones and the growth promoting effects of dietary protein on the exocrine pancreas have been attributed to these hormones, particularly cholecystokinin (CCK)⁷. Administration of exogenous CCK induces acinar cell hyperplasia in mice⁸. Similarly, feeding a trypsin inhibitor (TI), which increases endogenous CCK release^{9, 10}, is associated with cellular hyperplasia in rats and mice^{11, 12}. TI-induced acinar cell hyperplasia is absent in CCK¹² and CCK-A receptor deficient mice^{13, 14}. In contrast, pancreatic hypertrophy occurs during high protein feeding in rats administered a CCK-antagonist¹⁵. Similarly, a high protein diet induces pancreatic hypertrophy in CCK-deficient mice¹⁶. Recent studies from our laboratory have demonstrated that activation of both the protein phosphatase calcineurin and the protein kinase mTOR are required for acinar cell hyperplasia in response to TI feeding^{12, 17}. It is unknown however, whether activation of these same signaling pathways is required for acinar cell hypertrophy in response to dietary protein independent of CCK.

Despite the continued prevalence of protein malnutrition throughout the world, very little is known regarding molecular mechanisms whereby the loss of dietary protein results in pancreatic dysfunction and its return facilitates recovery. The purpose of this study was to 1) establish a mouse model of protein deficiency and determine changes in pancreatic morphology and function, 2) determine whether activation of the mTOR pathway is necessary for pancreatic growth following protein deficiency, and 3) determine whether activation of the mTOR pathway and/or pancreatic growth following protein deficiency is CCK dependent. These findings will be important in developing novel means of promoting recovery from protein malnutrition.

Methods

Animal care

Male ICR, C57BL/6, and homozygous CCK-null mice on C57BL/6 background age 6-9 weeks were maintained on a 12-hour light:dark cycle with free access to water and chow (Dyets Inc.; Bethlehem, PA) prior to experimentation. Animals were acclimated to control (AIN-93G; 3760 kcal/kg, 200 g/kg casein, 100 g/kg sucrose, 397 g/kg cornstarch, 70 g/kg soybean oil) chow for 3 days prior to experimental manipulation. Animals were divided into groups provided with AIN-93G chow or modified AIN-93G chow that was isocaloric but devoid of protein (3767 kcal/kg, 100 g/kg sucrose, 548 g/kg cornstarch, 70 g/kg soybean oil). After 4 days on the protein-deficient chow, animals were provided AIN-93G chow for up to 8 days. In separate experiments, mice received an intraperitoneal injection of 2% carboxymethylcellulose (CMC) in 10% ethanol (1 ml/100 g body weight), rapamycin (LC Laboratories; Woburn, MA; 0.2 mg/100 g body weight) suspended in 2% CMC, or FK506 (0.3 mg/100g body weight) in 10% ethanol 1 hour prior to being returned to control diet and then either daily injections of rapamycin or twice daily injections of FK506 for 4 days prior to tissue collection. The University of Michigan Committee on Use and Care of Animals approved the animal facilities and the experimental protocol used in these studies.

Assessment of pancreatic growth and morphology

Total pancreatic protein and DNA content were determined as described previously¹⁷. Blocks of pancreas were fixed for 2 h with a mixture of 2% glutaraldehyde and 2% formaldehyde in PBS, postfixed for 45 min with 1% OsO₄, and then dehydrated and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and images were recorded digitally using a Philips CM-100 electron microscope.

Pancreatic secretion

To measure pancreatic secretion *in vivo*, mice were anesthetized with isoflurane and a PE-10 catheter was inserted into the common bile-pancreatic duct using a dissecting microscope and glued in place. The mice were maintained under anesthesia and placed under a warming lamp. After a 15 min stabilization period, pancreo-biliary juice was collected for 15 min in a microcentrifuge tube containing 100 μ l of protease inhibitor solution. Pancreatic secretion was stimulated by infusion of the muscarinic cholinergic receptor agonist bethanechol (Carbamyl- β -methylcholine; Sigma, St. Louis, MO; 1.2 mg/kg over 10 min) through an intraperitoneal catheter and juice was collected for 15 min. The volume of the collected material was measured and the protein concentration determined. Previous studies have shown that muscarinic cholinergic receptor agonists do not stimulate bile protein output¹⁸. Cannulation of the pancreatic duct was confirmed at the end of the collection period by retrograde infusion of a methylene blue dye solution and subsequent coloration of the pancreas. In addition, equal volume samples of collected material were subjected to SDS-PAGE and the protein composition was visualized with Coomassie Blue stain.

Immunoblot analysis

Sample preparation and visualization was performed exactly as described previously¹⁷. β -actin or GAPDH were used as loading controls. 4E-BP1 antibody was obtained from Calbiochem (San Diego, CA), S6, GAPDH, and beclin 1 from Santa Cruz, Hsp70 from Stressgen (Ann Arbor, MI), LC3 from Novus Biologicals (Littleton, CO), amylase and β -actin from Sigma, chymotrypsinogen from Cortex (San Leandro, CA), pyruvate dehydrogenase from Molecular Probes (Eugene, OR), and all other antibodies from Cell Signalling (Beverly, MA).

Statistical analysis

Data are expressed as mean $\pm$ SEM. Data were analysed using GraphPad Prism (GraphPad Software Inc., San Diego, CA). Statistical significance was assessed using a one-way ANOVA and a Dunnett's post-test unless stated otherwise. *P* values < 0.05 were considered significant.

Results

Feeding a protein-deficient diet results in reversible pancreatic regression

Mice demonstrated an aversion to protein-deficient chow during the 1st day of feeding, but food consumption normalized thereafter (data not shown). Feeding protein-deficient chow was not associated with changes in plasma glucose concentrations, but was associated with a small decrease in plasma insulin concentrations and profound decreases in plasma amino acid concentrations (Supporting Information (SI) Table 1). There was a statistically significant decrease in body weight (SI Fig. 1A), from 31.7 ± 0.4 g to 28.3 ± 0.3 g, in mice fed protein-deficient chow for four days. The effect of protein deficiency on individual organ weights was, as reported previously¹⁹, extremely variable. For example, the weight of the heart was unchanged from 5.2 ± 0.1 mg/g body weight to 5.3 ± 0.2 mg/g body weight after 4 days on protein-deficient chow, but the weight of the pancreas (Fig. 1A) decreased to 76% of control values after 4 days. The observed decrease in pancreatic weight was not associated with any loss of pancreatic DNA (SI Fig. 1B). Most strikingly, pancreatic protein (Fig. 1B) was

significantly decreased after 2 days of feeding protein-deficient chow and decreased to almost 40% of initial values after 4 days. Pancreatic atrophy was readily reversed when mice were refed standard chow diet. While pancreatic DNA (SI Fig. 1B) remained unchanged, the depressed values of both the weight (SI Fig. 1A) and protein content (Fig. 1B) of the pancreas returned to initial values after 4 days on standard chow.

Changes in dietary protein intake affect mTOR signaling in the pancreas

The protein kinase mTOR integrates diverse nutritional and mitogenic signals to regulate cell growth^{20, 21}. An assessment of the phosphorylation state of an upstream kinase of mTOR, Akt, as well as two downstream targets of mTOR, the ribosomal protein S6 (S6) and the eukaryotic initiation factor 4E binding protein 1 (4E-BP1), was therefore made to determine whether changes in dietary protein intake affected mTOR signalling in the pancreas. The phosphorylation of Akt at Ser⁴⁷³, an indicator of Akt kinase activity²², was not affected by protein deficiency or by subsequent restitution of normal diet (data not shown). In contrast, the phosphorylation of both S6 (Fig. 2A) and 4E-BP1 (Fig. 2B) was significantly decreased in the pancreas of mice fed protein-deficient chow for four days in comparison to controls. The phosphorylation of S6 returned to control levels after one day, and was greater than control values after four days, of refeeding control chow. 4E-BP1 phosphorylation was greater than control values after a single day of refeeding and returned to control levels after four days. Interestingly, the total amount of 4E-BP1 (Fig. 2C) increased by almost 40% after four days of feeding protein-deficient chow and returned to control levels within a day of refeeding control chow. This increase in 4E-BP1 content does not appear to be a generalized effect of protein deficiency on proteins that modulate mRNA translation, as the total amount of eukaryotic initiation factor 4G and elongation factor 2 was not altered in these experiments (data not shown).

Stimulation of mTOR is required for growth of the pancreas following protein deficiency-induced atrophy

Both the protein kinase mTOR and the protein phosphatase calcineurin are necessary for hyperplastic growth of the pancreas in response to elevated levels of CCK^{12, 17}. To determine whether activation of either of these proteins is required for regrowth of the pancreas following protein deficiency, mice were fed protein-deficient chow for 4 days and then refed control chow while being given daily injections of the mTOR inhibitor rapamycin or the calcineurin inhibitor FK506. Recovery of pancreatic weight (Fig. 3A) was unaffected by FK506 administration, but was almost completely inhibited by rapamycin. Neither FK506 nor rapamycin affected pancreatic DNA content (data not shown) during recovery from protein deficiency. In accordance with its lack of effect on pancreatic weight, FK506 had no effect on recovery of pancreatic protein content (Fig. 3B). Despite its aforementioned effect on recovery of pancreatic weight, rapamycin had no effect on the recovery of pancreatic protein content; indicating that acinar cell growth and acinar cell protein content are differentially affected by rapamycin. To confirm that activation of the mTOR pathway is required for growth of individual acinar cells within the pancreas following protein deficiency, cell size was evaluated by counting the number of DAPI-stained acinar cell nuclei per unit area of the pancreas (SI Fig. 2A and 2B). Significant cellular atrophy was demarcated by a 49% increase in the number of nuclei per unit area from mice fed protein-deficient chow compared to controls. When mice were refed control chow for four days following protein deficiency, cell size returned to normal, however administration of rapamycin during the period of control chow refeeding was associated with a persistent increase in the number of nuclei per unit area and thus continuing acinar cell atrophy.

Feeding a protein-deficient diet results in morphological changes of the pancreas that are independent of mTOR activation

Under normal conditions, acinar cells contain large quantities of digestive enzymes packaged into zymogen granules. Electron micrographs of the pancreas (Fig. 4 A-D) demonstrated a large decrease in the size and number of zymogen granules in acinar cells after four days of feeding protein-deficient chow (Fig. 4B). Vesicular structures, whose appearance suggested their origin as decondensing zymogen granules, were also apparent after feeding protein-deficient chow. The general ultrastructure of acinar cells however, appeared unaffected by protein deficiency. For example, the content and organization of the mitochondria and endoplasmic reticulum was normal (SI Fig. 3A and 3B). Moreover, the expression of biomarkers of generalized and endoplasmic reticulum stress was unaltered after four days on protein-deficient chow (SI Fig. 3C). The observation that rapamycin administration inhibited regrowth of the pancreas, but not recovery of pancreatic protein content, following protein deficiency suggests that activation of the mTOR pathway is not necessary for digestive enzyme synthesis in response to dietary protein. This hypothesis is supported by our finding that zymogen granule size and number was significantly increased after refeeding of control chow for four days in both the absence (Fig. 4C) and presence (Fig. 4D) of rapamycin.

In some models, inhibition of mTOR signaling is associated with the induction of autophagy. However, autophagic vacuoles were not observed in acinar cells during protein deficiency or following rapamycin administration (Fig. 4B and Fig. 4D). Moreover, expression of the autophagy-associated proteins LC3-II²³ and beclin 1²⁴ was similar in all experimental conditions (SI Fig. 4A). Proteolysis is another possible mechanism underlying the observed pancreatic atrophy. In the current study, the cellular content of the FOXO1 transcription factor, which regulates the expression of ligases necessary for ubiquitin-mediated proteolysis^{25, 26}, was significantly increased during protein deficiency, but not in mice refed control chow in the presence of rapamycin (SI Fig. 4B).

Stimulation of the mTOR pathway is not required for increased secretory capacity of the pancreas following protein deficiency-induced atrophy

In accordance with our histological data, the amount of amylase and chymotrypsin per unit of pancreatic protein were decreased to 62 ± 8 and $55 \pm 6\%$ of control values, respectively, in mice fed protein-deficient chow for four days (SI Fig. 5A and 5B). Quantitative real-time PCR was performed using a protocol previously described by our laboratory to compare the expression of amylase, lipase, and chymotrypsin with that of 18s RNA and β -actin²⁷. Changes in digestive enzyme content were not associated with changes in mRNA content (data not shown), and thus appear to be translationally mediated. The translation of these mRNAs however, appears to be mTOR-independent as the content of amylase and chymotrypsin in the pancreas returned to control levels in after refeeding of control chow for four days in both the absence and presence of rapamycin (SI Fig. 5A and 5B).

The observed change in pancreatic digestive enzyme content during protein deficiency was associated with a functional difference in pancreatic secretory capacity. Under basal conditions, the volume of pancreo-biliary juice from mice fed protein-deficient chow was not different than that from control mice (data not shown). In contrast, the total amount of protein in collected pancreo-biliary juice (Fig. 5A) tended to be lower in mice fed protein-deficient chow compared to controls. After cholinergic stimulation, the juice volume increased slightly in both control mice and mice fed protein-deficient chow. However, the amount of protein in the juice of control mice increased nearly six-fold while that of the mice fed protein-deficient chow showed only a small increase, reaching a level that was not statistically different from control mice under basal conditions (Fig. 5A). Protein secretion returned to control levels in the juice from mice refed control chow for four days following protein deficiency under both basal and

bethanechol-stimulated conditions (Fig. 5A). Similar results were obtained from mice given daily injections of rapamycin during the four day recovery period; confirming that the observed increase in digestive enzyme content in the pancreas of these mice was not due to accumulation of digestive enzymes stemming from an unexpected effect of rapamycin on stimulus-secretion coupling. Western blotting of pancreatic juice samples confirmed that changes in total pancreatic juice protein were paralleled by changes in chymotrypsinogen content (Fig. 5B). Coomassie Blue staining (Fig. 5C) further demonstrated that changes in total pancreatic juice protein are due to changes in the secretion of all digestive enzymes, not solely through changes in protease content.

CCK is not required for growth of the pancreas following protein deficiency-induced atrophy

Growth of the pancreas in response to trypsin inhibitor feeding requires calcineurin activation¹². In the current study, administration of the calcineurin inhibitor FK506 did not prevent pancreatic regrowth during recovery from dietary protein deficiency (Fig. 3A). This finding led us to hypothesize that the return of the pancreas to its normal size following protein deficiency-induced atrophy does not require CCK. To test this hypothesis, CCK-null mice were fed protein-deficient chow and then refed control chow for four days. The initial weight of the pancreas (Fig. 6A) in CCK-null mice (6.1 ± 0.1 mg/g body weight) was significantly less than in controls (7.6 ± 0.4 mg/g body weight). The weight of the pancreas in CCK-null and control mice decreased in parallel when fed protein-deficient chow for four days and parallel increases occurred when refed normal chow for four days. These changes in pancreatic weight were not associated with any significant changes pancreatic DNA content (data not shown) and there was no statistical difference in initial pancreatic DNA content between control and CCK-null mice. The initial pancreatic protein content (Fig. 6B) was significantly greater in control mice than in CCK-null mice; however changes in pancreatic protein content occurred in parallel in response to changes in dietary protein in control and CCK-null mice, respectively. Similarly, changes in digestive enzyme content occurred in parallel in response to dietary protein between CCK-null and control mice, as demonstrated by changes in pancreatic chymotrypsin (Fig. 6C). Thus the changes in pancreatic weight, protein content, and digestive enzyme content observed during, and following, dietary protein deficiency are not dependent on the presence of CCK.

Conclusions

This study demonstrates that decreases in pancreatic weight are proportionally greater than those in body weight during short-term protein deficiency in mice. It also reveals that the decrease in pancreatic weight observed after four days of feeding a protein-deficient diet is due to exocrine pancreatic cell atrophy, and not cell death, as changes in pancreatic DNA content were not observed. Moreover, this study demonstrates that under these conditions, the pancreas readily returns to its initial size when protein is returned to the diet. This study did not determine whether the pancreas retains its ability to rapidly return to its original size following a more prolonged period of protein deficiency. Extensive studies by Fitzgerald et al.^{28, 29} however, have shown that the size of the pancreas, as well as digestive enzyme content, returns to normal within five days of refeeding control chow in rats fed protein-deficient chow for ten days. In the same experiments, rats fed protein-deficient chow for ten days with co-administration of the methionine analogue ethionine, to induce acinar cell necrosis, required almost a month for complete recovery. Thus, it appears the ability of the pancreas to regrow following protein deficiency is maintained unless significant cell death occurs during the period of protein deficiency.

Consumption of a protein-deficient diet for four days was associated with significant morphological changes in the pancreas. In particular, individual acinar cells were smaller and their zymogen granule content was dramatically decreased. Similar changes have recently been

reported in a study wherein rats received parenteral nutrition for one week³⁰. The current study also demonstrated that the capacity of the pancreas to secrete digestive enzymes is compromised after four days of feeding a protein-deficient diet. In contrast, the concentration of both amylase and chymotrypsin within the pancreas, as well as pancreatic secretory capacity, was completely reversed within four days of protein being returned to the diet. The observation that the effects of protein deficiency on secretory capacity are not mimicked by rapamycin administration to mice fed protein-replete chow demonstrates that loss of digestive enzyme content during protein deficiency is not due solely to downregulation of mTOR. To our knowledge, these results are also the first to demonstrate that inhibition of the mTOR pathway does not affect stimulus-secretion coupling or digestive enzyme synthesis in the exocrine pancreas *in vivo*.

Inhibition of mTOR signalling is associated with the induction of autophagy in some experimental models, particularly in cultured cells³¹. However, autophagy was not observed in the pancreas during protein deficiency. Under normal conditions, digestive enzymes constitute approximately 90% of all protein being synthesized in the adult pancreas³². The significant decreases in digestive enzyme synthesis during protein deficiency would limit amino acid utilization in the pancreas and may thereby prevent the necessity for autophagy-mediated recycling of amino acids for the maintenance of pancreatic ultrastructure. Moreover, protein deficiency was associated with increased levels of the mTOR effector 4E-BP1 in the pancreas. In its hypophosphorylated state, 4E-BP1 sequesters eukaryotic initiation factor 4E and thereby prevents cap-dependent mRNA translation³³. In a recent paper it was reported that 4E-BP1 expression is induced in pancreatic β cells under conditions of cell stress and that failure to induce its expression led to increased rates of β cell apoptosis³⁴. Induction of 4E-BP1 in the current study may therefore represent a mechanism to prevent acinar cell death, possibly by limiting the energetic and substrate demands of protein synthesis, during protein deficiency. Finally, the observation that FOXO1 expression is increased during protein deprivation indicates that amino acid recycling may occur in the pancreas during protein deprivation, but that it occurs primarily via ubiquitin-mediated proteolysis, rather than autophagy, *in vivo*.

Although pancreatic dysfunction in our model is not as severe as that observed in humans with symptomatic kwashiorkor, this model offers important insights into the regulation of pancreatic size and digestive enzyme synthesis by dietary protein. In the current study, decreased mTOR activity was associated with pancreatic atrophy. In contrast, increased mTOR activity was associated with regrowth of the pancreas. These findings support the notion of mTOR modulating protein accretion in the pancreas in response to dietary protein. By administering the mTOR inhibitor rapamycin during the recovery period following protein deficiency, we were able to prevent pancreatic regrowth and confirm that activation of mTOR is required for regrowth of the pancreas following protein deficiency. CCK and insulin affect mTOR activity through modulation of Akt activity^{35, 36}, while amino acids affect mTOR through an Akt-independent mechanism³⁷. In the current study, feeding protein-deficient chow was associated with a small decrease in plasma insulin concentrations. While this finding was not unexpected, as plasma amino acid levels were dramatically decreased in these mice and branched-chain amino acids are known stimulators of insulin release³⁸, our finding that the phosphorylation of Akt was unchanged in response to dietary protein intake indicates that insulin and Akt does not mediate the observed changes in mTOR activity. As such, these results suggest that modulation of mTOR activity in the current study is mediated directly by amino acids and is not due to hormonal changes associated with the consumption of dietary protein. Decreased mTOR signaling, and the resultant acinar cell atrophy, is likely to be a protective mechanism in the pancreas during protein deficiency. Failure to inactivate the mTOR pathway during cell stress results in increased rates of apoptosis³⁹. Thus, increased mTOR signaling during protein deficiency may result in significant cell death and, as discussed previously, delayed recovery

of pancreatic function when amino acids are returned to the diet. Interestingly, amino acids do not stimulate CCK release⁴⁰, but feeding large doses of purified amino acids stimulates protein synthetic pathways and pancreatic growth in the rodent pancreas^{15, 41}. Administration of a purified amino acid diet may therefore be useful for stimulating pancreatic regrowth in individuals suffering from protein malnutrition where initiating digestive enzyme secretion, gallbladder contraction, or other CCK-mediated events could prove deleterious to pancreatic recovery.

In conclusion, this study demonstrates that dietary protein deficiency is associated with significant pancreatic atrophy, but not cell death, and decreased pancreatic secretory capacity in the mouse. Conversely, returning protein to the diet reestablishes pancreatic size and secretory capacity. We have shown that mTOR signaling in the pancreas is modulated by dietary protein intake and that mTOR activation is required for regrowth of the pancreas following protein deficiency. Finally, we have demonstrated that CCK is not necessary to stimulate pancreatic growth following protein deficiency. Identifying the mechanism whereby amino acids and CCK differentially affects these modes of pancreatic growth will provide important insight into the management of patients with pancreatic dysfunction arising from such conditions as protein malnutrition, parenteral nutrition, or pancreatitis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

mTOR, mammalian target of rapamycin; DAPI, 4'-6-Diamidino-2-phenylindole.

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Fig 1.

Effect of dietary protein on pancreatic growth in mice. Time-course of changes in pancreas weight (A) and pancreatic protein content (B) during protein deficiency and subsequent return of dietary protein. NO PRO, 0 g/kg casein chow (dashed line); CON, 200 g/kg casein chow (solid line). Values represent means $\pm$ SE; $n = 5-11$. Statistical significance was assessed using a one-way ANOVA and Bonferroni's Multiple Comparison post-test. *significantly different from initial control value, †significantly different from 4 day NO PRO value, $P < 0.05$.

Fig 2.

Effect of dietary protein on mTOR pathway activation in the pancreas. Phosphorylation of ribosomal protein S6 (A), and 4E-BP1 (B), as well as the content of 4E-BP1 (C) was assessed by Western blotting in mice fed either 200 g/kg casein chow for 4 days (CON), 0 g/kg casein chow 4 days (NO PRO), CON chow for 1 day following 4 days on NO PRO chow (1D REC), and CON chow for 4 days following 4 days on NO PRO chow (4D REC). Insets: Representative immunoblots. S6(P), S6 phosphorylated on Ser^{240/244}; S6(T), total S6 content; BP1, total 4E-BP1 content; α, α-form of 4E-BP1; β, β-form of 4E-BP1; γ, γ-form of 4E-BP1; actin, β-actin (loading control). Values represent means ± SE; *n* = 6-8. *significantly different from control value, *P* < 0.05.

Fig 3.

Effect of rapamycin and FK506 on pancreatic growth following protein deficiency. Changes in pancreas weight (A) and protein content (B) in mice fed 200 g/kg casein chow for 4 days (CON), 0 g/kg casein chow for 4 days (NO PRO), NO PRO chow for 4 days and then CON chow for 4 days while administered vehicle daily (REC), NO PRO chow for 4 days and then CON chow for 4 days while administered daily i.p. injections of rapamycin (RAPA), or NO PRO chow for 4 days and then CON chow for 4 days while administered daily i.p. injections of FK506 (FK506). *significantly different from initial control value, $P < 0.05$.

Fig 4.

Effect of dietary protein on pancreatic morphology. Electron microscopy of ultrathin sections stained with uranyl acetate and lead citrate. Mice were fed either 200 g/kg casein chow for 4 days (A), 0 g/kg casein chow 4 days (B), 0 g/kg casein chow for 4 days and then 200 g/kg casein chow for 4 days while administered vehicle daily (C), 0 g/kg casein chow for 4 days and then 200 g/kg casein chow for 4 days while administered daily i.p. injections of rapamycin (D). Arrows point to acinar lumens; VS indicates vesicular structures.

Fig 5.

Effect of rapamycin on pancreatic secretory capacity following protein deficiency. Total amounts of protein in pancreo-biliary juice from mice under basal conditions and following bethanechol stimulation as determined by protein assay (A). The relative amount of chymotrypsinogen (B) and Coomassie Blue stained protein (C) per unit volume of pancreobiliary juice from mice following bethanechol stimulation. BASAL, unstimulated; STIMULATED, bethanechol-stimulated; CON, 200 g/kg casein chow for 4 days; NO PRO, 0 g/kg casein chow 4 days; REC, NO PRO chow for 4 days and then CON chow for 4 days while administered vehicle daily; RAPA, NO PRO chow for 4 days and then CON chow for 4 days while administered daily i.p. injections of rapamycin. *significantly different from basal control value, $P < 0.05$.

Fig 6.

Effect of dietary protein on pancreatic growth in CCK-deficient mice. Time-course of changes in pancreas weight (A), pancreatic protein content (B), and chymotrypsinogen content (C) during protein deficiency and subsequent return of dietary protein. WT, age-matched C57BL6 mice (black line); CCK -/-, CCK-deficient mice (grey line); CON, 200 g/kg casein chow (solid line); NO PRO, 0 g/kg casein chow (dashed line); C, CON chow for 4 days; N, NO PRO chow 4 days; R, NO PRO chow for 4 days and then CON chow for 4 days; Chymo, total chymotrypsinogen content; GAPDH (loading control). Values represent means $\pm$ SE; $n = 5-11$. Statistical significance was assessed using a one-way ANOVA and Bonferroni's Multiple Comparison post-test. *significantly different from respective control value, †significantly different from WT control value, $P < 0.05$.