ORIGINAL RESEARCH

CURRENT DEVELOPMENTS IN NUTRITION

Maternal and Pediatric Nutrition

Intermittent Bolus Feeding Enhances Organ Growth More Than Continuous Feeding in a Neonatal Piglet Model

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ABSTRACT

Background: Orogastric tube feeding is frequently prescribed for neonates who cannot ingest food normally. In a piglet model of the neonate, greater skeletal muscle growth is sustained by upregulation of translation initiation signaling when nutrition is delivered by intermittent bolus meals, rather than continuously.

Objectives: The objective of this study was to determine the long-term effects of feeding frequency on organ growth and the mechanism by which feeding frequency modulates protein anabolism in these organs.

Methods: Eighteen neonatal pigs were fed by gastrostomy tube the same amount of a sow milk replacer either by continuous infusion (CON) or on an intermittent bolus schedule (INT). After 21 d of feeding, the pigs were killed without interruption of feeding (CON; $n = 6$) or immediately before (INT-0; $n = 6$) or 60 min after (INT-60; $n = 6$) a meal, and fractional protein synthesis rates and activation indexes of signaling pathways that regulate translation initiation were measured in the heart, jejunum, ileum, kidneys, and liver.

Results: Compared with continuous feeding, intermittent feeding stimulated the growth of the liver (+64%), jejunum (+48%), ileum (+40%), heart (+64%), and kidney (+56%). The increases in heart, kidney, jejunum, and ileum masses were proportional to whole body lean weight gain, but liver weight gain was greater in the INT-60 than the CON, and intermediate for the INT-0 group. For the liver and ileum, but not the heart, kidney, and jejunum, INT-60 compared with CON pigs had greater fractional protein synthesis rates (22% and 48%, respectively) and was accompanied by an increase in ribosomal protein S6 kinase 1 and eukaryotic initiation factor 4E binding protein 1 phosphorylation.

Conclusions: These results suggest that intermittent bolus compared with continuous orogastric feeding enhances organ growth and that in the ileum and liver, intermittent feeding enhances protein synthesis by stimulating translation initiation. *Curr Dev Nutr* 2020;4:nzaa170.

Keywords: neonate, nutrition, pig model, liver, translation initiation, protein synthesis

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Abbreviations used: BW, body weight; C_S, protein synthetic capacity; CON, continuously; eEF2, eukaryotic elongation factor 2; eIF, eukaryotic initiation factor; INT, intermittently; K_{RNA}, protein synthetic efficiency; mTORC1, mammalian target of rapamycin complex 1; ODC, ornithine decarboxylase; rp, ribosomal protein; S6K1, ribosomal protein S6 kinase-1; SA, specific radioactivity; 4EBP1, 4E binding protein 1.

Introduction

Nutrition is a major factor that impacts the growth and survival of infants (1). Orogastric tube feeding, provided on either an intermittent bolus or continuous schedule, is routinely used to support infants who are not able to ingest food normally (1, 2). Whereas intermittent feeding is considered more advantageous (3) due to more favorable postprandial hormonal profiles (4, 5), continuous feeding is indicated for some infants who do not tolerate meal feeding (6, 7).

It is widely accepted that the high growth rates of infants (8) and neonatal animals (9–13) are largely driven by the efficient deposition of dietary amino acids into body proteins (8, 14–16). Due to ethical considerations that preclude studies in infants, we have used the piglet as a model for the human neonate. We showed that the postprandial rises in both plasma insulin and amino acid concentrations are potent stimulators of protein synthesis in skeletal muscles, whereas in other organs, like the liver and other visceral tissues, amino acids are the primary regulator of the feeding-induced stimulation of protein synthesis (17, 18).

Previously, we reported that intermittent bolus feeding promotes more protein deposition in skeletal muscle than continuous feeding in pigs over a 24-h period by stimulating translation initiation signaling and protein synthesis after each meal whereas degradation is unaffected by feeding modality (19, 20). Short-term intermittent feeding also enhances translation initiation signaling in visceral organs (21). Although the feeding-induced activation of the intracellular signaling pathways that regulate protein synthesis is rapid, it returns to baseline (fasting levels) before the next meal. To determine whether this increase in muscle protein deposition rate would translate into an increase in lean body mass and growth when sustained chronically, we recently conducted a long-term efficacy trial. We showed that intermittent feeding over 21 d promoted greater lean tissue accretion than feeding the same amount of nutrients as a continuous orogastric infusion, and this response was ascribed to the greater cumulative activation of anabolic pathways in skeletal muscle (22). However, whether other tissues responded similarly over the long term was not reported. We hypothesized that organ growth would be enhanced in neonatal pigs when they are fed the same total nutrient intake intermittently rather than continuously. Therefore, the objectives of this study were to: *1*) compare the effects of 3 wk of intermittent bolus feeding with continuous feeding on the growth of critical organs, and *2*) determine whether enhanced activation of translation initiation organ growth by intermittent feeding would be maintained after 3 wk.

Methods

Animals and surgeries

The experimental procedures in this study were approved by the Animal Care and Use Committee of Baylor College of Medicine and were described previously for the same group of pigs in El-Kadi et al. (22). The study was done in accordance with the NRC's *Guide for the Care and Use of Laboratory Animals*. Eighteen crossbred (Yorkshire × Landrace × Hampshire × Duroc) female pigs were obtained from the Agricultural Headquarters of the Texas Department of Criminal Justice. At 2 d old, pigs were placed under general anesthesia and implanted with indwelling catheters in the carotid artery and jugular vein and a gastrostomy tube as previously described (19, 22). Pigs were fitted with protective jackets and housed individually in environmentally controlled rooms maintained at 30°C and with a 12-h light/dark cycle. During the recovery period (3 d), pigs were fed a commercial sow milk replacer (Soweena, Litter Life; Merrik's).

Diet

Pigs were randomly assigned to 2 groups and given the same amount [240 mL · kg body weight (BW) $^{-1}$ · d $^{-1}$) of the experimental diet (**Table 1**) for 21 d either continuously (CON: 10 mL · kg BW $^{-1}$ · h $^{-1}$; $n = 6$), delivered by volumetric infusion pump (Abbott Laboratories), or intermittently (INT: 40 mL · kg BW $^{-1}$ · bolus $^{-1}$ delivered over 15 min every 4 h; $n = 12$), by gravity feed, as previously described (22). Briefly, the diet was mixed daily, stored at 4°C, and brought to room temperature when needed. BW was measured every third day and the diet amount was adjusted accordingly. We have previously demonstrated (20, 23) that ingestion of a meal rapidly stimulates protein synthesis in neonatal pigs, peaking at 1 h and returning to baseline before 4 h. Therefore, at the end of the study, half of the pigs in the INT group were killed immediately before a meal (INT-0; $n = 6$) and the other half were killed 60 min following a meal (INT-60; $n = 6$). At the same time CON pigs ($n = 6$) were killed without interruption of continuous feeding.

TABLE 1 Ingredients and nutrient composition of the experimental diet

Ingredient	Per kilogram diet as fed
Whey protein concentrate (80% CP),[1] g	73
Lactose, g	9
FatPak 80,[2] g	6
Corn oil, g	31
Water, g	868
Xanthan gum, g	2
Vitamin premix,[3] g	2
Mineral premix,[3] g	9
Calculated analysis	
Crude protein, g	58
Crude fat, g	40
Carbohydrates, g	13
Metabolizable energy, kcal	644

[1] Hilmar Ingredients.
[2] Milk Specialties Global Animal Nutrition.
[3] Dyets Inc. Vitamin premix provided (g/kg): thiamine HCl, 0.1; riboflavin, 0.375; pyridoxine HCl, 0.1; niacin, 1; calcium pantothenate, 1.2; folic acid, 0.13; biotin, 0.02; cobalamin B-12, 1.5; retinyl palmitate, 0.8; cholecalciferol, 0.05; tocopheryl acetate, 8.8; menadione sodium bisulfite, 0.08. Trace mineral premix provided (g/kg): calcium phosphate, dibasic, 187; calcium carbonate, 279, sodium chloride, 85; potassium phosphate monobasic, 155; magnesium sulfate, anhydrous, 44; manganous carbonate, 0.93; ferric citrate, 10; zinc carbonate, 1.84; cupric carbonate, 0.193; potassium iodate, 0.005; sodium selenite, 0.007.

Body composition

Body composition was determined on anesthetized pigs by DXA using a fan-beam densitometer (Hologic QDR4500A) in the infant whole-body scan mode (22). Briefly, body composition was determined on day −3 and day 18 relative to initiation of the feeding trial, and composition extrapolated to days 0 and 21 based on BW (22).

Fractional protein synthesis

A flooding dose of L-[4-^{3}H]phenylalanine was injected to determine tissue fractional synthesis rates, as previously described (11, 22). Piglets were killed using an overdose of pentobarbital (87 mg sodium pentobarbital/kg BW) 30 min following tracer infusion. Immediately following death, tissue samples were frozen in liquid nitrogen, and stored at −70° C until analyses. Specific activities of protein-bound and tissue-free phenylalanine were determined as previously described (14). Fractional protein synthesis (K_S) was calculated from the following equation:

$$K_S = \left[\left(SA_{\text{bound phe}}/SA_{\text{free phe}}\right) \times 1440/t\right] \times 100 \qquad (1)$$

where $SA_{\text{bound phe}}$ and $SA_{\text{free phe}}$ (dpm/nmol) are the tissue protein-bound and free phenylalanine specific radioactivities, respectively, t (minutes) is the labeling time after tracer administration, and 1440 is a conversion factor (minutes to days).

TABLE 2 Body weight, lean mass, fat percentage, and spine length of pigs fed for 21 d either continuously (CON) or intermittently (INT)[1]

	CON		INT			*P* values		
	0	21	0	21	SEM[2]	Treatment	Time	Interaction
Body weight, kg	2.3	4.2	2.4	5.6	0.11	<0.0001	<0.0001	<0.0001
Lean mass, kg	2.1	3.8	2.2	5.2	0.11	<0.0001	<0.0001	<0.0001
Fat, %	0.047	0.063	0.051	0.059	0.006	0.94	0.07	0.53
Spine length, cm	23	29	24	31	0.4	0.0045	<0.0001	<0.0001

[1]Pigs received 240 mL · kg BW^{-1} · d^{-1} of the experimental diet either continuously (CON: 10 mL · kg BW^{-1} · h^{-1}, $n = 6$) or intermittently (INT: 40 mL · kg BW^{-1} · bolus^{-1} delivered over 15 min every 4 h, $n = 12$) for 21 d. BW, body weight.
[2]Pooled standard error of the means.
Adapted with permission from reference 22.

Organ weights

The heart, jejunum, ileum, 1 kidney, and liver were dissected, and weighed before aliquots were frozen in liquid nitrogen to be used for fractional protein synthesis rate determination. Organ weight as a proportion of lean body mass was calculated from the ratio of organ weight to lean mass determined by DXA.

Protein synthetic capacity and efficiency

Total protein concentration was determined in tissue homogenates using the bicinchoninic acid assay (Catalog no. 23227; Thermo Scientific) and total RNA using the method of Munro and Fleck (24). Protein synthetic capacity (C_S; μg RNA · mg protein^{-1}) was calculated as the RNA-to-protein ratio, and protein synthetic efficiency (K_{RNA}; g protein · d^{-1} · g RNA^{-1}) as the total protein synthesized in a day per total RNA.

Protein expression and phosphorylation by western blot

The protein isolation and western blotting procedures have been described previously (19). Briefly, tissues were homogenized, the proteins separated by SDS-PAGE, transferred to polyvinylidene difluoride membranes, and immunoblotting performed using the following antibodies: eukaryotic elongation factor 2 (eEF2; total and phosphorylated Thr56; Cell Signaling Technology), eukaryotic initiation factor 2α (eIF2α; total and phosphorylated Ser51; Cell Signaling Technology), eIF4E binding protein 1 (4EBP1; total; Bethyl Laboratories, Inc.; phosphorylated Thr70; Cell Signaling Technology), ribosomal protein (rp) S6 kinase-1 (S6K1; total; Santa Cruz Biotechnology; phosphorylated Thr389; EMD Millipore). α-Tubulin (Santa Cruz Biotechnology) was used as a loading control, and phosphorylated proteins were normalized to their corresponding nonphosphorylated forms. An enhanced chemiluminescence kit (Catalog no. RPN 2232 and RPN 2235; GE Health Sciences) was used to visualize and analyze band intensity using a ChemiDoc-It Imaging System (Bio-Rad).

Hepatic rp mRNA expression

Polysomal fractions were separated using sucrose density gradients (25). The proportions of rpS4, rpS8, and ornithine decarboxylase (ODC) mRNAs in the polysomal fraction were determined by quantitative RT-PCR.

Statistics

Treatments were assigned to experimental units using a complete randomized block design, and data were analyzed using PROC MIXED of SAS (Version 9.4; SAS Institute). When a significant treatment effect was detected, means were compared using the Tukey–Kramer post hoc test. Data are presented as least square means ± SEM, and differences are considered significant at $P \leq 0.05$.

Results

Growth, body composition, substrates, and hormones

Detailed results on BW, body composition, and food and nutrient conversion efficiencies, and hormone and substrate concentrations are reported elsewhere (22) but are briefly described here to provide context for the current report. Briefly, after 21 d, BWs, lean mass, and spine length were greater in the INT than in the CON group ($P < 0.05$) (**Table 2**). There was no difference between groups in fat as a percentage of BW. Arterial plasma branched-chain amino acids, glucose, and insulin peaked by 60 min following a meal in the INT group but did not change in response to feeding in the CON group (**Table 3**).

Organ weights

Absolute weights of the heart, jejunum, ileum, kidney, and liver (**Figure 1**) were greater in the INT-0 and INT-60 groups compared with the CON group ($P < 0.05$). When normalized for lean mass (Figure 1) or BW (data not shown), liver weight was greater for pigs in the INT-60 compared with those in the CON group and was intermediate for those in the INT-0 group. However, the larger weights of the heart, jejunum, ileum, and kidney were proportional to the greater lean mass, and these proportions were not affected by feeding modality.

Fractional protein synthesis rates

Fractional protein synthesis rates of the heart, jejunum, and kidney were not affected by feeding modality (**Figure 2**). Ileal fractional protein synthesis rate was greater for the INT-60 compared with the INT-0 and CON groups. Liver fractional protein synthesis rate was greatest for the INT-60, intermediate for the INT-0, and lowest for the CON group ($P < 0.05$).

Protein synthetic capacity and efficiency

Protein synthetic capacity (C_S) of the heart, expressed as total RNA concentration corrected for protein content, was greater for the INT-0 and INT-60 groups compared with the CON group ($P < 0.05$; Figure 2). However, feeding frequency did not affect the protein synthetic capacity of the jejunum, ileum, and kidney. Protein synthetic capacity was greatest in the liver of the INT-0 pigs and least for those in the CON

TABLE 3 Arterial plasma branched-chain amino acids (BCAA), glucose, and insulin concentrations on day 21 of pigs fed the same diet either continuously (CON) or intermittently (INT)[1]

	BCAA, μmol/L		Glucose, mg/dL		Insulin, μU/mL	
Time, min	CON	INT	CON	INT	CON	INT
0	1228	970	112	132	5.5	4.0
15	1209	1025	116	148	5.7	27.8
30	1332	1333	116	158	5.3	48.4
45	1266	1504	121	154	6.3	42.7
60	1281	1763	118	152	6.8	34.4
75	1490	1701	122	153	7.5	21.6
90	1499	1640	121	143	7.0	16.7
120	1288	1430	121	142	6.0	10.4
150	1312	1380	117	142	5.2	7.4
180	1311	1152	121	135	4.0	5.3
210	1408	1058	117	131	4.7	4.2
240	1373	1027	117	135	3.5	4.1
SEM[2]	94		5.5		3.02	
P values						
Treatment	0.9888		0.0006		0.0002	
Time	<0.0001		<0.0001		<0.0001	
Interaction	<0.0001		0.0013		<0.0001	

[1]Pigs received 240 mL · kg BW^{-1} · d^{-1} of the experimental diet either continuously (CON: 10 mL · kg BW^{-1} · h^{-1}, $n = 6$) or intermittently (INT: 40 mL · kg BW^{-1} · $bolus^{-1}$ delivered over 15 min every 4 h, $n = 12$) for 21 d. Samples were taken starting immediately before a meal for the INT group and at the same time for the CON group over a 240-min period. BW, body weight.
[2]Pooled standard error of the means.
Adapted with permission from reference 22.

group, whereas those in the INT-60 group were intermediate. Protein synthetic efficiency (K_{RNA}) of the ileum was greater for the INT-60 compared with the INT-0 and CON groups ($P < 0.05$) but did not differ significantly among groups for the heart, jejunum, kidney, and liver.

Translation initiation and intracellular signaling

Phosphorylation of S6K1, 4EBP1, eIF2α, and eEF2 in the heart and kidney were not different among the groups (**Figure 3**). However, in the jejunum, ileum, and liver, phosphorylation of S6K1 and 4EBP1, but not eIF2α and eEF2, were enhanced in the INT-60 group compared with the CON and INT-0 groups ($P < 0.05$).

Rps

The expression of the ODC gene was used as a reference (**Figure 4**) and was similar among groups. The proportion of rpS8 mRNA in polysomes in the liver was greatest for the INT-60 group compared with the INT-0 and CON groups ($P < 0.05$). Expression of rpS4 tended to increase for the INT-60 group compared with the INT-0 and CON pigs ($P < 0.10$).

Discussion

Orogastric and nasogastric tube feeding using continuous and intermittent bolus feeding schedules are both used to support neonates who are unable to coordinate suckling, breathing, and swallowing. Although each method has some theoretical benefits, a 2011 Cochrane meta-analysis that included 511 infants in 7 trials concluded that the lack of evidence precluded recognizing the benefits of feeding frequency, largely due to clinical variables and methodological limitations (26). Since that time, 2 randomized control trials have concluded that the 2 feeding strategies are equally suitable for neonatal patients (27, 28). Despite the lack of clear clinical evidence, the majority (81%) of neonatal intensive care units in the United States and Canada administer enteral nutritional support by intermittent bolus feeds (29). However, continuous feeding can still be indicated in some neonates who cannot tolerate feeding on an intermittent bolus schedule (7).

Previously, we reported that short-term intermittent bolus feeding enhances protein synthesis in organs to a greater extent after each meal than continuous feeding (21). The aim of the current study was to determine whether those responses that we observed previously at the end of a 25.5-h feeding period could be maintained for a clinically relevant duration of 21 d and lead to improved growth. Available data for the normal range of organ weights in neonates are rare at best, and most data are generated from autopsy reports of critically ill infants (30–34), which might not represent normal populations (32). Although organ dimensions can be determined in healthy infants using noninvasive techniques, such as sonography (35), there is a lack of data on how feeding frequency affects organ growth and protein synthesis.

In the current study, organ weights after 21 d of feeding were greater for the intermittently fed pigs compared with those fed continuously (**Table 4**). This increase in the mass of the heart, jejunum, ileum, and kidney was proportional to that of lean BW, but the contribution of the liver to lean BW was greater in the intermittently fed compared with the continuously fed pigs. Jejunal weight as a percentage of BW was reported to be modestly increased in pigs fed intermittently compared with those fed continuously for 7 d (36). Although in that study the type of diet, frequency of feeding, and length of the experimental period were different from the current study, those data (36) suggest that the effect of feeding frequency on jejunal weight is transient. Although it is plausible that the increase in intestinal weight (36) could have

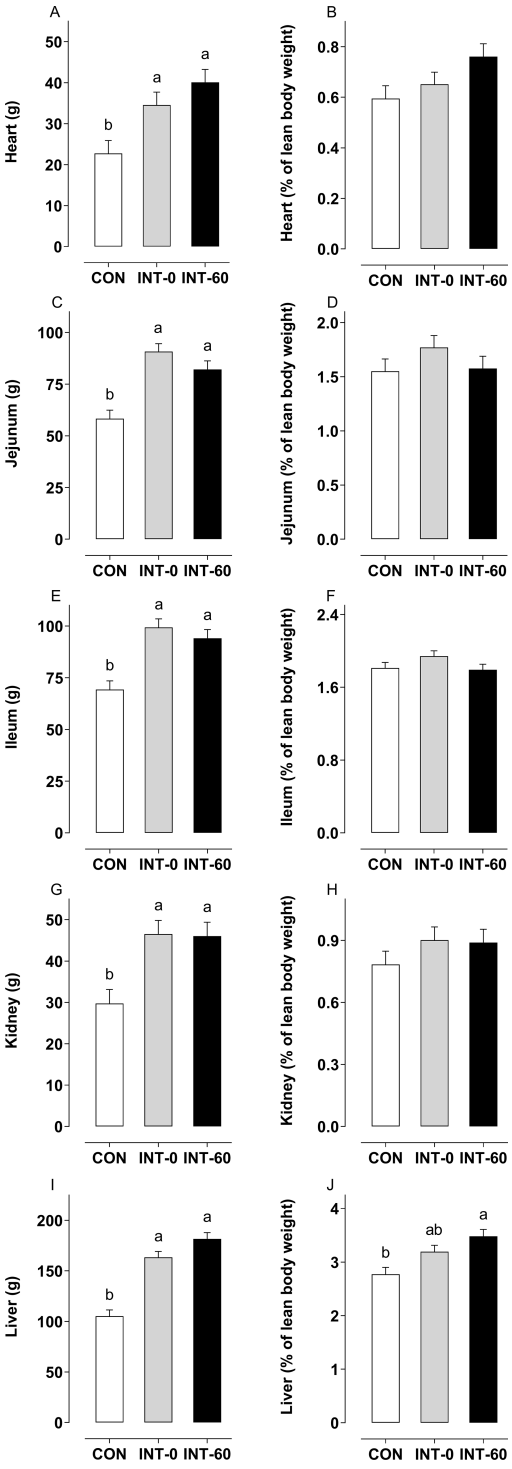

FIGURE 1 Absolute weights and as a percentage of lean body mass of the heart (A, B), jejunum (C, D), ileum (E, F), kidney (G, H), and liver (I, J) after 21 d of feeding neonatal pigs either continuously (CON) or intermittently (INT). Measurements were made without interruption of feeding in CON ($n = 6$) or immediately before (INT-0; $n = 6$) or 60 min after (INT-60; $n = 6$) a meal in INT. Statistical analyses were done using mixed-model ANOVA. When a significant effect was detected, means were compared using the Tukey–Kramer post hoc test. Values are means ± SEM; $n = 6$ per treatment.

occurred in response to greater expression of small intestinal trophic factors (37), production rates of gut peptides are similar in pigs fed continuously and intermittently, suggesting that trophic peptides do not play a role in modulating small intestinal mass in response to feeding frequency (38). The current data also suggest that the liver is more sensitive to the effect of feeding frequency than the other organs. Although we reported previously that intermittent bolus feeding stimulated protein synthesis rates in the heart, jejunum, liver, and kidney by 25%, 16%, 34%, and 27%, respectively, after 24 h of feeding (21), we speculated that this could have been an acute response and that a longer feeding period would be needed to establish whether this increase in protein synthesis could be sustained chronically to affect increases in mass. The current data indicate that after 21 d of feeding, ileum and liver fractional protein synthesis rates were 48% and 22% greater, respectively, in the intermittent bolus–fed group after a meal than in the continuously fed group, whereas the increase in protein synthesis in the heart, jejunum, and kidney observed after short-term intermittent bolus feeding was not maintained over the long term (Table 4). There are 2 possible explanations for the differences in protein synthesis among the different organs. One common characteristic that differentiates the intestines and liver from other tissues in the body is that in these tissues dietary amino acids are used for secretory and constitutive protein synthesis. Whereas in the small intestines secretory proteins (e.g., mucins and digestive enzymes) are subsequently recycled and reabsorbed as amino acids (39), liver-synthesized plasma proteins (e.g., albumin and fibrinogen) are released into the circulation (40). Therefore, the rate of protein synthesis in the intestines and liver represents not only protein accretion but also exported proteins. It is not possible to differentiate between secretory and constitutive protein synthesis in the current study because we used the flooding dose technique, which measures all proteins synthesized. However, data from neonatal pigs suggest that the proportion of dietary nitrogen that appears in small intestinal tissue, liver, and liver plasma proteins is greater than that observed in other organs like the kidneys (41). This does not exclude the second possibility that the extracellular amino acid profile that reaches peripheral tissues is different from that of the diet due to first-pass utilization of amino acids by the portal drained viscera and the liver (42). Thus, the profile and amounts of amino acids reaching the peripheral tissues might not match the amino acid pattern needed for maximal protein synthesis in peripheral tissues.

In the current study, circulating amino acid and insulin concentrations peaked 30 to 60 min following a meal in the INT group but remained low and constant in CON pigs (22). In this regard, we have demonstrated that short-term amino acid infusion using the pancreatic substrate clamp technique to raise plasma amino acids to concentrations observed after a meal increased protein synthesis in the liver; however, insulin infusion had no effect on protein synthesis (18, 43). In addition, we demonstrated that peripheral insulin infusion had only a modest effect in the heart, whereas neither peripherally infused amino acids nor insulin had an effect on protein synthesis in the jejunum (44). In contrast to the nonresponsiveness of jejunal protein synthesis to peripherally administered amino acid and insulin, enteral compared with parenteral feeding of an elemental diet at levels representing ≥60% of nutrient needs increased jejunal but not ileal fractional protein synthesis rates in neonatal pigs (44). We also have reported that enteral meals increase fractional protein synthesis in the jejunum of neonatal pigs (11, 20) but not in the heart (11) or kidney (20), and that this effect

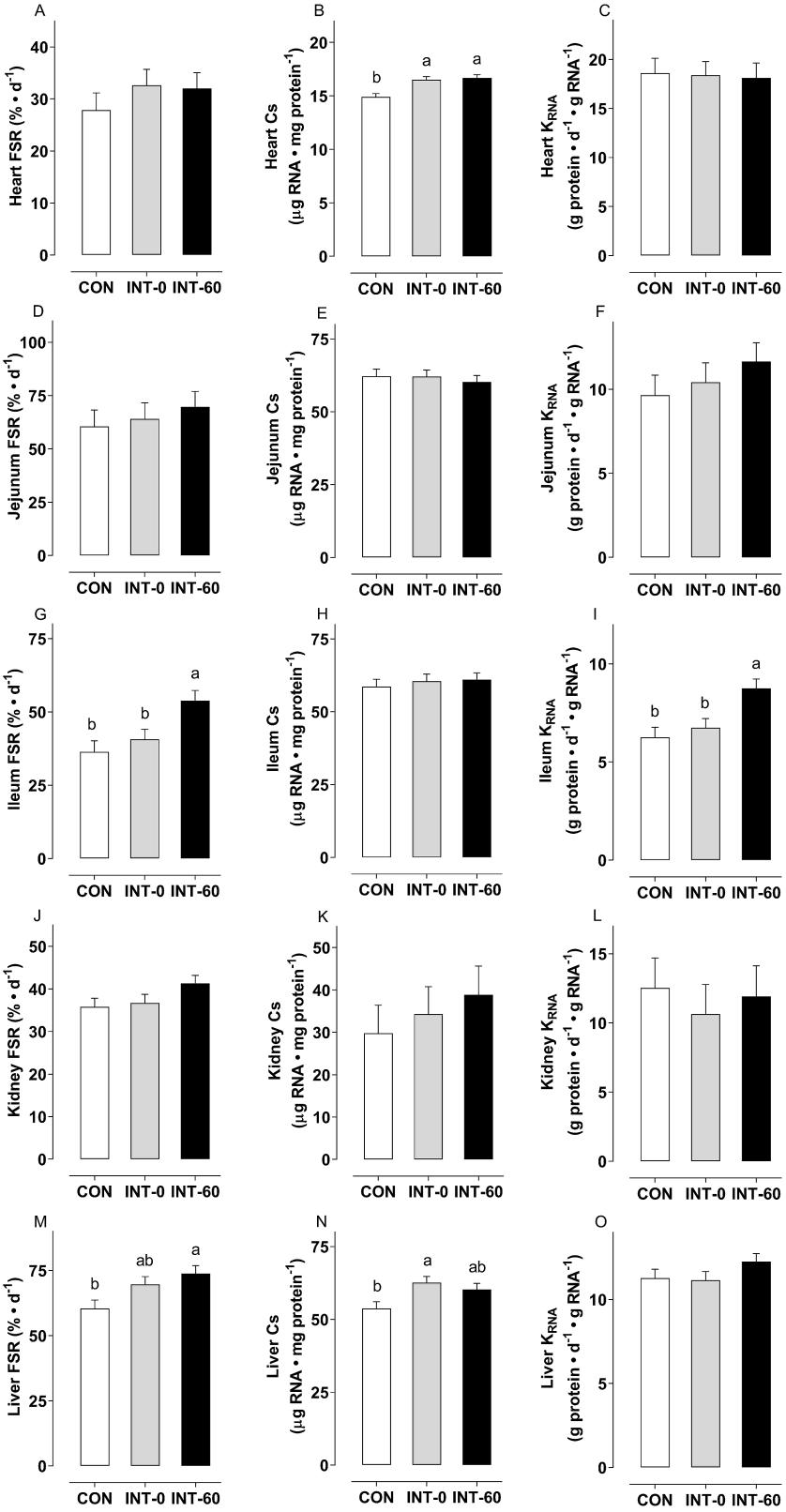

FIGURE 2 Fractional protein synthesis rate (FSR), RNA synthetic capacity (C_S), and RNA synthetic efficiency (K_{RNA}) of the heart (left ventricle; A–C), jejunum (D–F), ileum (G–I), kidney (J–L), and liver (M–O) after 21 d of feeding neonatal pigs either continuously (CON) or intermittently (INT). Measurements were made without interruption of feeding in CON ($n = 6$) or immediately before (INT-0; $n = 6$) or 60 min after (INT-60; $n = 6$) a meal in INT. Statistical analyses were done using mixed-model ANOVA. When a significant effect was detected, means were compared using the Tukey–Kramer post hoc test. Values are means ± SEM; $n = 6$ per treatment.

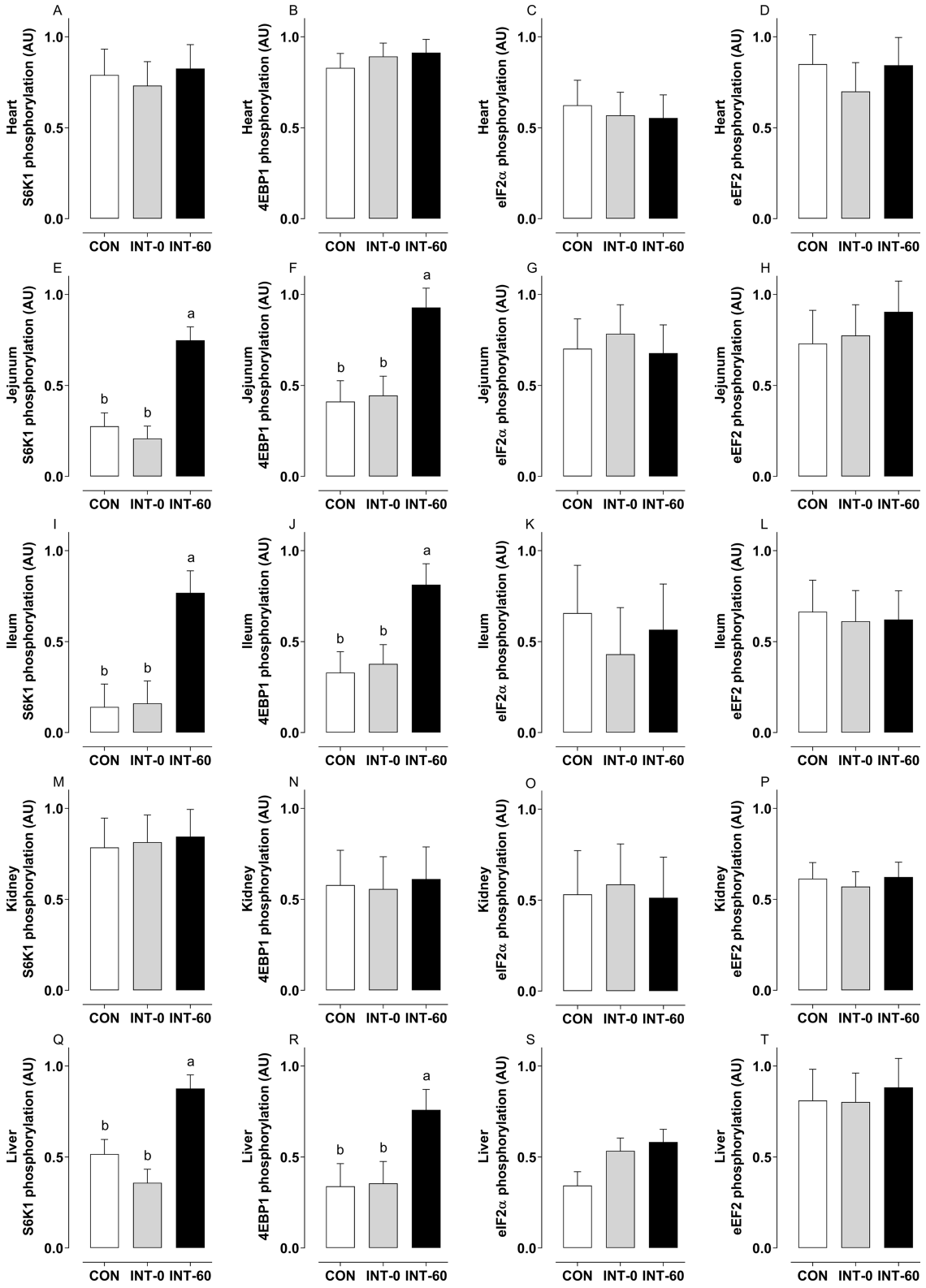

FIGURE 3 Phosphorylation of S6K1, 4EBP1, eIF2α, and eEF2 in the heart (left ventricle, A–D), jejunum (E–H), ileum (I–L), kidney (M–P), and liver (Q–T) after 21 d of feeding neonatal pigs either continuously (CON) or intermittently (INT). Measurements were made without interruption of feeding in CON ($n = 6$) or immediately before (INT-0; $n = 6$) or 60 min after (INT-60; $n = 6$) a meal in INT. Statistical analyses were done using mixed-model ANOVA. When a significant effect was detected, means were compared using the Tukey–Kramer post hoc test. Values are means ± SEM; $n = 6$ per treatment. AU, arbitrary units; eEF2, eukaryotic elongation factor 2; eIF2α, eukaryotic initiation factor 2α; S6K1, ribosomal protein S6 kinase-1; 4EBP1, 4E binding protein 1.

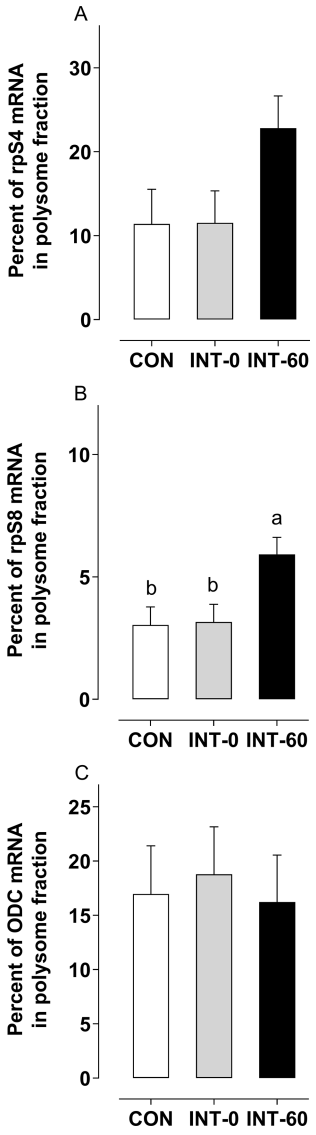

FIGURE 4 The proportion of ribosomal protein S4 (rpS4; A), ribosomal protein S8 (rpS8; B), and ornithine decarboxylase (ODC; C) mRNAs in the polysomal fraction of the liver after 21 d of feeding neonatal pigs either continuously (CON) or intermittently (INT). Measurements were made without interruption of feeding in CON ($n = 6$) or immediately before (INT-0; $n = 6$) or 60 min after (INT-60; $n = 6$) a meal in INT. Statistical analyses were done using mixed-model ANOVA. When a significant effect was detected, means were compared using the Tukey–Kramer post hoc test. Values are means ± SEM; $n = 6$ per treatment.

is greater for pigs fed intermittently compared with those fed continuously (21). Thus, our findings of the long-term effects of intermittent feeding on protein synthesis in the different tissues in the current study are largely consistent with previous findings of the differential effects of amino acids and insulin on protein synthesis in these organs, and that for the intestine, protein synthesis may be influenced by the route of delivery of nutrients (44).

The rapid stimulation of protein synthesis in organs of neonatal pigs following a feed is mediated via activation of mammalian target of rapamycin complex 1 (mTORC1), but this response is transient, and protein synthesis returns to the prefeeding level by 2 h postprandially (20). We reported that the greater organ protein synthesis rates in pigs fed intermittently for 24 h compared with those fed continuously were associated with activation of translation initiation through the mTORC1 signaling pathway (21). Two regulatory processes control translation initiation downstream of mTORC1. The first is mediated by eIF2, which facilitates the binding of methionyl-tRNA to the 40S ribosomal subunit to form the 43S preinitiation complex. The second is mTORC1 and its downstream mediators, S6K1 and 4EBP1, that allows the formation of the eIF4E·eIF4G complex that promotes binding of the 43S preinitiation complex to mRNA (45, 46). Consistent with our previous short-term feeding data (21), feeding frequency had no effect on eIF2α phosphorylation in any tissue in the current study (Table 4). However, the enhanced phosphorylation of S6K1 and 4EBP1 we observed in the heart, jejunum, liver, and kidney after short-term bolus compared with continuous feeding (21) occurred only in the jejunum, ileum, and the liver of the pigs fed intermittently long term, with no effect of feeding modality on S6K1 or 4EBP1 phosphorylation in the heart or kidney (Table 4).

There is also evidence that protein synthesis rates can be regulated through peptide chain elongation and mediated by eEF2 in an mTORC1-dependent mechanism (47, 48). However, we have shown that under normal physiological conditions, amino acids (49, 50), insulin (50), or high protein feeding (51) do not affect eEF2 phosphorylation in skeletal muscles and organs of neonatal pigs despite activation of mTORC1 and its downstream effectors, S6K1 and 4EBP1. In the current study, eEF2 phosphorylation was similar in all groups and supports our previous findings that protein synthesis in visceral organs (21) and skeletal muscles (22) is mainly controlled by translation initiation rather than peptide elongation (Table 4).

Protein synthetic capacity and ribosomal translational efficiency regulate protein synthesis rates (14). Protein synthetic capacity is a function of ribosome abundance, and synthesis of rps is tightly correlated to activation of mTORC1 and its downstream effector, S6K1 (52). In the current study, there was a greater proportion of rp mRNA in the polysomal fraction in the liver of intermittently fed pigs after a meal compared with those fed continuously, suggesting enhanced rp synthesis in response to intermittent feeding. Previously, we reported that feeding frequency does not influence the synthesis of rpS4 and rpS8 in skeletal muscles of neonatal pigs over the long term (22). In the liver, synthesis of rpS4 and S8 was enhanced 30 min following a single meal and returned to baseline by 4 h (20). Similarly, a greater proportion of rpS4 mRNA was present in the polysomal fraction in the liver of short-term intermittent bolus–fed pigs compared with those fed continuously or fasted overnight (21). The current long-term study supports our previous reports that the expression of rp mRNA was greater in the livers of intermittently fed pigs after a meal compared with those fed continuously, and that the greater expression of rp mRNA in the liver was associated with greater protein synthetic capacity. The greater expression of rp following a meal compared with continuously or intermittently fed pigs before a meal would suggest that ribosome abundance in the liver is more sensitive to feeding/fasting cycles than other tissues like muscles. Although it is not possible from the current data to discern the cause for such differences, these could be attributed to diurnal oscillations in mass and ribosome numbers, which have been reported in the liver even

TABLE 4 Summary of the changes in weight, fractional synthesis rate, protein synthetic capacity and efficiency, and translation initiation factor activation in organs of pigs fed the same diet intermittently (INT) vs. continuously (CON) for 21 d[1]

	Heart	Jejunum	Ileum	Kidney	Liver
Weight, g	↑	↑	↑	↑	↑
Weight, % lean BW	↔	↔	↔	↔	↑
FSR, %/d	↔	↔	↑	↔	↑
C_S, μg/mg	↑	↔	↔	↔	↑
K_{RNA}, g/g	↔	↔	↑	↔	↔
p-S6K1, AU	↔	↑	↑	↔	↑
p-4EBP1, AU	↔	↑	↑	↔	↑
p-eIF2α, AU	↔	↔	↔	↔	↔
p-eEF2, AU	↔	↔	↔	↔	↔

[1]Pigs received 240 mL · kg BW^{-1} · d^{-1} of the experimental diet either continuously (CON: 10 mL · kg BW^{-1} · h^{-1}, $n = 6$) or intermittently (INT: 40 mL · kg BW^{-1} · $bolus^{-1}$ delivered over 15 min every 4 h, $n = 12$) for 21 d. Samples in the INT group were obtained immediately before (INT-0; $n = 6$) or 60 min after (INT-60; $n = 6$) a meal and without interruption of feeding in CON ($n = 6$). ↑, INT-60 > CON; ↔, INT-60 = CON. AU, arbitrary units; BW, body weight; C_S, protein synthetic capacity; eEF2, eukaryotic elongation factor 2; eIF2α, eukaryotic initiation factor 2α; FSR, fractional synthesis rate; K_{RNA}, protein synthetic efficiency; p, phosphorylation; S6K1, ribosomal protein S6 kinase-1; 4EBP1, 4E binding protein 1.

of mice fed ad libitum, and that this response was unique to the liver and not other tissues (53). In addition, phosphorylation of S6K1 and 4EBP1 in the liver, jejunum, and ileum was enhanced in intermittently fed pigs after a meal compared with those continuously fed, and resulted in greater protein synthetic efficiency in the ileum (40%), whereas for the jejunum and liver this increase in protein synthetic efficiency was modest and nonsignificant (8% and 20%, respectively). Taken together, the data suggest that the greater fractional protein synthesis rate in the liver was driven by an increase in ribosomal abundance and mTORC1-dependent translation initiation, whereas in the ileum protein synthesis was only associated with enhanced mTORC1-dependent translation initiation.

In conclusion, the results of the current study suggest that intermittent bolus compared with continuous feeding promotes organ growth that is largely proportional to whole body growth. Although protein synthesis rates in the liver, jejunum, kidney, and heart are enhanced with short-term intermittent bolus feeding, this increase in protein synthesis is maintained over the long term only in the liver and ileum. These greater protein synthesis rates in the liver and ileum can be ascribed, in part, to the activation of mTORC1-dependent translation initiation signaling. These data, together with our previous finding of enhanced skeletal muscle and lean growth in intermittent bolus–fed piglets, provide supportive evidence that, if tolerated, intermittent bolus feeding is preferable to continuous feeding for providing nutritional support to neonates who are unable to feed normally. Although the use of continuous feeding is reserved for situations where intermittent bolus feeding is precluded, our data underscore the need for more studies that focus on the development of nutritional management strategies that could improve neonatal growth in infants who cannot tolerate intermittent bolus feeding and must be fed continuously.

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