

1 **Modulation of intestinal protein synthesis and**  
2 **protease mRNA by luminal and systemic nutrients**

3  
4 OLASUNKANMI A. J. ADEGOKE<sup>1</sup>, MICHAEL I. McBURNEY<sup>1</sup>, SUSAN E. SAMUELS<sup>2</sup>,  
5 AND VICKIE E. BARACOS<sup>1</sup>

6  
7 <sup>1</sup>*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB,*  
8 *T6G 2P5, Canada; and <sup>2</sup>Food, Nutrition & Health, The University of British Columbia,*  
9 *Vancouver, BC, V6T 1Z4, Canada*

10

11

12 Running Title: Nutrients and mucosal protein metabolism

13

14 KEY WORDS: amino acids; intestine; protein breakdown; glutamine; ubiquitin

15

16

17 Corresponding Author: Vickie E. Baracos, Department of Agricultural, Food and Nutritional  
18 Science, 4-10 Agriculture-Forestry Centre, University of Alberta, Edmonton, Alberta T6G 2P5,  
19 Canada.

20 Telephone: (780) 492-7664 Fax: (780) 492-9130 E-mail: [vickie.baracos@ualberta.ca](mailto:vickie.baracos@ualberta.ca)

**1 ABSTRACT**

2 Route of nutrient supply is important in regulation of intestinal protein metabolism, as total  
3 parenteral nutrition, in comparison with enteral feeding, leads to profound atrophy. The  
4 participation of protein synthesis (Ks) and degradation in regulation of gut protein balance and  
5 their possible modulation by specific nutrients are the focus of our work. We developed an *in*  
6 *situ* experimental system that allows controlled exposure of intestinal mucosa to nutrients  
7 systemically, luminally or both. We examined the effects of systemic glucose and amino acid  
8 (AA) infusion in overnight-fasted piglets. Jejunal segments within each piglet were  
9 simultaneously luminally perfused with solutions containing various amino acids or glucose.  
10 Intravenous infusion of glucose increased mucosal Ks by 16% ( $P < 0.05$ ), while intravenous  
11 infusion of AA had no effect on Ks. Systemic glucose infusion had no effect on mRNA levels for  
12 components of the ubiquitin-proteasome proteolytic pathway. However, levels of these mRNA  
13 were reduced by intravenous or luminal AA supply. This effect was greatest (-50%) when  
14 highest tissue concentrations of amino acids were achieved by the simultaneous infusion of AA  
15 by both routes ( $P < 0.05$ ). Our findings suggest that the modulation of protein balance in the  
16 intestine in response to nutrients is only in part attributable to anabolic stimulation of protein  
17 synthesis initiated by the systemic appearance of glucose, but that a fall in protein degradation is  
18 also a likely contributor. Amino acids appear to be a key factor required to reduce expression of  
19 genes connected with proteolysis.

## 1 INTRODUCTION

2 Feeding, fasting and specific nutrients participate in regulating small intestinal protein  
3 metabolism and mass. Mucosal protein synthesis and mass increase after feeding, and decrease  
4 during deprivation of food (8, 18, 22). Route of nutrient supply is also important as total  
5 parenteral nutrition (TPN), in comparison with enteral feeding, leads to profound atrophy and  
6 decreased protein synthesis rates (13, 20, 23). While this response may be attenuated by the  
7 addition of specific nutrients such as glutamine or short chain fatty acids to parenteral nutrition,  
8 full restoration of intestinal protein mass is not achieved (20, 23).

9 We had hypothesized that luminal nutrients *per se* may be needed to maintain intestinal  
10 protein mass, by activation of protein synthesis, suppression of protein degradation, or both.  
11 To test the specific roles of individual luminal nutrients in regulating intestinal protein turnover,  
12 we developed an *in situ* experimental system that allows exposure of mucosa to nutrients on the  
13 apical side without exerting systemic effect (1). Using jejunal segments subjected to acute (40-90  
14 min) luminal infusion (2), we showed that neither energy fuels such as glucose or short chain  
15 fatty acids, nor a mixture of amino acids or glutamine reproduced the rise in protein synthesis  
16 associated with oral feeding (8, 18). The difference between oral feeding and our luminal  
17 perfusion approach is the appearance of nutrients and hormones in the systemic circulation. In  
18 the study reported here, we test the hypothesis that the regulation of intestinal protein  
19 metabolism is also dependent on systemic signals reflecting overall nutritional status and that  
20 such signals would permit elevations of protein synthesis as seen after feeding *in vivo*. We  
21 selected two potential candidates, amino acids and glucose, as the systemic nutrient regulators.  
22 The plasma concentrations of these nutrients rise after feeding (8, 12, 19, 24). These were

1 infused intravenously, with or without simultaneous perfusion with luminal nutrients to  
2 additionally test for interaction between luminal and systemic treatments.

3 The regulation of intestinal proteolysis by feeding and in response to specific nutrients is  
4 considerably less well known compared with protein synthesis. While no direct method for  
5 estimating intestinal protein catabolism is presently available, we measured mRNA levels of  
6 components of different proteolytic systems to examine the potential regulation of proteolysis at  
7 this level (22). We previously observed that mucosal mRNA levels for several elements of the  
8 ubiquitin–proteasome dependent proteolytic system decreased after luminal exposure to amino  
9 acids (2), suggesting that protein degradation may be an important determinant of intestinal  
10 protein mass. Thus a second objective of this study was to assess gene expression within the  
11 ubiquitin-proteasome system, in response to the systemic and luminal treatments.

12

## 13 **MATERIALS AND METHODS**

14 ***Study design.*** Our prior work had focused exclusively on the provision of luminal nutrients  
15 in the absence of systemic effects (1, 2). In the current study, we examined intravenously  
16 administered nutrients. In study 1, intravenous infusion of glucose was compared with saline; in  
17 study 2, intravenous infusion of amino acids was compared with saline. Within both of these  
18 main experiments, we examined several luminal nutrient treatments (saline, 30 mM amino acids ±  
19 50 mM glucose, 30 mM glutamine) that we had used in our prior studies, to determine if there  
20 were interactions between the luminal and systemic provision of nutrients.

21 ***Chemicals.*** Sterile 75% glucose and physiological saline were purchased from Baxter  
22 (Deerfield, IL). L-[2,6-<sup>3</sup>H]phenylalanine (2.15 TBq/mmol; radiochemical purity: 99.6%) was

1 purchased from Amersham International (Amersham Place, Little Chalfont, Bucks, UK). Other  
2 chemicals were from Sigma Chemical (St. Louis, MO).

3 ***Animals and surgical and perfusion procedures.*** All studies were performed in accordance  
4 with the Canadian Council on Animal Care Guidelines and were authorized by the institutional  
5 Animal Policy and Welfare Committee. Six-week old male piglets, weighing ~12 kg,  
6 (Camborough X Canabrid Pig Improvement Company crosses) were obtained through the  
7 University of Alberta Health Sciences Laboratory Animal Services. Piglets were weaned at four  
8 weeks of age and maintained on a wheat/oatgroat-soybean/whey powder starter diet (crude  
9 protein 205 g/kg; digestible energy 15.07 kJ/g). Animals were food deprived overnight before  
10 experimentation but water was available at all times.

11 Piglets were anesthetized as previously described (1). Catheters were placed in the right and  
12 left jugular veins for intravenous infusion and blood sampling, respectively. The catheters were  
13 filled with heparinized saline while the intestinal cannulation was done. Insertion of cannulae and  
14 luminal perfusion of 6-cm intestinal segments were done as described previously (1, 2).

### 15 ***Treatment Groups***

16 ***Study 1: Intravenous glucose infusion.*** Piglets were randomly allocated to receive an  
17 intravenous infusion of either glucose or saline (n = 6 / treatment). Piglets were injected with 5  
18 mL of saline or 5 mL of a 75 % glucose solution through the infusion catheter and then infused  
19 with saline or 75% glucose solution at a rate of 2.4 mL/ kg body weight/ h for 75 min. The  
20 amount of glucose infused was chosen to substantially increase plasma glucose levels in order to  
21 test our hypothesis that systemic signals are required to stimulate protein synthesis. A  
22 preliminary experiment, using this rate of glucose infusion, showed a plateau in glucose and

1 insulin concentrations between 40 and 60 min of the start of infusion (data not shown). Two  
2 piglets were studied each day, one infused with glucose and the other with saline.

3 Four jejunal segments within each animal in the glucose or saline infused piglets were  
4 independently but simultaneously perfused for 75 min with either phosphate buffered saline  
5 (PBS: 126 mM NaCl, 14.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 1 mM NaH<sub>2</sub>PO<sub>4</sub>.H<sub>2</sub>O, pH 7.4), or different nutrient  
6 solutions, including (i) 30 mM amino acid mixture + 50 mM glucose, (ii) 30 mM amino acid  
7 mixture, or (iii) 30 mM glutamine. The amino acid mixture, with or without 50 mM glucose, was  
8 chosen to simulate some of the components of a meal, and was formulated based on published  
9 composition of jejunal digesta (3, 14, 16). This mixture contained (mM) aspartate (0.67), serine  
10 (2.03), glutamate (2.34), glutamine (1.84), proline (3.15), glycine (3.75), alanine (2.00), cystine  
11 (0.30), tyrosine (0.79), histidine (0.59), arginine (1.29), asparagine (0.73), threonine (1.34), valine  
12 (1.66), methionine (0.55), isoleucine (1.14), leucine (2.09), phenylalanine (2.00), lysine (1.58),  
13 and tryptophan (0.19). A treatment with glutamine was included because this amino acid is a  
14 preferred fuel of intestinal mucosal cells (28). All perfusates were made iso-osmotic to a level  
15 typical of jejunal digesta in the piglet (300 mosm/L) in a PBS buffer.

16 ***Study 2: Intravenous amino acid infusion.*** This study focused specifically on systemic  
17 and luminal effects of amino acids. Piglets were randomly allocated to receive an intravenous  
18 infusion of either saline or amino acids (see Table 1). The infusion was intended to deliver a bolus  
19 of amino acids approximating 1/3 of daily protein requirements (i.e. a large protein meal). This  
20 was specifically intended to substantially increase plasma amino acid concentrations to test our  
21 hypothesis that systemic signals modulate intestinal protein metabolism. The infusate was based  
22 on the complete amino acid mixture for feeding piglets orally or intravenously used by Bertolo et

1 al. (6) to which we also added glutamine (Table 1). Piglets (n = 6 / treatment) were injected with  
2 5 mL of saline or 5 mL of amino acid solution (119 g/L) (Table 1) through the infusion catheter  
3 and then infused with saline or the amino acid solution at a rate of 7.5 mL / kg body weight/h for  
4 90 min. Two piglets were studied each day, one infused with amino acids and the other with  
5 saline. Within each intravenous infusion group, two intestinal segments within each piglet were  
6 luminally perfused with: (i) phosphate-buffered saline, or (ii) 30 mM amino acid mixture, as  
7 described above. The overall duration of intravenous and luminal treatments was 90 min.

8 ***Measurement of protein synthesis and tissue sampling.*** During the last 15 min of perfusion,  
9 protein synthesis was measured using the luminal flooding dose technique (1). We validated this  
10 experimental system and showed that: 1) the rate of protein synthesis was the same for luminal  
11 and i.v. flooding, 2) the phenylalanine free specific radioactivity was constant over the labeling  
12 period, 3) the tissue free phenylalanine specific radioactivity rapidly rose to a level  
13 indistinguishable from that of the perfusate, and 4) the tissue free specific radioactivity in  
14 adjacent jejunal tissue not perfused with isotope was insignificant. We also showed that luminal  
15 perfusion of 30 mM amino acid mixtures  $\pm$  50 mM glucose did not affect free phenylalanine  
16 specific radioactivity compared with saline perfusion (2).

17 Tissue phenylalanine concentrations and specific activity were determined in every segment  
18 where protein synthesis was determined. Under conditions of luminal and / or intravenous amino  
19 acid perfusion phenylalanine specific activity was not different among treatments and  
20 furthermore not different from that of the perfusate (Table 2).

21 In the present study, intestinal segments were emptied and perfused with the test solutions  
22 containing 2 mM L-[2,6-<sup>3</sup>H]phenylalanine (specific activity = 42 kBq/nmol) for 15 min. In all

1 experiments the final phenylalanine concentration was 2 mM. Segments were then rapidly  
2 removed, emptied, and flushed with three changes of ice-cold saline. Mucosa was scraped on an  
3 ice-cold surface, frozen in liquid N<sub>2</sub> and stored at -80°C until analyzed. Animals were then killed  
4 by cardiac injection of Euthanyl (1 mL/kg body weight; MTC Pharmaceuticals, Cambridge, ON  
5 Canada).

6 Sample processing for the measurement of protein synthesis was as described (1).  
7 Fractional rate of protein synthesis (K<sub>s</sub>), expressed as % per day, was calculated according to  
8 McNurlan and Garlick (18):

$$9 \quad K_s = 100 S_b / S_f t,$$

10 where S<sub>b</sub> is the specific radioactivity (sr) of protein-bound phenylalanine, t is the duration of  
11 isotope perfusion in days, and S<sub>f</sub> is the intracellular free phenylalanine sr in tissue samples.

12 ***Northern hybridization.*** We examined the effects of the various treatments on mRNA levels  
13 of critical components of the ubiquitin-proteasome and the calcium-activated proteolytic  
14 systems. Northern hybridization was done for m-calpain (calcium activated system) and for  
15 ubiquitin, the ubiquitin conjugating enzyme 14-kDa E2, known to be involved in ubiquitin  
16 conjugation of substrates in skeletal muscles prior to proteolysis, and for the C8 and C9 subunits  
17 of the 20S proteasome, the proteolytic core of the 26S proteasome (4). mRNA levels for various  
18 components of proteolytic systems have been used as an indirect approach to study proteolysis  
19 in rodent muscle (27, 31) and intestine (22). In skeletal muscle, these mRNA levels correlate  
20 with rates of protein degradation measured *in vitro* (27, 29). Since there are no *in vivo* techniques  
21 available to measure mucosal proteolysis, the rate of this process in the mucosa remains  
22 unknown. Although relative changes in mRNA do not necessarily imply changes in the levels of

1 proteins encoded by those mRNA, treatment differences may suggest directional changes in  
2 protein degradation as well as indicate possible transcriptional regulation.

3 Total RNA was isolated from mucosal samples with Trizol<sup>TM</sup> Reagent (Life Technologies,  
4 Burlington, ON Canada) according to the manufacturer's instructions. Fifteen micrograms of  
5 total RNA were electrophoresed in 1% agarose-formaldehyde gels containing ethidium bromide  
6 for 5 h at 100V. RNA was checked visually for integrity of 28S and 18S ribosomal RNA and  
7 was transferred to nylon membranes (GeneScreen<sup>TM</sup>, NEN, Boston, MA) by capillary transfer  
8 and cross linked to membranes under ultraviolet light using Stratalinker (Stratagene, La Jolla, CA).  
9 Membranes were hybridized with cDNA probes derived from the genes mentioned above (2, 22).  
10 Because the results from Study 1 showed that mRNA for ubiquitin and 14-kDa E2 were  
11 consistently suppressed by luminal amino acids, in Study 2 hybridization was carried out only  
12 with probes for these two genes. Hybridization signals were quantified with a phosphorimager  
13 (Molecular Dynamics, Sunnyvale, CA). Differences in RNA loading were corrected for by  
14 stripping membranes and reprobing with <sup>32</sup>P-labeled cDNA probe for glyceraldehyde phosphate  
15 dehydrogenase (GAPDH) (22). GAPDH mRNA levels did not differ among treatments ( $P >$   
16 0.05).

17 ***Other analyses.*** Plasma glucose concentration was measured using Glucose (Trinider Kits  
18 (Sigma, St. Louis, MO). Plasma insulin concentration was determined using the Enzymum-T  
19 (Boehringer Mannheim Immunodiagnosics, Laval, PQ Canada). Plasma and tissue amino acid  
20 concentrations were determined using high performance liquid chromatography (1).

21 ***Statistical analyses.*** Data are expressed as means  $\pm$  SE or pooled SE. Data were analyzed  
22 using a two-way ANOVA (SAS, Version 6.02, SAS Institute, Cary, NC) with luminal and

1 intravenous treatments as independent variables. Intravenous treatments served as the blocks.  
2 When no significant interaction between luminal and intravenous treatments was present,  
3 statistical comparisons were only made for main effect parameters (luminal and intravenous  
4 treatments) and data were pooled. Significant differences ( $P < 0.05$ ) between means were  
5 examined using Fisher's Protected Least Significant Difference test.

6

## 7 **RESULTS**

### 8 ***Study 1: Intravenous Glucose Infusion***

9 ***Plasma glucose and insulin levels.*** Pre-infusion plasma glucose and insulin levels did not  
10 differ significantly (glucose:  $6.0 \pm 0.9$  vs.  $5.0 \pm 0.3$  mM; insulin:  $8.5 \pm 0.7$  vs.  $6.5 \pm 1.14$   $\mu$ U/mL,  
11 for glucose- and saline-infused piglets, respectively). Both plasma insulin and glucose levels were  
12 increased by 300% by the end of the infusion ( $P < 0.05$ , Fig. 1). The increase in glucose was  
13 rapid and plasma glucose remained constant during the last 30 min of infusion, whereas the  
14 increase in insulin did not occur until the last 15 min of infusion. As expected, eak plasma  
15 glucose concentrations were higher than in fed piglets ( $\sim 7.5$  mM) (25). Plasma insulin  
16 concentration at this time ( $20 \pm 2$   $\mu$ U/mL) was lower than in fed 4-wk old piglets (36  $\mu$ U/mL)  
17 (8).

18 ***Mucosal protein synthesis.*** Analysis of variance revealed no interactions between the effects  
19 of intravenous and luminal treatments ( $P > 0.05$ ). Pooled data for mucosal protein synthesis in  
20 response to intravenous treatments and in response to luminal treatments are shown in Fig. 2.  
21 Intravenous glucose infusion increased mucosal protein synthesis by 16% ( $P < 0.05$ ,  $n = 6$ ).  
22 Irrespective of intravenous treatments, the different luminal nutrient solutions (30 mM amino

1 acids with or without 50 mM glucose, or 30 mM glutamine) suppressed mucosal protein  
2 synthesis by 10% relative to segments perfused with PBS ( $P < 0.05$ ,  $n = 12$ , Fig. 2). These data  
3 suggest that the systemic supply of glucose increases the rate of mucosal protein synthesis  
4 independent of luminal nutrients.

5 ***mRNA levels for elements of proteolytic systems.*** We measured the mRNA levels of  
6 components of ATP-ubiquitin-proteasome and calcium-activated proteolytic systems. Analysis  
7 of variance revealed no interactions between the effects of intravenous and luminal treatments ( $P$   
8  $> 0.05$ ). Intravenous glucose infusion exerted no effects on the expression of mRNA for m-  
9 calpain, ubiquitin, 14-kDa E2 or proteasome subunits C8 and C9 ( $P > 0.05$ ; data not shown).  
10 Perfusion of intestinal segments (Fig. 3) with 30 mM amino acid mixture or 30 mM glutamine  
11 suppressed mRNA levels of total ubiquitin, the 1.2 kb transcript of 14-kDa E2, and the  
12 proteasome subunit C9 by ~30% ( $P < 0.05$ ) relative to PBS. Although there are two bands in the  
13 14-kDa E2 northern blot, only the 1.2 kDa band was suppressed; this transcript has been shown  
14 to be regulated by different nutritional manipulations in skeletal muscle (29, 30). However, the  
15 suppression of mRNA was not seen in segments perfused with 30 mM amino acids plus glucose.  
16 There were no effects of luminal treatments on mRNA for proteasome subunit C8 or m-calpain  
17 ( $P > 0.05$ , data not shown).

18 ***Plasma and mucosal free amino acid concentrations.*** To examine the possible mechanisms  
19 by which the different treatments regulate mucosal Ks and gene expression, we measured the  
20 effects of the intravenous glucose and luminal treatments on plasma and mucosal tissue free  
21 amino acid concentrations. Intravenous glucose infusion had no effect on plasma amino acid  
22 concentrations compared with saline infused piglets (data not shown). Intravenous glucose

1 infusion had no effect on mucosal free amino acid concentrations compared with saline infused  
2 piglets, therefore these data were pooled with saline infused piglets (Table 3). The effects of  
3 luminal treatments on mucosal free amino acids (Table 3) may be summarized as follows:

- 4 1. when 30 mM amino acids or 30 mM glutamine was luminally perfused, the concentrations of  
5 most amino acids in tissue were significantly elevated in a manner that reflected their  
6 concentrations in the perfusates,
- 7 2. the presence of 50 mM glucose plus 30 mM amino acids in perfusates significantly reduced  
8 intracellular levels of most amino acids when compared with perfusion of 30 mM amino acids  
9 alone, and,
- 10 3. 30 mM glutamine perfusion alone increased concentrations of aspartate, glutamate, arginine,  
11 alanine, and ornithine relative to PBS perfusion.

12 We observed lower mucosal free phenylalanine concentrations in segments perfused with 30 mM  
13 amino acids compared with segments perfused with saline containing 2 mM phenylalanine. This  
14 was likely due to increased competition for amino acid uptake. This however would not be  
15 expected to interfere with the estimation of protein synthesis as free phenylalanine specific  
16 radioactivity was not different among luminal treatments.

### 17 ***Study 2: Intravenous Amino Acid Infusion***

18 ***Plasma and mucosal free amino acid levels.*** With the exception of citrulline and asparagine,  
19 plasma concentrations of all measured amino acids increased significantly ( $P < 0.05$ ) during  
20 infusion of the complete amino acid mixture (Fig. 4). Plasma amino acid levels were on average  
21 about twice higher than in continuously intragastrically fed piglets as expected (6). Intravenous  
22 amino acid infusion resulted in a significant increase in plasma and tissue free glutamine (+29%;  $P$

1 < 0.05); this increase paralleled a 28% increase in concentration of tissue total free amino acids ( $P$   
2 < 0.05; Table 4). Tissue levels of most amino acids were elevated ( $P < 0.05$ ) after intravenous  
3 amino acid infusion compared with saline infusion; only levels of glycine, aspartate and glutamate  
4 did not change (Table 4). Total tissue free amino acids increased by 25% during intravenous  
5 infusion, by 52% during luminal perfusion and by 72% when both intravenous and luminal amino  
6 acids were supplied together.

#### 7 *Mucosal protein synthesis and mRNA levels for ubiquitin-proteasome proteolytic system.*

8 By contrast to intravenous glucose infusion, intravenous amino acid infusion did not affect  
9 mucosal protein synthesis, even though tissue free amino acid levels were significantly increased  
10 by this treatment (Fig. 5). Intravenous and luminal amino acid infusion independently decreased  
11 levels of mRNA encoding ubiquitin and the 1.2 kb transcript of the 14-kDa E2 ( $P < 0.05$ ). When  
12 amino acids were delivered simultaneously through both routes, mRNA level decreased further  
13 (Fig. 6). The decrease in mRNA levels paralleled the increase in total mucosal free amino acid  
14 concentrations (see Fig. 5 and Table 4). Thus, when mucosal amino acids were elevated to the  
15 greatest extent by simultaneous luminal and intravenous amino acid delivery the effect on mRNA  
16 levels was the greatest.

17

## 18 **DISCUSSION**

19 Our experimental system was developed to allow a clear discrimination between the effects  
20 of nutrients applied luminally and intravenously on intestinal mucosal protein metabolism.  
21 Because the size of the perfused segments is small, luminal perfusion is not associated with any  
22 detectable systemic accumulation of perfused substrate (1). This is therefore a unique “first

1 pass” system, that affords the opportunity to identify any direct influence of luminal nutrients  
2 on metabolism.

3 The results of this study point to a fascinating degree of multiplicity in the regulation of  
4 intestinal protein turnover. We demonstrated that the effects of glucose and amino acids on  
5 mucosal protein synthesis and mRNA levels for proteolytic systems were rapid, nutrient-  
6 specific, and depended on route of delivery. We have observed surprisingly few effects of  
7 luminal nutrients, which led us to speculate that the appearance of systemic nutrients could  
8 acutely modulate mucosal protein metabolism. Intravenous glucose stimulated mucosal protein  
9 synthesis. However, when delivered luminally by itself (our previous observations, (Ref (2)) or  
10 with amino acids (this study), glucose had no effect on protein synthesis, suggesting that its  
11 effect might be indirect. An intravenous amino acid mixture, on the other hand, had no effect on  
12 protein synthesis but decreased mRNA levels for components of the ubiquitin-proteasome  
13 system, an effect that was augmented when luminal amino acids were also provided. This  
14 suggests that amino acids may play an important and direct role in reducing mucosal proteolysis.  
15 Collectively, our data indicate a cooperative role for glucose and amino acids in regulating  
16 intestinal protein balance.

17 ***Regulation of intestinal mucosal protein turnover by glucose.*** The stimulatory effect of  
18 feeding on tissue (such as skeletal muscle) and whole body protein mass in young growing  
19 animals is well documented (8, 12). However the precise role of nutrients in regulating intestinal  
20 protein synthesis and catabolism is both complex and poorly understood. Our novel finding that  
21 luminal energy fuels or amino acids did not reproduce the effect of oral feeding on protein  
22 synthesis (2), led us to examine whether additional (systemic) factors are needed to reproduce the

1 feeding-induced stimulation of protein synthesis. Our observation that intravenous glucose  
2 infusion increased protein synthesis, whether or not nutrients were present in the lumen, is  
3 consistent with the effects of feeding on protein synthesis, and with the known anabolic effects  
4 of glucose in other tissues (19, 24). Given that feeding is associated with an increase in mucosal  
5 protein synthesis of ~18% (8), this study suggests that the elevated systemic level of glucose is a  
6 major contributor to augmented mucosal protein synthesis and therefore the anabolic effect of  
7 feeding.

8       The systemic effects of glucose may be mediated by the increased plasma insulin levels in  
9 the glucose infused piglets, a hypothesis consistent with increased jejunal protein synthesis and  
10 plasma insulin levels observed during refeeding (8). The regulation of intestinal protein  
11 metabolism via systemic nutrients is complex. By contrast to the highly controlled conditions of  
12 luminal exposure, intravenous infusion of nutrients unleash a host of metabolic changes, including  
13 secretion of hormones and growth factors, and changes in substrate concentrations secondary to  
14 their appearance making it difficult to explain how systemic nutrients precisely regulate intestinal  
15 protein metabolism. Experiments specifically designed to manipulate hormone and substrate  
16 concentrations (i.e. clamp experiments) could be employed to resolve the identity of the  
17 individual factors that may act systemically. Using clamp experiments, elevation of plasma  
18 insulin concentrations, in the absence of increased plasma glucose or amino acid concentrations,  
19 failed to affect protein synthesis in fasted piglets (9, 10), suggesting the effect of systemic  
20 glucose is not mediated by insulin and / or that the presence of both glucose and insulin are  
21 required. IGF-1 also failed to stimulate jejunal protein synthesis under these conditions (11).

1 Further research is required to ascertain the mechanism by which systemic glucose increases  
2 mucosal protein synthesis.

3 The contribution of proteolysis to intestinal protein balance has not been extensively  
4 studied. However, two recent studies examined the effect of somatotropin or GLP-2 on  
5 intestinal protein metabolism and concluded that proteolysis may be a major determinant of  
6 intestinal protein balance, because protein balance changed without alteration in the rates of  
7 protein synthesis (7, 26). In our investigation, the intravenous supply of glucose had no effect  
8 on mucosal mRNA levels for proteolytic systems, suggesting that glucose (and / or the associated  
9 elevated plasma insulin and hormone concentrations) may not influence mucosal proteolysis.  
10 Larbaud et al. (15) showed that a 6 h infusion of insulin had no effect on the mRNA levels for  
11 various proteolytic systems in caprine jejunum; this is consistent with our results.

12 ***Regulation of intestinal mucosal protein turnover by amino acids.*** Amino acids may also  
13 be mediators of the feeding-induced up-regulation of protein synthesis and net anabolism in the  
14 intestine, because plasma and tissue amino acid levels are increased with feeding (8, 19, 22).  
15 Intravenous amino acid infusion did not stimulate mucosal protein synthesis. This lack of effect  
16 was not due to sub-optimal levels of systemic amino acids because the plasma concentrations  
17 achieved exceeded those seen after intragastric feeding (6). Davis et al. (10) showed that i.v.  
18 infusion of amino acids (euinsulinemic) or amino acids plus insulin failed to stimulate jejunal  
19 protein synthesis in euglycemic fasted piglets. Intravenous infused glutamine also does not  
20 stimulate intestinal protein synthesis in dogs (17). These results are consistent with ours.  
21 However, luminal provision of amino acids modestly but consistently reduced protein synthesis  
22 (2). This result was surprising. This was not due to methodological problems because we

1 extensively validated our experimental system (1). In the present experiment, the intracellular  
2 phenylalanine free specific radioactivity was not different among all luminal and systemic  
3 nutrient treatments including saline and was indistinguishable from that of the perfusate. The  
4 circumstances under which decreased protein synthesis was observed, is a model “first pass”  
5 system, where luminal influences can be studied without systemic alterations in concentrations of  
6 the same nutrients. Consequently, it is impossible to compare our results with any previous  
7 studies, since observations of this kind have never been made before. This may not correspond  
8 directly to any specific event in the cycles of feeding and fasting other than the first few  
9 moments of refeeding. Regardless, amino acids do not directly stimulate mucosal protein  
10 synthesis.

11 Irrespective of the route of delivery, amino acids were effective in suppressing mRNA levels  
12 for the ubiquitin-proteasome proteolytic system - the effect being maximal when amino acids  
13 were given both intravenously and luminally. This effect is not due to a non-specific regulation  
14 of gene expression, as we did not observe any changes in mRNA levels of GAPDH in response  
15 to luminal amino acid perfusion. The ability of luminal amino acids to decrease gene expression  
16 implies a direct mechanism, since our perfusion is without apparent systemic accumulation of  
17 free amino acids or changes in hormone levels. Furthermore, the effect of amino acids on gene  
18 expression could not be ascribed to any one single amino acid. A consistent observation was that  
19 the higher the concentration of mucosal intracellular amino acids, the lower the mRNA levels.  
20 This may explain why TPN fails to maintain intestinal protein balance because TPN is associated  
21 with modest rises in plasma and tissue free amino acid concentrations compared with oral feeding  
22 (5, 6). If protease gene expression in mucosa is a function of amino acid concentration achieved

1 in the tissue, it may be that intravenous feeding simply cannot sufficiently raise amino acid  
2 concentrations to suppress the expression of genes involved in degradative processes.

3 The luminal co-perfusion of glucose and amino acids blunted the effects of amino acids on  
4 the expression of components of the ubiquitin-proteasome proteolytic system. Total mucosal  
5 free amino acid concentrations were significantly lower in intestinal segments perfused with a  
6 mixture of both amino acids and glucose, compared with segments perfused with the amino acid  
7 mixture alone. This is consistent with our supposition that high mucosal amino acid  
8 concentrations are required to decrease protease gene expression. The reduced intracellular amino  
9 acid levels in intestinal segments perfused with both glucose and amino acids may be due to a  
10 reduced amino acid uptake. This hypothesis is supported by the fact that 1) the mucosal  
11 intracellular concentrations of citrulline and ornithine, amino acids not included in the perfusates  
12 but which the intestine can synthesize (28), were not affected and 2) the presence of luminal  
13 sugars may limit the inward transport of amino acids (21). Taken together, the coordinated  
14 suppression of mRNA of components of ubiquitin-proteasome proteolytic pathway by  
15 systemic and luminal amino acids suggests that amino acids, irrespective of route of delivery,  
16 may directly decrease gene expression and play a role in controlling intestinal protein mass.

17 **Significance.** The regulation of jejunal protein synthesis and gene expression depends on  
18 direct effects of nutrients and on systemic signals reflecting the overall nutritional status. In the  
19 fed state, with elevated circulating nutrients, hormones and growth factors, the intestine is  
20 capable of responding to systemic anabolic stimuli; systemic glucose may initiate this response.  
21 Amino acids appear to directly reduce expression of genes connected with proteolysis. The  
22 observed changed in gene expression, if accompanied by alterations in protein degradation of a

1 similar magnitude, may be of considerable physiologic importance in regulation of gut protein  
2 mass. A disruption in the mechanism by which nutrients regulate protein synthesis and  
3 catabolism could explain the wasting observed in the small intestine during many disease states  
4 and with intravenous feeding.

1 **FOOTNOTES**

2

3 Address for reprint requests and other correspondence: V.E. Baracos, Department of  
4 Agricultural, Food and Nutritional Science, 4-10 Agriculture-Forestry Centre, University of  
5 Alberta, Edmonton, Alberta T6G 2P5, Canada (E-mail: [vickie.baracos@ualberta.ca](mailto:vickie.baracos@ualberta.ca)).

6

7 Present address for O.A.J. Adegoke: Polypeptide Hormone Laboratory, Department of  
8 Medicine, McGill University, 3640 University Street, Montreal, Quebec H3A 2B2, Canada.

9

10 Present address for M.I. McBurney: W. K. Kellogg Institute for Food and Nutrition Research, 2  
11 Hamblin Avenue East, Battle Creek MI 49016-3232.

## 1 ACKNOWLEDGMENTS

2

3 The authors are grateful to Dr. Chantal Farges for technical assistance and to Ms. Jody Aldrich,  
4 Ms. Abha Dunichand-Hoedl and staff of Edmonton Research Station, Metabolic Swine Unit, for  
5 animal care and surgical assistance. We also thank Dr. Simon Wing (McGill University,  
6 Montreal, Canada) for generous provision of plasmids containing cDNA sequences encoding rat  
7 14-kDa E2 ubiquitin conjugating enzyme and to Dr. Keiji Tanaka (Tokyo Institute for Medical  
8 Research, Tokyo, Japan) for the gift of plasmids containing the cDNA sequence of the rat C8 and  
9 C9 proteasome subunits. We are grateful to Dr. Robert Hardin for valuable advice regarding  
10 statistical analysis. This research was supported by grants from the Alberta Agricultural  
11 Research Institute to VEB and from the Natural Sciences and Engineering Research Council of  
12 Canada to VEB and to SES.

1   **REFERENCES**

- 2   1   **Adegoke OAJ, McBurney MI, and Baracos VE.** Jejunal mucosal protein synthesis:  
3       validation of luminal flooding dose method and effect of luminal osmolarity. *Am J Physiol*  
4       *Gastrointest Liver Physiol* 276: G14-G20, 1999.
- 5   2.   **Adegoke OAJ, McBurney MI, Samuels SE, and Baracos VE.** Luminal amino acids  
6       acutely decrease intestinal mucosal protein synthesis and protease mRNA in piglets. *J Nutr*  
7       129: 1871-1878, 1999.
- 8   3.   **Adibi SA, and Mercer D.** Protein digestion in human intestine as reflected in luminal,  
9       mucosal and plasma amino acid concentrations after meals. *J Clin Invest* 52: 1586-1594,  
10      1973.
- 11  4.   **Attaix D, Aurousseau E, Combaret L, Kee A, Larbaud D, Ralliere C, Souweine B,**  
12      **Taillandier D, and Tilignac T.** Ubiquitin-proteasome-dependent proteolysis in skeletal  
13      muscle. *Reprod Nutr Dev* 38: 153-165, 1998.
- 14  5.   **Bertolo RF, Chen CH, Pencharz PB, and Ball RO.** Intestinal atrophy has a greater  
15      impact on nitrogen metabolism than liver by-pass in piglets fed identical diets via gastric,  
16      central venous or portal venous routes. *J Nutr* 129: 1045-52, 1999.
- 17  6.   **Bertolo RFP, Pencharz PB, and Ball RO.** Organ and plasma amino acid concentrations are  
18      profoundly different in piglets fed identical diets via gastric, central venous or portal venous  
19      routes. *J Nutr* 130: 1261-1266, 2000.
- 20  7.   **Burrin DG, Stoll B, Jiang R, Petersen Y, Elnif J, Buddington RK, Schmidt M, Holst**  
21      **JJ, Hartmann B, and Sangild PT.** GLP-2 stimulates intestinal growth in premature TPN-

- 1 fed pigs by suppressing proteolysis and apoptosis. *Am J Physiol Gastrointest Liver Physiol*  
2 279: G1249-G1256, 2000.
- 3 8. **Davis TA, Burrin DG, Fiorotto ML, and Nguyen HV.** Protein synthesis in skeletal muscle  
4 and jejunum is more responsive to feeding in 7- than in 26-day-old pigs. *Am J Physiol*  
5 *Endocrinol Metab* 270: E802-E809, 1996.
- 6 9. **Davis TA, Fiorotto ML, Beckett PR, Burrin DG, Reeds PJ, Wray-Cahen D, and**  
7 **Nguyen HV.** Differential effects of insulin on peripheral and visceral tissue protein  
8 synthesis in neonatal pigs. *Am J Physiol Endocrinol Metab* 280: E770-E779, 2001.
- 9 10. **Davis TA, Fiorotto ML, Burrin DG, Reeds PJ, Nguyen HV, Beckett PR, Vann RC, and**  
10 **O'Connor PMJ.** Stimulation of protein synthesis by both insulin and amino acids is unique  
11 to skeletal muscle in neonatal pigs. *Am J Physiol Endocrinol Metab* 282: E880-E890, 2002.
- 12 11. **Davis TA, Fiorotto ML, Burrin DG, Vann RC, Reeds PJ, Nguyen HV, Beckett PR, and**  
13 **Bush JA.** Acute IGF-1 infusion stimulates protein synthesis in skeletal muscle and other  
14 tissues of neonatal pigs. *Am J Physiol Endocrinol Metab* 283: E638-E647, 2002.
- 15 12. **Davis TA, Fiorotto ML, Nguyen HV, and Reeds PJ.** Enhanced response of muscle protein  
16 synthesis and plasma insulin to food intake in suckled rats. *Am J Physiol Reg Integ Compar*  
17 265: R334-R340, 1993.
- 18 13. **Dudley MA, Wykes LJ, Dudley Jr. AW, Burrin DG, Nichols BL, Rosenberger J,**  
19 **Jahoor F, Heird WC, and Reeds PJ.** Parenteral nutrition selectively decreases protein  
20 synthesis in the small intestine. *Am J Physiol Gastrointest Liver Physiol* 274: G131-G137,  
21 1998.

- 1 14. **Ferraris RP, Yasharpour S, Lloyd KC, Mirzayan R, and Diamond JM.** Luminal glucose  
2 concentrations in the gut under normal conditions. *Am J Physiol Gastrointest Liver Physiol*  
3 259: G822-G837, 1990.
- 4 15. **Larbaud D, Debras E, Taillandier D, Samuels SE, Temparis S, Champredon C,**  
5 **Grizard J, and Attaix D.** Euglycemic hyperinsulinemia and hyperaminoacidemia decrease  
6 skeletal muscle ubiquitin mRNA in goats. *Am J Physiol Endocrinol Metab* 271: E505-E512,  
7 1996.
- 8 16. **Low GA.** Studies on digestion and absorption in the intestines of growing pigs 6.  
9 Measurements of the flow of amino acids. *Br J Nutr* 41: 147-156, 1979.
- 10 17. **Marchini JS, Nguyen P, Deschamps J-Y, Maugère P, Krempf M, and Darmaun D.**  
11 Effect of intravenous glutamine on duodenal mucosa protein synthesis in healthy growing  
12 dogs. *Am J Physiol Endocrinol Metab* 276: E747-E753, 1999.
- 13 18. **McNurlan MA, and Garlick PJ.** The contribution of rat liver and gastrointestinal tract to  
14 whole-body protein synthesis in the rat. *Biochem J* 186: 381-383, 1980.
- 15 19. **Moldawer LL, O'Keefe JD, Bothe Jr A, Bistrain BR, and Blackburn GL.** In vivo  
16 demonstration of nitrogen-sparing mechanisms for glucose and amino acids in the injured rat.  
17 *Metabolism* 29: 173-180, 1980.
- 18 20. **O'Dwyer ST, Smith RJ, Hwang TL, and Wilmore DW.** Maintenance of small bowel  
19 mucosa with glutamine-enriched parenteral nutrition. *J Parent Enteral Nutr* 13: 579-585,  
20 1989.

- 1 21. **Rerat A, Simoes-Nunes C, Mendy F, Vaissade P, and Vaugelade P.** Splanchnic fluxes of  
2 amino acids after duodenal infusion of carbohydrate solutions containing free amino acids or  
3 oligopeptides in the non-anaesthetized pig. *Br J Nutr* 68: 111-138, 1992.
- 4 22. **Samuels SE, Taillandier D, Aurousseau E, Cherel Y, Le Maho Y, Arnal M, and**  
5 **Attaix D.** Gastrointestinal protein synthesis and mRNA levels of proteolytic systems in  
6 adult fasted rats. *Am J Physiol Endocrinol Metab* 271: E232-E238, 1996.
- 7 23. **Stein TP, Yoshida S, Schluter MD, Drews D, Assimon SA, and Leskiw MJ.**  
8 Comparison of intravenous nutrients on gut mucosal protein synthesis. *J Parent Enteral*  
9 *Nutr* 18: 447-452, 1994.
- 10 24. **Tessari P, Barazzoni R, Zanetti M, Kiwanuka E, and Tiengo A.** The role of substrates  
11 in the regulation of protein metabolism. *Bailliere's Clin Endocrinol Metab* 10: 511-532,  
12 1996.
- 13 25. **Tumbleson ME, and Schmidt DA.** Swine clinical chemistry *In Swine in Biomedical Research*  
14 (MT Tumbleson, ed), Plenum Press, New York, 1986,
- 15 26. **Vann RC, Nguyen HV, Reeds PJ, Burrin DG, Fiorotto ML, Steele NC, Deayer DR,**  
16 **and Davis TA.** Somatotropin increases protein balance by lowering body protein  
17 degradation in fed, growing pigs. *Am J Physio. Endocrino. Metab* 278: E477-E483, 2000.
- 18 27. **Voisin L, Breuille D, Combaret L, Pouyet C, Taillandier D, Aurousseau E, Obled C,**  
19 **and Attaix D.** Muscle wasting in a rat model of long lasting sepsis results from the  
20 activation of lysosomal , Ca<sup>2+</sup>-activated and ubiquitin-proteasome proteolytic pathways. *J*  
21 *Clin Invest* 97: 1610-1617, 1996.

- 1 28. **Windmueller HG.** Glutamine utilization by the small intestine. *Adv Enzymol Relat Areas*  
2 *Mol Biol* 201-237, 1982.
- 3 29. **Wing SS, and Banville D.** 14-kDa ubiquitin conjugating enzyme: structure of the rat gene  
4 and regulation upon fasting and by insulin. *Am J Physiol Endocrinol Metab* 267: E39-E48,  
5 1994.
- 6 30. **Wing SS, and Bedard N.** Insulin-like growth factor I stimulates degradation of a mRNA  
7 transcript encoding the 14 kDa ubiquitin conjugating enzyme. *Biochem J* 319: 455-461, 1996
- 8 31. **Wing SS, and Goldberg AL.** Glucocorticoids activate the ATP-ubiquitin-dependent  
9 proteolytic system in skeletal muscle during fasting. *Am J Physiol Endocrinol Metab* 264:  
10 E668-E676, 1993.

## 1 **FIGURE LEGENDS**

2 **Fig. 1.** Intravenous glucose infusion increased plasma glucose and insulin concentrations. Piglets  
3 were intravenously infused with a 75% glucose solution (2.4 mL/kg body weight/h, n = 6) or  
4 saline for 90 min. Blood samples collected before and at 15-min intervals during infusion were  
5 analyzed. Glucose concentrations with different letters are significantly different from one  
6 another ( $P < 0.05$ ). \*insulin concentration at this time point is significantly different from the  
7 pre-infusion value ( $P < 0.05$ ).

8  
9 **Fig. 2.** Intravenous glucose infusion increases mucosal fractional rate of protein synthesis  
10 independent of the effects of luminal treatments. Piglets were intravenously infused with a 75%  
11 glucose solution (2.4 mL/kg body weight/h or saline for 75 min. Within each infusion group, one  
12 of four intestinal segments within each piglet was perfused with phosphate buffered saline (PBS,  
13 pH 7.4), 30 mM amino acid (AA) mixture (with or without 50 mM glucose), or 30 mM  
14 glutamine for 75 min. Protein synthesis was then measured in the last 15 min of perfusion.  
15 Glucose infusion increased mucosal Ks ( $P < 0.05$ , n = 6), independent of intravenous treatments.  
16 Luminal nutrients modestly but significantly suppressed mucosal protein synthesis relative to  
17 control PBS, independent of glucose infusions ( $P < 0.05$ , n = 12).

18  
19 **Fig. 3.** Luminal amino acids decrease mRNA levels for components of the ubiquitin-proteasome  
20 proteolytic system. Treatments were as described in the legend to Fig. 2. Northern  
21 hybridizations were carried out for ubiquitin, 14-kDa ubiquitin conjugating enzyme and, C9  
22 subunit of the proteasome. Data are expressed as arbitrary units after phosphorimage analysis.

1 \*significant decrease in mRNA levels after luminal treatments with amino acids or glutamine  
2 alone ( $P < 0.05$ ;  $n = 12$ ).

3  
4 **Fig. 4.** Intravenous amino acid infusion raises plasma concentrations of amino acids. Piglets  
5 were infused intravenously with saline or a mixture of amino acids (Table 1) for 90 min. Except  
6 for asn and cit, i.v. amino acid infusion increased the plasma concentrations of all amino acids ( $P$   
7  $< 0.05$ ;  $n = 6$ ).

8  
9 **Fig 5.** Intravenous amino acids raise tissue amino acid levels but do not activate protein  
10 synthesis. Piglets were intravenously infused with saline or a complete amino acid mixture ( $n =$   
11 6). Data are means  $\pm$  SE. Within each infusion group, intestinal segments were perfused  
12 luminally with a complete amino acid mixture (Table 1) or PBS. Mucosa samples harvested from  
13 perfused segments were used to determine protein synthesis (Ks) and free amino acid  
14 concentrations. \*significant increase in amino acid levels ( $P < 0.05$ ;  $n = 6$ ).

15  
16 **Fig 6.** Luminal and systemic amino acids additively suppressed the expression of ubiquitin and  
17 14-kDa E2 in a manner dependent on the total tissue free amino acids. Piglets were intravenously  
18 infused with saline or a complete amino acid mixture ( $n = 6$ ). Data are means  $\pm$  SE. Within each  
19 infusion group, intestinal segments were perfused luminally with a complete 30 mM amino acid  
20 mixture or PBS as described under Materials and Methods. RNA from mucosa samples was  
21 analyzed for the expression of ubiquitin and the 14-kDa E2. The bottom panel shows the  
22 changes in intracellular total free amino acids. <sup>a,b,c</sup>different alphabetic superscripts denote  
23 significant differences ( $P < 0.05$ ;  $n = 6$ ).

1 Table 1. *Amino acid composition of intravenous infusate*

<b>Amino acid</b>	<b>mg / g total amino acids</b>
Histidine	29
Methionine	18
Tryptophan	20
Phenylalanine	46
Valine	49
Isoleucine	43
Leucine	97
Threonine	49
Lysine	96
Aspartate	56
Glutamate	97
Cysteine	14
Proline	77
Serine	30
Glutamine	97
Glycine	7
Arginine	74
Taurine	4
Alanine	99
Tyrosine	4
Ornithine	-
Citrulline	-
<b>Total</b>	<b>1000</b>

- 1 The amino acid solution was prepared in sterile PBS and contained 119 g total amino acids/L and
- 2 infused at a rate of 7.5 mL/kg body weight/h. The mixture was based on a formula described by
- 3 Bertolo et al. (8), except that glutamine was also added at the level indicated.

1 Table 2 *Mucosal free phenylalanine specific radioactivity after intravenous and luminal amino*  
 2 *acid treatments.*

3

<b>Intravenous treatment</b>	<b>Saline</b>	<b>Saline</b>	<b>Amino acids</b>	<b>Amino acids</b>
<b>Luminal treatment</b>	<b>Saline</b>	<b>Amino acids</b>	<b>Saline</b>	<b>Amino acids</b>
Phe specific radioactivity (dpm/nmol)	625 ± 14	617 ± 33	581 ± 11	577 ± 35
% of perfusate specific activity	100 ± 2.3	98.8 ± 5.3	93.0 ± 1.8	92.3 ± 5.6

4  
 5 Piglets were infused intravenously with saline (n = 6) or an amino acid solution (n = 5). Two  
 6 intestinal segments within each piglet were perfused with phosphate buffered saline or with a 30  
 7 mM amino acid solution as described in Materials and Methods. During the last 13 minutes of  
 8 the experiment <sup>3</sup>H-phenylalanine was added to the luminal perfusates in all treatments. Luminal  
 9 perfusates and mucosal samples from perfused segments were analyzed for specific  
 10 radioactivity.

1 Table 3. *Effects of luminal nutrient treatments on tissue free amino acid concentrations in jejunal*  
 2 *mucosa tissue*

	PBS	Amino Acids plus glucose	Amino Acids	Glutamine	Pooled SE
Methionine	28*	107†	160‡	35*	6
Tryptophan	13*	22†	29‡	17*	1
Phenylalanine	597*	221†	305‡	238†	21
Valine	204*	434†	618‡	217*	28
Isoleucine	108*	267†	397‡	115*	18
Leucine	96*	293†	434‡	103*	17
Threonine	235*	288*	367*	207*	57
Lysine	32*	65†	69†	41*	4
<b>Total EAA</b>	<b>1319*</b>	<b>1717†</b>	<b>2405‡</b>	<b>952*</b>	<b>126</b>
Aspartate	392*	495*	731†	1136‡	54
Glutamate	1849*	2218*†	2512†	4372‡	200
Asparagine	78*	332†	558‡	108*	28
Serine	185*	842†	1355‡	208*	54
Glutamine	310*	809*	569*	10548†	644
Glycine	1884*	3290†	3832†	1528*	227
Citrulline	184*	288*†	394†	279*†	48
Arginine	126*	357†	521‡	207§	24
Taurine	3274*	2179†	2235†	1957†	269
Alanine	475*	1114†	1497‡	1096†	78
Tyrosine	65*	153†	226‡	94§	9
Ornithine	3*	4†	5†	4†	0.3
<b>Total NEAA</b>	<b>8824*</b>	<b>12082†</b>	<b>15679‡</b>	<b>22273§</b>	<b>1031</b>

---

<b>Total</b>	<b>10183*</b>	<b>13840†</b>	<b>18317‡</b>	<b>23317§</b>	<b>1069</b>
--------------	---------------	---------------	---------------	---------------	-------------

---

1  
2 Values are in nmol/g wet mucosa. Piglets were perfused intravenously with saline or glucose.  
3 Within each piglet, intestinal segments were perfused with phosphate buffered saline (PBS, pH  
4 7.4), or 30 mM amino acid mixture (with or without 50 mM glucose), or 30 mM glutamine for 75  
5 min as described in Materials and Methods. Since there were no differences between intravenous  
6 treatments, data for the two infusion groups were pooled. Mucosal samples from perfused  
7 segments were analyzed for amino acids. \*†‡§Values within a row with different symbols differ  
8 from one another ( $P < 0.05$ ,  $n = 6$ ). EAA, essential amino acids; NEAA, non-essential amino  
9 acids.

1 Table 4. *Intravenous and luminal amino acid perfusions raise free amino acid concentrations in*  
 2 *jejunal mucosa*

Amino acid	Intravenous saline		Intravenous amino acids		Pooled SE
	Luminal saline	Luminal AA	Luminal saline	Luminal AA	
Methionine	24*	113†	76‡	174§	12
Tryptophan	25*	41†	66.5‡	91§	6
Phenylalanine	682*	401†	1010‡	549*	60
Valine	244*	540†	500†	823‡	45
Isoleucine	108*	317†	282†	493‡	30
Leucine	129*	498†	528†	911‡	60
Threonine	309*	538†	497‡	731§	32
Lysine	99*	226†	332	510	34
Aspartate	846	1010	794	1008	40
Glutamate	2193	2300	2211	2280	58
Asparagine	66.22*	318†	50*	250†	28
Serine	210*	975‡	298†	903 ‡	71
Glutamine	350*	523‡	451†	680§	29
Glycine	2319*	4186‡	2043*	3598†	198
Citrulline	206*	409†	372†	483‡	24
Arginine	129*	353‡	293†	540	33
Taurine	1775*	1500†	1862*	1653†	58
Alanine	608*	1369†	1227†	1864‡	103
Tyrosine	106*	253‡	131†	278‡	17
Histidine	48*	119†	112†	256‡	20
Ornithine	88*	111†	103†	162‡	8

---

<b>Total</b>	<b>10555*</b>	<b>16095‡</b>	<b>13237†</b>	<b>18225</b>	<b>666</b>
--------------	---------------	---------------	---------------	--------------	------------

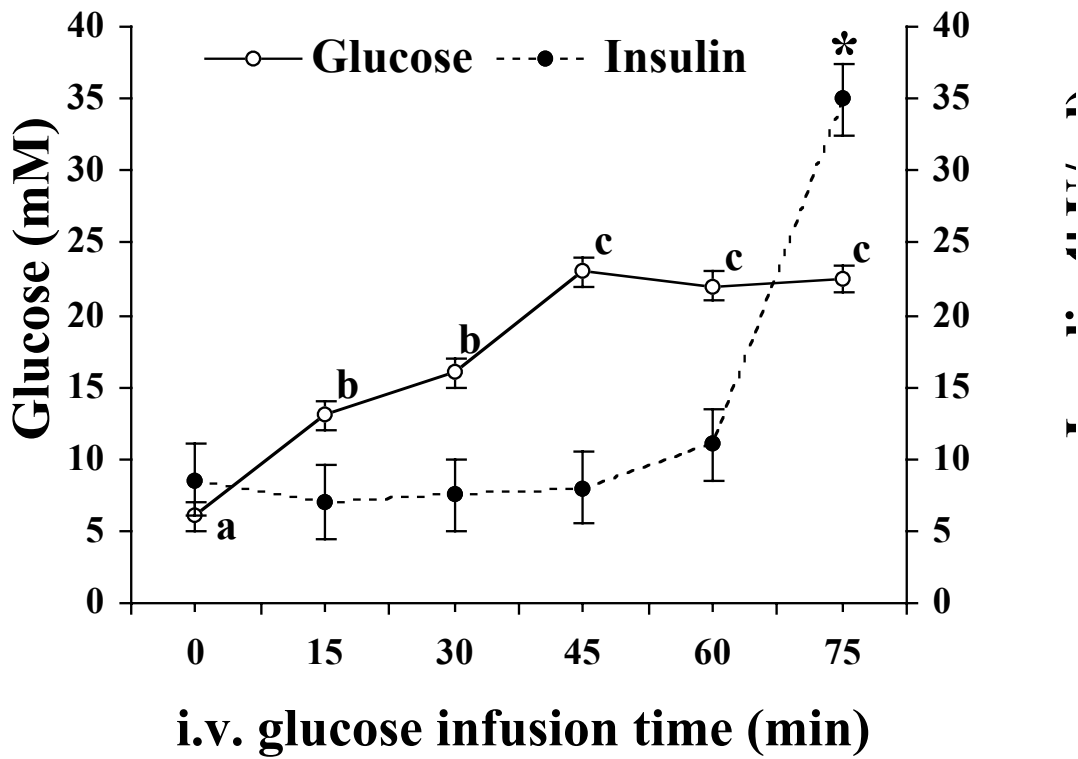
---

1

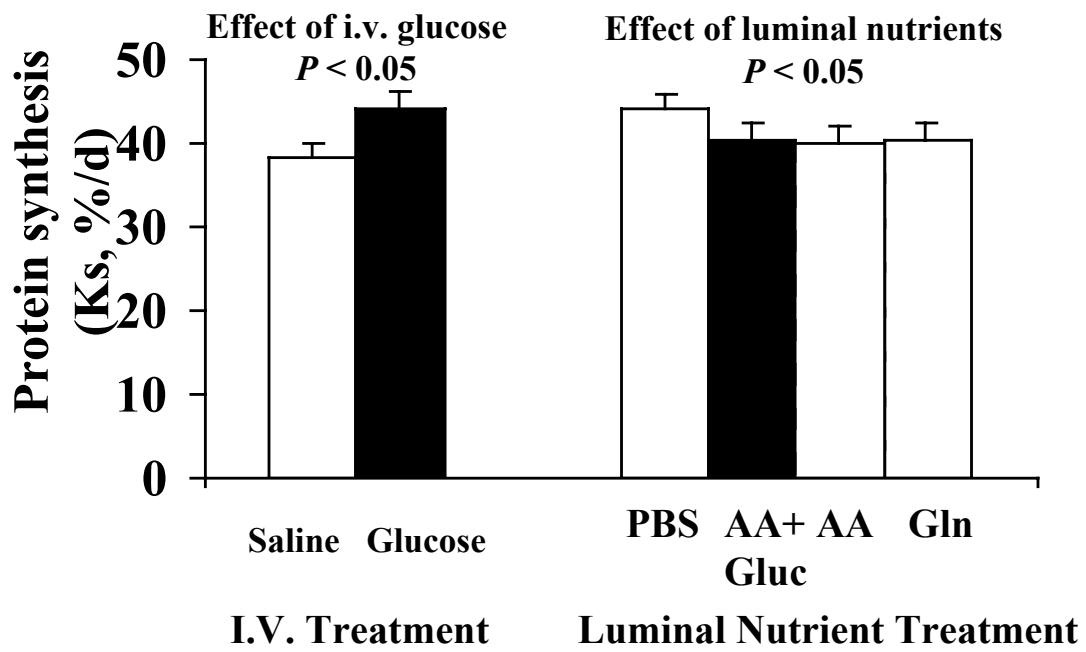
2 Values are in nmol/g wet mucosa. Piglets were infused intravenously with saline (n = 6) or an  
3 amino acid solution (n = 5) (Table 1). Two intestinal segments within each piglet were perfused  
4 with phosphate buffered saline or with a 30 mM amino acid solution as described in Materials  
5 and Methods. Mucosal samples from perfused segments were analyzed for amino acids.

6 \*†‡§ Values within a row with different symbols differ from one another ( $P < 0.05$ ).

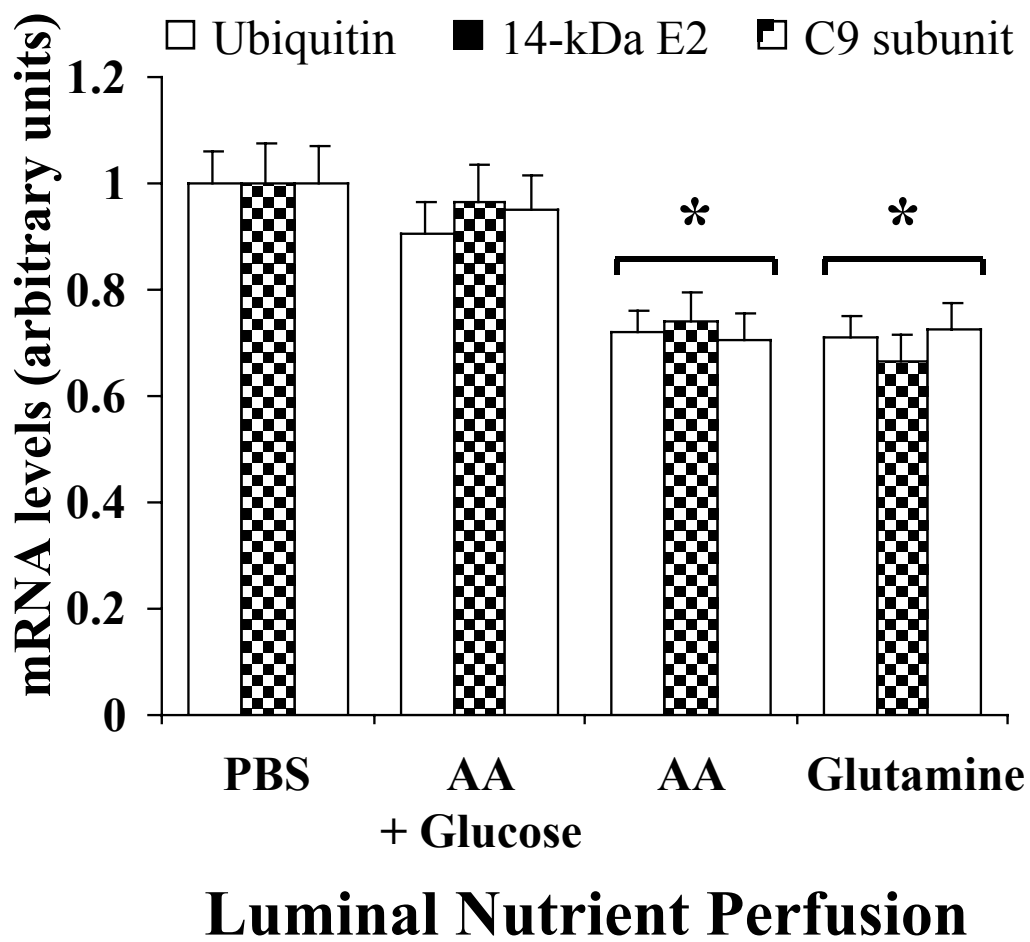
1 Fig. 1



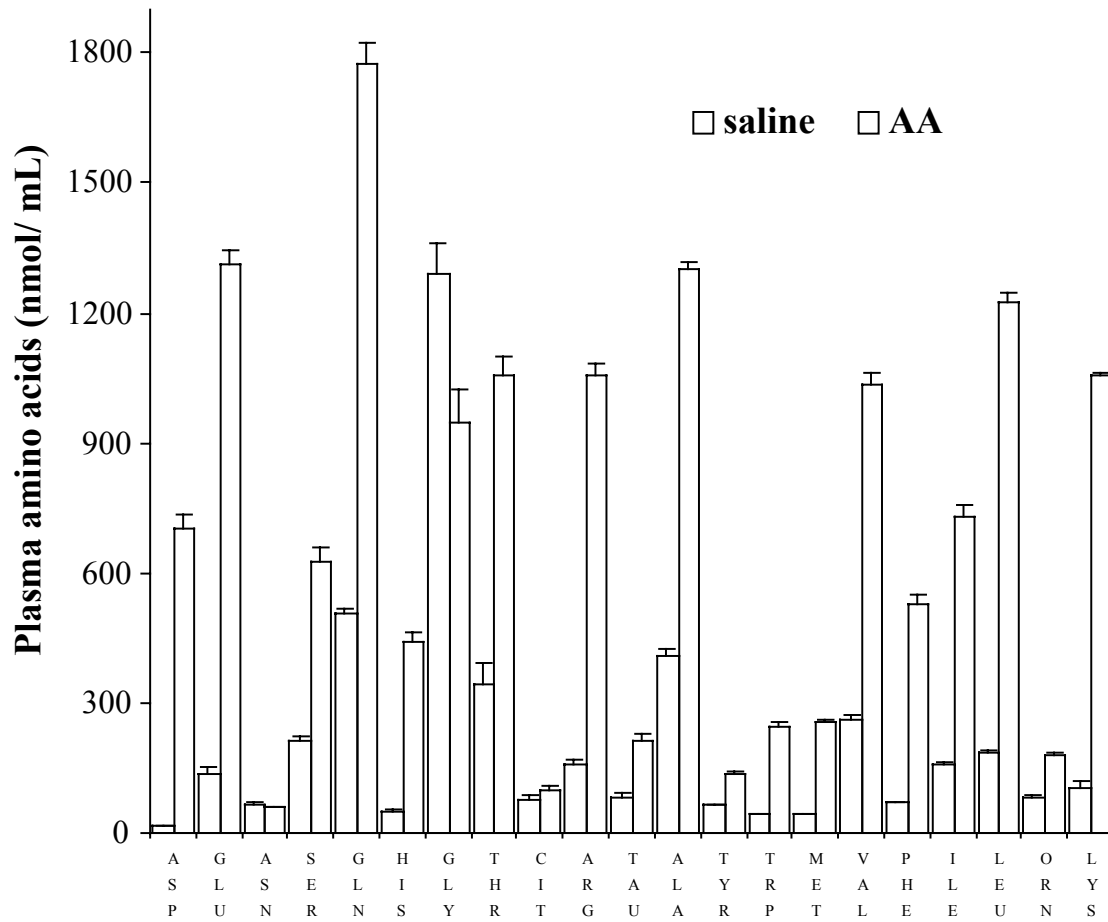
1 Fig. 2



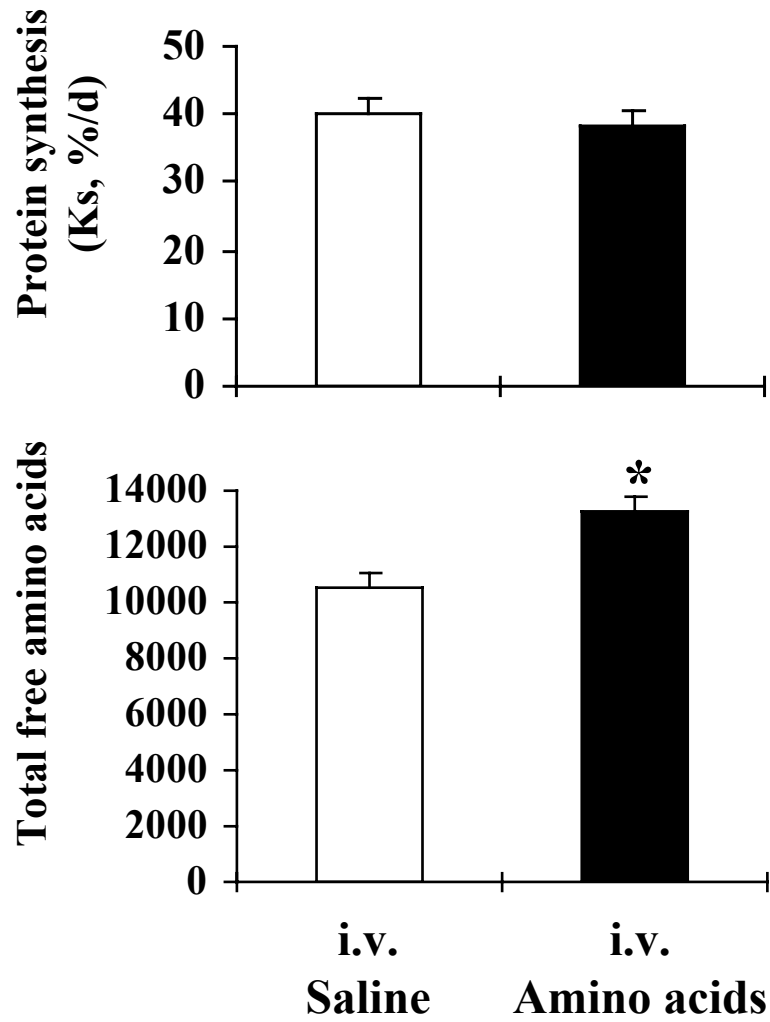
1 Fig. 3



1 Fig. 4



1 Fig. 5



1 Fig. 6

