Short-Term Ingestion of a High Protein Diet Increases Liver and Kidney Mass and Protein Accretion but not Cellularity in Young Pigs (43598)

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Abstract. **Increased visceral organ mass raises the energy cost of maintenance in animals. To determine the nutritional factors that affect organ size during growth and development, we studied 12 genetically obese 4-week-old pigs for 14 days. The piglets had free access to either a control (17% protein) or a high protein (34%) diet. They were sacrificed after 14 days and their empty gastrointestinal tracts, livers, and kidneys were weighed and samples were analyzed for protein and DNA concentrations. The absolute and relative (percentage of body weight) weights of liver and kidneys were greater in high protein than control piglets: liver (313 vs 246 g, SD = 24, $P < 0.09$; 3.61% vs 3.18%, SD = 0.04, $P < 0.01$); kidneys (57 vs 41 g, SD = 4, $P < 0.04$; 0.66% vs 0.55%, SD = 0.02, $P < 0.01$). Protein content was greater in high protein than control pigs in both liver (48.2 vs 34.0 g, SD = 3.4, $P < 0.03$) and kidneys (6.0 vs 4.6 g, SD = 0.5, $P < 0.06$). Liver and kidney total DNA were unaffected by diet in both groups. The protein to DNA ratio was greater in high protein than control pigs in both liver (45.4 vs 39.0, SD = 0.6, $P < 0.01$) and kidneys (26.6 vs 24.9, SD = 0.4, $P < 0.02$). We conclude that when weaned pigs have free access to a high protein diet (2 × requirement) for 2 weeks, liver and kidney protein accretion increases, suggesting cell hypertrophy, with no clear evidence of cell hyperplasia.** [P.S.E.B.M. 1993, Vol 203]

The ingestion of high protein diets by growing animals increases the mass of visceral organs, including the gastrointestinal tract, liver, and kidneys (1–3). The high correlation between visceral organ mass and fasting heat production in growing animals (4–8) and human infants (9, 10) has important implications for dietary conditions that induce increased mass of these organs. Visceral organ mass, which contributes less than 10% of total body mass, accounts for more than 20% of body heat production in growing animals (11–13). The mechanism of action of the observed increase in liver and kidney mass in response to high dietary protein is not clearly understood, but we hypothesize that it would be due to an increase in total protein mass.

The objectives of our study were to determine the relative response of the gastrointestinal tract organs, liver, and kidneys to the short-term *ad libitum* ingestion by the piglet of a diet containing twice the recommended level of protein and to ascertain whether the increased organ mass is a result of cellular hypertrophy or hyperplasia.

Materials and Methods

Twelve (two litters of six pigs each) genetically obese piglets were assigned randomly within litter at 6 weeks of age to an adequate control (17% protein) diet or a high protein (34% protein) diet (Table I). The piglets were penned in groups of three (two pens of three pigs fed each diet) and had free access to their respective diets for 14 days. The body weight of each pig was recorded on Days 1, 8, and 15 of the experiment and feed consumption by each pen of pigs was recorded

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Table I. Composition of Diets

Ingredient	Control (%)	High protein (%)
Corn (ground)	10.0	10.0
Corn oil (Mazola)	2.0	
Corn starch	54.1	19.1
Soybean meal (44%)	20.0	50.0
Blood meal	10.0	15.0
Calcium phosphate	2.4	2.4
Calcium carbonate	0.5	0.5
Sodium chloride	0.4	0.4
Trace mineral mix[a]	0.2	0.2
Vitamin mix[b]	0.2	0.2
Choline chloride	0.2	0.2
Total	100.0	100.0
Calculated protein	17.2	34.2

[a] Trace mineral mix supplied the following (mg/kg diet): Cu (as $CuSO_4$), 10; Fe (as $FeSO_4 \Sigma 6H_2O$), 160; Mn (as $MnSO_4$), 20; and Zn (as ZnO), 100. Ground limestone was used as carrier.
[b] Vitamin mix supplied the following (units/kg mixed diet): vitamin A (retinylpalmitate), 5280 IU; vitamin D_3 (cholecalciferol), 704 IU; vitamin E (α-tocopherylacetate), 35.2 IU; vitamin K (Menadione), 3.52 mg; vitamin B_{12}, 26.4 μg; riboflavin, 5.28 mg; niacin, 28.16 mg; D-pantothenic acid, 21.12 mg; biotin, 88 μg; and thiamin, 2.2 mg.

throughout the experiment. On Day 15, all pigs were sacrificed with ketamine (50 mg/kg) and acepromazine (0.5 mg/kg) administered intramuscularly. Liver, kidneys, and the entire gastrointestinal tract were removed. The stomach, small intestine, and large intestine were emptied of their contents, and all organs were weighed.

A 5- to 10-g sample of liver, kidney, and jejunum (100 cm distal to pylorus) was frozen in liquid nitrogen and stored at −70°C until analysis for protein (14) and DNA (15) concentrations. Dry matter concentration was determined on a subsample of each tissue by weighing before and after lyophilization. Data were analyzed by two-way analysis of variance with diet and litter as main effects; main effects and diet × litter interaction were tested in the model.

Results

Initial (control: 4.56 ± .36 kg; high protein: 4.39 ± .49 kg) and final (control: 7.71 ± .72 kg; high protein: 8.70 ± .70 kg) body weight were not affected by the high protein diet. Feed intake, calculated as the total feed consumed by each piglet over the 2-week experimental period, was also not different between the two groups (control: 8.94 ± .29 kg/piglet; high protein: 8.95 ± .39 kg/piglet). Liver weight ($P < 0.09$) and kidney weight ($P < 0.05$), but not small intestine weight, were increased by high dietary protein; the percentage of dry matter was not affected by diet in any of the three tissues (Table II). The relative weights (percentage of body weight) of liver and kidney were greater in pigs fed the high protein diet than in controls ($P < 0.01$), but relative weights of stomach, small intestine, and large intestine were unaffected by diet (Table II). There were no diet × litter interactions for any organ, except liver ($P < 0.05$); one litter responded more dramatically to the high protein diet than the other. The relative weight of large intestine was greater ($P < 0.05$) in one litter than in the other.

Protein and DNA concentrations and the total amount of each constituent in liver, kidneys, and small intestine are summarized in Table III. Liver protein concentration and total content (organ weight × concentration) were increased by the high protein diet ($P < 0.05$), but DNA concentration and total content were unaffected. The protein to DNA ratio was greater in the livers of pigs fed the high protein diet than in livers of controls ($P < 0.05$). Kidney protein concentration was not affected by diet, but total protein was increased by the high protein diet ($P < 0.10$). DNA concentration was lower in the kidneys of pigs fed the high protein diet than in kidneys of controls ($P < 0.05$), but total kidney DNA was similar in the two groups. The protein to DNA ratio was greater ($P < 0.05$) in the kidneys of pigs fed the high protein diet than in kidneys of controls. Jejunal protein and DNA concentrations and the protein to DNA ratio were unaffected by diet; total protein and DNA in the small intestine, assuming uniform concentrations of each constituent throughout the small intestine, also were unaffected by diet.

Discussion

Our study has shown increased protein accretion in the liver and kidneys of young pigs in response to the short-term ingestion of high levels of dietary protein. These results are in agreement with the significant enlargement observed in the mass of liver and kidneys in growing pigs (approximately 4–5 months of age, 55 kg) that had free access to a high protein diet for 17 days. Our results also support the finding of increases in the mass of liver and kidneys of pigs (similar to those in the present study, approximately 2.5 months of age, 27 kg) fed similar high and low protein diets for 28 days (2). The nature of the increased mass of liver and kidneys in response to high dietary protein, however, was not evaluated in those reports. One of our litters responded to the high protein diet by increasing liver size so dramatically that a diet × litter interaction was statistically significant. While all pigs were genetically similar, this response suggests that some genetic variability exists in the response of the liver to an high protein diet. The established positive relationship between the energy cost of whole animal maintenance and visceral organ mass, of which the liver is a major constituent, underscores the importance of determining that liver and kidney hypertrophy is the result of increased protein accretion.

We verified an increase in protoplasmic mass, rather than a mere increase in tissue water content, through the similar dry matter concentration of liver

Table II. Effect of High Protein Diet on Body Weight and Organ Weights of Young Pigs

	Control	High protein	SD	*F*-value		
				Diet	Litter	Diet × litter
Liver						
Absolute wt (g)	246	313	61	3.72[a]	2.30	0.14
Relative wt (% of body wt)	3.18	3.60	0.11	46.64[b]	0.01	7.5[c]
Dry matter (%)	27.8	27.8	1.3	0.02	0.49	0.04
Kidney						
Absolute wt (g)	41	57	11	6.20[b]	2.44	0.00
Relative wt (% of body wt)	0.55	0.66	0.04	24.58[b]	0.01	0.04
Dry matter (%)	20.6	19.9	1.5	2.08	3.07	0.32
Small intestine						
Absolute wt (g)	398	424	65	0.38	0.03	0.02
Relative wt (% of body wt)	5.26	5.02	0.76	0.25	3.69	0.01
Dry matter (%)	20.0	19.7	1.3	0.05	0.78	1.84

[a] $P < 0.09$.
[b] $P < 0.05$.
[c] $P < 0.01$

Table III. Liver Protein and DNA Concentration and Total Content of Liver, Kidneys, and Jejunum in Pigs Fed an Adequate Control or High Protein Diet[a]

	Control ($n = 6$)	High protein ($n = 6$)
Liver		
Protein		
Concentration (mg/g)	138 ± 0.3	154 ± 3[b]
Content (g)	34 ± 3	48 ± 4[c]
DNA		
Concentration (mg/g)	3.6 ± 0.1	3.4 ± 0.1
Content (g)	0.87 ± 0.07	1.06 ± 0.08
Protein:DNA ratio	39.0 ± 0.8	45.4 ± 1.0[c]
Kidney		
Protein		
Concentration (mg/g)	108 ± 1	106 ± 1
Content (g)	4.6 ± 0.5	6.0 ± 0.5[d]
DNA		
Concentration (mg/g)	4.33 ± 0.03	3.98 ± 0.08[c]
Content (g)	0.18 ± 0.02	0.22 ± 0.02
Protein:DNA ratio	24.9 ± 0.2	26.6 ± 0.05[c]
Jejunum		
Protein		
Concentration (mg/g)	104 ± 4	104 ± 2
Content[e] (g)	42 ± 3	44 ± 3
DNA		
Concentration (mg/g)	5.9 ± 0.2	5.9 ± 0.3
Content[e] (g)	2.4 ± 0.2	2.5 ± 0.2
Protein:DNA ratio	17.6 ± 0.2	18.0 ± 0.6

[a] Data are expressed as mean ± SE.
[b] $P < 0.01$.
[c] $P < 0.05$.
[d] $P < 0.10$.
[e] Jejunal protein and DNA concentrations were assumed to be representative of the entire small intestine; the value for content is for total small intestine (jejunal concentration × small intestine mass).

and kidneys of animals fed high or normal protein diets. The greater protein concentration in liver ($P < 0.01$) and greater total protein content of liver ($P < 0.05$) and kidneys ($P < 0.06$) of pigs fed a high protein, rather than control, diet showed clearly the increased protein accretion in these organs in response to excessive protein intake. Our findings also support the observation in rats (16) that relative liver weight increases during adaptation to a high protein diet. Baldwin *et al.* (4) estimated that kidney and liver functions contribute a total of 11%–17% of the energy expenditure for service functions, i.e., functions performed by tissues for the benefit of the integrated organism, including respiration, integrative nerve functions, and heart, kidney, and liver metabolism. Therefore, increases in the mass of these two organs have a disproportionately large effect on total maintenance energy requirements.

DNA concentration was decreased in kidneys ($P < 0.01$) but not in liver by the high protein diet; total DNA was not changed in either organ by the high protein content of the diet. The observation that protein accretion increased in the absence of a change in cellularity (indicated by DNA content) was supported by the increase in protein to DNA ratio ($P < 0.05$) in both organs in pigs fed the high protein diet. The increased protein to DNA ratio and the absence of an increase in the DNA content of liver and kidneys of pigs fed the high protein diet indicated that the enlarged organ mass was due to cellular hypertrophy rather than hyperplasia. Although the trophic agents are unknown, the increased amounts of nitrogenous metabolites processed by the liver and kidneys in animals ingesting excessive protein appeared to result in the availability of nutrients to accommodate the hypertrophic response.

The failure of the protein and DNA concentrations of the jejunum and total protein content of the small intestine to increase in pigs fed the high protein diet may be related to the fact that the measurement was made on a cross-section of the entire jejunum, rather than on the mucosa; the mucosa would be expected to show an immediate hyperplastic cellular response to

high protein in the lumen (17). The response of muscular serosal tissue, which might have been expected to be more refractory to dietary protein manipulation, may have masked any effect on mucosal hyperplasia. In fact, if the 6.5% increase in small intestine weight was due only to mucosal hyperplasia and the mucosa account for 35% of small intestine weight (18), then the mucosa would have actually increased 18.6%.

We conclude that the dramatic increase in the mass of liver and kidneys during a 2-week period of a dietary protein intake twice that of the recommended allowance (19) was directly linked with an increase in protoplasmic mass and protein accretion without a concomitant change in cellularity in either organ. We suggest that the increase in mass and protein content of these two metabolically active tissues has important effects on basal energy requirements in young, growing, early-weaned piglets. The magnitude of the response has important implications for neonatal animals and infant humans whose diets contain levels of protein that exceed metabolic requirements for normal growth.

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