Colitis Increases Albumin Synthesis at the Expense of Muscle Protein Synthesis in Macronutrient-Restricted Piglets1,2

Michelle L. Mackenzie, Mark R. Warren and Linda J. Wykes3

School of Dietetics and Human Nutrition, McGill University, Montreal, Quebec, Canada

ABSTRACT Our aim was to examine the effect of acute inflammation localized in the colon and early macronutrient restriction on protein synthesis in a piglet model. In a 2 x 2 factorial design, piglets (n = 32) were fed an adequate or macronutrient-restricted diet with or without dextran sulfate–induced colitis for 7 d. The stable isotope tracer L-[5,5,5-2H3]leucine was infused to determine protein kinetics at the whole-body level and synthesis of tissue and plasma proteins. In the well-nourished state, colitis did not affect weight gain or protein kinetics except for an increase in albumin synthesis (P < 0.05). Macronutrient restriction alone caused a general slowing of protein metabolism including decreased weight gain (P < 0.0004), whole-body protein turnover (P < 0.0001), and liver (P < 0.01) and plasma protein (P < 0.03) synthesis. However, in the presence of macronutrient restriction, colitis compromised weight gain further (P < 0.02) and decreased muscle protein synthesis (P < 0.05) due to a redistribution of protein metabolism that supported enhanced synthesis of plasma proteins. The increased contribution of plasma protein synthesis to whole-body protein turnover was attributable mainly to increased synthesis of albumin (P < 0.006). Concentrations of plasma proteins were unaffected despite dramatic changes in their synthesis rates, thereby underestimating the effects of malnutrition and colitis on protein metabolism. Increased synthesis of plasma proteins, particularly the negative acute phase reactant albumin, compromises weight gain and muscle protein synthesis only when macronutrient intake is inadequate, underscoring the role of adequate nutrition in preventing growth impairment and muscle wasting in acute inflammation. These results suggest that the hypoalbuminemia of inflammatory bowel disease should not be attributed to decreased synthesis.


KEY WORDS: piglets • protein synthesis • inflammation • albumin • malnutrition

Growth failure, muscle wasting and hypoalbuminemia are frequent complications of chronic inflammatory conditions such as inflammatory bowel disease (IBD)4 (1,2). Depletion of muscle mass (3) and impaired muscle function (4) as well as reduced height velocity and compromised adult stature (5) are important long-term sequelae of recurrent inflammatory episodes. Elevated whole-body protein synthesis and breakdown in IBD (6,7) contribute to the increased metabolic demands including energy expenditure during active gastrointestinal inflammation (8,9). Concurrently, dietary intake is frequently compromised in IBD due to cytokine-induced anorexia (10) and food avoidance (11). Therefore, metabolic demands of the inflammatory response, including the synthesis of acute phase proteins, are complicated by inadequate dietary intake during gastrointestinal inflammation.

The metabolic response to infections and injury typically includes increased hepatic production of acute phase proteins and impaired muscle protein synthesis. However, the early systemic response to inflammation localized in the colon is not known. Increased concentrations of positive acute phase plasma proteins such as fibrinogen and decreased concentrations of negative acute phase proteins such as albumin are hallmark signs of the systemic inflammatory response in IBD (1,12); however, the mechanisms are unclear. Changes in the concentrations of plasma proteins can be mediated by shifts in the balance among synthesis, degradation and extravascular loss. The hypoalbuminemia of IBD has been attributed to either a decrease in synthesis (13) or an increase in catabolism (14), yet albumin synthesis in IBD has never been measured directly. It has been shown in a piglet model that malnutrition and systemic inflammation decrease plasma albumin concentration by different mechanisms (15). Chronic protein deficiency reduces albumin concentration by decreasing its synthesis, whereas systemic inflammation in the protein-malnourished state exacerbates hypoalbuminemia despite a dramatic increase in its synthesis rate (15). The independent influences of malnutrition and acute inflammation...
flammmation on protein metabolism in IBD are difficult to study clinically; therefore we adapted a rodent model of dextran sulfate (DS)-induced colitis (16,17) to the piglet. The piglet has a well-characterized amino acid and protein metabolism that is similar to that of humans (18); piglets respond rapidly to dietary inadequacies (19) and have been used previously to study the effect of protein malnutrition with and without inflammation on protein kinetics (15). To distinguish the effects of gastrointestinal inflammatory stress from that of macronutrient restriction (MR), we used a stable isotope tracer method to measure protein synthesis rates in tissue and plasma protein pools, including fibrinogen and albumin. Because increased synthesis of acute phase proteins in inflammatory states increases amino acid demands and energy expenditure (20), we hypothesized that gastrointestinal inflammation would increase protein synthesis to support the acute phase response, and that this priority response would compromise body weight gain and protein synthesis particularly in muscle when macronutrient intake was reduced.

**MATERIALS AND METHODS**

**Experimental protocol.** Piglets (n = 32; Landrace × Yorkshire) of either sex were obtained from Macdonald Farm, McGill University, at 7 d of age and housed in individual cages. Piglets were randomly assigned to one of four groups in a 2 × 2 factorial design: nutrition (well-nourished control vs. MR) and inflammation (water vs. DS-induced colitis). Catheters were implanted aseptically (19) under isoflurane anesthesia (MTC Pharmaceuticals, Cambridge, Canada) into the jugular vein for infusion of stable isotopes, femoral vein for access to the diet and, to account for potential in

**Diet.** All piglets were weaned to a corn and soy meal–based diet (21) composed of 23% protein, 61% carbohydrate and 5% fat, with vitamins and minerals designed to meet the requirements of growing piglets (22). Well-nourished piglets receiving DS were offered free and that this priority response would compromise body weight gain and protein synthesis particularly in muscle when macronutrient intake was reduced.

**Materials and methods**

**Experimental protocol.** Piglets (n = 32; Landrace × Yorkshire) of either sex were obtained from Macdonald Farm, McGill University, at 7 d of age and housed in individual cages. Piglets were randomly assigned to one of four groups in a 2 × 2 factorial design: nutrition (well-nourished vs. MR) and inflammation (water vs. DS-induced colitis). Catheters were implanted aseptically (19) under isoflurane anesthesia (MTC Pharmaceuticals, Cambridge, Canada) into the jugular vein for infusion of stable isotopes, femoral vein for access to the diet and, to account for potential inflammation-induced anorexia, well-nourished control piglets were pair-fed on a per kg body basis. Malnourished piglets received 15 g/kg of the MR (macronutrient-restricted) diet twice a day. Previously, this degree of feed restriction has been used to reduce weight gain and yet maintain the health of young piglets (23). Additional micronutrients were added to the MR diet to maintain micronutrient intake at the level of the well-nourished group. Metabolizable energy content of both diets was 15.9 MJ/(kg day). All piglets were weaned to a corn and soy meal–based diet (21) composed of 23% protein, 61% carbohydrate and 5% fat, with vitamins and minerals designed to meet the requirements of growing piglets (22). Well-nourished piglets receiving DS were offered free and that this priority response would compromise body weight gain and protein synthesis particularly in muscle when macronutrient intake was reduced.

**Diet.** All piglets were weaned to a corn and soy meal–based diet (21) composed of 23% protein, 61% carbohydrate and 5% fat, with vitamins and minerals designed to meet the requirements of growing piglets (22). Well-nourished piglets receiving DS were offered free and that this priority response would compromise body weight gain and protein synthesis particularly in muscle when macronutrient intake was reduced.

**Diet.** All piglets were weaned to a corn and soy meal–based diet (21) composed of 23% protein, 61% carbohydrate and 5% fat, with vitamins and minerals designed to meet the requirements of growing piglets (22). Well-nourished piglets receiving DS were offered free and that this priority response would compromise body weight gain and protein synthesis particularly in muscle when macronutrient intake was reduced.
using the SAS correlation procedure and Pearson correlation coefficients, mixed procedure with the following factorial model:

\[ \text{action effects} = \text{diet effects} \times \text{pair-fed controls} \]

1. **Statistics.** SAS (Version 8.1, SAS Institute, Cary, NC) was used for all statistical analysis. Statistical significance was considered at \( P < 0.05 \) and data were expressed as means ± SEM. Data were log transformed if not normally distributed and multiple variances were used if group variances were not homogenous. Treatment and interaction effects were determined using two-way ANOVA in the SAS mixed procedure with the following factorial model:

\[ y = \mu + \text{diet} + (\text{diet} \times \text{group}) + e_{ij} \]

where \( \mu \) is the overall mean effect; \( \text{diet} \) is the diet group (well-nourished or MR); \( d_i \) is the treatment group (water or DS); \( \text{group} \times d_i \) is the interaction; and \( e_{ij} \) is the random error. Groups were compared using Student’s t test. Correlations were determined using the SAS correlation procedure and Pearson correlation coefficients.

**RESULTS**

**Feed intake and weight gain.** Well-nourished piglets given DS and pair-fed controls consumed equal amounts of feed [58 ± 4 g/(kg · d)]. Their metabolizable energy intake [922 ± 63 kJ/(kg · d)] exceeded their estimated metabolizable energy requirement of 858 kJ/(kg · d) (22). Piglets consuming the MR diet had 50% reductions in macronutrient and energy intakes [MR, 29 ± 0.5 g/(kg · d); MR + DS, 30 ± 0.5 g/(kg · d)]. Macronutrient intake for both well-nourished and MR piglets met or exceeded requirements for growing piglets (22), with the exception of calcium and phosphorus. Although urine volume was not measured, no difference was noted in urination during either the 7-d treatment or the stable isotope study when the piglets were monitored intensively. Over the 7-d protocol, piglets administered DS developed progressively looser stools, which tested positive for occult blood on d 7. In contrast, piglets not receiving DS had solid feces that were negative for occult blood.

Initial body weight (3.1 ± 0.6 kg) was not different among groups. Well-nourished piglets gained 30% of initial body weight over the 7 d of treatment (0.98 ± 0.21 kg) (Fig. 1). DS did not affect weight gain in well-nourished piglets (0.90 ± 0.22 kg). In contrast, MR alone attenuated weight gain by 60% (0.41 ± 0.06 kg, \( P < 0.0004 \)). Weight gain was further impaired by the addition of DS (0.20 ± 0.06 kg, \( P < 0.02 \)) despite a similar feed intake.

**Whole-body protein turnover.** In well-nourished piglets, DS did not affect protein metabolism at the whole-body level (Table 1). The reduction of both leucine flux (\( P < 0.0001 \)) and whole-body protein turnover (\( P < 0.0001 \)) induced by MR was not exacerbated by the addition of DS.

**Tissue fractional synthesis rates.** Hepatic protein synthesis was rapid, i.e., ~50% of mixed hepatic proteins were newly synthesized each day (Fig. 2). In contrast, the turnover of mixed skeletal muscle protein was <10%/d. In well-nourished piglets, DS did not affect liver or muscle FSR. Adaptation to MR reduced liver protein synthesis by 60% (\( P < 0.01 \)); however, superimposed DS substantially elevated liver protein synthesis (\( P < 0.03 \)) to the rates of well-nourished piglets. Protein synthesis in muscle was attenuated only by DS superimposed on MR (\( P < 0.05 \)). Liver and muscle protein FSR (Fig. 3) were positively correlated in well-nourished piglets (\( r = 0.72; \ P < 0.004 \)); however, this correlation was no longer evident in MR piglets (\( r = -0.39; \ P < 0.15 \)).

**Plasma proteins.** The rate of incorporation of the leucine tracer into the intravascular plasma protein pool was linear over the 6-h infusion (Fig. 4). DS did not affect the concentration or synthesis rate of the total plasma protein pool in well-nourished piglets (Table 1). In MR piglets, pool size was maintained despite lowered fractional and absolute synthesis rates (\( P < 0.03 \)). In contrast, DS superimposed on MR increased absolute and fractional synthesis rates by 77% (\( P < 0.03 \)).

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>WN</th>
<th>WN + DS</th>
<th>MR</th>
<th>MR + DS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Whole body</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine flux, ( \mu \text{mol/(kg} \cdot \text{d)} )</td>
<td>1005 ± 65a</td>
<td>1113 ± 148a</td>
<td>611 ± 76b</td>
<td>595 ± 45b</td>
</tr>
<tr>
<td>Protein turnover, ( g/(kg} \cdot \text{d) )</td>
<td>38.5 ± 2.5a</td>
<td>42.7 ± 5.7a</td>
<td>23.4 ± 2.9b</td>
<td>22.8 ± 1.7b</td>
</tr>
<tr>
<td><strong>Total plasma protein pool</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration, ( g/L )</td>
<td>47.8 ± 1.8</td>
<td>43.3 ± 2.2</td>
<td>46.6 ± 1.7</td>
<td>44.9 ± 1.4</td>
</tr>
<tr>
<td>FSR, %/d</td>
<td>43.7 ± 7.4a,ab</td>
<td>54.1 ± 9.7a</td>
<td>30.2 ± 3.5b</td>
<td>53.1 ± 7.3a</td>
</tr>
<tr>
<td>ASR, ( mg/(kg} \cdot \text{d) )</td>
<td>1156 ± 221a</td>
<td>1400 ± 280a</td>
<td>712 ± 81b</td>
<td>1320 ± 146a</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, \( n = 8 \) per group. Means with superscripts without a common letter differ, \( P < 0.05 \).
2 FSR, fractional synthesis rate; ASR, absolute synthesis rate.
< 0.002) and 100% (P < 0.02) respectively, but without achieving an increase in plasma protein concentration.

Albumin concentration and synthesis rates were unaffected by MR alone (Fig. 5). The DS-induced increases in albumin absolute (P < 0.05) and fractional (P < 0.04) synthetic rates were not compromised by superimposition of MR (P < 0.006 and P < 0.003, respectively). Despite increased synthesis rates, plasma albumin concentration did not differ among the dietary groups.

As with albumin, neither concentration nor synthesis of fibrinogen was affected by MR alone (Fig. 6). DS increased the fractional (P < 0.04) and absolute (P < 0.04) synthesis rates in MR piglets such that >100% of the fibrinogen pool was newly synthesized each day. However, MR piglets given DS had a lower fibrinogen concentration (P < 0.01) compared with well-nourished piglets given DS.

In well-nourished piglets, 3% of whole-body protein turnover was attributed to plasma protein synthesis and this was
unaffected by DS-induced inflammation (Fig. 7). Plasma protein synthesis and whole-body protein turnover were lowered proportionally in MR piglets; thus, the relative contribution of total plasma protein synthesis was not changed except for a slightly greater contribution from albumin (P < 0.05). In contrast, DS superimposed on MR doubled the proportion of whole-body protein turnover that was attributed to plasma protein synthesis (P < 0.05), which included greater contributions from fibrinogen (P < 0.02) and albumin (P < 0.001) synthesis. The contribution of albumin to total plasma protein synthesis increased from 21 to 44% and the contribution to whole-body protein turnover increased from 0.7 to 2.5%, compared with well-nourished controls.

**DISCUSSION**

Our objective in developing a piglet model of dextran sulfate—induced colitis was to understand the interactive effects on protein metabolism of the acute inflammation and reduced dietary intake that are such an integral part of IBD. To understand the mechanisms of how changes in nutritional status are affected, we measured protein synthesis in muscle, liver and plasma protein pools. Plasma protein concentrations were unchanged despite dramatic increases in their rates of synthesis, illustrating that measurement of concentration alone underestimates the effect of colitis on protein metabolism. In the well-nourished state, colitis did not compromise weight gain, rates of protein synthesis in liver and muscle or the overall plasma protein pool. As expected, short-term macronutrient restriction alone resulted in a general slowing of protein metabolism, including a reduction in weight gain, whole-body protein turnover, and liver and plasma protein synthesis. However, superimposition of colitis in the macronutrient-restricted state compromised muscle protein synthesis and weight gain due to a redistribution of protein synthesis to support an increase in albumin and fibrinogen synthesis. Overall, our results demonstrate the critical role of adequate protein-energy nutrition in preventing the compromised protein nutritional status characteristic of IBD.

The failure of malnourished piglets with colitis to gain weight at the rate of their noninflamed counterparts suggests a redistribution of metabolic processes away from growth during acute inflammation when macronutrient intake is below requirements. Similar to our findings with weight gain, young rats with acute colitis have impaired linear growth in which only 30–40% can be accounted for by reduced feed intake (33), and rats with Escherichia coli infection show greater body weight loss than pair-fed controls (34). Solomons and colleagues (35) hypothesized that the metabolic demands required to support a chronic acute phase response compromise growth in undernourished children. Indeed, this notion is supported by the observations of growth impairment before diagnosis in 80–90% of children and adolescents with IBD (36), as well as the elevated energy expenditure of malnourished adolescents with IBD compared with adolescents with the uncomplicated malnutrition of anorexia nervosa (8).

Previous studies of chronic protein deficiency in piglets showed that the dampening effect of malnutrition on hepatic protein synthesis (29) conflicts with the stimulatory effect of the systemic inflammatory response (15,37). In contrast, both stimuli elicit inhibitory effects on protein synthesis in muscle (15). The protein kinetic responses in liver and skeletal muscle to short-term macronutrient restriction and acute colitis are consistent with this pattern and with the effects of chronic colitis in rats (38). Adequate dietary intake during the stress of acute colitis was essential to maintaining the positive relationship between protein synthesis in muscle and liver. However, the differential response of muscle and liver during macronutrient restriction eliminated the positive relationship between protein synthesis in the two organs. It is not surprising that the total plasma protein synthetic response, which represents a
The weighted average of the synthesis of all plasma proteins, paralleled the total hepatic protein synthetic response because the majority of plasma proteins are synthesized within the liver. During uncomplicated macronutrient restriction, plasma protein synthesis was reduced in proportion to the reduction in whole-body protein economy; however with the superimposition of colitis, the proportion of whole-body protein turnover attributable to plasma protein synthesis doubled. This prioritization of hepatic protein synthesis to produce circulating plasma proteins critical for tissue repair and protection diverts the limited supply of amino acids away from muscle, ultimately contributing to muscle wasting and growth failure.

Maintenance of muscle mass is dependent on a balance between muscle protein synthesis and proteolysis, a balance that must be positive if growth is to occur. Our results support a critical role for compromised protein synthesis in the muscle wasting of IBD; however, one must also consider the effect of a critical role for compromised protein synthesis in the muscle between muscle protein synthesis and proteolysis, a balance plasma proteins critical for tissue repair and protection diverts the limited supply of amino acids away from muscle, ultimately contributing to muscle wasting and growth failure.

During a classic acute phase response, the concentration of inflammation on muscle proteolysis. Rather than showing a reduction in proteolysis corresponding to decreased synthesis, there is evidence that breakdown of muscle proteins is increased during cachectic and inflammatory conditions (39,40). As Reeds et al. (41) suggested, skeletal muscle can be viewed as an amino acid reservoir that can supply amino acids when dietary protein is not adequate to meet elevated metabolic demands. Increasing muscle proteolysis to mobilize amino acids to support an acute phase response is an inherently inefficient process because these two classes of proteins differ dramatically in their amino acid composition. For example, the tryptophan content of fibrinogen is 3 times that of muscle protein, and the phenylalanine and cysteine content of albumin are 1.7 and 4 times as high, respectively (42,43). Thus excessive muscle proteolysis would occur to supply the necessary amino acids for acute phase protein synthesis during inflammation or injury. The lack of these particular amino acids especially during periods of reduced nutrient intake would limit reutilization of the other amino acids released for protein synthesis, ultimately leading to the well-known increase in nitrogen excretion in cachetic states (44). Manary et al. (45,46) showed that when children with severe protein energy malnutrition and infection were fed a protein source similar in composition to acute phase proteins, rates of urea appearance and amino acid oxidation were reduced, suggesting that excessive amino acid loss was attenuated. Our study provides evidence that maintaining protein and energy intake over the short term prevents the decrease in muscle protein synthesis and growth associated with inflammation. Therefore, improving nutritional support in terms of both protein quantity and quality has an important role in supporting the acute phase response while maximizing protein synthesis and minimizing amino acid loss.

During a classic acute phase response, the concentration of positive acute phase proteins such as fibrinogen increase, whereas the concentration of negative acute phase reactants, mainly albumin, decrease (1). Our stable isotope data reveal striking changes in the protein synthetic response of these plasma proteins that is not evident from observing changes in concentrations. The colitis-induced increase in fibrinogen synthesis demonstrates that short-term macronutrient restriction did not compromise the acute phase response. The fact that the increase in synthesis did not translate to increased concentration indicates that the rate of disappearance was correspondingly increased. The high molecular weight of fibrinogen makes significant extravascularization unlikely. More likely is an increased utilization for blood clotting and tissue repair in the damaged colon. However, increased synthesis of this rap-idly turning over protein has a minimal effect on whole-body protein turnover because of its small pool size.

Most striking was the colitis-induced doubling of albumin synthesis that occurred regardless of nutritional status. The increased contribution of this classical negative acute phase protein to total plasma protein synthesis (44 vs. 21% in well-nourished controls) and to whole-body protein turnover (2.5 vs. 0.7%) indicates a redistribution of protein metabolism not demonstrated by measurements of plasma protein concentrations. A similar prioritization of albumin synthesis was found in piglets with chronic protein deficiency coupled with systemic inflammation (15), and in patients with cancer (47) or head trauma (48). The piglet studies show that the increased albumin synthesis was associated with a corresponding reduction in muscle protein synthesis (15). Hence, the elevation and prioritization of albumin synthesis during acute inflammation might contribute to muscle wasting and growth impairment in IBD and other conditions with chronic inflammatory activity. Prioritized albumin synthesis was prioritized in its plasma concentration suggests increased loss or catabolism during colitis. An early study of albumin kinetics measured with 131I-albumin in hypoalbuminemic patients with IBD demonstrated that increased synthesis of albumin was not sufficient to compensate for the more severely increased rate of removal from circulation through gastrointestinal loss (14). Because 131I was present in the feces both in the free and albumin-bound forms, some of the albumin lost into the gut may have been hydrolyzed and amino acids made available for protein synthesis. Albumin catabolism may be enhanced in IBD to provide the amino acids required to meet an increased metabolic demand. Albumin is catabolized throughout the body (49) and has a high sulfur amino acid content (42), making it a good amino acid source in periods of oxidative stress. However, little is understood about the regulation of albumin catabolism and its function as an amino acid reservoir. Extravascularization of albumin into interstitial fluid during an acute phase response may contribute to albumin loss (50). However, increased albumin in hepatic extravascular fluid would inhibit rather than stimulate albumin synthesis (50). Therefore, the increase in albumin synthesis in our study was sufficient to balance the colitis-induced albumin loss whether this loss was through protein-losing enteropathy, increased catabolism or extravascularization. On the basis of previous studies of albumin synthesis in malnutrition in both piglets and humans, it is doubtful whether this balance could be maintained during chronic colitis.

In summary, traditional markers of malnutrition and inflammation, including plasma protein concentrations and whole-body protein turnover, underestimated the metabolic effect of colitis in this piglet model of DS-induced acute colitis and macronutrient restriction. Protein synthesis was reprioritized to support albumin synthesis at the expense of muscle protein synthesis and weight gain. Our data provide strong evidence for the critical role of adequate dietary protein-energy intake, and the potential for early dietary intervention, in preventing muscle wasting and growth impairment during active gastrointestinal inflammation.

**LITERATURE CITED**


PROTEIN SYNTHESIS IN PIGLETS WITH COLITIS


Downloaded from https://academic.oup.com/jn/article-abstract/133/6/1875/4688183 by guest on 28 February 2019