Threonine Utilization for Synthesis of Acute Phase Proteins, Intestinal Proteins, and Mucins Is Increased during Sepsis in Rats

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Abstract

We hypothesized that the dietary threonine demand for the anabolic response may be increased more than that of other essential amino acids during sepsis. Using a flooding dose of either L-[1-13C]valine or L-[U-13C]threonine, we measured valine and threonine utilization for syntheses of plasma proteins (minus albumin), and wall, mucosal, and mucin proteins of the small intestine in infected (INF; d 2 and d 6 of postinfection) and control pair-fed (PF) rats. At d 2, the protein absolute synthesis rate (ASR) of INF rats was 21% (mucins) to 41% (intestinal wall) greater than that of PF when measured using valine as tracer, and 45% (mucosa) to 113% (mucins) greater than that of PF when measured with threonine as tracer. Plasma protein ASR was higher in INF than in PF rats, reaching 5- to 6-fold the value of PF. The utilization of both amino acid tracers for the protein synthesis was significantly increased by the infection in all compartments studied. The daily increased absolute threonine utilization for protein synthesis in gut wall plus plasma proteins was 446 mol/d compared with 365 μmol/d for valine, and it represented 2.6 times the dietary threonine intake of rats at d 2. Most changes in protein ASR and threonine utilization observed at d 6 of postinfection were limited. In conclusion, sepsis increased the utilization of threonine for the anabolic splanchnic response. Because this threonine requirement is likely covered by muscle protein mobilization, increasing the threonine dietary supply would be an effective early nutritional management for patients with sepsis. J. Nutr. 137: 1802–1807, 2007.

Introduction

Sepsis is a major cause of death throughout the world. It is associated with important metabolic disorders leading to a negative nitrogen balance and a redistribution of body proteins (1). More specifically, the net catabolism in muscle allows the mobilization of amino acids that are required to supply the net protein anabolism and associated oxidation in the liver and the intestine (2,3). This anabolism in the splanchnic area constitutes a defense response of the body against the aggression. In a situation of low food intake, the sepsis-associated amino acid demand has to be met using the patient’s protein store, especially at the expense of muscle mass and function (4). Protein anabolism in the liver reflects the acute-phase response, i.e., the increased synthesis of both resident and exported acute-phase proteins (2,5,6). In the intestine, protein anabolism reflects the immune response and the nonimmune defense mechanisms, including the synthesis of mucins aimed at maintaining an effective gut barrier integrity and function (7).

The metabolic perturbations associated with sepsis lead to additional amino acid requirements. The composition of acute-phase proteins and mucosal proteins may be responsible for tissue wasting in sepsis. As hypothesized by Reeds et al. (8), using a theoretical approach based on amino acid composition of muscle and acute-phase proteins, a high proportion of the body nitrogen net loss may result from the excessive demand of specific amino acids for acute-phase protein synthesis. Aromatic amino acids would be the most important, but a high quantity of threonine is also necessary to support acute-phase protein synthesis (8). Threonine is also of great importance for the maintenance of the gut barrier integrity and function (9–12). Intestinal mucins, key glycoproteins protecting the epithelium from injury, are particularly enriched in threonine (~30% of their amino acid composition (13)). Their synthesis is either stimulated or maintained to control levels in several animal models of intestinal inflammation (12,14,15). Thus, threonine requirements may be strongly increased for both acute-phase proteins and mucosal proteins synthesis during sepsis. This hypothesis is partly confirmed by our observation in septic rats that threonine and cysteine contents are more increased than that of other essential amino acids in liver, and less decreased at the whole body level (16), even if threonine requirements cannot be extrapolated from the measurement of organ contents in threonine.

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Moreover, that study was focused on whole body and liver compartments, and no measurement was performed in the intestine, a key organ in the anabolic response to stress (17).

We hypothesized that, with infection, the threonine demand would be increased more than that of other essential amino acids such as valine, because the intestinal and liver proteins involved in anabolic processes contain particularly high levels of threonine. We quantified the effect of infection on the threonine utilization for acute-phase and intestinal protein synthesis and extrapolate that to threonine requirements. For that, we established a dynamic approach to quantify threonine utilization for the synthetases of plasma proteins, minus albumin, which is considered representative of positive acute-phase proteins (18) and of wall, mucosal, or mucin proteins. Our approach consisted of an assessment of protein synthesis and contents and threonine concentrations in these different compartments. The threonine utilization for protein synthesis was compared with that of valine, which was chosen as a reference isobar as valine concentration in body proteins is approximately the same as the mean concentration of all amino acids (19). Therefore, the protein synthesis rate was measured by a flooding dose of either L-[U-13C]threonine or L-[1-13C]valine (20).

Materials and Methods

Animal experiment. All procedures were in accordance with the guidelines formulated by the European Community for the use of experimental animals (L358–86/609/EEEC). Male Sprague-Dawley rats (Iffa Credo), with body weight between 260 and 270 g, were individually housed in wire-bottomed cages in a temperature-controlled room (22–23°C) with a 12h:12h light:dark cycle. The rats were fed a diet previously described by Faure et al. (12) containing 12% protein (5.7 g/kg diet) and previously shown to sustain normal growth in rats (8). The rats were acclimatized for 8 d and at 300 g body weight, the rats received an injection of saline (pair-fed (PF), CS31A (21)), as described previously (21). The rats of the second group received an injection of L-[1-13C]valine or L-[U-13C]threonine, were used separately. Both amino acids were converted to their N-ethoxycarbonyl-ethyl ester derivative as detailed previously (27). The chromatographic separation was carried out using a TA DB Wax (Agilent) capillary column (60 m in length with an i.d. of 0.25 mm. The fractional synthesis rate (FSR) of plasma proteins minus albumin, defined as the percentage of proteins synthesized per day (%/d), was calculated as previously described using the enrichment of free valine or threonine in the liver as the precursor pools (26). FSR of gut wall, mucosa, and mucin proteins was calculated as previously detailed (11), using L-[1-13C]valine or L-[U-13C]threonine as tracers and the enrichment of free valine or threonine in the different intracellular precursor pools in the gut wall and the corresponding intestinal mucosa. The corresponding absolute synthesis rate (ASR), defined as the quantity of proteins or mucins synthesized per day by the whole small intestinal tissue (mg/d), was determined by multiplying FSR by total protein content in gut wall, mucosa, or mucins. For plasma proteins minus albumin, ASR was calculated from the concentration of plasma proteins minus albumin and plasma volume previously determined (26,28).

Utilization of valine and threonine for the synthesis of plasma proteins minus albumin, small intestinal wall, mucosa, and mucin proteins. The amino acid composition in plasma proteins (minus albumin), small intestinal wall, mucosal proteins, and purified mucins was analyzed as previously detailed (16,25). The utilization of valine and threonine for protein synthesis was calculated by multiplying the respective ASR values for each protein compartment by respective protein concentrations in valine or threonine, and expressed as μmol of valine or threonine utilized per day. The utilization of valine and threonine for other purposes than protein synthesis (e.g., oxidation) was not measured.

Methodological control: effect of a flooding dose of L-[U-13C] threonine on synthesis of mucosal and mucin proteins. Because the synthesis of intestinal mucins is particularly sensitive to dietary threonine supply (11,12), one could argue that a flooding dose of threonine could stimulate the protein synthesis per se, and thus lead to an overestimation of threonine utilization. To check our methodological approach, 4 additional groups, 2 IN at d 0 postinfection (n = 8) and 2 FF

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4 Abbreviations used: ASR, absolute synthesis rate; FSR, fractional synthesis rate; INF, infected; MPE, mol percentage excess; PF, pair-fed.
Mucin content,

mg

Protein content in

time effect (d 2 vs. d 6) was not tested.

Mucosa weight,3

Protein content in

Comparison of the groups injected with L-[1-13C]valine + threonine with those injected with L-[1-13C]valine alone tested the effect of the threonine flooding dose on the measurement of protein FSR in the small intestinal mucosa and mucins, using valine as tracer.

Statistics. Data are presented as means ± SEM. The significance of differences was analyzed by Student's t test for unpaired data or by 2-way ANOVA, with treatment (INF or PF) and tracer (threonine or valine) used as variables, followed by Fisher's protected least significant difference (PLSD) multiple comparison test (Statview) when appropriate. Significance was determined at \( P < 0.05 \). Time points (d 2 vs. d 6) were not compared.

Results

Food intake and animal weight. Two days after the E. coli i.v. injection, the food intake of rats was only 11.5% of intake before infection. This anorexia induced a dramatic body weight loss until d 4 (33 g cumulative loss in INF rats, 32 g in PF rats). From d 4 postinfection, the food intake of INF rats was progressively restored and by d 6 was 76% of intake observed before infection. Consequently, during this period, body weight of INF rats was restored and by d 6 was 76% of the intake observed before infection. This anorexia induced a dramatic body weight loss that was significantly higher in INF than in PF rats (14 ± 1 g).

Tissue weights and protein and mucin contents in the small intestine. At d 2 and d 6, the tissue weights and protein contents in the small intestine and mucosa of INF rats were significantly greater than those of PF rats (Table 1). The mucosal mucin content of INF rats did not differ from that of PF rats at d 2, but tended to be greater (\( P = 0.08 \)) at d 6 (Table 1).

Protein synthesis in plasma proteins minus albumin. At d 2, plasma proteins minus albumin FSR was significantly higher in INF rats, reaching 2- to 3-fold the value of PF rats (Table 2). The absolute synthesis rate (ASR) of plasma proteins minus albumin was also increased in presence of infection but to a greater extent (5- to 6-fold the value of PF rats). At d 6, plasma proteins minus albumin FSR and ASR remained significantly greater in INF rats and were, respectively, −1.5- and 3-fold the value of PF rats (Table 2). For both FSR and ASR, a significant effect of the tracer was observed only at d 2, but the interaction between the infection and the tracer was not significant.

Effect of a flooding dose of L-[U-13C]threonine on the synthesis of small intestinal mucosal proteins and mucins. The concomitant injection of unlabeled threonine and L-[1,13C]valine did not significantly modify mucin FSR in either the INF rats [235 ± 23%/d (valine) and 210 ± 16%/d (valine + threonine)] or in the PF rats [195 ± 8%/d (valine) and 217 ± 15%/d (valine + threonine)]. The same was found for the FSR of small intestinal mucosal proteins in INF rats [101 ± 5%/d (valine) and 97.7 ± 5.9%/d (valine + threonine)] and PF rats [(92.8 ± 3.4%/d (valine) and 89.3 ± 3.4%/d (valine + threonine)].

Protein synthesis in small intestinal wall, mucosa, and mucins. At d 2, the protein FSR and ASR were significantly higher in INF than in PF rats in all intestinal compartments studied (Table 2). A significant effect of the tracer was observed only in the mucosa. The interaction between the infection and the tracer was significant only for mucin FSR (\( P = 0.003 \)) and ASR (\( P = 0.013 \)). In particular, mucin FSR (+70%) and ASR (+113%) was significantly greater in INF rats than in PF rats when measured with threonine as tracer, whereas it did not differ significantly (+14% and +21%, respectively) when measured using valine as tracer. At d 6, the infection significantly increased the protein FSR in the gut wall and mucins, and the protein ASR in all intestinal compartments studied (Table 2). In contrast to d 2, there was no interaction between the infection and the tracer, and the extent of overall modifications at d 6 (~20%) was limited.

Valine and threonine utilization for protein synthesis in the small intestinal wall, mucosa, mucins, and in plasma proteins minus albumin. At d 2, the utilization of the tracer was significantly greater in INF rats than PF rats in all compartments studied, and a significant effect of the tracer was observed (Table 3). The interaction between the infection and the tracer was significant for mucins and plasma proteins minus albumin. In particular, the utilization of threonine for the synthesis of mucins was significantly greater in INF rats (+70%) than in PF rats, whereas that of valine did not differ with infection. At d 2, the increased absolute amino acid utilization for plasma protein minus albumin synthesis due to infection was +371 μmol for threonine and +302 μmol for valine. At d 6, the utilization of the tracer remained significantly higher in INF rats than in PF rats in all compartments studied except in mucins, and a significant effect of the tracer was observed (Table 3). The interaction between the infection and the tracer was, however, not significant, although it tended to be significant, for plasma proteins not including albumin (\( P = 0.065 \)).

Discussion

We hypothesized that, with infection, the threonine demand would be more significantly increased than that of other essential amino acids such as valine, insofar as the intestinal and liver proteins involved in anabolic processes contain particularly high levels of threonine (8,13,25). To our knowledge, our study is the first to quantify increased threonine utilization for the syntheses of plasma proteins minus albumin [representative of acute-phase proteins (18)] and of proteins from various intestinal compartments (wall, mucosa, and mucins) in septic rats compared with...
Different from corresponding valine,

3 Plasma proteins minus albumin.

1 Values are means ± SEM; d 2, n = 6 (INF) or n = 7 (PF); d 6, n = 7 (INF and PF); (2-way ANOVA followed by Fisher’s PLSD test). *Different from corresponding PF, P < 0.05; **Different from corresponding valine, P < 0.05 (Fisher’s PLSD test). The time effect (d 2 vs. d 6) was not tested.

2 Measured with valine or threonine as tracer.

3 Tr, tracer; Int, Interaction.

4 Plasma proteins minus albumin.

PF control rats. Our hypothesis could be confirmed only by measuring protein turnover using 2 amino acid tracers. Indeed, the synthesis rate of a protein compartment evaluated through the use of tracers depends on the relative proportion of the different proteins synthesized, the proportion of the amino acid used as tracer in these proteins, and the rate of synthesis of each protein. A high content of the amino acid tracer in newly synthesized proteins with rapid turnover will give a high protein FSR, reflecting a high utilization of this amino acid. The absolute amino acid utilization for protein synthesis would be lower when measured using an amino acid tracer less represented in these proteins. We assumed that an increase in threonine utilization for protein synthesis in 2 major compartments of the splanchnic area during sepsis would reflect an increased threonine requirement. However, as the other tissues and organs were quantitatively less involved and impaired during sepsis, we did not check whether their threonine utilization for protein synthesis was impaired or not, as suggested by our previous study (16).

During the acute phase of infection (d 2), small intestinal protein FSR and ASR were stimulated, confirming previous works (29,30). This stimulation was to a variable extent and depended on the intestinal compartment and the tracer used, with the highest stimulation using threonine as tracer (+113% for ASR) and limited stimulation using valine as tracer (+21% for ASR). The stimulation of mucin synthesis was not due to the threonine flooding dose per se, because mucin FSR, measured with L-[1-13C]valine, was not influenced by the concomitant addition of unlabeled threonine. The difference between the 2 tracers reflects the particularly high threonine content in intestinal mucins (~30% of their amino acid composition vs. 3% for valine (13,25)). It suggests the synthesis of mucins types richer in threonine during infection than in healthy conditions, and could reflect an increased synthesis of the main secreted intestinal mucin 2, which is particularly rich in threonine (>30%) compared with other intestinal mucins (13–20%) (31).

Increased threonine utilization may also originate from liver protein anabolism, to support acute-phase protein synthesis (8). Plasma proteins minus albumin were considered representative of positive acute-phase proteins (18). At d 2, the absolute synthesis rate of these plasma proteins was greater in INF rats, reaching 5- to 6-fold the value of PF, independently of the amino acid tracer used. This suggests that plasma proteins minus albumin synthesized during the acute-phase reaction would not contain more threonine-rich proteins than in healthy conditions.

**TABLE 2**

FSR and ASR of proteins in the small intestinal wall (excluding duodenum), mucosa, mucins, and in plasma proteins minus albumin in PF and INF rats at d 2 and d 6 postinfection.

<table>
<thead>
<tr>
<th></th>
<th>d 2</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>d 6</th>
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<th></th>
<th></th>
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<tr>
<td></td>
<td>Valine2</td>
<td>Threonine</td>
<td>P-value</td>
<td>Valine4</td>
<td>Threonine</td>
<td>P-value</td>
<td>Valine4</td>
<td>Threonine</td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td>PF</td>
<td>INF</td>
<td></td>
<td>PF</td>
<td>INF</td>
<td>Treatment</td>
<td>Tr3</td>
<td>Int</td>
<td>PF</td>
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<tr>
<td>Wall</td>
<td>66 ± 1</td>
<td>79 ± 4</td>
<td></td>
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<td>NS</td>
<td></td>
<td></td>
<td>68 ± 2</td>
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<tr>
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<td>79 ± 4</td>
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<td>0.0003</td>
<td>0.009</td>
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<td>62 ± 5</td>
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<td>93 ± 6</td>
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<td>103 ± 9</td>
<td>127 ± 7</td>
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<td></td>
<td></td>
<td>87 ± 5</td>
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<tr>
<td></td>
<td>173 ± 7</td>
<td>197 ± 15</td>
<td></td>
<td>133 ± 9**</td>
<td>227 ± 9*</td>
<td></td>
<td></td>
<td></td>
<td>135 ± 12</td>
</tr>
<tr>
<td>Plasma</td>
<td>42 ± 2</td>
<td>135 ± 7</td>
<td></td>
<td>60 ± 3</td>
<td>138 ± 5</td>
<td></td>
<td></td>
<td></td>
<td>51 ± 2</td>
</tr>
</tbody>
</table>

**TABLE 3**

Utilization of valine and threonine for protein synthesis in the small intestinal wall (excluding duodenum), mucosa, mucins, and in plasma proteins minus albumin in PF and INF rats at d 2 and d 6 postinfection.

<table>
<thead>
<tr>
<th></th>
<th>d 2</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>d 6</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Valine2</td>
<td>Threonine</td>
<td>P-value</td>
<td>Valine4</td>
<td>Threonine</td>
<td>P-value</td>
<td>Valine4</td>
<td>Threonine</td>
<td>P-value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PF</td>
<td>INF</td>
<td></td>
<td>PF</td>
<td>INF</td>
<td>Treatment</td>
<td>Tr3</td>
<td>Int</td>
<td>PF</td>
<td>INF</td>
</tr>
<tr>
<td>Wall</td>
<td>153 ± 5</td>
<td>216 ± 13</td>
<td></td>
<td>110 ± 6</td>
<td>185 ± 17</td>
<td></td>
<td>&lt;0.0001</td>
<td>0.003</td>
<td>NS</td>
<td>173 ± 9</td>
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<tr>
<td></td>
<td>80 ± 7</td>
<td>122 ± 9</td>
<td></td>
<td>0.001</td>
<td>0.039</td>
<td>NS</td>
<td></td>
<td></td>
<td>88 ± 6</td>
<td>102 ± 8</td>
</tr>
<tr>
<td></td>
<td>0.113 ± 0.0112 ± 0.140.200 ± 0.080 ** 0.120 ± 0.188***</td>
<td>0.0086</td>
<td>&lt;0.0001</td>
<td>0.0005</td>
<td>0.080 ± 0.070 ± 0.152 ± 0.016 ± 0.006 ± 1.035 ± 0.197 ± 1.276 ± 0.115</td>
<td>NS</td>
<td>0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>64 ± 3</td>
<td>365 ± 15*</td>
<td></td>
<td>103 ± 11</td>
<td>474 ± 26**</td>
<td></td>
<td>&lt;0.0001</td>
<td>0.0004</td>
<td>0.046</td>
<td>92 ± 3</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM; d 2, n = 6 (INF) or n = 7 (PF); d 6, n = 7 (INF and PF); (2-way ANOVA followed by Fisher’s PLSD test). *Different from corresponding PF, P < 0.05; **Different from corresponding valine, P < 0.05 (Fisher’s PLSD test). The time effect (d 2 vs. d 6) was not tested.

2 Measured with valine or threonine as tracer.

3 Plasma proteins minus albumin.

Increased threonine utilization during sepsis

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At d 6 postinfection, and independently of the tracer used, the protein ASR remained significantly higher in INF rats than in PF rats in all compartments studied and was limited in the mucosa (+28%) and mucins (+20%) while still high in plasma proteins minus albumin (+240%). The absence of a strong stimulation of protein synthesis in intestinal compartments containing the highest threonine level (mucosal and mucins) may reflect a low availability of threonine that could become limiting.

In the healthy, and to a greater extent in gastrointestinal inflammatory situations, threonine is highly retained by the gut to maintain its integrity and function (9–12). Our results indicate that this also occurs during systemic infections. Indeed, at d 2, the stimulation of threonine utilization for protein synthesis in INF rats (small intestinal wall, 68%; mucosa, 52%; mucins, 71%) was higher than that of valine (small intestinal wall, 41%; mucosa, 27%; mucins, did not differ). This means there is a greater dietary demand of threonine than valine, especially for mucins which exhibit a high turnover and are enriched in threonine. In contrast, the higher threonine utilization (+360%) for the synthesis of plasma proteins minus albumin in INF rats than PF rats (d 2) was in the same range as that of valine (+472%). This means a greater utilization of both amino acids for the synthesis of plasma proteins minus albumin during infection. However, because we observed that the plasma protein minus albumin response is quantitatively important at the whole body level [depending on the tracer used, 650–710 mg of protein/d synthesized in INF rats compared with 100–150 mg/d in PF rats (Table 2)], the threonine demand for liver protein anabolism likely contributes to a high extent to whole-body greater threonine requirements due to infection. The demand for aromatic amino acids could also be increased as suggested by previous work (8), but this was not measured in our study.

A calculation of the net daily increased threonine and valine utilization for protein synthesis due to sepsis is required to better evaluate the contribution of each amino acid to defense processes. We calculated the differences in the utilization of each amino acid for protein synthesis between INF rats and PF rats, and then summed the increased utilization in the gut wall and plasma proteins minus albumin. The acute infection (d 2) induced an additional daily use of 446 μmol threonine (gut wall, 75 μmol; plasma proteins minus albumin, 371 μmol) and 365 μmol valine (gut wall, 63 μmol; plasma proteins minus albumin, 302 μmol) compared with PF rats. At d 2, this additional daily threonine demand represented 2.6-fold the daily dietary threonine intake of INF rats (150 μmol/d) compared with 1.9-fold for valine. At d 6, the additional daily amino acid utilization for protein synthesis was +321 μmol for threonine and +263 μmol for valine. Over the 6 d of infection, the availability of threonine (and perhaps other amino acids) to support normal growth may have been restricted due to the anorexia and increased demands for mucin and acute-phase protein synthesis. Therefore, to meet the challenge of the infection and maintain normal anabolism, the animals would require even more threonine than the actual estimates of rate calculated above. A diet supplemented in threonine may better meet the specific amino acid demand resulting from infection and could contribute to reduce muscle mobilization. Indeed, according to respective threonine and valine contents in muscle proteins, 1.15 g of muscle proteins [i.e., >30% of muscle losses at whole body level, according to our previous work (2,21,29)] is net catalyzed to supply the higher threonine utilization for plasma proteins minus albumin and intestinal protein syntheses during infection vs. 0.77 g to supply the higher valine requirements.

In conclusion, in pathological situations such as sepsis, the defense and repair processes dramatically increase the demand of amino acids, but especially threonine. We showed that the threonine utilization for syntheses of small intestinal and, to a greater extent, of plasma proteins minus albumin, was particularly increased and represented more than twice the threonine intake. Thus, increasing threonine dietary supply could better meet specific demands related to the defense mechanisms taking place in the splanchic area and, consequently, could limit muscle wasting.

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Literature Cited


