Combined enteral infusion of glutamine, carbohydrates, and antioxidants modulates gut protein metabolism in humans1–3

Moïse Coe¨ffier, Sophie Claeyssens, Stéphane Lecleire, Jonathan Leblond, Aude Coquard, Christine Bôle-Feysot, Alain Lavoinne, Philippe Ducrotté, and Pierre Déchelotte

ABSTRACT

Background: Available data suggest that nutrients can affect intestinal protein metabolism, which contributes to the regulation of gut barrier function.

Objective: We aimed to assess whether an oral nutritional supplement (ONS) containing glutamine (as the dipeptide Ala-Gln), carbohydrates, and antioxidants would modulate duodenal protein metabolism in healthy humans.

Design: Thirty healthy control subjects were included and, over a period of 5 h, received by nasogastric tube either normal saline or ONS providing 11.7 kcal/kg as 0.877 g Ala-Gln/kg, 3.9 g carbohydrates/kg, and antioxidants (29.25 mg vitamin C/kg, 9.75 mg vitamin E/kg, 195 μg β-carotene/kg, 5.85 mg Se/kg, and 390 μg Zn/kg) or glutamine (0.585 g/kg, 2.34 kcal/kg). Simultaneously, a continuous intravenous infusion of L-[1-13C]-leucine was done until endoscopy. Leucine enrichment was assessed by using gas chromatography–mass spectrometric analysis, and mucosal fractional synthesis rate was calculated by using intracellular amino acid enrichment as precursor. Mucosal proteolytic pathways were also evaluated.

Results: ONS infusion resulted in a doubling increase (P < 0.01) of duodenal fractional synthesis rate and a significant (P < 0.05) decrease in cathepsin D-mediated proteolysis compared with saline, whereas proteasome and Ca2+-dependent activities were unaffected. ONS infusion significantly (P < 0.01) decreased duodenal glutathione but not glutathione disulfide concentrations or the ratio of glutathione to glutathione disulfide. Insulinemia increased after ONS infusion, whereas plasma essential amino acids decreased. Infusion of glutamine alone did not reproduce ONS effects.

Conclusions: ONS infusion improves duodenal protein balance in healthy humans. Further investigations are needed to study the origin of these effects and to evaluate ONS supply in stressed persons. Am J Clin Nutr 2008;88:1284–90.

INTRODUCTION

The gut barrier plays a critical role in the defense of the organism, and an alteration of intestinal barrier and antioxidant defense may contribute to the incidence of infection by impairing gut integrity and the function of gut-associated lymphoid tissue (GALT) (1–4). Nutritional interventions with key nutrients such as glutamine (5, 6) or antioxidants (7) improve clinical outcomes (5–7). A targeted early enteral supplementation with high amounts of these combined pharmaconutrients is well tolerated (8), and it may help counteract gastrointestinal complications.

The intestinal barrier is regulated by a balance, on the one hand, between cell proliferation and apoptosis and, on the other hand, between protein synthesis and degradation. Duodenal protein fractional synthesis rates (FSRs) have been shown to approach 50%/d (9, 10), a value much higher than those in other major tissues, such as liver or muscle. Protein degradation processes are involved in the regulation of many cellular pathways, eg, cell removal, apoptosis, or inflammation (11). Previous studies reported that nutritional states (9, 12, 13), refeeding (14), or specific nutrient supplementation (15–20) can affect gut protein metabolism, but the influence of a combination with several key nutrients had not been evaluated. The aim of the present study was thus to evaluate the effects of an oral nutritional supplement (ONS) containing glutamine, carbohydrates, and antioxidants on duodenal protein metabolism and intestinal glutathione content in healthy humans.

SUBJECTS AND METHODS

Subjects and experimental design

Thirty volunteers (22 M, 8 F) participated in the study; they were in good general health and had no hepatic, renal, or cardiac dysfunction or any medical or surgical digestive history. Their mean ± SD age was 23.8 ± 5.5 y, and they had a body mass index (BMI; in kg/m2) of 21.7 ± 1.3.

Over a period of 3 d, all subjects consumed a controlled diet providing 30 kcal and 1.2 g protein kg/d. The meals were prepared by the hospital dietary unit. The study was begun at 0800 on day 4, after a 12-h overnight fast. The subjects were divided in 3 groups of 10 subjects each, who, over a period of 5 h, received by nasogastric feeding tube either normal saline (saline group),...
Evaluation of the activities of the proteolytic pathway

Endoscopic samples were homogenized in ice-cold lysis buffer containing 30 mmol/L Tris-HCl/L (pH 7.2), 1 mmol di-thiothreitol/L, and 1% Triton X-100. They were placed on ice for 15 min and then centrifuged for 15 min at 4 °C and 12 000 rpm.

Lysosomal cathepsin D activity

Cathepsin D activity was quantified as previously described (21, 22) with InnoZyme Cathepsin D Immunocapture Activity Assay Kit Fluorogenic (Calbiochem, Nottingham, United Kingdom). After being captured by coated antibody anti-cathepsin D, standards and lysates were simultaneously incubated at 37 °C for 1 h with 50 µL of the fluorogenic peptide H$_2$N-RKLRFFLPK-GCA as substrate. Measurements of proteolysis (unquenched MCA peptide) were obtained in a microtiter plate fluorometer (SpectraMax Gemini XS; Molecular Devices, Sunnyvale, CA; excitation: 320 nm; emission: 405 nm). Activity values were expressed in relative fluorescence units · 60 min$^{-1}$ · mg total protein$^{-1}$.

Calpain and proteasome activities

For activities of calpains (1 and 2) and chymotrypsin-like protease in cytosolic extracts, 20 µg protein was incubated with 20 nmol Tris-HCl/L (pH 7.2), 0.5 mmol EDTA/L, 0.035% sodium dodecylsulfate, and 70 µmol/L of the fluorogenic protease substrate N-succinyl-Leu-Leu-Val-Tyr-MCA (Calbiochem) at 37 °C for 1 h, as described previously (21, 22). For each sample, incubation was also performed in the presence of the specific inhibitors Calpain Inhibitor X (2.4 µmol/L) (Calbiochem) or clasto-lactacystin β-lactone (10 µmol/L; Calbiochem). Measurements of proteolysis (unquenched MCA peptide) were carried out in a microtiter plate fluorometer (Mithras LB 940; Berthold Technologies, Bad Wildbad, Germany; excitation: 355 nm; emission: 460 nm). The activity values shown were derived by subtracting the fluorescence obtained in the presence of the specific inhibitor from the value obtained in its absence; they are expressed in RFU/60 min.

Evaluation of protein expression by Western blotting analysis

Endoscopic samples were homogenized in ice-cold lysis buffer containing 50 mmol Hepes/L, (pH 7.5), 150 mmol NaCl/L, 10 mmol EDTA/L, 10 mmol β-glycerophosphate/L, 100 mmol NaF/L, 2 mmol sodium orthovanadate/L, 1 mmol phenylmethysulfonylfluoride/L, 20 µmol leupeptin/L, 100 U aprotinin/mL, and 1% Triton X-100. Vials were placed on ice for 15 min and then centrifuged for 15 min at 4 °C and 12 000 rpm. The supernatant fluid was collected and stored at −80 °C until analysis. For immunoblotting, extracts (25 µg proteins/lane) were added to lauryl dodecyl sulfate sample buffer and boiled for 10 min. After separation in 4–12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel (Invitrogen, Cergy-Pontoise, France), samples were transferred onto nitrocellulose (GE Healthcare, Orsay, France) or polyvinylidene difluoride (Bio-Rad, Hercules, CA) membranes, according to the antibodies, by voltage gradient transfer. Membranes were blocked in 5% skimmed milk for 1.5 h and then submitted to several washes [Tris-buffered saline-Tween (TBS-T); Sigma Aldrich, St-Quentin Fallavier, France]. Then membranes were incubated overnight in TBS-T and 5% skimmed milk with various specific

Analysis of fractional protein synthesis rate

Mucosal tissue samples were processed as described previously (17, 18). Briefly, mucosal tissue samples were quickly rinsed in ice-cold 0.9% NaCl and immediately ground. Proteins were precipitated with 10% trichloroacetic acid (TCA), and free amino acids were then taken with the supernatant fluid. The supernatant fluid was prepared as plasma for the measurement of free amino acid enrichment. The protein pellet containing isotopically enriched proteins was dissolved in 1 mol NaOH/L and then hydrolyzed in 6 mol HCl/L at 110 °C for 18 h to allow analysis of the enrichment of amino acid released from protein hydrolysis.

The enrichments of [13C]-leucine were measured in the mucosal intracellular free amino acid pools and in the mucosal proteins by using gas chromatography–mass spectrometry (GC-MS) MSD 5972; Hewlett-Packard, Palo Alto, CA) with tert-butyldimethylsilyl (t-BDMS) derivatives as described previously (17). Appropriate standard curves were run simultaneously for measurement of the enrichments.

The FSR of duodenal mucosal protein was calculated by using the follows equation:

\[
FSR(\% / d) = \left( \frac{(Et - Eo)}{Ep} \right) \times \frac{1}{t} \times 24 \times 100 \quad (J)
\]

where Et is the enrichment in tissue protein at time t (in %); Eo is the natural abundance of the labeled amino acid in intestinal mucosal protein (in %); Ep is the enrichment of the precursor pool at plateau (in %); and t is the duration of the tracer infusion (in h). The baseline isotopic enrichment was measured in biopsies of healthy duodenal tissue from ambulatory patients undergoing endoscopy for medical reasons. The precursor pool used was the intracellular free amino acid pool.
primary antibodies (goat polyclonal antibody anti-cathepsin D (1:500; Santa Cruz Biotechnology, Tebu-bio, Le Perray en Yvelines, France), rabbit polyclonal antibody anti-proteasome 20S β subunit (1:1000; Biomol, Tebu-bio); and rabbit polyclonal antibody anti-proteasome 20S α1 subunit (1:1000) and anti-ubiquitin (1:1000; both: Calbiochem), as previously described (21). Horseradish peroxidase–conjugated antibodies, goat anti-rabbit (Euromedex, Mundolsheim, France), and rabbit anti-goat (Rockland, Gilbertsville, PA) antibodies were used as secondary antibodies (all at 1:1000). Immunoreactive proteins were visualized by using an enhanced chemiluminescence (ECL) technique and autoradiography (Hyperfilm ECL; both: GE Healthcare). Quantification of protein expressions was estimated by the ratio of intensity signals to protein concentration samples. Intensity signals of immunoreactive proteins were estimated with IRIS software (version 5.10; IRIS, Buil, France).

**Plasma concentrations**

Plasma amino acids concentrations were measured with the use of an amino acid analyzer (LC3000-Eppendorf; Biotronik, Munich, Germany). Plasma concentrations of insulin and insulin-like growth factor-I (IGF-I) were analyzed by routine radioimmunoassay.

**Mucosa-reduced and oxidized glutathione concentrations**

Mucosa-reduced glutathione (GSH) and oxidized glutathione disulfide (GSSG) concentrations were assayed as previously described (23). Frozen mucosal tissue samples were homogenized in glass Dounce homogenizer (Fisher Scientific Bioblock, Illkirch, France) with 0.5 mol/L chloric acid/L, and the homogenate was centrifuged at 12 000 × g at 4 °C for 10 min. The glutathione concentration in the supernatant fluid was assayed with the use of an HPLC-electrochemical method with an ESA Coulochem II detector using a Model 5010 analytic cell and a 5020 guard cell (ESA Inc, Bedford, MA). Because glutathione concentrations may be affected by the well-known glutamine effect that is induced by cell swelling, the mucosal glutathione concentration was expressed per mg tissue protein. Thus, the tissue pellet remaining after acid precipitation was dissolved in 1 mol NaOH/L at 37 °C and protein content was measured by using the Lowry method (24).

**Statistical analysis**

Results are expressed as mean ± SEM and compared by using GRAPHPAD PRISM software (version 5.0; GraphPad Software, Inc, San Diego, CA). To evaluate the effects of glutamine, ONS or saline over time (plasma data), statistical analysis consisted of a 2-factor analysis of variance for repeated measures and Bonferroni tests as post tests. To evaluate the effects of glutamine, ONS, or saline at hour 5 (data from biopsies), statistical analyses were assessed by using one-factor analysis of variance with the Tukey test as the post hoc test if comparable variances were observed. In other cases, nonparametric tests were used. For all, P < 0.05 was considered significant.

**RESULTS**

Histologic examination of the duodenal tissue that underwent biopsy showed no signs of mucosal lesions in any subjects. Biologic characteristics of healthy volunteers at inclusion are shown in Table 1; they did not differ between groups.

**TABLE 1**

Biological characteristics of healthy volunteers before and after 5-h infusions

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Time</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycemia (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>4.8 ± 0.1±^1</td>
<td>4.6 ± 0.1</td>
</tr>
<tr>
<td>ONS</td>
<td>5.0 ± 0.1</td>
<td>5.5 ± 0.3^4±^6</td>
</tr>
<tr>
<td>Glutaminemia (μmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>643 ± 33</td>
<td>1128 ± 61^5^6</td>
</tr>
<tr>
<td>ONS</td>
<td>721 ± 45</td>
<td>1128 ± 61^5^6</td>
</tr>
<tr>
<td>Plasma EAA (μmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>821 ± 32</td>
<td>842 ± 23</td>
</tr>
<tr>
<td>ONS</td>
<td>788 ± 41</td>
<td>550 ± 19^4±^6</td>
</tr>
<tr>
<td>Plasma NEAA (μmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>1989 ± 85</td>
<td>1838 ± 43</td>
</tr>
<tr>
<td>ONS</td>
<td>1959 ± 92</td>
<td>3229 ± 123^4±^6</td>
</tr>
<tr>
<td>Plasma insulin (pmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>39 ± 6.8</td>
<td>47 ± 9.0</td>
</tr>
<tr>
<td>ONS</td>
<td>13.0 ± 3.7</td>
<td>178 ± 23^4±^6</td>
</tr>
<tr>
<td>Plasma IGF-1 (μg/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>296 ± 15</td>
<td>314 ± 27</td>
</tr>
<tr>
<td>ONS</td>
<td>265 ± 16</td>
<td>300 ± 27</td>
</tr>
</tbody>
</table>

^1 n = 10. ONS, oral nutritional supplement; Gln, glutamine; H0, hour 0; H5, hour 5; EAA, essential amino acid; NEAA, nonessential amino acid; IGF-I, insulin growth factor-I.

^2 Results were compared by using 2-factor ANOVA with Bonferroni post tests. No difference between groups was observed at H0.

^3 Significantly different from H0, P < 0.05.

^4 Significantly different from saline, P < 0.05.

^5 Significantly different from glutamine, P < 0.05.
ONS infusion resulted in a significant 2-fold increase in duodenal FSR compared with saline infusion (Figure 1) and in a decrease in cathepsin D expression and activity (Figure 2). In contrast, chymotrypsin-like proteasome and calpain activities remained unaffected by ONS infusion (Table 2).

To assess the contribution of glutamine to the effects of ONS, 10 volunteers received Gln only at the same rate as that contained in ONS. Gln infusion increased duodenal FSR ≈20% compared with saline infusion (Figure 1), but this difference was not significant. Nevertheless, after ONS infusion, FSR was significantly higher than that measured after Gln infusion ($P < 0.001$). Regardless of the pathway studied, Gln infusion had no effect on proteolytic activities compared with saline infusion (Figure 2 and Table 2). Consequently, cathepsin D expression and activity were lower after ONS infusion than after Gln infusion. The expression of ubiquitin, and α1 and β5 20S proteasome subunits remained unaffected by ONS or Gln infusions (data not shown).

ONS infusion resulted in a significant decrease in duodenal GSH compared with saline infusion (Table 2). Gln infusion resulted only in a trend to decrease duodenal GSH and GSSG. The ratio of GSH to GSSG was not influenced compared with saline infusion.

Plasma total essential amino acid (EAA) concentrations were not affected by saline or Gln infusions but were significantly decreased by ONS infusion (Table 1). Plasma total nonessential amino acid (NEAA) concentrations were increased by Gln and ONS infusions, but in more marked manner by ONS infusions (Table 1). Only plasma serine, glycine, alanine (data not shown), and glutamine (Table 1) concentrations were significantly increased. Plasma IGF-I concentrations decreased after all infusions (Table 1). Plasma insulin remained unchanged after saline or Gln infusion and increased markedly after ONS infusion (Table 1). In addition, strong positive correlations between plasma insulin and plasma glutamine ($r = 0.53$, $P < 0.01$), between plasma insulin and duodenal FSR ($r = 0.47$, $P < 0.01$), and between plasma glutamine and duodenal FSR ($r = 0.42$, $P < 0.01$) were observed. A positive correlation was also observed between plasma total NEAA concentrations and duodenal FSR ($r = 0.44$, $P < 0.02$); in contrast, a negative correlation was observed between plasma total EAA concentration and duodenal FSR ($r = -0.63$, $P < 0.01$).

**DISCUSSION**

Previous data underline the beneficial effects of immunonutrition or pharmaconutrition in critically ill patients and in surgical patients. Glutamine (25) or trace element supplementations (7) improve clinical outcome—eg, infectious complications and length of stay. More recently, it has been suggested that these nutrients could be combined to gain synergistic effects (26). However, there are only a few studies of the intestinal protein metabolism in response to fasting or to feeding in humans.

In the present study, the ONS containing glutamine, antioxidants, and carbohydrates markedly modified the protein metabolism of the duodenal mucosa in healthy volunteers. Indeed,
ONS approximately doubled the duodenal mucosal FSR, which was already as high as 50%/d in control conditions (9, 10). ONS also reduced cathepsin D–mediated proteolysis measured with an in vitro assay, probably by a decrease of cathepsin D expression. Because it is required in the activation of other cathepsins (27), cathepsin D is used in the present study as a marker of lysosomal proteolysis. The lysosomal pathway and, in particular, cathepsin D have been reported to be involved in intestinal damage in several models (22, 28, 29), and they may be featured as therapeutic targets to prevent intestinal damage (30). These results should be confirmed by an evaluation of gut proteolysis in vivo. Because intestinal integrity plays a key role in the defense of the organism against infections and complications in critically ill and postoperative patients, an improvement of gut protein metabolism by ONS may contribute to its potential beneficial effects.

In the present study, ONS infusion supplied 11.7 kcal/kg and has been compared with the saline infusion, which could be considered as fasting. In some (31–33) but not all (34–36) studies in animals, protein metabolism in the small intestine has been reported to be sensitive to the nutritional state. In humans, gut protein synthesis did not differ significantly between 13 and 36 h of fasting (9). In addition, in maldnourished patients, duodenal protein synthesis was not different from that in controls, but it increased after ~3 wk of nutritional support (14). To our knowledge, the present study shows for the first time that the supply of an ONS improves gut protein metabolism within a few hours in humans.

In previous studies (17, 18, 37), we studied the in vivo effect of amino acids and, notably, of Gln on the duodenal mucosa protein metabolism in healthy subjects; we found that enteral Gln stimulated protein synthesis and decreased ubiquitin mRNA expression (18). Therefore, in the present study, we compared ONS infusion with infusion of Gln alone to ascertain whether the effects of ONS on gut protein FSR were related to Gln only or to other constituents. The gut protein synthesis rate increased ~20% after Gln infusion; this difference was not significant, but it increased ~100% after ONS infusion. It is interesting that glutaminemia and insulinemia were significantly correlated with each other and that each was significantly correlated with duodenal FSR. However, the infusion of Gln alone did not reproduce ONS effects. In the present study, the improvement in protein metabolism was associated with increases in plasma insulin and NEAA concentrations. Similar data were previously obtained after an intravenous infusion of amino acid plus insulin (38). In that study, it was concluded that the infusion of insulin alone had no effect on splanchnic protein metabolism and that amino acids largely determine protein metabolism in the splanchnic bed. Nevertheless, insulin has been shown to be required for the maintenance of protein synthesis rate in the small intestinal mucosa of patients with type 1 diabetes (39). In addition, the injection of insulin is associated with a decrease in lysosomal enzymes—ie, cathepsin D—in the liver and kidney (40). All of these data suggest that greater insulinemia and amino acidemia may contribute to ONS effects. In contrast, the drop in plasma EAA concentrations may reflect the decrease in tissue amino acid release in the systemic circulation and the increase in the tissue amino acid uptake.

Because ONS supplies a complex mixture of nutrients, we cannot exclude the possibility that other components may also contribute to its effects on protein metabolism. To our knowledge, there are no data evaluating the effects of a carbohydrate load on gut protein metabolism in humans. However, in piglets, intravenous glucose was shown to increase jejunal protein synthesis (16). In fibroblasts, glucose regulates lysosomal proteolysis without affecting autophagic sequestration and nonlysosomal proteolytic systems—ie, calpains and proteasome (41). ONS also provides large amounts of some trace elements. Recently, trace element supplementation was reported to modulate local protein metabolism in burned skin by limiting protein catabolism (42), but, to our knowledge, the effect of trace elements on gut protein turnover has not yet been evaluated. In the present study, we cannot exclude the possibility that trace elements contribute to the effects of ONS both by limiting proteolysis and also by stimulating protein synthesis. Indeed, this hypothesis is consistent with recent data showing that selenium supplementation up-regulates gene expression that encodes proteins involved in lymphocyte protein biosynthesis (43). Nevertheless, we are not able to determine the origin of ONS effects that may be due to either energy intake or specific nutrients or a combination of those factors. This uncertainty warrants further investigations evaluating an isoenergetic solution without nutrients—ie, amino acids or antioxidants.

GSH is essential to the function and structural integrity of the gut. Indeed, GSH-deficient mice show severe functional injury of the jejunum and colonic mucosa (44). GSH is well known to play a major role as antioxidant, and it scavenges free radicals—namely, reactive oxygen species (ROS) that are produced as a result of aerobic metabolism (45). In the present study, mucosal glutathione concentrations are near to the previously reported
values in humans (23, 46). We show that ONS infusion is associated with a 78% decrease in the mucosal duodenal GSH concentration. This decrease may result from different mechanisms: a decreased synthesis, an increased utilization or loss, or both. Indeed, a decrease in GSH synthesis has been reported in dog duodenal mucosa after enteral glutamine infusion (47). In contrast, ONS infusion is associated with a high rate of mucosal protein synthesis, a high energy-consuming metabolic pathway. This energy is likely provided by the activation of the aerobic metabolism and ATP production after an ONS infusion that supplies an amount of carbohydrates similar to that provided by a meal as well as a high quantity of Ala-Gln. The activation of the aerobic metabolism may contribute to greater ROS production, and GSH may be used and oxidized in GSSG. Because GSSG is able to diffuse out of the cells mainly into the lumen to maintain the equilibrium of the GSH and GSSG concentrations (48), these mechanisms may explain the acute intracellular GSH loss, without an increase in GSSG, after an ONS infusion. Accordingly, after the infusion of glutamine alone, mucosal protein synthesis only tended to increase, and mucosal duodenal GSH concentration only tended to decrease.

In conclusion, an ONS containing glutamine as the dipeptide Ala-Gln, carbohydrates, and antioxidants improves gut protein metabolism in healthy humans by increasing protein synthesis and by limiting cathepsin D–mediated proteolysis. This modulation of gut protein metabolism may contribute to the improvements in sequential organ failure assessment scores recently reported in critically ill patients after early enteral supplementation with a similar diet also including butyrate (49). The beneficial effects of ONS on gut protein metabolism in this short-term pilot study warrant further investigations of long-term treatment in clinical situations with altered gut barrier. Inhibition of cathepsin D expression and activity may be especially interesting during Crohn disease (30) and drug-induced enterocolitis (22), in both of which enhanced cathepsin D–related proteolysis was recently described.

We thank Brigitte Maurer (Laboratory of Medical Biochemistry) for assistance with the enrichment and glutathione analysis and the staffs of the Endoscopy Unit for their assistance with volunteer inclusion and infusion procedures and with the endoscopic procedure, respectively.

The authors’ responsibilities were as follows—MD and SC: contributed to the writing of the manuscript. PD has received honoraria from Fresenius Kabi and biorel. SL and PDu: endoscopy and sampling; AC: preparation of enteral and protein synthesis, glutathione experiments, and analysis of biological vari-

equally to this work; MC, JL and CBF: proteolysis analysis; SC and AL: assistance with volunteer inclusion and infusion procedures and with the enrichment and glutathione analysis and the staffs of the

REFERENCES


