Does Enteral Glutamine Modulate Whole-Body Leucine Kinetics in Hypercatabolic Dogs in a Fed State?

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To determine whether enteral glutamine alters whole-body leucine metabolism in a state of hypercatabolism, 6 dogs adapted to a normocaloric, low-protein diet received intramuscular dexamethasone (0.44 mg · kg^{-1} · d^{-1}) for 1 week, during 2 separate study periods. On the last day of each period, intravenous infusions of L-[1-^{13}C]leucine and L-[2-^{15}N]glutamine were performed to assess whole-body leucine and glutamine metabolism, and duodenal biopsies were obtained to determine gut protein fractional synthesis rate (FSR), while dogs were receiving enteral nutrition. The nutrient mixture supplied 6.2 kcal · h^{-1} nonprotein energy per $kg^{0.75}$ of body weight (84% glucose, 16% fat) and 0.2 g amino acid per $kg^{-0.75}$ · h^{-1}; the nutrient mixture was glutamine-free on the "control day," and supplemented with 1,150 μmol · kg^{-1} · h^{-1} natural L-glutamine on the "glutamine day." Glutamine supplementation induced an approximately 56% rise in plasma glutamine appearance rate ($P < .05$), and was associated with an approximately 26% reduction in leucine oxidation ($P < .05$) with no change in leucine release from protein breakdown or nonoxidative leucine disposal, an index of whole-body protein synthesis. Glutamine supplementation improved net leucine balance (protein synthesis – protein breakdown) (-26 ± 4 *v* -48 ± 11 μmol · kg^{-1} · h^{-1}; $P < .05$). In addition, glutamine enhanced intestinal protein FSR by approximately 22% in the 4 dogs where it was assessed. We conclude that, in hypercatabolic adult dogs in the fed state, enteral glutamine supplementation acutely decreases leucine oxidation and improves net leucine balance, and may thus preserve body protein.

ALTHOUGH GLUTAMINE can be synthesized de novo, and is therefore classified as a nonessential amino acid, it may play a role in the modulation of nitrogen balance. Depletion of the muscle glutamine pool is observed in stress-induced protein wasting in humans, and intravenous glutamine improved nitrogen balance after major surgery[1,2] or bone marrow transplantation.[3] A doubling in plasma glutamine concentration—achieved via enteral glutamine administration—acutely suppressed leucine oxidation, and enhanced nonoxidative leucine disposal in healthy humans in the postabsorptive state.[4] Similarly, enteral glutamine decreased leucine oxidation and whole-body protein breakdown in children with Duchenne muscular dystrophy.[5] The site of the putative anabolic effect of glutamine remains uncertain. Although skeletal muscle is the main site of glutamine de novo synthesis, the gut is a potential candidate as a "target tissue" for glutamine's putative anabolic effect. Glutamine is indeed extensively extracted by the small intestine.[6] Moreover, glutamine stimulated protein synthesis in isolated rat enterocytes,[7] and a trend towards an increased duodenal protein synthesis was observed in humans made catabolic by a 3-day treatment with prednisone, when they received enteral glutamine in the fasted state,[8] although the trend failed to reach statistical significance.

Whether glutamine promotes protein anabolism remains, however, the matter of debate. Oral glutamine failed to alter whole-body leucine kinetics when given in supplement to the diet in healthy young men,[9] and intravenous glutamine does not affect either whole-body leucine kinetics in hypercatabolic adult dogs[10] or duodenal protein synthesis in healthy growing dogs in the fasting state.[11] We speculated that, in the latter studies,[10,11] the intravenous route of glutamine delivery and/or the fasting state, which reduces amino acid availability for protein synthesis, may have prevented glutamine from exerting its putative anabolic response. Little is known about the effect of exogenous glutamine on glutamine kinetics in dogs.

The first aim of this study was therefore to determine whether a short-term enteral glutamine infusion, given in the fed state, would modulate whole-body leucine metabolism in a model of hypercatabolism that produces in the dog[10] most of the alterations in leucine kinetics observed in critically ill humans.[12] The second aim was to test whether glutamine would exert an anabolic effect on duodenal mucosa in this model.

MATERIALS AND METHODS

Animals

All studies were conducted in accordance with current guidelines from the French Department of Agriculture for the care and use of animals in biological research. Six adult Beagle dogs were studied. Each animal underwent 2 isotope infusion studies (as described below) at least 1 month apart, and in a randomized order. Only healthy animals that had a hematocrit greater than 38%, a good appetite, normal stools, normal body temperature (38.5 to 39.5°C), and that were on no medications were enrolled.

Materials

Natural L-glutamine was obtained from Sigma (St Louis, MO), L-[1-^{13}C]leucine (99% ^{13}C) from MassTrace (Woburn, MA), and L-[2-^{15}N]glutamine (99% ^{15}N) from Cambridge Isotope Laboratories (Andover, MA). Tracer solutions were determined to be pyrogen-free (limulus lysate assay), sterile (plate culture), and 99% chemically and

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optically pure before use. Stable isotope tracers were weighed on a high-precision scale and dissolved in known volumes of sterile 0.9% NaCl. Solutions were prepared no earlier than 24 hours before study, sterilized by passing through a 0.22-μm filter, stored in sterile sealed containers, and kept at 4°C until used.

Protocol Design

For 3 weeks prior to the first isotope infusion, and throughout the experimental period, dogs were fed a diet providing 132 kcal $\cdot$ d^{-1} $\cdot$ kg^{-1} metabolic weight (as defined as body $weight^{0.75}$, a measure commonly accepted as an approximation of the metabolically active part of the body weight in dogs, and used for the calculation of energy supply in dogs[13]) with amino acids accounting for 12% of total energy intake, versus 24% in the maintenance diets. This experimental diet was designed to mimic the dietary protein restriction that invariably occurs following trauma or severe illness; this intake nevertheless meets the assumed minimal protein requirements of healthy Beagle dogs, as defined by the Association of American Feed Control Officials (AAFCO).[13] During the week preceding each isotope infusion, each dog received daily intramuscular injections of dexamethasone at the dose of 0.44 mg $\cdot$ kg^{-1}. The combination of dietary protein restriction with the dexamethasone treatment has been shown to induce a hypercatabolic state,[10] which produces several of the alterations of protein metabolism that occur in critically ill humans.[12] During the isotope infusion day, dogs received continuous nutrition via a nasogastric feeding tube. The design allowed for each dog to be studied under 2 conditions, in randomized order: (1) enteral nutrition alone, and (2) enteral nutrition supplemented with natural L-glutamine (1,150 $\pm$ 24 μmol $\cdot$ kg^{-1} $\cdot$ h^{-1}). This dose is higher than those used in clinical trials in humans,[3] and was chosen to take into account the 3-fold higher protein turnover observed in dogs (when expressed per unit of body weight) compared to humans.

Isotope Infusion Protocol

Each dog was studied after overnight fasting. On the night before each infusion day, a nasogastric tube was inserted into the animal's stomach through one nostril, after a short general anesthesia with Imalgène 1000 (Mérial, Lyon, France), and the tube remained in place throughout the following day until 8 PM. On the day of each isotope infusion at 8:30 AM dogs were weighed, and 2 short intravenous catheters (Vasocan 20 gauge, Braun Medical, Emmenbrücke, Germany) were placed using an aseptic technique: one in the cephalic vein of the forelimb for isotope infusion, and another in the contralateral forelimb for blood sampling. Each dog was maintained in a cage equipped with an infusion set including a metal tether and a dog-adapted jacket (Harvard Apparatus, Les Ulis, France), in order to prevent the animal from compromising the infusion lines.

At 8:45 AM, a baseline 10-mL blood sample was collected in EDTA tube for determination of background isotope enrichment in plasma-free glutamine and α-ketoisocaproate (KIC). To determine background $^{13}CO_2$ enrichment in expired air, a baseline breath sample was obtained by having the dog expire into a 2-L Douglas rubber bag through a 2-way valve-equipped mask. Duplicate aliquots of 10-mL expired air were immediately transferred into evacuated glass tubes (Hexatainer; Labco, Buckinghamshire, UK) for subsequent determination of $^{13}CO_2$ enrichment.

Starting at 9 AM, each dog received 3 stable isotope infusions. First, at 9 AM, a priming dose of L-[1-^{13}C]leucine (7.5 μmol $\cdot$ kg^{-1}) was injected; the prime was immediately followed by a continuous 7-hour intravenous infusion of L-[1-^{13}C]leucine (9.8 $\pm$ 0.2 and 9.6 $\pm$ 0.4 μmol $\cdot$ kg^{-1} $\cdot$ h^{-1} on control and glutamine study day, respectively) delivered by means of a calibrated Bioblock (Fisher Scientific, Illkirch, France) syringe pump. The labeled leucine infusion was designed to assess both whole-body leucine metabolism and duodenal mucosa protein fractional synthesis rate (FSR). Second, at 11 AM, a priming dose of L-[2-^{15}N]glutamine (85 μmol $\cdot$ kg^{-1}) was injected, and immediately followed by a continuous 5-hour intravenous infusion of L-[2-^{15}N]glutamine (38.9 $\pm$ 5.6 and 46.5 $\pm$ 4.5 μmol $\cdot$ kg^{-1} $\cdot$ h^{-1}, control and glutamine study day, respectively) to assess plasma glutamine appearance rate. Third, a priming dose of $NaH^{13}CO_3$ (6 μmol $\cdot$ kg^{-1}) was injected, followed immediately by a continuous 3-hour intravenous infusion of $NaH^{13}CO_3$ (3.44 $\pm$ 0.20 and 3.11 $\pm$ 0.15, control and glutamine study day, respectively) performed between 5 PM and 8 PM to estimate CO_2 production rate, based on the recovery of ^{13}C in expired air.[14,15]

Simultaneous with tracer infusion, a nasogastric infusion of liquid diet (with or without glutamine supplementation) was initiated at 9 AM, and continued throughout the isotope infusion studies until 8 PM. Enteral diets were designed to mimic the low-protein diet, and were isoenergetic. They were comprised of 10% D-glucose (Aguettant, Lyon, France), a 20% long-chain triglyceride/glycerol emulsion (Intralipide; Fresenius-Kabi France, Sèvres, France), and a mixture of free amino acids. The amino acid mix was Nutrilamine 16 (Braun-Bruneau Laboratories, Boulogne-Billancourt, France), a glutamine-free amino acid mixture commonly used for intravenous nutrition in Europe. Dogs received either Nutrilamine as their only source of amino acids on 1 study day (control day), or Nutrilamine plus natural L-glutamine (1,150 μmol $\cdot$ kg^{-1} $\cdot$ h^{-1}) on the other study day (glutamine day). The enteral mixture infused provided an hourly intake equal to 1/24 of the total daily energy intake in the weeks preceding the study.

Five-milliliter venous blood samples were collected in EDTA tubes 280, 300, 320, 340, and 360 minutes after the start of the labeled leucine infusion. Tubes were kept on ice until centrifugation at 4°C at 5,000 $\times$ *g* for 10 minutes. Plasma was immediately separated and frozen at -20°C. Aliquots of expired air were collected at 20-minute intervals during the last hour of labeled leucine infusion.

During the last hour of labeled leucine and labeled glutamine, ie, at 4 PM, duodenal biopsies were taken under direct peroral fibroscopy after a general anesthesia with Imalgène 1000. A dozen of samples of different sites of duodenal mucosa were pooled to obtain approximately 80 mg of tissue. Biopsies were immediately washed 2 times into 0.9% NaCl, weighed, and frozen at −80°C until analysis.

Aliquots of expired air were collected at 10-minute intervals during the last hour of the labeled bicarbonate infusion.

Analytical Methods

The ^{13}C-enrichment in plasma KIC was used as an index of intracellular leucine enrichment. Plasma KIC was isolated from plasma by cation exchange chromatography, and converted to its oxime-tert-butyl, dimethyl silyl (TBDMS) derivative as described.[16] Isotopic enrichment was measured by electron impact ionization gas chromatography–mass spectrometry (GCMS) on a 5890 Series II gas chromatograph coupled with a 5971 mass selective detector (Hewlett-Packard, Palo Alto, CA) by monitoring ions at m/z 316 and 317, using a DB-1 capillary column (30 m $\times$ 0.25 mm id, 0.25-μm film thickness; J&W Scientific, Folsom, CA) operated in the splitless mode. The temperature of the injection port was 250°C and the temperature program was initially set at 80°C, and then ramped at 10°C $\cdot$ min^{-1} up to 220°C.

Plasma leucine, KIC, and glutamine concentrations were determined by selected ion monitoring GCMS using norleucine, ketocaproate, and ^{15}N-glutamine as internal standards, respectively, as described.[14,17] For determination of plasma glutamine concentration, 2 measurements were made, in replicate plasma samples with and without internal standard respectively, to assess and subtract plasma ^{15}N-glutamine enrichment arising from the ^{15}N-glutamine intravenous infusion. Glutamine was separated from glutamate by anion exchange as described,[18] derivatized to its heptafluorobutyramide propyl ester deriv-

atives (PHFBA), and analyzed by GCMS using electron impact (EI) ionization. Ions at m/z 340 and 341 were selectively monitored.

To isolate amino acids in gut tissue, biopsy samples were homogenized in 1 mL 0.9% NaCl, and a 100-μL aliquot of 5 mmol $\cdot$ L^{-1} L-[1-^{13}C]glutamine added as an internal standard to measure intracellular free glutamine concentration.[18] The protein of 300 μL of the homogenate was then precipitated with 2 mL of 12% (wt/vol) trichloroacetic acid (TCA), and samples were centrifuged at 2,000 $\times$ *g* for 20 minutes. The supernatant containing intracellular free amino acids was removed and dried under nitrogen flux. The pellet containing proteins was washed 3 times with 2 mL of 12% TCA, and one time with diethylether to remove lipids. The pellet was hydrolyzed in 6 mol $\cdot$ L^{-1} HCl at 110°C for 24 hours, and then dried under nitrogen flux.

The amino acids derived from the hydrolysis of the pellet protein were isolated by cation-exchange chromatography, and leucine derivatized to its N-acetyl, n-propyl ester (NAP) derivative. The low isotopic enrichments of leucine derived from the hydrolysis of the pellet protein were determined by gas chromatography–combustion-isotope ratio mass spectrometry (GC-C-IRMS) using a 5890 series II GC (Hewlett-Packard) equipped with an ultra-1 capillary column (25 m $\times$ 0.32 mm id, 0, 17-μm film thickness; Hewlett-Packard), and coupled with a Delta S IRMS (Finnigan MAT, Bremen, Germany, as previously described[11,19]). The temperature of the injection port was 250°C. The initial oven temperature was set on 80°C, and increased at 8°C $\cdot$ min^{-1} up to 160°C and then at 40°C $\cdot$ min^{-1} up to 230°C, where it was kept for 2.5 minutes.

To estimate baseline body protein ^{13}C-leucine labeling, 50 μL of plasma sampled before the first isotope infusion was used, as described.[11] Briefly, the plasma aliquot was treated with 12% TCA; the mixed plasma protein pellet was then hydrolyzed, and ^{13}C-leucine enrichment was measured by GC-C-IRMS in this hydrolyzate, and used as baseline in the calculation of mucosal protein synthetic rate.

To derivatize the free leucine in the supernatants containing duodenal mucosa intracellular free amino acids, 200 μL of acetonitrile and 100 μL of N-tert-butyldimethylsilyl-*N*-methyL-trifluoroacetamide (MTBSTFA) were added to the dry supernatant, and leucine derivatized to its TBDMS derivative. Samples were incubated at 60°C for 30 minutes, and then dried under nitrogen flux. The isotopic enrichment was measured using EI ionization GCMS equipped with a DB-1 capillary column (30 m $\times$ 0.25 mm id, 0.25-μm film thickness, J&W Scientific), in the splitless mode. The temperature of the injection port was 250°C and the temperature program was initially set at 80°C, and then ramped at 10°C $\cdot$ min^{-1} up to 210°C, and then at 50°C $\cdot$ min^{-1} from 210°C to 280°C. Ions at m/z 302 and 303 were selectively monitored.

Duodenal mucosa intracellular free glutamine concentration was determined by selected ion-monitoring GCMS using ^{13}C-glutamine as internal standard and by derivatizing glutamine to its heptafluorobutyryl, acetyl (HFBA) derivative. Ions at m/z 307 and 308 were selectively monitored. Although both ^{13}C-glutamine (internal standard) and ^{15}N-glutamine present in the mucosa contribute to the ion at m/z 308, the very large amount of ^{13}C-glutamine added as an internal standard largely exceeded the contribution of ^{15}N-glutamine arising from the intestinal uptake of glutamine from systemic circulation during the infusion of ^{15}N-glutamine. We estimated the potential error in the assessment of glutamine concentration in duodenal mucosa at about 3%.

Expired air $^{13}CO_2$ enrichments were determined by gas chromatography–isotope ratio-mass spectrometry (GC-IRMS) on a Breathmat GC-IRMS (Finnigan, Bremen, Germany). Because the enteral nutrition regimen contained maize-derived glucose—and was therefore "naturally enriched" in ^{13}C—the breath $^{13}CO_2$ enrichment resulting from the oxidation of enteral nutrients per se was assessed in a preliminary study in 2 dogs. When the enteral regimen was administered alone, without any isotope infusion, the rise observed in breath $^{13}CO_2$ was 0.00148 $\pm$ 0.00012 mole percent excess (MPE), and was not different between study days, whether the enteral nutrition mixture contained glutamine or not. In all of our subsequent experiments, baseline breath $^{13}CO_2$ was assessed before enteral nutrition started; this 0.00148 value was therefore subtracted from the observed breath $^{13}CO_2$ enrichment during ^{13}C-leucine infusion or during ^{13}C-bicarbonate infusion in each isotopic study. This correction represented less than 1/10 of the rise in breath enrichment associated with the ^{13}C-leucine and ^{13}C-bicarbonate infusions.

Calculations

Isotopic steady state in plasma leucine and KIC was established by demonstrating by linear regression that the slope of the isotope enrichment against time was not different from zero.

Leucine appearance rate *(Ra_{Leu}, $\mu mol \cdot kg^{-1} \cdot h^{-1}$).* The Ra_{Leu} into plasma was calculated as:

$$Ra_{Leu} = i \cdot (Ei_{Leu}/Ep_{KIC} - 1)$$

where i is the tracer infusion rate (μmol $\cdot$ kg^{-1} $\cdot$ h^{-1}), Ei_{Leu} and Ep_{KIC} are the isotopic enrichments in the infused leucine, and plasma KIC at plateau, respectively.

Protein breakdown (B, $\mu mol \cdot kg^{-1} \cdot h^{-1}$). In the fed state, ie, when some leucine arises from the absorption of the enterally supplied amino acid mixture, Ra_{Leu} represents the sum of the leucine flux from protein breakdown (B) and leucine flux derived from enteral supply. Then:

$$Ra_{Leu} = B + I$$

where I is the flux of leucine coming from the enteral infusion of the diet. This parameter was equal to the flux of leucine supplied enterally minus the fraction of this leucine that was retained by the splanchnic bed, and thus does not appears in systemic plasma. Therefore:

$$I = (1 - f) \cdot I_{Dietary\ leucine}$$

where f is the fraction of dietary leucine taken up in the first pass in the splanchnic bed and $I_{Dietary\ leucine}$ the rate of enteral infusion of leucine in the diet.

Thus,

$$B = Ra_{Leu} - (1 - 0.35) \cdot I_{Dietary\ leucine}$$

where 0.35 is the fraction of dietary leucine taken up in the first pass in the splanchnic bed in dogs receiving enteral feeding with a mixture of free amino acids.[20] The enteral leucine intake was 58.3 $\pm$ 0.4 and 58.0 $\pm$ 0.7 μmol $\cdot$ kg^{-1} $\cdot$ h^{-1}, on the control and glutamine day, respectively.

Total CO_2 production (Ra_{CO2}, $\mu mol \cdot kg^{-1} \cdot h^{-1}$). Total CO_2 production was determined by isotope dilution, based on the appearance of $^{13}CO_2$ in expired air over the course of the primed, 3-hour infusion of $H^{13}CO_3Na$, as described.[14,15]

$$Ra_{CO2} = i_{Bicarb} \cdot [(Ei_{Bicarb}/E_{CO2\text{-}Bicarb}) - 1]$$

where i_{Bicarb} is the $H^{13}CO_3Na$ infusion rate (μmol $\cdot$ kg^{-1} $\cdot$ h^{-1}), and Ei_{Bicarb} and $E_{CO2\text{-}Bicarb}$ are the ^{13}C enrichments (MPE) in the infused bicarbonate solution and in expired air at steady state during the last hour of the labeled bicarbonate infusion, respectively.

Leucine oxidation (Ox_{Leu}, $\mu mol \cdot kg^{-1} \cdot h^{-1}$). Ox_{Leu} was calculated as:

$$Ox_{Leu} = Ra_{CO2} \cdot E_{13CO2\text{-}Leu} \cdot [1/Ep_{KIC} - 1/Ei_{Leu}]$$

where $E_{13CO2\text{-}Leu}$ is the steady-state $^{13}CO_2$ enrichment in breath over the last hour of labeled leucine infusion.

Whereas the method does not calculate the actual recovery rate of bicarbonate in breath, it overestimates the absolute value for CO_2 production by the same amount that labeled bicarbonate is not recovered in breath.[15] If we assume that the recovery of $^{13}CO_2$ does not vary

between the ^{13}C-labeled bicarbonate infusion and the ^{13}C-leucine infusion carried out on the same day, a distinct advantage of this approach is that it corrects for any potential change in the recovery of ^{13}C in breath between the 2 nutritional regimens.

Nonoxidative leucine disposal (NOLD, $\mu mol \cdot kg^{-1} \cdot h^{-1}$). NOLD was calculated as:

$$NOLD = Ra_{Leu} - Ox_{Leu}$$

Fractional synthesis rate (FSR, $\% \cdot d^{-1}$). The FSR of duodenal mucosa protein was calculated according to the precursor/product relationship:

$$FSR = 100 \times 24 \times (E_{t,prot} - E_{0,prot}) / (E_{precursor} \times t)$$

where $E_{0,prot}$ and $E_{t,prot}$ are the ^{13}C enrichments in the bound leucine residues in total plasma protein at time 0 and in duodenal protein at time t, respectively. $E_{precursor}$ is the ^{13}C enrichment of the precursor pool (intracellular free leucine or plasma KIC), and t is the time (h) of the biopsy (time 0 being the time of beginning of the labeled leucine infusion).

Total glutamine appearance rate *(Ra_{Gln}, $\mu mol \cdot kg^{-1} \cdot h^{-1}$).* Ra_{Gln} into plasma was calculated as:

$$Ra_{Gln} = i_{Gln} \cdot (Ei_{Gln}/Ep_{Gln} - 1)$$

where i_{Gln} is the tracer infusion rate ($\mu mol \cdot kg^{-1} \cdot h^{-1}$), and Ei_{gln} and Ep_{Gln} are the isotopic enrichment in the infused glutamine, and plasma glutamine at plateau, respectively. This Ra_{Gln} includes both endogenously produced glutamine, and the fraction of exogenous glutamine that escapes first-pass extraction in splanchnic bed.[21]

Statistical Analysis

Data are presented as means ± SE. Data were compared between treatments using a paired Wilcoxon nonparametric test. Significance was established at $P < .05$.

RESULTS

The dogs' body weight did not change significantly between the 2 experimental days (12.8 ± 0.4 and 13.1 ± 0.7 kg, control and glutamine day, respectively).

Over the last 60 minutes of each isotope infusion, a steady state was observed in the plasma concentrations of glutamine, leucine, and KIC, as well as in the ^{13}C-enrichments of breath CO_2 (Fig 1) and plasma KIC, and in the ^{15}N-enrichment of plasma glutamine.

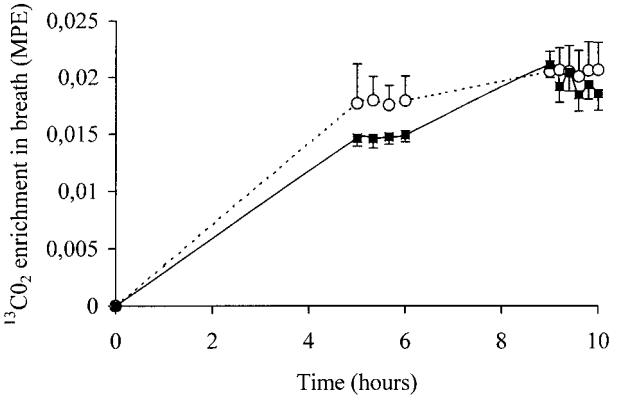

Fig 1. $^{13}CO_2$ enrichments in breath, expressed in mole percent excess (MPE), in adult Beagle dogs maintained on a low-protein diet with dexamethasone treatment during continuous enteral nutrition: effects of glutamine-supplemented diet (■) *v* control diet (○). Each point represents the mean ± SE of 6 dogs.

Table 1. Plasma Leucine, KIC, and Glutamine Concentrations; Plasma ^{15}N-Glutamine and ^{13}C-Leucine Enrichments; and Intestinal Glutamine Concentration in 6 Dogs Receiving an Enteral Infusion of a Control Diet or a Glutamine-Supplemented Diet

	Control	Glutamine
Plasma [glutamine] ($\mu mol \cdot L^{-1}$)	762 ± 45	1,347 ± 55*
Plasma ^{15}N-glutamine enrichment (MPE)	3.62 ± 0.67	2.77 ± 0.37
Intestinal glutamine ($\mu mol \cdot g^{-1}$ tissue wet weight)	5.44 ± 0.37	5.27 ± 0.79
Plasma KIC ($\mu mol \cdot L^{-1}$)	51 ± 3	61 ± 8
Plasma leucine ($\mu mol \cdot L^{-1}$)	137 ± 11	149 ± 15
Plasma ^{13}C-leucine enrichment (MPE)	8.01 ± 0.96	6.40 ± 0.75

NOTE. Data expressed as means ± SE.
*$P < .05$, glutamine day *v* control day.

As expected, intragastric glutamine infusion resulted in a significant, approximately 77% ($P < .05$) rise in plasma glutamine concentration (Table 1), and an approximately 56% rise in plasma glutamine appearance rate (1,669 ± 116 *v* 1,068 ± 73 $\mu mol \cdot kg^{-1} \cdot h^{-1}$, glutamine day *v* control day; $P < .05$). Intestinal mucosa-free glutamine concentration was unaffected by the 6-hour enteral glutamine infusion at the rate of 1,150 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ (5.3 ± 0.8 *v* 5.4 ± 0.4 $\mu mol \cdot g^{-1}$ wet tissue weight, glutamine *v* control, respectively; P = not significant [NS]) (Table 1), and remained higher than observed in untreated dogs (1.25 ± 0.14 $\mu mol \cdot g^{-1}$ wet tissue weight, unpublished data, Humbert et al).

Even though leucine metabolism was assessed in the fed state in the current study, our dogs remained in negative leucine balance (NOLD-B) on both study days (Fig 2). This is most likely due to the protein wasting effect of glucocorticoids.[10] Glutamine supplementation failed to affect plasma KIC or leucine concentrations ($P = .11$ and $P = .67$, respectively), and the trend toward a decline in both leucine Ra (259 ± 11 *v* 275 ± 17 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ on the glutamine and control day, respectively) or leucine release from protein breakdown (222 ± 11 *v* 237 ± 17 $\mu mol \cdot kg^{-1} \cdot h^{-1}$), did not reach statistical significance. In contrast, glutamine supplementation was associated with a significant decrease in leucine oxidation (63 ± 3 *v* 85 ± 11 $\mu mol \cdot kg^{-1} \cdot h^{-1}$; $P < .05$), compared with the control diet. Whereas NOLD was not significantly affected by glutamine supplementation (196 ± 12 *v* 189 ± 17 $\mu mol \cdot kg^{-1} \cdot h^{-1}$; P = NS), leucine balance (NOLD-B) was significantly improved on the glutamine study day (-26 ± 4 *v* -48 ± 11 $\mu mol \cdot kg^{-1} \cdot h^{-1}$; $P < .05$) (Fig 2).

Duodenal protein FSR was not affected by glutamine supplementation when using plasma KIC as the precursor pool (45 ± 3 *v* 44 ± 2% $\cdot d^{-1}$, glutamine *v* control day, respectively; NS). Due to technical difficulties in measuring ^{13}C-enrichements in the small intracellular free leucine pool, intracellular free leucine enrichment data were available for only 4 of the 6 dogs studied. In these 4 dogs, glutamine seemed to enhance gut FSR by approximately 22% when using intracellular free leucine as a precursor pool (67 ± 11 *v* 55 ± 7% $\cdot d^{-1}$,

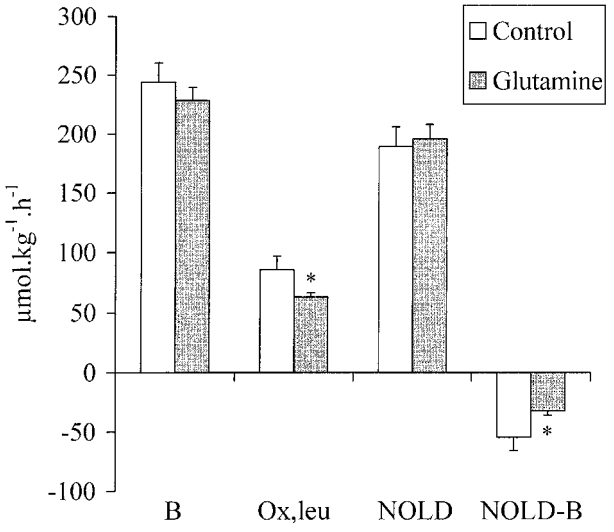

Fig 2. Leucine release from protein breakdown (B), leucine oxidation (Ox,leu), nonoxidative leucine disposal (NOLD), and leucine balance (NOLD-B; all in μmol · kg^{-1} · h^{-1}) in adult Beagle dogs maintained on a low-protein diet with dexamethasone treatment during continuous enteral nutrition: effects of glutamine-supplemented diet *v* control diet. Each bar represents the mean ± SE of 6 dogs. *$P < .05$.

glutamine *v* control day, respectively; NS) (Fig 3). When the average intracellular ^{13}C-leucine / plasma ^{13}C-KIC ratio measured in the remaining dogs was used to estimate the missing free leucine enrichment data, gut protein FSR increased significantly (~20%) on the glutamine day (65 ± 7 *v* 54 ± 5% · d^{-1}, glutamine *v* control day, respectively; $P < .05$) (Fig 3).

DISCUSSION

The findings of the current study suggest that, even in a state of protein wasting, the enteral route can be used to supply glutamine not only to the small intestine, but to other tissues as well, since both plasma glutamine concentration and glutamine appearance into systemic plasma rose substantially upon enteral glutamine supplementation. In addition, the results suggest that glutamine may have a protein-sparing effect, as glutamine supplementation was associated with a reduction in the rate of leucine oxidation, an index of protein oxidation, and improved leucine balance. Finally, the data suggest that the small intestine could be a site of protein accretion in response to glutamine, as there was a trend towards an increase in duodenal mucosa protein synthesis on the glutamine infusion day.

Very little is known on glutamine turnover rate in the dog. In the current study, glutamine Ra (with the glutamine-free regimen) was approximately 78% higher than measured by others in dogs under baseline conditions (1,068 ± 73 *v* 600 ± 36 μmol · kg^{-1} · h^{-1}).[22] Differences in experimental setting could easily account for the difference, since in the current study, (1) glutamine kinetics were measured in the fed rather than the fasting state, and (2) corticoid treatment presumably enhanced glutamine production in our dogs, as it does in humans.[17]

Earlier work established that the splanchnic bed extracts 50% to 75% of an enteral load of glutamine in healthy humans,[21,23] and that most of the extracted glutamine undergoes oxidation in that territory.[24] We further observed that the fraction of enteral glutamine that underwent splanchnic extraction was enhanced in prednisone-treated volunteers,[25] a model of stress-induced protein wasting. Despite that extensive extraction, enteral glutamine enhanced plasma glutamine concentration and the appearance of glutamine into systemic circulation in the fasting state. In the current study, splanchnic glutamine extraction could not be quantified, as this would have required the concomitant infusion of a second labeled glutamine tracer via the enteral route. Yet to our knowledge, this study is the first to demonstrate that enteral glutamine administration is an effective means of elevating glutamine appearance rate and glutamine concentration in systemic plasma in the fed state in protein wasting dogs. This suggests that enteral nutrition regimens supplemented with free glutamine may in fact be a source of bioavailable glutamine not only for the gut, but for other tissues as well.

Supplementation of the enteral regimen with 1,150 μmol · kg^{-1} · h^{-1} glutamine was associated with significant alterations in whole-body leucine metabolism in the current study. Indeed, glutamine supplementation was associated with a trend (NS) toward a decline in leucine release from protein breakdown, compared to the control regimen. This is similar to the decline in protein breakdown observed upon oral glutamine administration in children suffering from Duchenne muscular dystrophy,[5] a disease characterized by progressive, relentless muscle protein wasting. It should nevertheless be kept in mind that the calculation of the leucine release from protein breakdown is dependent on the fractional splanchnic uptake of dietary

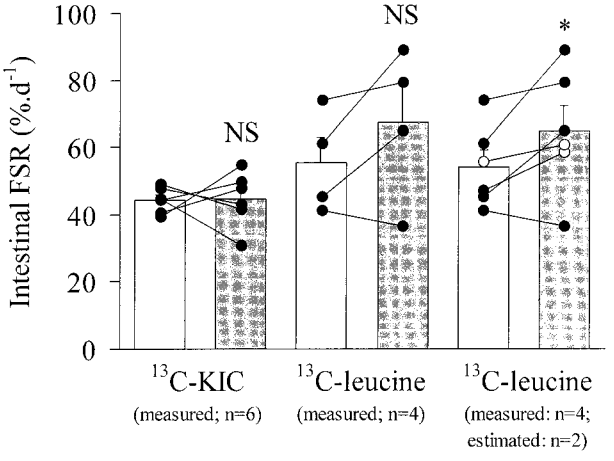

Fig 3. Intestinal protein fractional synthesis rate (FSR, % per day)—calculated using plasma ^{13}C-KIC (left graph), and intestinal free ^{13}C-leucine as precursor pool (middle and right graphs)—in adult Beagle dogs maintained on a low-protein diet and dexamethasone treatment during enteral nutrition: effects of glutamine-supplemented diet (solid bars) *v* control diet (open bars). Middle graph: n = 4 dogs, FSR based on *measured* intracellular free ^{13}C- leucine. Right graph: n = 6 dogs, FSR values calculated from either measured (●) or *estimated* free ^{13}C-leucine enrichments (○)—the latter estimates based on the average ^{13}C-leucine /^{13}C-KIC ratio; each bar represents the mean ± SE of the individual FSR values represented by the circles. *$P < .05$; NS, not significant.

Table 2. ^{13}C Enrichments in Plasma KIC, Intestinal Intracellular Free Leucine, and Intestinal Protein-Bound Leucine in 4 Dogs Receiving an Enteral Infusion of a Control or Glutamine-Supplemented Diet

	^{13}C Enrichment (MPE)					
	Plasma KIC		Intestinal Intracellular Free Leucine		Protein-Bound Leucine	
Dog	Control	Glutamine	Control	Glutamine	Control	Glutamine
1	3.194	3.480	3.477	2.912	0.394	0.317
2	3.161	3.630	2.742	3.069	0.338	0.530
3	4.114	3.009	2.266	1.817	0.487	0.398
4	3.742	4.021	2.727	2.243	0.425	0.517
5	2.903	3.124	3.030	ND	0.401	0.344
6	3.699	3.996	ND	ND	0.506	0.482
Mean ± SE	3.469 ± 0.186	3.543 ± 0.174	2.848 ± 0.199	2.510 ± 0.292	0.425 ± 0.025	0.431 ± 0.037

Abbreviation: ND, not determined.

leucine, which ranges between 23% and 50% in humans,[26] and a set value of 35% was used for calculations in the current study. As splanchnic leucine was not assessed in this study, we cannot exclude that our protocol design failed to detect a significant effect of glutamine on leucine release from protein breakdown.

Consistent again with earlier studies in humans,[4,5,27] enteral glutamine was associated with a significant 26% decline in the rate of leucine oxidation in our hypercatabolic dogs. As opposed to protein breakdown, determination of leucine oxidation solely relies on plasma ^{13}C-KIC and breath $^{13}CO_2$ excretion, and therefore does not depend on the knowledge of splanchnic leucine uptake. Although the 2 regimens did not provide the same nitrogen intake, the decline in leucine oxidation induced by glutamine is unlikely to result from the higher total nitrogen intake since leucine oxidation rose when amino acid intake was increased in children receiving graded intakes of an amino acid mixture intravenously.[28] The drop in leucine oxidation observed in our dogs is similar to the 37%, 35%, and 26% declines induced by enteral glutamine in fasting humans,[4] children with Duchenne muscular dystrophy,[5] and prednisone-treated adult volunteers,[27] respectively. The mechanism(s) by which glutamine decreases leucine oxidation remain(s) unclear. In theory, glutamine could inhibit leucine oxidation by interfering with the uptake of leucine by tissues; a reduction in cellular leucine uptake could, in turn, decrease the subsequent intracellular oxidation of leucine. This is, however, unlikely since, in the current study, there was no decline in the plasma ^{13}C-KIC/plasma ^{13}C-leucine ratio on the glutamine day (0.575 ± 0.045 *v* 0.445 ± 0.040, glutamine *v* control, $P = .07$). As this ratio reflects the equilibration of the leucine tracer between intracellular and extracellular milieus,[29] this suggests that glutamine does not alter leucine uptake in tissues in vivo.

Whereas our studies performed in healthy volunteers demonstrated a small but significant stimulation of non oxidative leucine disposal, an index of whole-body protein synthesis, by enteral glutamine, this was not the case in the current study. This again is consistent with our earlier studies performed in prednisone-treated volunteers, in whom glutamine affected leucine oxidation without affecting NOLD .[27]

Finally, the improvement in net leucine balance observed on the glutamine infusion day in the current study is consistent with the improved nitrogen balance associated with intravenous glutamine supplementation in surgical patients.[2] The improved leucine balance observed in the current study suggests that more leucine was indeed retained in the body on the glutamine day. As storage of free leucine in the body is negligible, any "retained" leucine must have been retained as protein-bound leucine. NOLD, however, is a calculated parameter, and the precision in its determination therefore hampered by propagation of errors, as shown by Matthews et al.[30] Relative lack of precision may thus have prevented us from detecting changes in our index of whole-body protein synthesis.

Although anesthesia is known to induce protein catabolism[31] and could thus contribute to the state of hypercatabolism observed in our dogs, we used a paired study design, and anesthesia was performed on each study day (ie, both before the control day and the glutamine day) under the same conditions. The difference in whole-body leucine kinetics between the study days is therefore unlikely to result from anesthesia per se.

The site of the glutamine's putative anabolic effect remains unclear, and several tissues are potential candidates. Although muscle could be a major target site, muscle protein synthesis was not assessed in the current study. In fact, due to the slow turnover rate of muscle protein (≈1% per day), the relatively short duration of the labeled leucine infusion and the use of singly labeled leucine would have yielded very low isotope enrichments—at the limit of sensitivity of our GC-C-IRMS system—in protein-bound leucine. The current findings, however, suggest that duodenal mucosa is a potential candidate. As shown by other workers, in the fed state, the calculated rates of intestinal protein FSR are dependent on the choice of the precursor pool.[32] In the current study, which was performed in the fed state, intracellular free leucine and plasma KIC (Table 2) could not be used interchangeably for calculation of duodenal protein synthesis, in contrast to previous data obtained in fasting dogs.[11] Cayol et al suggested that the use of an intracellular precursor pool (eg, very–low-density lipoprotein [VLDL] apolipoprotein B100) is preferable to that of a circulating precursor pool (eg, plasma ^{13}C-leucine or ^{13}C-KIC) to calculate hepatic protein synthesis.[33] Regarding intestinal protein FSR, the choice of apolipoprotein B48 or apolipoprotein A-IV, which are exclusively synthesized in the gut, would have been the more appropriate precursor pool, but their separation is technically difficult. Intracellular ^{13}C-leucine seemed to be the most logical choice, and when using it, glutamine did

stimulate gut protein FSR in 3 dogs (by 8%, 43%, and 46%) and decreased it slightly in the fourth dog. Even though data obtained in only 4 dogs do not allow a clear conclusion, they nevertheless are consistent with recent work by Bouteloup-Demange et al in prednisolone-treated humans.[8] Whereas interspecies differences may account for the difference in the absolute values for gut FSR between studies, the relative change in duodenal protein FSR was of the same order of magnitude in both studies. Indeed, based on intracellular ^{13}C-leucine enrichments, gut protein FSR rose by approximately 26% (from 100 to 126% · d^{-1}) in Bouteloup-Demange's study, compared to about 22% (from 55 to 67% · d^{-1}) in the current study. If we assume that small intestine accounts for 4.3% of body weight,[34] and that protein makes up 20% of gut tissue, then the 22% rise in gut protein FSR would account for 12 g of protein per day. On the other hand, on the glutamine infusion day, leucine balance improved by approximately 22 μmol · kg^{-1} · h^{-1} (Fig 2), which translates into the sparing of 11 g of protein per day. As these presumed protein gains are of the same magnitude, it is tempting to speculate that the gut may account for a significant fraction of the protein accretion induced by glutamine. However, this conclusion is tentative, as it is dependent on which precursor pool is used for the calculation of gut protein FSR.

Although the doses of glutamine infused in the current study were large enough to elevate plasma glutamine, intracellular glutamine concentration remained unaltered on the glutamine study day. An increased rate of glutamine utilization in enterocytes could account for this finding, since dexamethasone is known to enhance glutaminase mRNA expression in the gut.[35] This suggests that, if glutamine indeed affects gut protein synthesis, glutamine's putative anabolic effect may be mediated by a metabolite of glutamine rather than by glutamine itself.

In conclusion, the current data suggest that in a model of protein hypercatabolism, enteral glutamine may attenuate body protein wasting as it decreases the rate of oxidation of leucine, an essential amino acid. The role of the small intestine as a potential target of glutamine's protein sparing clearly deserves further investigation.

REFERENCES

1. Morlion BJ, Stehle P, Wachtler P, et al: Total parenteral nutrition with glutamine dipeptide after major abdominal surgery: A randomized, double-blind, controlled study. Ann Surg 227:302-308, 1998

2. Stehle P, Zander J, Mertes N, et al: Effect of parenteral glutamine peptide supplements on muscle glutamine loss and nitrogen balance after major surgery. Lancet 1:231-233, 1989

3. Ziegler TR, Young LS, Benfell K, et al: Clinical and metabolic efficacy of glutamine-supplemented parenteral nutrition after bone marrow transplantation. A randomized, double-blind, controlled study. Ann Intern Med 116:821-828, 1992

4. Hankard RG, Haymond MW, Darmaun D: Effect of glutamine on leucine metabolism in humans. Am J Physiol Endocrinol Metab 271: E748-E754, 1996

5. Hankard RG, Hammond D, Haymond MW, et al: Oral glutamine slows down whole body protein breakdown in Duchenne muscular dystrophy. Pediatr Res 43:222-226, 1998

6. Klimberg S, Souba WW, Salloum RM, et al: Intestinal glutamine metabolism after massive small bowel resection. Am J Surg 159:27-32, 1990

7. Higashiguchi T, Hasselgren PO, Wagner K, et al: Effect of glutamine on protein synthesis in isolated intestinal epithelial cells. J Parenter Enteral Nutr 17:307-317, 1993

8. Bouteloup-Demange C, Claeyssens S, Maillot C, et al: Effects of enteral glutamine on gut mucosal protein synthesis in healthy humans receiving glucocorticoids. Am J Physiol 278:G677-681, 2000

9. Hiramatsu T, Cortiella J, Marchini JS, et al: Source and amount of dietary nonspecific nitrogen in relation to whole-body leucine, phenylalanine, and tyrosine kinetics in young-men. Am J Clin Nutr 59:1347-1355, 1994

10. Humbert B, Le Bacquer O, Nguyen P, et al: Protein restriction and dexamethasone as a model of protein hypercatabolism in dogs; effect of glutamine on leucine turnover. Metabolism 50:293-298, 2001

11. Marchini JS, Nguyen P, Deschamps JY, et al: Effect of intravenous glutamine on duodenal mucosa protein synthesis in healthy growing dogs. Am J Physiol Endocrinol Metab 276:E747-E753, 1999

12. Van Goudoever JB, Wattimena JD, Carnielli VP, et al: Effect of dexamethasone on protein metabolism in infants with bronchopulmonary dysplasia. J Pediatr 124:112-118, 1994

13. Dzanis DA: The Association of American Feed Control Officials Dog and Cat Food Nutrient Profiles: Substantiation of nutritional adequacy of complete and balanced pet foods in the United States. J Nutr 124:2535S-2539S, 1994

14. Liet JM, Piloquet H, Marchini JS, et al: Leucine metabolism in preterm infants receiving parenteral nutrition with medium-chain compared with long-chain triacylglycerol emulsions. Am J Clin Nutr 69: 539-543, 1999

15. Spear ML, Darmaun D, Sager BK, et al: Use of [^{13}C]bicarbonate infusion for measurement of CO_2 production. Am J Physiol Endocrinol Metab 268:E1123-E1127, 1995

16. Salman EK, Haymond MW, Bayne E, et al: Protein and energy metabolism in prepubertal children with sickle cell anemia. Pediatr Res 40:34-40, 1996

17. Darmaun D, Matthews DE, Bier DM: Physiological hypercortisolemia increases proteolysis, glutamine, and alanine production. Am J Physiol Endocrinol Metab 255:E366-E373, 1988

18. Darmaun D, Manary MJ, and Matthews DE: A method for measuring both glutamine and glutamate levels and stable isotopic enrichments. Anal Biochem 147:92-102, 1985

19. Yarasheski KE, Smith K, Rennie MJ, et al: Measurement of muscle protein fractional synthetic rate by capillary gas chromatography/combustion isotope ratio mass spectrometry. Biol Mass Spectrom 21:486-490, 1992

20. Yu YM, Wagner DA, Tredget EE, et al: Quantitative role of splanchnic region in leucine metabolism: L-[1-^{13}C,^{15}N]leucine and substrate balance studies. Am J Physiol Endocrinol Metab 259:E36-E51, 1990

21. Hankard RG, Darmaun D, Sager BK, et al: Response of glutamine metabolism to exogenous glutamine in humans. Am J Physiol Endocrinol Metab 269:E663-E670, 1995

22. Halseth AE, Rhéaume N, Messina AB, et al: Regulation of hepatic glutamine metabolism during exercise in the dog. Am J Physiol Endocrinol Metab 275:E655-E664, 1998

23. Matthews DE, Marano MA, Campbell RG: Splanchnic bed utilization of glutamine and glutamic acid in humans. Am J Physiol Endocrinol Metab 264:E848-E854, 1993

24. Haisch M, Fukagawa NK, Matthews DE: Oxidation of glu-

tamine by the splanchnic bed in humans. Am J Physiol Endocrinol Metab 278:E593-E602, 2000

25. Darmaun D, Welch S, Haymond MW: Splanchnic glutamine metabolism during prednisone-induced protein wasting in humans. Gastroenterology 114:A545, 1998 (abstr G2396)

26. Boirie Y, Gachon P, Beaufrère B: Splanchnic and whole-body leucine kinetics in young and elderly men. Am J Clin Nutr 65:489-495, 1997

27. Darmaun D, Welch S, Sager BK, et al: Can glutamine alter the protein catabolic response to prednisone in humans? Clin Nutr 17:62-63, 1998 (abstr)

28. Goulet O, DePotter S, Salas J, et al: Leucine metabolism at graded amino acid intakes in children receiving parenteral nutrition. Am J Physiol Endocrinol Metab 265:E540-E546, 1993

29. Matthews DE, Schwarz HP, Yang RD, et al: Relationship of plasma leucine and alpha-ketoisocaproate during a L-[1-^{13}C]leucine infusion in man: A method for measuring human intracellular leucine tracer enrichment. Metabolism 31:1105-1112, 1982

30. Matthews DE, Motil KJ, Rohrbaugh DK, et al: Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-^{13}C]leucine. Am J Physiol Endocrinol Metab 238:E473-E479, 1980

31. Horber FF, Krayer S, Rehder K, et al: Anesthesia with halothane and nitrous oxide alters protein and amino acid metabolism in dogs. Anesthesiology 69:319-326, 1988

32. Bouteloup-Demange C, Boirie Y, Déchelotte P, et al: Gut mucosal protein synthesis in fed and fasted humans. Am J Physiol Endocrinol Metab 274:E541-E546, 1998

33. Cayol M, Boirie Y, Prugnaud J, et al: Precursor pool for hepatic protein synthesis in humans: Effects of tracer route infusion and dietary proteins. Am J Physiol Endocrinol Metab 270:E980-E987, 1996

34. Rivers JP, Burger IH: Allometric considerations in the nutrition of dogs, in Burger IH, Rivers JP (eds): Nutrition of the Dog and Cat. Cambridge, UK, Cambridge University Press, 1989, pp 67-112

35. Sarantos P, Abouhamze Z, Copeland EM, et al: Glucocorticoïds regulate glutaminase gene expression in human intestinal epithelial cells. J Surg Res 57:227-231, 1994