Protein Restriction and Dexamethasone as a Model of Protein Hypercatabolism in Dogs: Effect of Glutamine on Leucine Turnover

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To determine (1) whether protein restriction, combined with glucocorticosteroid treatment, can be used as a hypercatabolic model and (2) if so, whether glutamine attenuates protein wasting in this model, the effects of protein restriction, dexamethasone, and glutamine on leucine metabolism were assessed in dogs. A control group (n = 8) received a maintenance diet; another group (n = 8) received a protein-restricted diet either (1) alone; (2) along with a 7-day corticoid treatment; or (3) along with a 7-day corticoid treatment and a 7-hour intravenous (IV) glutamine infusion. The last day of each regimen, dogs underwent an IV isotope infusion in the fasting state, with a 3-hour $NaH^{13}CO_3$ infusion to assess CO_2 production, and immediately thereafter, a 3-hour ^{13}C-leucine infusion to assess leucine appearance rate (Ra), oxidation (Ox), and nonoxidative leucine disposal (NOLD), expressed as $\mu mol \cdot kg^{-1} \cdot h^{-1}$. Protein restriction was associated with a 24% decline in leucine Ra (223 ± 16 *v* 298 ± 17; *P* < .01), an index of whole body proteolysis, and a 29% decline in NOLD (180 ± 15 *v* 223 ± 13; *P* < .01), an index of whole body protein synthesis. In the protein-restricted group, dexamethasone treatment was associated with a 32% increase in Ra, (295 ± 28 *v* 223 ± 16; *P* < .05), a 186% increase in Ox (120 ± 14 *v* 43 ± 4; *P* < .001), with no change in NOLD, when compared with the protein-restricted alone. After protein restriction + dexamethasone, glutamine infusion induced a 40% increase in plasma glutamine (1,090 ± 70 *v* 780 ± 29 $\mu mol \cdot L^{-1}$; *P* < .01), but failed to alter Ra, Ox, or NOLD. These results suggest that (1) in dogs, protein restriction combined with a 7-day course of dexamethasone results in alterations in leucine kinetics similar to those observed in stress-induced protein wasting in humans, and (2) in that model, a 7-hour IV glutamine infusion in the fasting state does not significantly attenuate protein wasting.

PROTEIN WASTING COMMONLY accompanies critical illness and can adversely affect prognosis, as it impairs wound healing, immune response, and pulmonary function.[1] As it has long been known that glucocorticoid excess induces protein wasting, increased cortisol secretion is believed to mediate some or many of the alterations of protein metabolism associated with stress.[2] During human illness, however, mild to severe restriction of dietary protein intake commonly occurs as well. An animal model can thus be of interest to delineate the separate effects of corticoids and protein restriction in the protein wasting associated with stress. In addition, a large animal model of hypercatabolism that would allow for repeated paired studies could serve as a tool to investigate the mechanism of action of putative protein anabolic agents, such as glutamine.

The first aim of the current study was therefore to develop an animal model of protein hypercatabolism in dogs using infusion of ^{13}C-leucine, a technique that has become the method of reference to assess whole body protein metabolism.[3] Although the effects of corticosteroids and dietary protein restriction on leucine metabolism have been evaluated in several species, and although leucine kinetics have been assessed under many experimental conditions in dogs, the separate effects of dietary protein restriction and corticoids on leucine metabolism have not, however, to our knowledge, been previously evaluated in the dog.

Although glutamine is the most abundant free amino acid in the body and can be synthesized de novo, depletion of muscle glutamine pool is consistently observed in stress-induced protein wasting in humans, and glutamine administration was associated with improvements in nitrogen balance after major surgery[4,5] or bone marrow transplantation.[6] Another objective of this study was to test whether glutamine can acutely attenuate protein wasting in a model of hypercatabolism in a large animal. The aims of the current study were therefore to determine (1) whether protein restriction, combined with glucocorticosteroid treatment, induces protein wasting in dogs as it does in humans; and (2) if so, whether glutamine acutely attenuates protein wasting 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. After approval by the National School of Veterinary Medicine at Nantes, 2 groups of 8 adult Beagle dogs were studied. Only healthy animals that had a hematocrit greater than 38%, a leukocyte count less than 18,000/mm^{-3}, a good appetite, normal stools, and body temperature (38.5 to 39.5°C), and 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) was obtained from MassTrace (Woburn, MA). Tracer solutions were determined to be pyrogen-free (limulus lysate assay), sterile (plate culture), and 99% chemically and optically

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Submitted April 3, 2000; accepted September 18, 2000.

Supported, in part, by grants from the National Institutes of Health (RO1 Grant No. DK51477 to Dominique Darmaun). B.H. was supported, in part, by grants from the European Society for Parenteral and Enteral Nutrition (ESPEN), the Société Francophone de Nutrition Entérale et Parentérale (SFNEP), and the Conseil Régional des Pays-de-la-Loire.

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0026-0495/01/5003-0005$35.00/0
doi:10.1053/meta.2001.21018

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

One group of 8 dogs (group 1) was studied under baseline conditions with a maintenance 24% protein diet providing 132 kcal $\cdot$ d^{-1} $\cdot$ kg^{-1} metabolic weight (as defined by body weight$^{0.75}$) (control). Another group of 8 dogs (group 2) was studied after protein restriction. For 3 weeks before the first isotope infusion and throughout the experimental period, dogs of the protein-restricted group were fed a diet providing 132 kcal $\cdot$ d^{-1} $\cdot$ kg^{-1} metabolic weight, with a 50% restriction in dietary protein (12% protein *v* 24% in usual diets). This experimental diet was designed to mimic the dietary protein restriction that invariably occurs after 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) recommendations.[7] During the week preceding each isotope infusion, each dog of group 2 received either no treatment or daily intramuscular injections of dexamethasone (0.44 mg $\cdot$ kg^{-1} $\cdot$ d^{-1}). The dose and duration of the dexamethasone treatment were selected based on earlier studies suggesting a catabolic effect of that regimen, as judged from the switch from a positive to a negative nitrogen balance, and a large decrease in total nitrogen content in skeletal muscle.[8] During the isotope infusion day, each dog received an intravenous (IV) infusion at a rate of 4 mL $\cdot$ kg^{-1} $\cdot$ h^{-1} of either 0.45 g $\cdot$ dL^{-1} NaCl or 200 mmol $\cdot$ L^{-1} natural L-glutamine. The dose and duration of the glutamine infusion were selected a priori, based on doses of glutamine found to acutely suppress leucine oxidation in healthy adult humans or children with Duchenne muscular dystrophy.[9,10] The design thus allowed for each protein-restricted dog to be studied under 3 different experimental conditions, in randomized order, and with a 3-week interval between infusions: (1) saline infusion at baseline (restricted); (2) saline infusion after 7 days of dexamethasone treatment (restricted + dex); (3) IV glutamine infusion after 7 days of dexamethasone (restricted + dex + gln).

Isotope Infusion Protocol

Each dog was studied after 24 hours of fasting. On the day of each isotope infusion at 8:30 AM, each dog was weighed, and 2 short IV catheters (Vasocan, 20 gauge, Braum Medical, Emmenbrücke, Germany) were placed using aseptic technique: 1 in the cephalic vein of the forelimb for isotope infusion and infusion of saline or natural glutamine and another 1 in the contralateral forelimb for blood sampling. At 8:45 AM, a baseline blood sample was obtained to determine background isotope enrichment in plasma-free leucine and α-ketoisocaproate (KIC). To determine background $^{13}CO_2$ enrichment in breath, a baseline breath sample was obtained by having the dog expire for 1 minute into a 2-L Douglas rubber bag (Fisher Scientific, Illkirch, France) through a 1-way valve. Duplicate aliquots of 10-mL of breath gas were immediately transferred into evacuated glass tubes (Hexatainer, Labco, Buckinghamshire, UK) for subsequent determination of $^{13}CO_2$ enrichment. Starting at 9:00 AM each dog received 2 consecutive stable isotope infusions. First, at 9:00 AM, a priming dose of $NaH^{13}CO_3$ (6 μmol $\cdot$ kg^{-1}) was injected; the prime was immediately followed by a continuous 3-hour IV infusion of $NaH^{13}CO_3$ (3 μmol $\cdot$ kg^{-1} $\cdot$ h^{-1}) delivered by means of a calibrated Bioblock syringe-pump (Fisher Scientific). The labeled bicarbonate infusion was designed to estimate CO_2 production rate, based on the recovery of ^{13}C in expired air.[11,12] Second, at 1:00 PM, ie, 1 hour after the discontinuation of labeled bicarbonate, a primed, continuous infusion of L-[1-^{13}C]leucine (15 μmol $\cdot$ kg^{-1}, 10 μmol $\cdot$ kg^{-1} $\cdot$ h^{-1}) was started and continued at a constant rate for 3 hours until 4:00 PM to assess whole body leucine metabolism. Simultaneous with tracer infusion, a 4-mL $\cdot$ kg^{-1} $\cdot$ h^{-1} IV infusion of 0.45 g $\cdot$ dL^{-1} saline or unlabeled 200 mmol $\cdot$ L^{-1} L-glutamine was initiated at 9:00 AM and continued until 4:00 PM. Five-milliliter venous blood samples were taken in EDTA tubes 120, 140, 160, and 180 minutes after the start of the labeled leucine. The tubes were kept on ice until centrifugation at 4°C at 5,000*g* for 10 minutes. Plasma was immediately separated and frozen at −20°C. Aliquots of breath gas were collected at 10-minute intervals during the last hour of the labeled bicarbonate infusion, and at 20-minute intervals during the last hour of labeled leucine infusion. A percutaneous needle biopsy of approximately 15 mg of the vastus lateralis muscle was obtained after a short general anesthesia with Imalgène 1000 (Mérial, Lyon, France) at the end of the isotope infusion study to determine muscle-free glutamine concentration.

Analytical Methods

^{13}C-enrichment in plasma KIC was used as an index of intracellular leucine enrichment.[13] Plasma KIC was isolated from plasma by cation exchange chromatography and converted to its oxime-ter-butyl dimethyl-silyl (TBDMS) derivative as described.[14] Isotopic enrichment was measured by electron impact ionization gas chromatography-mass spectrometry (GCMS) using a DB-1 capillary column (30 m × 0.25 mm id, 0.25 mm 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/min up to 220°C. Ions at m/z 317 and 316 were selectively monitored. Plasma leucine, KIC, glutamine, and muscle glutamine concentrations were determined by selected ion monitoring GCMS using norleucine, ketocaproate, and ^{15}N-glutamine, respectively, as internal standards, as previously described.[11,15]

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).

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 0.

Leucine appearance into plasma (Ra_{Leu}, μmol $\cdot$ kg^{-1} $\cdot$ h^{-1}) was calculated as: $Ra_{Leu} = i[(Ei_{Leu}/E_{p\text{-}KIC}) - 1]$, in which i is the tracer infusion rate (μmol $\cdot$ kg^{-1} $\cdot$ h^{-1}), Ei_{Leu} and $E_{p\text{-}KIC}$ are the isotopic enrichment in the infused leucine, and plasma KIC at plateau, respectively.

In an additional experiment performed in 1 dog, natural, unlabeled glutamine was infused intravenously without any tracer to determine whether glutamine per se would alter breath $^{13}CO_2$ enrichment: we observed that IV unlabeled glutamine induced a 2.4% ± 0.1% Pee Dee Belemnite (δ PDB) increase in breath $^{13}CO_2$ enrichment. This value was subtracted from the $^{13}CO_2$ enrichments measured at the end of both ^{13}C-leucine and ^{13}C-bicarbonate infusions in the experiments involving glutamine infusion.

Ra_{CO2}, the rate of 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 $NaH^{13}CO_3$, as described.[11,12]

$Ra_{CO2} = i_{Bicarb}[(E_{13CO2\text{-}Bicarb}/E_{Bicarb}) - 1]$, in which i_{Bicarb} is the $NaH^{13}CO_3$ infusion rate (μmol $\cdot$ kg^{-1} $\cdot$ h^{-1}), and $E_{13CO2\text{-}Bicarb}$ and E_{Bicarb} are the ^{13}C enrichments (mole % excess) in expired air at steady state during the last 30 minutes of the labeled bicarbonate infusion and in the infused bicarbonate solution, respectively.

Leucine oxidation (Ox_{Leu}, μmol $\cdot$ kg^{-1} $\cdot$ h^{-1}) was calculated as: $Ox_{Leu} = Ra_{CO2} \cdot E_{13CO2\text{-}Leu} \cdot [1/E_{KIC} - 1/Ei_{Leu}]$, in which $E_{13CO2\text{-}Leu}$ is the steady state $^{13}CO_2$ enrichment in breath over the last hour of labeled leucine infusion.

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

Statistical Analysis

Data are presented as means ± SE. Data were compared between treatment using repeated measures analysis of variance (ANOVA), and unpaired and paired Student's *t* test where appropriate. Significance was established at $P < .05$.

RESULTS

Over the last 60 minutes of each isotope infusion, a steady state was observed in the plasma concentrations of leucine and KIC, as well as in the ^{13}C-enrichments of plasma KIC and breath CO_2 (data not shown).

Effects of Protein Restriction

Although energy intake was the same (132 $kcal \cdot d^{-1} \cdot kg^{-1}$ metabolic weight) in the control and protein-restricted groups, protein restriction was associated with a slightly, but not significantly lower body weight (12.7 ± 0.6 after protein restriction *v* 13.6 ± 0.7 kg after control diet, respectively; $P = .32$). Protein restriction induced approximately a 49% decrease in plasma leucine concentration (77 ± 9 *v* 151 ± 10 $\mu mol \cdot L^{-1}$ after protein restriction period *v* control diet period, $P < .001$) (Table 1). Leucine Ra, an index of whole body protein breakdown, as determined from steady state plasma ^{13}C-KIC enrichments, was lower in the protein-restricted group than in controls (223 ± 16 *v* 298 ± 17 $\mu mol \cdot kg^{-1} \cdot h^{-1}$, $P < .01$). Such was also the case for nonoxidative leucine disposal, an index of whole body protein synthesis, which decreased with protein restriction and 180 ± 15 versus 253 ± 13 $\mu mol \cdot kg^{-1} \cdot h^{-1}$, protein-restricted group versus nonrestricted group, $P < .01$, respectively. Leucine oxidation was not affected by protein restriction (43 ± 4 *v* 46 ± 4 $\mu mol \cdot kg^{-1} \cdot h^{-1}$, protein-restricted group *v* nonrestricted group, not significant [NS]) (Fig 1). Protein restriction increased plasma glutamine concentrations by approximately 38% (892 ± 31 *v* 648 ± 30 $\mu mol \cdot L^{-1}$, protein restricted group *v* nonrestricted group, $P < .001$) (Table 1). There was a trend toward a higher muscle glutamine concentration after protein restriction, but this change did not reach statistical significance (8.4 ± 1.4 *v* 5.3 ± 0.6 $\mu mol \cdot g^{-1}$ wet weight, protein-restricted group *v* nonrestricted group, NS).

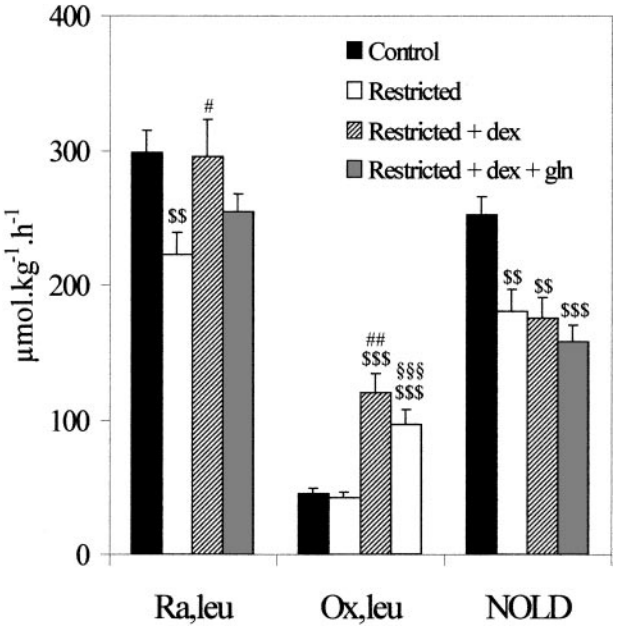

Fig 1. Leucine appearance rate (Ra, leu), oxidation (Ox, leu), and nonoxidative leucine disposal (NOLD; all in $\mu mol \cdot kg^{-1} \cdot h^{-1}$) in 8 adult Beagle dogs maintained on a normal protein diet (control), on a protein-restricted diet (restricted), on a restricted diet with dexamethasone treatment (restricted + dex), and on a protein-restricted diet with dexamethasone treatment and IV glutamine (restricted + dex + gln). Each data represents the mean ± SE of 8 dogs. ($, compared with control; #, compared with restricted; §, compared with restricted + dex. One symbol, $P < .05$; 2 symbols, $P < .01$; 3 symbols, $P < .001$).

Table 1. Leucine and Glutamine Concentrations

Regimen	Plasma [leu] ($\mu mol \cdot L^{-1}$)	Plasma [gln] ($\mu mol \cdot L^{-1}$)	Muscle [gln] ($mmol \cdot kg^{-1}$ wet weight)
Control	151 ± 10	648 ± 30	5.26 ± 0.57
Restricted	77 ± 9*	892 ± 31*	8.42 ± 1.36
Restricted + dex	119 ± 12†	780 ± 29‡	10.08 ± 1.32§
Restricted + dex + gln	93 ± 9§	1,090 ± 70‖	9.97 ± 1.38§

NOTE. Effect of dietary protein restriction (restricted), alone or combined with dexamethasone (restricted + dex) or dexamethasone plus glutamine (restricted + dex + gln), on plasma leucine and glutamine concentrations (in $\mu mol \cdot L^{-1}$) and muscle-free glutamine concentration (in $mmol \cdot kg^{-1}$ wet weight) in Beagle dogs. Each column represents the mean ± SE of 8 dogs.

*Compared with normal protein diet (control), $P < .001$.

†Compared with restricted diet, $P < .01$.

‡Compared with restricted diet (control), $P < .05$.

§Compared with normal protein diet (control), $P < .01$.

‖Compared with restricted + dex, $P < .01$.

Effects of Dexamethasone Treatment

The 7-day dexamethasone treatment was not associated with any change in the dogs' body weight (12.5 ± 0.6 *v* 12.7 ± 0.6 kg after dexamethasone and after control period under protein restriction conditions, respectively, NS). Plasma leucine concentrations increased approximately 55% (119 ± 12 *v* 77 ± 8 $\mu mol \cdot L^{-1}$, $P < .01$) (Table 1) after glucocorticosteroid treatment. This increase can be attributed to an increase in leucine release from protein breakdown, because leucine Ra increased approximately 32% after dexamethasone compared with protein restriction alone: 295 ± 28 versus 223 ± 16 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ ($P < .05$). Dexamethasone treatment produced a dramatic, approximately 186% increase in leucine oxidation: 120.0 ± 14.0 versus 43 ± 4 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ ($P < .001$); yet NOLD remained unaltered: 175 ± 15 versus 180 ± 15 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ ($P = .78$) (Fig 1). Finally, whereas plasma glutamine declined approximately 13% with dexamethasone treatment (780 ± 29 *v* 892 ± 30 $\mu mol \cdot L^{-1}$, $P < .05$), there was no significant change in muscle glutamine concentration (10.1 ± 1.3 *v* 8.4 ± 1.4 $\mu mol \cdot g^{-1}$ wet weight, $P =$ NS) (Table 1).

Effect of IV Glutamine Infusion

As expected, IV glutamine infusion resulted in a significant, approximately 40% increase in plasma glutamine concentration (1,090 ± 70 *v* 780 ± 29 μmol · L^{-1}, $P < .01$). In contrast, the concentration of free glutamine failed to increase in skeletal muscle (10.0 ± 1.4 *v* 10.1 ± 1.3 μmol · g^{-1} wet weight). Glutamine treatment produced a slight, but not significant, decline in plasma leucine level (93.2 ± 9.1 *v* 119.1 ± 12.0 μmol · L^{-1}, NS) (Table 1). Glutamine infusion failed to significantly affect leucine kinetics (Fig 1). Indeed, leucine Ra was 295 ± 28 and 255 ± 14 μmol · kg^{-1} · h^{-1} during the dexamethasone + saline, and dexamethasone + glutamine study days, respectively. Similarly, although a tend toward a decrease in both leucine Ra and oxidation (≈−14% and ≈−16%, respectively) could be observed on the glutamine day, glutamine failed to significantly alter leucine oxidation (120 ± 14 *v* 101 ± 14 μmol · kg^{-1} · h^{-1} during the dexamethasone + saline, and dexamethasone + glutamine study days, respectively; NS) or nonoxidative leucine disposal (175 ± 15, and 153 ± 14 μmol · kg^{-1} · h^{-1} during the dexamethasone + saline, and dexamethasone + glutamine study days, respectively; P = NS).

DISCUSSION

The present study used continuous infusion of stable isotopes to examine the effects of (1) protein restriction alone, (2) protein restriction combined with a 7-day glucocorticoid treatment, and (3) a short-term IV glutamine infusion on whole body leucine metabolism in dogs submitted to both protein restriction and glucocorticoid treatment. The results show that protein restriction and glucocorticoid excess have distinct effects on whole body leucine metabolism. Furthermore, they show that adult dogs receiving a low protein diet and a 7-day course of dexamethasone display alterations in leucine kinetics similar to those observed in critical illness-associated protein wasting in humans, suggesting that this regimen could be used as a experimental animal model for hypercatabolism. Finally, although a trend toward both lower proteolysis and amino acid oxidation could be observed under the experimental setting chosen, a 7-hour infusion of IV glutamine in the fasting state did not significantly attenuate protein wasting.

The protein-restricted diet provided an intake of 2.6 g · kg^{-1} · d^{-1} of protein versus 5.2 g · kg^{-1} · d^{-1} of protein in the control group. Although this amounts to a 50% restriction in protein intake, such a protein supply without energy restriction is still presumed to cover the minimal protein requirements of healthy Beagle dogs as defined by AAFCO recommendations.[7] Protein restriction was associated with a reduction in both protein breakdown and protein synthesis, but failed to affect amino acid oxidation. Although most studies performed in humans describe a correlation between plasma leucine concentration and leucine oxidation,[16] we observed that although protein restriction was associated with a 50% decrease in plasma leucine concentration, it failed to alter leucine oxidation. We speculate that the restricted diet may have supplied insufficient amounts of some indispensable amino acids, such as lysine and tryptophan. Indeed, if the digestibility of the protein in the regular diet was poorer than expected, this would lead to a deficiency in some indispensable amino acids. This deficiency may, in turn, account for the 25% reduction in leucine turnover (both in leucine Ra and NOLD) we observed after adaptation to the protein-restricted diet when compared with control diet values. The reduction in protein synthesis would eventually lead to the accumulation and oxidation of other essential amino acids (eg, leucine) and thus maintain a high rate of leucine oxidation. These data suggests that when dogs are submitted to protein restriction with very minimal indispensable amino acids supplies, adaptive mechanisms operate to slow down protein turnover so that the overall net leucine balance remains unaltered. Similar mechanisms are believed to operate in humans submitted to dietary protein restriction.[17,18]

We document that protein restriction results in an increase in plasma glutamine in dogs. We speculate that the increase in plasma glutamine and the trend toward an increase in muscle glutamine could result from an adaptive regulation involving a higher rate of de novo glutamine synthesis in the protein-restricted dog. Matthews et al[19] have indeed shown enhanced rates of glutamine de novo synthesis in protein-restricted humans.

The doses of dexamethasone selected in the current study (0.44 mg · kg^{-1} · d^{-1}) were equivalent to 11 mg · kg^{-1} · d^{-1} hydrocortisone, or approximately 9 times the basal rate of cortisol secretion in healthy dogs. Increases in endogenous cortisol secretion of that magnitude are known to occur during stress.[20] The dramatic increase in leucine Ra and leucine oxidation, indices of whole body protein breakdown and oxidation, respectively, when compared with the restricted diet alone data, reflects a significant protein wasting effect. Assuming body protein contains 8-g leucine per 100-g protein, such an increase in proteolysis and protein oxidation, if sustained in the fed state as well, would result in approximately a 3 g · kg^{-1} · d^{-1} loss of body protein. If we assume muscle tissue is about 20% protein by weight, this implies a lean tissue loss of 1.35 kg for a 13-kg dog over the 7 days of dexamethasone. In this study, dexamethasone did not induce any weight loss. Two factors may account for the lack of weight loss: (1) although glucocorticoids have a weak mineralocorticoid effect, the latter effect may not have been negligible with the high doses used, and muscle wasting may have been compensated by sodium and water retention, but this is unlikely because dexamethasone has essentially no mineralocorticoid activity; and (2) a gain in fat mass may have occurred compensating for the presumed loss in lean body mass. Although we did not assess body composition, the determination of nitrogen balance performed in 8 dogs (−0.3 g N kg^{-1} · d^{-1}, ie, −1.9 ± 0.3 g protein · kg^{-1} · d^{-1} after dexamethasone; data not shown) confirmed by another independent method that dexamethasone induced loss of body protein. Losses of that magnitude are commonly observed during critical illness.

The current findings are consistent with the effects of glucocorticoids in humans. Indeed, treatment of healthy humans with cortisol or prednisone, a synthetic glucocorticosteroid, produced a dramatic increase in leucine appearance rate (Ra), an index of whole body protein breakdown,[15,21] and leucine oxidation (Ox),[21] an index of protein oxidation, and these alterations are similar to those observed in severe illness. In earlier studies performed in dogs, Mc Allister et al[22] observed

that ACTH treatment caused a marked increase in protein breakdown, as assessed with ^{3}H-labeled leucine infusion. Yet the current study is, to our knowledge, first to document that in dogs, glucocorticoids dramatically enhance leucine oxidation as well. Obtaining these data was a prerequisite if we were to use that animal as a putative catabolic model. The current study indeed suggests that protein restriction associated with dexamethasone treatment produces a hypercatabolic state with high rates of proteolysis and amino acid oxidation; this model may thus be used as a model of stress-induced protein wasting.

Studies performed in humans during major surgery, multiple trauma, or sepsis suggest that a profound depletion of muscle glutamine pool is associated with protein wasting in severe acute diseases.[5,23,24] In the current study, although plasma glutamine declined slightly in protein-restricted dexamethasone-treated dogs when compared with dogs submitted to protein restriction alone, the corticosteroid treatment failed to deplete muscle-free glutamine pool, because muscle-free glutamine remained unaltered after dexamethasone. This contrasts with earlier work by Mühlbacher et al,[8] who reported an approximate 47% decline in muscle glutamine content after 7 to 14 days of dexamethasone. Several differences may account for this discrepancy. Compared with the dogs studied by Mühlbacher et al,[8] our dogs had a lower muscle-free glutamine at baseline before either protein restriction or corticosteroid treatment (5.3 *v* 8.4 and 10.1 μmol $\cdot$ g^{-1} wet weight). Second, dietary protein intake differed between the 2 studies. Finally, the site of the muscle biopsy may affect the response of muscle glutamine to glucocorticoids, as the response glutamine synthetase activity differed from 1 muscle group to the next in corticoid-treated rats.[25]

Although more and more complete amino acid mixtures have been used in parenteral nutrition (PN), conventional PN still is devoid of glutamine. This is mainly because (1) glutamine has relatively poor solubility and stability in an aqueous solution in vitro and thus cannot be heat sterilized and (2) until recently, glutamine was believed to be the classic example of a nonessential amino acid. Over the last decade, more than 50 clinical trials have assessed the potential benefit of IV glutamine supplementation in various clinical settings. Although glutamine supplementation failed to show any benefit in stable patients receiving home parenteral nutrition,[26] glutamine was shown to improve nitrogen balance after elective surgery for cholecystectomy or colon cancer[4,5,27] and to shorten hospital stay in surgical patients.[4,28] Similarly, IV glutamine decreased the incidence of sepsis and the length of hospital stay in patients with hematologic malignancies receiving bone marrow transplantation,[6] yet later studies failed to substantiate that effect.[29] Recently, glutamine-supplemented PN was found to decrease long-term mortality in a study of critically ill trauma patients.[30] Conflicting data have appeared with regard to enteral glutamine supplementation. Although Jensen et al[31] failed to observe any improvement in nitrogen balance when trauma patients received enteral glutamine supplements, Houdijk et al[32] reported a significant reduction in the incidence of sepsis in a similar population of patients receiving more than 5 days of enteral glutamine supplements compared with an isonitrogenous, isocaloric, glutamine-free regimen. The same decline in the incidence of sepsis was observed in preterm infants receiving glutamine-supplemented nutrition, as compared with regular formula.[33] Taken together, published studies suggest that in the absence of liver or renal failure, glutamine is safe; they furthermore support a benefit of glutamine supplementation in some, but not all, clinical situations associated with stress and protein wasting. Several issues regarding the potential benefit of glutamine remain pending, including (1) the dose-response curve of its effects, (2) the specific clinical indications of glutamine supplements, and (3) the mechanism(s) by which glutamine may exert its effects. For instance, does glutamine affect gut trophicity and prevent germ translocation,[34] or does it affect immune status, does it work through its role as a precursor of glutathione and thus protect redox balance,[35] or does glutamine enhance whole body protein anabolism? The current study attempted to address the latter issue in a specific animal model.

A 7-hour intravenous infusion of glutamine failed to significantly alter whole body leucine oxidation and nonoxidative leucine disposal in the current study. Although there was a trend toward lower rates of leucine appearance and leucine oxidation, the present results suggest that short-term IV glutamine did not have any anabolic effect. This finding contrasts with the approximately 40% inhibition of leucine oxidation and approximately 40% increase in the nonoxidative leucine disposal observed upon glutamine infusion in healthy adults.[9] Although this discrepancy may reflect interspecies differences, differences in the dose, duration, timing, and route of glutamine supply may account for the failure of glutamine to elicit a protein anabolic effect. Indeed, the dose used (800 μmol $\cdot$ kg^{-1} $\cdot$ h^{-1}) in the current study was identical to the dose that increased NOLD in human studies[9,10] and close to doses that were found to improve nitrogen balance in severely ill humans.[6] We have since observed that glutamine turnover rate is 2 to 3 times faster in dogs as it is in humans, when expressed per kg of body weight (Humbert et al, unpublished data). Although larger doses of glutamine might have had an effect, the use of such doses would hardly be feasible with IV free glutamine. Indeed, due to the relatively low solubility of glutamine ($\approx$ 2.6 g $\cdot$ dL^{-1}), larger doses would imply the infusion of a very large fluid volume that would be poorly tolerated by the animals. Moreover, skeletal muscle is one of the main sites both of leucine oxidation and of the putative protein anabolic effect of glutamine, and other investigators have shown a striking correlation between the concentration of free glutamine in skeletal muscle and the rate of muscle protein synthesis in various models of stress in the rat.[36,37] If the concentration of free glutamine in situ is indeed a protein anabolic signal, then the failure of IV glutamine infusion to raise muscle glutamine concentration in our dogs could explain the lack of protein anabolic effect of glutamine observed in the current study. The route of glutamine infusion may have been inappropriate as well, because glutamine-induced inhibition of leucine oxidation has, to our knowledge, only been reported upon enteral glutamine infusion.[9,10] Finally, because the dogs were studied after adaptation to a low protein diet and in the fasted state, limited amino acid availability may have blunted the protein anabolic response to glutamine. To test the latter hypothesis, further studies addressing the effect of enteral glutamine in the fed state would be warranted.

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