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The effect of glutamine- and alanine-enriched total parenteral nutrition on postburn proteolysis in the rat

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This study compared the effects of total parenteral nutrition (TPN) supplemented with either glutamine (GLN) or alanine (ALA) on postburn proteolysis in the rat. Thirty adult Sprague-Dawley rats underwent catheterization of the superior vena cava and placement of a swivel apparatus. One group (CONTROL, N = 18) was then returned to their cages while the remaining animals (BURN, N = 12) received a full-thickness scald burn (approximately 20% BSA). CONTROL and BURN rats were then randomly assigned to receive TPN supplemented with either GLN or ALA. TPN consisted of dextrose (250 Kcal/Kg BW/day) and crystalline amino acids (0.9 gN/kg BW/day) composed of all essential amino acids, histidine, arginine, glycine, and either ALA or GLN (1.2 g/100 mg). TPN was administered for 3 days after the burn. Urinary 3-methyl histidine (3MH) and nitrogen excretions were determined, and blood amino acids were measured.

Both BURN groups had significantly higher 3MH excretion and blood levels of branched chain amino acids (BCAA) and alanine than both CONTROL groups. In both BURN and CONTROL groups, GLN-TPN did not significantly affect 3MH excretion or the blood levels of BCAA or alanine compared with ALA-TPN. In BURN animals, GLN-TPN resulted in significantly higher urinary nitrogen excretion than ALA-TPN resulting in lower nitrogen retention. Compared with ALA-TPN, GLN-TPN produced greater urinary nitrogen losses and did not reduce protein catabolism. (J. Nutr. Biochem. 9:28–30, 1998) © *Elsevier Science Inc. 1998*

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One of the goals in the nutritional support of stressed patients is to minimize the loss of body protein. During metabolic stress there is an increase in nitrogen losses associated with increased muscle proteolysis.[1] Alanine (ALA) and glutamine (GLN) account for nearly 50% of the amino acids released by muscle during stress.[2] In the liver, the carbon skeletons of these amino acids serve as precursors for gluconeogenesis, whereas the amino nitrogen can be used for the synthesis of either protein or urea.[3]

The metabolic response to stress has been classically regarded as enhanced flux of amino acids from muscle to the liver to supply carbons for gluconeogenesis. However, recent investigations have shown that the gastrointestinal tract also plays an active role in this response.[4] Part of the GLN released by muscle is taken up by the intestine and used as a fuel by the epithelial cells.[5]

Because the intestinal uptake of GLN is increased during stress, it has been suggested that the increased consumption of GLN by the intestine may induce increased protein breakdown during hypercatabolic states.[6] Moreover, the intravenous administration of the dipeptide GLN-ALA during stress has been shown to reduce the release of GLN from muscle.[7] Thus, in theory, if the increased intestinal consumption of GLN can be met with the exogenous administration of GLN, endogenous protein may be spared.

The purpose of this study was to determine whether the administration of TPN supplemented with GLN would reduce post-trauma proteolysis in the rat as compared with TPN with an isonitrogenous amount of ALA.

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Table 1 Composition of the TPN formula

I. Amino acids—0.9 g N/kg BW/day
a. Essentials (g% w/v)
ILE 0.46 MET 0.73
LEU 0.72 PHE 0.73
VAL 0.53 TRY 0.16
LYS 0.53 THR 0.33
HIS 0.43
b. Non-essentials (g% w/v)
ARG 1.40 GLY 1.30
ALA 1.46 *(OR)* GLN 1.20
II. Dextrose—250 Kcal/Kg BW/day
III. Minerals and vitamins

Amino acids provided as Aminosyn-RF™ (Abbott, N. Chicago IL). Amino acids added to the TPN solution (see Methods).

Materials and methods

Thirty male Sprague-Dawley rats (275–300 g) were housed in individual metabolic cages and acclimated to the laboratory for 48 hr. All rats were then anesthetized (Ketamine, 80 mg/kg BW; i.p.) and had placement of a Silastic catheter (Dow Corning Corp., Corning, NY USA) into the superior vena cava via the jugular vein. The catheter was exteriorized in the interscapular area within a spring and connected to a swivel apparatus. Eighteen rats (CONTROL) were returned to their cages. The remaining 12 rats (BURN) had the hair on their dorsum clipped and were then secured within a mold that exposed an area of skin that represented approximately 20% of the total body surface area. Full-thickness scald burns were produced by immersing the exposed area into boiling water for 10 sec.[8] CONTROL and BURN rats were then randomly assigned to receive total parenteral nutrition (TPN) supplemented with either GLN or ALA.

The TPN solution consisted of dextrose (250 Kcal/Kg BW/day) and crystalline amino acids (0.9 gN/kg BW/day) plus minerals and vitamins to meet the requirements of the rat.[9] The TPN solution for this study was formulated to provide isonitrogenous amounts of GLN or ALA, maintaining the same essential to nonessential amino acid ratio. The stock TPN solution consisted of dextrose (250 Kcal/Kg BW/Day) and crystalline amino acids, including histidine. Arginine was supplemented to the solutions because it is considered semi-essential for rats. Additional nonessential amino nitrogen was included as glycine to reach the same essential:nonessential amino acid ratio used in complete amino acid formulas (1:1.1). GLN or ALA were added to the solution to provide 14% of all dietary nitrogen (*Table 1*). These amino acids were added as the L-forms (Sigma, St. Louis, MO USA) using aseptic techniques.

Rats received TPN for 3 days after the burn and were restricted of any p.o. intake, i.e., water and chow. Urine and feces were collected on Days 2 and 3 of TPN administration. All rats were anesthetized and had blood drawn by cardiac puncture within 1 min from stopping the TPN infusion for amino acid determinations.

Urinary 3-methyl histidine (3MH) was measured using high-performance liquid chromatography (HPLC).[10] Amino acids were measured in whole blood by HPLC using a lithium ion-exchange column (Pickering Laboratory Incorporated, Mountain View, CA USA). Total dietary, urinary, and fecal nitrogen were determined by the Kjeldahl method.[11] Statistical analyses were performed using a two-way analysis of variance (ANOVA) for each dependent variable. When the ANOVA indicated a significant effect, comparisons between groups were made using Student's *t*-test for independent groups.

Table 2 Urinary 3 methyl histidine excretion and blood amino acid levels

	Control		Burn	
	ALA	GLN	ALA	GLN
	5	8	5	6
Urinary 3MH (μM)	0.52 (0.64)	0.55 (0.76)	14.27[a] (5.70)	14.13[a] (5.37)
Blood AA (% total AA)				
Branched Chain	8.3 (1.4)	7.4 (2.7)	11.2[a] (3.0)	12.2[a] (1.3)
Alanine	19.0 (3.0)	19.6 (3.2)	23.9[a] (3.0)	21.7[a] (2.8)
Glutamine & Glutamate	16.5 (1.3)	17.8 (2.0)	15.4 (1.8)	16.3 (1.6)

[a]$P < 0.05$ vs Control groups.
Data are presented as mean/standard deviation.

Results

All animals survived the study period, but six animals (4 CONTROL ALA, 1 CONTROL GLN, and 1 BURN ALA) developed superior vena cava thrombosis, which precluded the administration of TPN and, therefore, were excluded from data analysis.

Both BURN groups, ALA and GLN, excreted significantly more urinary 3MH than their respective CONTROL groups (*Table 2*). The excretion of 3MH did not differ significantly between ALA and GLN animals within either the BURN or CONTROL groups. Similarly, both BURN groups showed significant increases in blood branched chain amino acids (BCAA), and ALA as compared with their respective CONTROL groups. The blood levels of BCAA, GLN, and ALA did not differ significantly between ALA and GLN animals within either the BURN or the CONTROL group. Thus, the blood levels of ALA and GLN were not significantly affected by the intravenous infusion of either ALA or GLN.

Nitrogen intake and fecal nitrogen excretion did not differ significantly among the four groups (*Table 3*). Urinary nitrogen excretion, however, was significantly higher in the BURN GLN group compared to the BURN ALA group. This increased nitrogen loss for the BURN GLN group was associated with negative nitrogen retention in the BURN GLN group.

Table 3 Nitrogen balance

	Control		Burn	
mg Nitrogen X/(SD)	ALA	GLN	ALA	GLN
Intake	1733 (76)	1857 (133)	1730 (64)	1755 (100)
Urinary excretion	1556 (270)	1527 (115)	1554 (196)	1937[a] (236)
Fecal excretion	141 (47)	119 (66)	73 (22)	51 (11)
Net balance	+34 (317)	+209 (288)	+101 (211)	−233[b,c] (312)

[a]$P < 0.05$ vs BURN ALA.
[b]$P < 0.05$ vs CONTROL.
[c]$P = 0.06$ vs BURN ALA.

Discussion

In this rat model of stress the significant increase in urinary 3MH excretion is an indication of increased protein catabolism. In addition, the burn wound was associated with significantly elevated blood levels of BCAA, which also serves as a marker of a significant metabolic stress. Neither the urinary excretion of 3MH nor the blood levels of BCAA and tyrosine was significantly affected by the supplementation of GLN in TPN. In burn animals GLN-TPN was associated with significantly higher urinary nitrogen excretion than ALA-TPN, resulting in negative nitrogen retention, although this difference did not reach statistical significance. Thus, in this model of metabolic stress using the dietary formulation described, GLN-TPN does not reduce catabolism of endogenous protein and results in greater nitrogen losses than ALA-TPN.

Urinary nitrogen losses result from the net balance between whole-body protein synthesis and protein breakdown. The amino nitrogen released from amino acids can be transferred to alpha-keto acids and form new amino acids, or it can be incorporated into urea and lost in urine. During stress there is an increase in gluconeogenesis, and secondarily, an increase in proteolysis, resulting in greater ureagenesis.[3] GLN and ALA are the main products of muscle proteolysis and both serve as precursors for gluconeogenesis.[12]

GLN serves as a respiratory fuel for the intestinal epithelium, kidney, brain, liver, and white blood cells.[5] In the intestine, the nitrogen products of GLN metabolism are ammonia (37.9%), citrulline (27.6%), ALA (24.4%), proline (7.2%), glutamate (1.8%), and ornithine (1.1%).[5] These products of GLN metabolism are transported by the portal system to the liver where they become precursors for ureagenesis. Because protein catabolism, assessed by urinary 3MH excretion, did not differ between the ALA and the GLN groups, it seems that the increased nitrogen losses may be secondary to increased ureagenesis.

Catabolic hormones such as glucocorticoids and glucagon exert significant effects on GLN metabolism.[12] During glucocorticoid infusion the release of GLN from the muscle increases as the uptake of GLN by the intestine also increases.[13] Glucagon stimulates hepatic gluconeogenesis[14] and renal ammonia-genesis[15] from GLN. The effects of glucagon on glutamine metabolism seem to be secondary to an increased activity of glutaminase.[16] In a rat model of burn injury similar to the one used in this study the rate of intestinal glutaminase activity was increased.[17] However, in a model of sepsis Ardawi and collaborators found a decrease in the rate of intestinal glutaminase activity.[18] Although, in this study we did not measure the rate of intestinal glutaminase activity, it is known that the route of nutrition can influence the rate of intestinal glutaminase activity. The rate of glutaminase activity decreases as the metabolic demands to the intestine decrease when maintained at rest.[19] In addition, parenteral nutrition can produce a subclinical state of sepsis by allowing bacterial translocation through an increased intestinal permeability.[20]

In conclusion, in this rat model of stress combined with bowel rest, greater nitrogen loss results from the intravenous supplementation of GLN as compared with ALA. Because no differences were detected in the sparing of proteolysis with supplementation of either GLN or ALA, we suggest that post-traumatic protein catabolism is not elicited by a need for GLN.

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