Amelioration of the Protein Metabolic Response in Spermidine-Supplemented Trauma Rats

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Polyamines are biologically active, small, positively charged ubiquitous compounds that play an important role in initiating adaptive changes in cell proliferation, cell growth, and synthesis of proteins and nucleic acids. The potential for exogenous dietary polyamine to significantly contribute to whole-body growth and health has not been explored. This study evaluates the efficacy of feeding a liquid diet supplemented with 0.05% spermidine in injured rats. Rats traumatized by bilateral femur fracture (n = 12) and pair-fed uninjured controls (n = 12) were starved for 2 days and then refed for 4 days with a liquid diet containing 0.05% spermidine or an isonitrogenous control diet. Daily urine and body weight data were collected. At the end of refeeding, the rats were killed, and blood, cerebrospinal fluid (CSF), muscle, and brain tissue were collected. Spermidine supplementation (0.05%) was found to be tolerated well by the rats and (1) did not affect voluntary food intake by traumatized rats, (2) did not alter the growth rate, (3) increased protein utilization efficiency, and (4) decreased leucine, isoleucine, and valine levels in plasma and muscle. The profound effect of trauma on plasma amino acid metabolism seen in rats fed the basal diet was absent in spermidine-supplemented rats. Depletion of plasma glutamine (GLN) levels due to trauma was significantly less in rats with spermidine supplementation (11% *v* 33%), indicating a beneficial effect to counteract trauma responses. The results suggest that spermidine supplementation improves protein utilization efficiency and ameliorates trauma effects on amino acid levels.

SEVERE INJURY ELICITS profound metabolic changes to meet the increased demand. Hormones, cytokines, and endogenous fuel substrates modulate these changes, and their interaction is important for an early recovery. Efficient management of this early, crucial injury period with a more effective way of preserving the lean body mass is imperative for early uncomplicated recovery. Optimal nutritional support plays a beneficial role in normalization of the injury effects. The use of novel substrates with potential benefits to severely injured patients has recently been explored in an effort to meet specific organ or tissue needs, since the efficacy of nutrition cannot be easily improved by quantitative modifications alone. Adjuvant use of an anabolic stimulus during intensive nutritional therapy is needed in the catabolic "flow phase" of severe trauma.[1] Several adjuvant therapies such as pharmacologic doses of insulin,[2] anabolic steroids,[3,4] ornithine α-ketoglutarate,[5,6] branched-chain amino acids (BCAA),[7,8] dipeptides,[9] glutamine (GLN),[10] growth hormone,[11-13] insulin-like growth factor-1,[14,15] and epidural blockade[16] have been used, supplementing conventional nutrition to improve nitrogen economy. Exogenous polyamines are another promising source to improve nutritional outcome. Polyamines are known to play an important role in the growth process of a number of species, including man.

Putrescine, spermidine, and spermine are the three biologically active polyamines widely distributed in living organisms. They promote anabolic events such as synthesis of DNA, RNA, and protein and increased amino acid uptake by cells.[17] The potential for exogenous dietary polyamines to significantly contribute to growth and health has been recently reviewed.[18] Any process of rapid regeneration or regrowth of tissues is associated with elevated polyamine levels in plasma and urine. Urinary polyamine levels are indicated as valid biomarkers of injury response in multiple-trauma victims.[19] Elevated urine polyamine levels in trauma patients correlate well with other measures of the protein metabolic response to injury and changes during nutritional therapy.[20] For optimal growth, intracellular polyamine levels are regulated within a relatively narrow range; the upper limits are seemingly determined by the potential cellular toxicity of polyamine excess, and the lower limits by the polyamine requirement of cell growth. Feeding of spermidine at moderate intake (spermidine 0.05% of dietary nitrogen) is not toxic in growing, normal rats.[21] Its dietary effects following trauma are not known.

Polyamine requirements that are not met by biosynthesis must be provided by exogenous polyamines derived from food or supplementation. Exogenously supplied dietary polyamines are readily taken up by the gastrointestinal tract and enter the systemic circulation.[18] The relative efficacy of dietary putrescine,[22] spermidine,[23] and spermine[24] on whole-body levels has recently been investigated in normal growing chicks.

Spermidine protects intestinal mucosa from lipid-induced injury, suggesting a physiologically relevant role in modulating mucosal integrity postprandially.[25] Moreover, spermidine accelerated the healing of gastric mucosal stress ulcerations better than putrescine.[26] In addition, the polyamine requirements of mammalian cells cannot be met by putrescine until it is converted to spermidine. Thus, spermidine would be the logical polyamine choice to evaluate for exogenous administration and dietary efficacy. It is expected to show beneficial metabolic and nutritional outcome.

The objective of this study is to investigate the nutritional efficacy of spermidine as a dietary supplement in growing rats following bilateral femoral fracture. This may be the first attempt in a mammalian trauma situation to test the dietary efficacy of a polyamine.

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Submitted April 28, 1997; accepted July 21, 1997.

Presented at the Experimental Biology 95 meeting of the Federation of American Societies for Experimental Biology (FASEB), Atlanta, GA, April 9-13, 1995, and published as an abstract in FASEB J 9:A574, 1995 (abstr 3328).

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0026-0495/98/4702-0019$03.00/0

MATERIALS AND METHODS

Young male Sprague-Dawley rats (ACE Animals, Boyertown, PA) weighing 250 to 270 g were housed in individual metabolic cages (Plas-Labs, Lansing, MI) and kept in our well-ventilated temperature (23° ± 1°C)- and humidity (45% ± 5%)-regulated vivarium with controlled 12-hour light-dark cycles. They were adapted to freely available liquid-diet feeding and water for 3 days. The animal research facility at St. Joseph's Hospital and Medical Center (Phoenix, AZ) is accredited by the American Association of Accreditation of Laboratory Animal Care. The research protocol was reviewed and approved by the Institutional Animal Care and Use Committee, and the study adhered to the National Research Council's *Guide for the Care and Use of Laboratory Animals.* Twenty-four animals were divided into two groups: control (n = 12) and trauma (n = 12). Animals in each group were weight-matched and then assigned to one of two isonitrogenous diets: basic diet or test diet. Food intake was determined daily by weighing the liquid-diet feeding tubes at the beginning and end of each 24-hour period, and urine output was collected from 8:00 AM to 8:00 AM for determination of 24-hour excretion of nitrogen, creatinine, and urea. Each day between 8:30 and 9:30 AM, the animals were weighed, urine output was measured and removed, and the feeding tubes were changed. All rats had free access to water.

On day 1, all rats were anesthetized with an intraperitoneal injection of ketamine hydrochloride 0.1 mg/g body weight (Ketalar 50 g/L; Parke-Davis, Morris Plains, NJ), and the animals were weight-matched. Half of the animals (n = 12) received closed bilateral femur fractures involving standard-force fracture at the midshaft of the femur by twice releasing the arm of a common spring-loaded rat trap onto the leg. This ensured uniform closed fracture and soft-tissue damage. The other half (n = 12) acted as weight-matched, pair-fed controls for each traumatized animal. The animals were then returned to individual cages and had free access to water but were deprived of food for 2 days. This condition was imposed to simulate the conditions of human subjects treated in trauma intensive care units.

On the third day, feeding was started and continued for 4 days. One set of 12 rats (six control and six trauma) were given a casein-based basic oral liquid diet (#F1259; BioServ, Frenchtown, NJ) that provided N 6.5 g L and 4,187 kJ (1,000 kcal)/L. The diet contained 18% protein, 35% fat, and 47% carbohydrate. The remaining set of 12 animals were fed the oral liquid test diet. This isonitrogenous test diet contained the basic diet in which 32.5 mg N/L was replaced by nitrogen from spermidine. The test diet therefore had 0.50% of the nitrogen replaced by spermidine nitrogen. In terms of spermidine base, the test diet contains 0.05% spermidine (weight per dry diet weight). For a daily intake of 500 mg N by a 250-g rat, this corresponds to an intake of 60 μmol spermidine per day from the test diet. This dose rate was selected on the basis of observations in chicks.[23] Control and trauma rats tolerated this dosage of spermidine.

In each weight-matched group, control rats were pair-fed with trauma animals using the BioServ ARF/Israel simultaneous pair-feeding system (BioServ product no. F7378; US Patent No. 4628866). This eliminated the gorging-fasting syndrome commonly associated with liquid-diet experiments.[27] All animals had free access to water. On day 6 at the end of 4 days of oral feeding, food was withdrawn for 2 hours and the rats were anesthetized. Blood was collected by cardiac puncture, and the separated plasma was stored at −80°C for amino acid analysis. The cerebrospinal fluid (CSF) was also collected by ventricular puncture. Forearm muscle and brain tissue were excised, and the blood was wiped out immediately, frozen by liquid N, and then stored at −80°C until analysis. The tissues were freeze-dried to constant weight.

Total daily urinary nitrogen was determined using a chemiluminescence digital analyzer (model 7000; Antek Instruments, Houston, TX). Urine urea and creatinine concentrations were determined using standard procedures (urease method for urea and picric acid method for creatinine) with the MicroCentrifugal Analyzer (Multistat Plus; Instrumentations Laboratory, Lexington, MA). Individual free amino acids in plasma, CSF, and dry tissues were determined by the automated ion-exchange method with an amino acid analyzer (model 7300; Beckman Instruments, Palo Alto, CA). Briefly, 500 μL 4% (40 g/L) sulfosalicylic acid containing the internal standard amino-ethyl-L-cysteine was added to 500 μL plasma or CSF or 20 to 30 mg dry tissue and vortexed slowly. It was then centrifuged at 10,000 *g* for 10 minutes at 4°C. The supernatant was filtered through a 0.22-μm filter, and a 50-μL aliquot sample was injected for analysis. A calibration standard was analyzed after every six samples. The coefficient of variation for multiple analyses was less than 3%.

Apparent daily nitrogen balance was calculated by subtracting urinary nitrogen excretion from dietary nitrogen intake. Because fecal output was small and constant, nitrogen losses from this source were considered small and negligible and were not included in nitrogen-balance calculations.[28] The apparent daily nitrogen balance was normalized to the intake of nitrogen. The effects of trauma in relation to dietary supplementation were analyzed between corresponding groups.

Unless otherwise noted, values listed are the mean ± SEM. Statistical analyses were performed using two-way ANOVA, with the factors defined as group status (control and trauma) and diet (basal and test).[29] A P value of .05 or less was considered statistically significant.

RESULTS

Metabolic and nutritional parameters for the animals with growth indices are summarized in Table 1. Control rats were

Table 1. Growth and Metabolic Parameters in the Rat Groups

	Basal Diet (−0.05% SD)		Test Diet (+0.05% SD)	
Parameter	Control	Trauma	Control	Trauma
Body weight (g)				
Initial	262 ± 3	265 ± 4	264 ± 4	262 ± 3
2 d starvation	231 ± 3	232 ± 3	233 ± 3	226 ± 3
4 d feeding	275 ± 4	262 ± 5	277 ± 5	262 ± 6
Body weight gain				
g/d	12.2 ± 1.7	10.7 ± 1.8	11.9 ± 1.6	9.4 ± 1.9
g/g N consumed	24.3 ± 3.0	19.7 ± 3.2	22.8 ± 3.1	19.4 ± 3.9
Food intake				
mg N/d	501 ± 20	523 ± 20	518 ± 12	496 ± 13
mg N/g body weight	1.91 ± 0.09	2.02 ± 0.08	2.01 ± 0.04	1.92 ± 0.04
Total N excretion (g N/d)	287 ± 11	315 ± 18	220 ± 11*	254 ± 12†‡
Urea N excretion (g N/d)	250 ± 10	274 ± 12	184 ± 9*	230 ± 13†‡
N balance				
mg N/d	227 ± 15	228 ± 16	312 ± 12*	259 ± 13‡
mg N/g N consumed	439 ± 21	440 ± 24	600 ± 18*	519 ± 19†‡
Creatinine				
mg/d	8.9 ± 0.3	8.1 ± 0.3	7.2 ± 0.3*	6.7 ± 0.3†
μg/g body weight	33.8 ± 1.1	31.7 ± 1.2	27.3 ± 1.0*	26.2 ± 0.9†
Urea N/total N (g/100 g)	87.6 ± 20	81.9 ± 2.8	84.6 ± 3.3	90.3 ± 2.5†

NOTE. Results are the mean ± SEM; n = 6 per group. All results except the first 3 rows are the average of 4 days of feeding.

Abbreviation: SD, spermidine.

*$P \leq .05$ *v* basal diet (−0.05% SD) control.

†$P \leq .05$ *v* basal diet (−0.05% SD) trauma.

‡$P \leq .05$ *v* test diet (+0.05% SD) control.

pair-fed to respective weight-matched trauma rats. The starting weight of the animals was 263 ± 3 g. Daily body weight in the growing rats is illustrated in Fig 1. Food deprivation for 2 days with free access to water resulted in a 13% loss of body weight in control and trauma rats. The trauma rats were not immobilized at any time and displayed little hindrance to motion from bilateral femur fractures except in the ability to exert force with the hindlimbs. Four days of refeeding after 2 days of fasting resulted in a gain of more body weight in control rats (12.0 g/d) than in trauma rats (10.1 g/d) irrespective of the diet, although control rats were pair-fed to the respective trauma rats. Relative daily food intake (1.97 ± 0.06 mg N/g body weight) was similar for all rats. On the first day of feeding, all control and trauma rats were able to ingest about 90% of their 4-day average consumption. Changes in daily nitrogen balance (milligrams N per day) are shown in Fig 2. The majority of nitrogen loss during fasting occurred during the first day. The first day of feeding reverted the nitrogen balance to positive. The protein efficiency ratio (PER), defined as the gain in body weight per gram of nitrogen consumed, was 17% lower in trauma rats of each dietary group, and there was no diet-induced change in PER. None of the rats showed any sign of discomfort, lethargy, or toxicity due to 0.05% spermidine supplementation.

Total N and urea N excretion were significantly lower in spermidine-fed control and trauma rats. This resulted in a better retention of nitrogen in rats supplemented with spermidine. Net protein utilization ([NPU] percentage of ingested nitrogen to retained nitrogen) was significantly higher in spermidine-fed control ($P = .001$) and trauma ($P < .025$) rats compared with the respective basal diet–fed rats (Fig 3). This finding demonstrated the beneficial nutritional efficacy of the spermidine-supplemented diet. In general, trauma rats excreted more urea (10% to 20%) and less creatinine (7%) than control rats. The ratio of urinary urea nitrogen to total nitrogen excreted did not change with trauma. Rats fed spermidine, in general, excreted significantly less creatinine, urea, and total nitrogen.

Free amino acid levels in plasma and CSF are listed in Table 2; muscle and brain tissue amino acid levels are shown in Table 3. CSF data were available for rats fed the test diet only and are provided for analysis of the trauma effect. Free amino acid levels in CSF show a profound change from plasma levels and are affected by trauma in humans.[30] CSF amino acid levels and dietary manipulations in the rat have not been investigated before, and this report includes simultaneous changes in CSF and brain amino acid levels. Hypoaminoacidemia of trauma was more pronounced in basal diet–fed rats than in spermidine-supplemented rats. Plasma total amino acid (TAA) levels were decreased by 27% due to trauma in basal diet–fed rats, compared with only a 2% decrease in spermidine-supplemented rats. Changes in TAAs due to trauma in CSF, muscle, and brain were not significant in both groups. Generally, trauma-induced changes in amino acids individually and in groups (BCAA, essential amino acids [EAA], and nonessential amino acids [NEAA]) were minimal and nonsignificant in spermidine-supplemented rats. This demonstrated that spermidine supple-

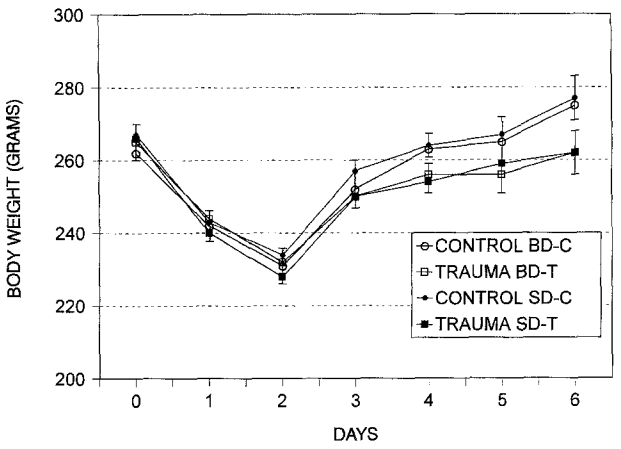

Fig 1. Body weight change in growing rats with and without injury. Rats were starved for the first 2 days and then refed for 4 days. ○, control basal diet without spermidine (SD); □, trauma rats fed basal diet without SD; ●, control rats fed test diet with SD; ■, trauma rats fed test diet with SD. Results are the mean ± SEM (n = 6 per group).

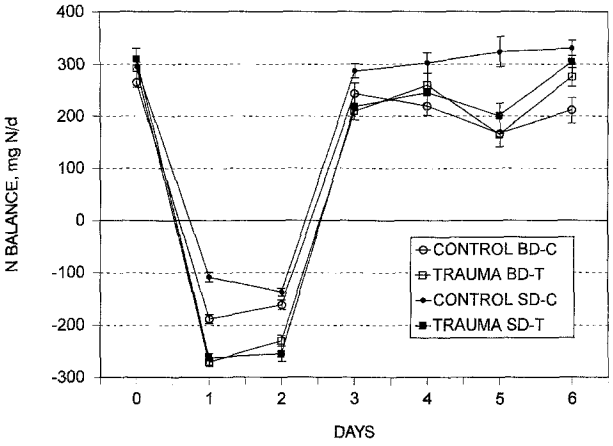

Fig 2. Daily nitrogen balance (mg N/d) in control and trauma rats fed basal (without 0.05% spermidine [SD]) or test (SD 0.05%) diet. Rats were starved for 2 days initially and then refed for 4 days. Results are the mean ± SEM (n = 6 per group). See Fig 1 for symbol definitions.

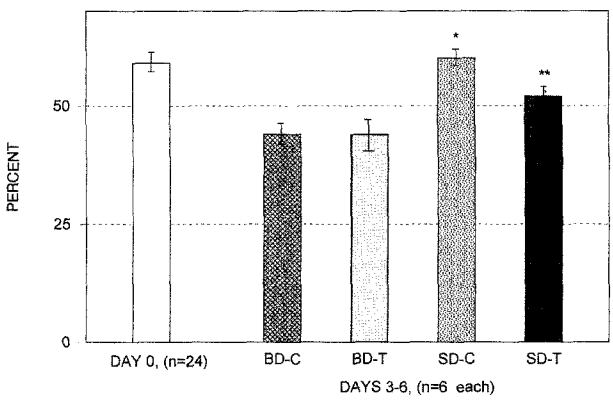

Fig 3. Relative nitrogen retention (% nitrogen intake retained in the body) in control (C) and trauma (T) rats fed basal (−0.05% SD [BD]) or test (+0.05% SD) diet. *$P = .001$, BD-C *v* SD-C; **$P < .025$, BD-T *v* SD-T. Results are the mean ± SEM (n = 6 per group for days 3 to 6; n = 24 for day 0).

Table 2. Plasma and CSF Free Amino Acid Levels (μmol/L) in Normal and Trauma Rats

	Plasma					
	Basal Diet (−0.05% SD)		Test Diet (+0.05% SD)		CSF Test Diet (+0.05% SD)	
Parameter	Control	Trauma	Control	Trauma	Control	Trauma
EAA						
VAL	229 ± 20	149 ± 10*	145 ± 11*	140 ± 7	22.7 ± 6.3	16.6 ± 3.0
LEU	153 ± 17	98 ± 7*	104 ± 10*	101 ± 6	18.1 ± 7.0	10.8 ± 0.6
ILE	92 ± 8	60 ± 6*	65 ± 6*	65 ± 3	10.5 ± 3.0	6.3 ± 0.7
PHE	51 ± 6	39 ± 2	40 ± 2	40 ± 2	8.8 ± 2.6	6.4 ± 0.6
THR	324 ± 19	229 ± 24*	343 ± 21	302 ± 15†	17.5 ± 3.2	6.0 ± 0.7‡
TRP	108 ± 6	95 ± 17	60 ± 4*	62 ± 3†	18.5 ± 6.1	18.7 ± 9.2
MET	59 ± 5	46 ± 3*	37 ± 2*	39 ± 1†	6.7 ± 1.9	4.6 ± 0.3
LYS	602 ± 38	487 ± 34*	446 ± 17*	484 ± 22	193 ± 15	181 ± 11
NEAA						
ALA	508 ± 41	365 ± 29*	524 ± 18	521 ± 19†	88 ± 21	110 ± 8
GLY	245 ± 22	188 ± 8*	252 ± 9	252 ± 11†	36 ± 9	21.5 ± 2.7
SER	299 ± 17	197 ± 21*	267 ± 7	253 ± 9†	83 ± 19	85 ± 13
GLN	536 ± 66	360 ± 14*	553 ± 19	490 ± 15†‡	525 ± 32	498 ± 23
PRO	209 ± 43	184 ± 29	247 ± 18	258 ± 17	5.4 ± 0.0	8.1 ± 0.8
ARG	127 ± 9	103 ± 11	100 ± 8*	125 ± 5	40.6 ± 4.2	41.4 ± 3.1
HIS	78 ± 6	61 ± 3*	128 ± 14*	100 ± 8†	18.5 ± 2.4	13.5 ± 1.2
TAU	196 ± 24	135 ± 12*	164 ± 18	179 ± 26	92 ± 13	75 ± 7
GLU	134 ± 28	84 ± 13	136 ± 12	106 ± 6†	20.4 ± 5.8	18.0 ± 6.1
TYR	79 ± 6	57 ± 4*	63 ± 3*	75 ± 3†	12.1 ± 2.8	10.3 ± 1.2
ORN	53 ± 6	38 ± 2*	73 ± 7*	59 ± 6†	4.5 ± 0.4	4.7 ± 0.2
CIT	109 ± 8	91 ± 2*	76 ± 4*	73 ± 4†	14.1 ± 2.1	6.8 ± 1.7‡
ASN	61 ± 5	43 ± 4*	53 ± 3	58 ± 2†	11.6 ± 2.3	9.3 ± 0.9
CYS	52 ± 8	38 ± 2	51 ± 6	60 ± 8†	6.0 ± 2.4	2.9 ± 0.2
ASP	14 ± 2	9 ± 1*	26 ± 11	33 ± 10†	50 ± 16	36 ± 20
ΣBCAA	473 ± 45	307 ± 23*	314 ± 27*	306 ± 16	46 ± 14	31 ± 4
ΣEAA	1,617 ± 111	1,202 ± 70*	1,239 ± 64*	1,232 ± 32	277 ± 33	243 ± 14
ΣNEAA	2,684 ± 188	1,942 ± 112*	2,713 ± 68	2,643 ± 43†	820 ± 130	754 ± 108
ΣTAA	4,301 ± 295	3,144 ± 178*	3,952 ± 128	3,875 ± 71†	1,097 ± 156	997 ± 117
BCAA/EAA	0.290 ± 0.009	0.255 ± 0.007	0.251 ± 0.009	0.248 ± 0.010	0.153 ± 0.025	0.127 ± 0.015
BCAA/TAA	0.109 ± 0.003	0.098 ± 0.004	0.079 ± 0.004*	0.079 ± 0.004†	0.039 ± 0.006	0.032 ± 0.003
EAA/NEAA	0.604 ± .014	0.620 ± 0.016	0.456 ± 0.014*	0.466 ± 0.008†	0.272 ± 0.031	0.277 ± 0.037
EAA/TAA	0.376 ± 0.005	0.382 ± 0.006	0.313 ± 0.007*	0.318 ± 0.004†	0.393 ± 0.066	0.419 ± 0.088
PHE/TYR	0.638 ± 0.044	0.682 ± 0.045	0.638 ± 0.035	0.540 ± 0.020	0.482 ± 0.092	0.479 ± 0.063
VAL/GLY	0.939 ± 0.045	0.796 ± 0.063	0.584 ± 0.051*	0.564 ± 0.043†	0.887 ± 0.165	0.825 ± 0.101
ARG/ORN	2.474 ± 0.112	2.690 ± 0.169	1.427 ± 0.156*	2.215 ± 0.180‡	8.02 ± 0.58	8.82 ± 0.62
OHPRO	24 ± 5	37 ± 2*	40 ± 2*	42 ± 3	—	—

NOTE. Results are the mean ± SEM; n = 6 per group.

Abbreviations: OHPRO, orthohydroxyproline; SD, spermidine.

*$P \leq .05$ *v* basal diet (−0.05% SD) control.

†$P \leq .05$ *v* basal diet (−0.05% SD) trauma.

‡$P \leq .05$ *v* test diet (+0.05% SD) control.

mentation tends to ameliorate the injury effect on protein metabolism.

The trends for changes in plasma and muscle GLN levels in the growing rats are illustrated in Fig 4. GLN comprised 12%, 48%, 16%, and 15% of TAA in plasma, CSF, muscle, and brain, respectively. The well-established depletion of plasma and muscle GLN levels due to stress states[31,32] was confirmed here in trauma rats. Plasma and muscle GLN levels in injured rats decreased significantly and independently of diet; however, in spermidine-supplemented trauma rats, plasma GLN decreased significantly less (11% *v* 33%; Fig 4) than in the control rats. Glutamate is the major amino acid (37% of TAA) of brain tissue, compared with 3%, 2%, and 6% of TAA in plasma, CSF, and muscle, respectively. Glutamate levels showed a trend to decrease due to injury in basal diet–fed rats. However, this trauma effect was not seen in spermidine-supplemented rats.

Although taurine is the major amino acid (36% of TAA) in muscle, it was not affected either by trauma or by spermidine supplementation. However, plasma taurine levels were significantly ($P < .05$) reduced by 31% due to trauma in basal diet–fed rats, but were not affected by spermidine feeding. Plasma levels of the BCAA valine, leucine, and isoleucine were significantly decreased due to trauma in basal diet–fed rats. Although this same trend was seen in muscle tissue, the differences were not statistically significant. BCAA levels in brain tissue were not affected by injury or dietary spermidine supplementation. Total BCAA levels were significantly ($P < .025$) reduced by 35% in plasma and 45% in muscle due to

Table 3. Muscle and Brain Free Amino Acid Levels (μmol/g dry tissue) in Normal and Trauma Rats

	Muscle				Brain			
	Basal Diet (−0.05% SD)		Test Diet (+0.05% SD)		Basal Diet (−0.05% SD)		Test Diet (+0.05% SD)	
Parameter	Control	Trauma	Control	Trauma	Control	Trauma	Control	Trauma
EAA								
VAL	1.39 ± 0.14	1.10 ± 0.16	0.94 ± 0.08*	1.13 ± 0.05	0.77 ± 0.04	0.77 ± 0.07	0.78 ± 0.06	0.79 ± 0.04
LEU	0.82 ± 0.20	0.57 ± 0.08	0.33 ± 0.05*	0.45 ± 0.03	0.64 ± 0.03	0.68 ± 0.08	0.55 ± 0.05	0.56 ± 0.06
ILE	0.49 ± 0.10	0.37 ± 0.05	0.20 ± 0.03*	0.31 ± 0.01	0.33 ± 0.01	0.32 ± 0.04	0.27 ± 0.02*	0.28 ± 0.02
PHE	0.35 ± 0.09	0.27 ± 0.03	0.16 ± 0.02	0.22 ± 0.01	0.33 ± 0.01	0.37 ± 0.04	0.29 ± 0.02	0.28 ± 0.03
THR	6.60 ± 0.57	4.78 ± 0.51*	6.67 ± 0.53	5.20 ± 0.30	4.25 ± 0.09	3.82 ± 0.31	4.91 ± 0.39	4.08 ± 0.09
TRP	0.18 ± 0.06	0.17 ± 0.04	0.18 ± 0.04	0.14 ± 0.02	0.11 ± 0.02	0.15 ± 0.02	0.10 ± 0.01	0.08 ± 0.01
MET	0.28 ± 0.05	0.24 ± 0.03	0.14 ± 0.02*	0.16 ± 0.01†	0.17 ± 0.02	0.17 ± 0.02	0.17 ± 0.01	0.15 ± 0.02
LYS	5.90 ± 0.93	4.88 ± 0.52	4.38 ± 0.38	5.06 ± 0.58	2.06 ± 0.16	2.17 ± 0.17	2.1 ± 0.13	1.81 ± 0.17
NEAA								
ALA	14.51 ± 1.21	11.91 ± 1.05*	13.47 ± 0.37	13.75 ± 0.69	4.89 ± 0.19	4.36 ± 0.36	5.13 ± 0.45	5.10 ± 0.15
GLY	20.90 ± 2.44	17.33 ± 1.58	18.48 ± 1.24	21.06 ± 1.5	5.54 ± 0.28	6.23 ± 0.56	4.95 ± 0.49	4.23 ± 0.20†
SER	8.14 ± 0.61	5.36 ± 0.56*	6.85 ± 0.57	5.82 ± 0.38	5.67 ± 0.34	4.88 ± 0.45	6.54 ± 0.56	6.24 ± 0.25†
GLN	24.96 ± 2.53	17.28 ± 1.59*	27.62 ± 3.07	19.11 ± 1.23‡	21.17 ± 1.36	18.85 ± 1.41	28.55 ± 2.43	24.62 ± 0.67†
PRO	4.44 ± 0.39	3.60 ± 0.54	3.42 ± 0.20*	3.67 ± 0.18	0.51 ± 0.06	0.57 ± 0.05	0.56 ± 0.05	0.57 ± 0.05
ARG	1.27 ± 0.15	1.05 ± 0.06	0.73 ± 0.07*	0.94 ± 0.09	0.84 ± 0.09	1.11 ± 0.13	0.58 ± 0.04*	0.56 ± 0.06†
HIS	1.19 ± 0.10	0.97 ± 0.10	1.09 ± 0.06	1.02 ± 0.08	1.24 ± 0.22	1.09 ± 0.11	1.17 ± 0.02	0.98 ± 0.16
TAU	57.0 ± 5.2	54.2 ± 4.0	55.5 ± 4.8	57.5 ± 2.14	24.90 ± 2.05	21.86 ± 1.53	24.22 ± 1.43	24.17 ± 0.65
GLU	9.53 ± 0.88	8.20 ± 0.88	6.28 ± 0.03*	6.36 ± 0.27	50.76 ± 2.58	43.20 ± 2.98	54.26 ± 3.81	52.21 ± 1.04†
TYR	0.81 ± 0.09	0.64 ± 0.05	0.51 ± 0.04*	0.73 ± 0.04	0.52 ± 0.02	0.48 ± 0.03	0.54 ± 0.04	0.54 ± 0.01
ORN	0.29 ± 0.03	0.26 ± 0.02	0.24 ± 0.01	0.27 ± 0.02	0.62 ± 0.07	0.58 ± 0.03	0.60 ± 0.03	0.48 ± 0.11
CIT	1.76 ± 0.20	1.25 ± 0.13	1.43 ± 0.07	1.20 ± 0.06	0.19 ± 0.01	0.15 ± 0.02	0.16 ± 0.02	0.16 ± 0.02
ASN	1.21 ± 0.09	1.01 ± 0.08	1.04 ± 0.03	0.92 ± 0.05	—	—	—	—
ASP	3.02 ± 0.45	2.38 ± 0.17	2.19 ± 0.18	1.49 ± 0.47	12.33 ± 0.28	12.78 ± 0.17	14.45 ± 1.00	13.61 ± 0.24†
ΣBCAA	2.69 ± 0.39	2.04 ± 0.29	1.47 ± 0.16*	1.89 ± 0.09	1.73 ± 0.07	1.77 ± 0.18	1.60 ± 0.13	1.63 ± 0.12
ΣEAA	12.05 ± 1.24	10.68 ± 1.07	12.99 ± 1.00	12.67 ± 0.79	8.60 ± 0.18	8.45 ± 0.56	9.16 ± 0.63	8.02 ± 0.26
ΣNEAA	147 ± 11	123 ± 7	137 ± 6	134 ± 6	129 ± 6	109 ± 9	141 ± 10	133 ± 2
ΣTAA	159 ± 10	134 ± 8	150 ± 7	146 ± 5	138 ± 6	117 ± 10	151 ± 10*	141 ± 2†
BCAA/EAA	0.223 ± 0.023	0.199 ± 0.033	0.113 ± 0.010*	0.152 ± 0.011	0.201 ± 0.005	0.208 ± 0.013	0.174 ± 0.006*	0.203 ± 0.009
BCAA/TAA	0.017 ± 0.003	0.015 ± 0.002	0.010 ± 0.001	0.013 ± 0.001	0.013 ± 0.001	0.015 ± 0.001	0.011 ± 0.001	0.012 ± 0.001
EAA/NEAA	0.087 ± 0.015	0.088 ± 0.009	0.094 ± 0.004	0.094 ± 0.003	0.063 ± 0.002	0.073 ± 0.002	0.061 ± 0.002	0.057 ± 0.002†
EAA/TAA	0.079 ± 0.012	0.081 ± 0.008	0.086 ± 0.003	0.086 ± 0.008	0.067 ± 0.003	0.079 ± 0.003	0.065 ± 0.002	0.060 ± 0.002†
PHE/TYR	0.410 ± 0.049	0.421 ± 0.026	0.306 ± 0.022	0.306 ± 0.016	0.660 ± 0.029	0.771 ± 0.064	0.546 ± 0.031	0.525 ± 0.056†
VAL/GLY	0.072 ± 0.011	0.064 ± 0.008	0.054 ± 0.008	0.056 ± 0.005	0.141 ± 0.011	0.126 ± 0.010	0.161 ± 0.008	0.188 ± 0.005‡
ARG/ORN	5.16 ± 1.01	4.16 ± 0.51	3.00 ± 0.19	3.48 ± 0.20	1.21 ± 0.17	1.93 ± 0.21*	0.98 ± 0.09	2.42 ± 1.25

NOTE. Results are the mean ± SEM; n = 6 per group.
Abbreviation: SD, spermidine.
*$P \leq .05$ *v* basal diet (−0.05% SD) control.
†$P \leq .05$ *v* basal diet (−0.05% SD) trauma.
‡$P \leq .05$ *v* test diet (+0.05% SD) control.

dietary spermidine supplementation. No trauma effects on BCAA levels were seen in plasma, CSF, muscle, and brain during spermidine supplementation.

Plasma levels of tryptophan, methionine, lysine, histidine, tyrosine, citrulline, and EAA were significantly reduced due to trauma and spermidine supplementation in control rats. The reduced levels of these amino acids in spermidine-supplemented control rats were not further affected by trauma. Trauma significantly reduced plasma alanine, glycine, serine, NEAA, and TAA levels in basal diet–fed rats, but not in spermidine-supplemented rats. Plasma levels of ornithine, the direct precursor amino acid of the polyamine putrescine, were significantly ($P < .05$) increased in spermidine-supplemented control (38%) and trauma rats (55%) compared with the respective basal diet–fed rats. This, along with the reduction in urea production, seems to indicate the diversion of ornithine from polyamine synthesis.

DISCUSSION

The nutritional value of the constituent protein in the diet is expressed as the PER (weight gained per gram of nitrogen consumed) and also as NPU (percentage of ingested nitrogen to retained nitrogen). The mean PERs in these casein-based liquid diet–fed rats did not show any change due to spermidine supplementation; however, in trauma rats, PERs were 17% lower than in the uninjured rats, showing the altered metabolic adjustment due to injury. NPU values were significantly improved due to dietary spermidine supplementation in control rats (43.9% ± 2.1% *v* 60.0% ± 1.8%, $P < .001$) and in trauma rats (44.0% ± 2.4% *v* 51.9% ± 1.9%, $P < .025$). The basal diet

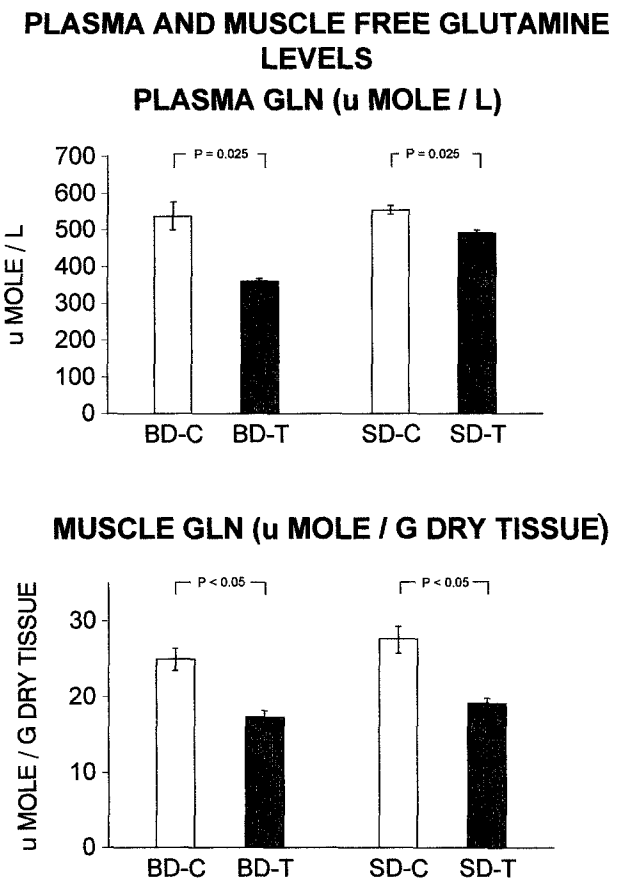

Fig 4. Plasma (μmol/L) and muscle (μmol/g dry tissue) GLN levels in rats. BD-C, basal diet (−0.05% SD) control rats; BD-T, basal diet (−0.05% SD) trauma rats; SD-C, test diet (+0.05% SD) control rats; SD-T, test diet (+0.05% SD) trauma rats. Results are the mean ± SEM (n = 6 per group). $P = .005$, plasma BD-T *v* SD-T.

did not change NPU due to trauma, whereas NPU was significantly ($P < .005$) decreased by 14% in spermidine-fed rats. These data suggest that spermidine supplementation results in better utilization of dietary protein in control and injured states, with no significant effect on body weight.

The potential for exogenous dietary polyamines to significantly contribute to growth and health has recently been reviewed.[18] Polyamines can promote growth by enhancing nutrient uptake from the intestinal tract. Feeding 0.2% putrescine, the least cationic of polyamines, has been shown to promote whole-body growth in chicks, but levels of 0.8% and higher proved toxic.[22] Dietary spermine, the most cationic of polyamines, has been shown to be much more toxic than putrescine, although growth-promoting potential was seen at very low levels.[24] Chicks fed 0.05% supplemental spermidine had increased growth.[23] We were unable to detect any body weight gain with a 0.05% spermidine-supplemented diet in starved and refed rats; however, a significant improvement in NPU was found. The absence of weight loss and death in spermidine-supplemented rats indicates that this dosage is not toxic. Perhaps a lower rather than higher level of supplemental spermidine may be more beneficial (our unpublished data).

The metabolic response to trauma demonstrated a trend for increased nitrogen and urea excretion and decreased creatinine excretion. Control and trauma groups of spermidine-supplemented rats excreted less creatinine with increased nitrogen retention. It is generally accepted that urinary creatinine excretion is significantly correlated with lean body mass or muscle mass and is associated with the degree of muscle damage.[33,34] There is a parallel relationship of the urinary excretion of nitrogen and creatinine in response to the catabolic stress of injury and serious illness.[33] It seems possible that the decreased creatinine excretion in spermidine-supplemented rats is due to a decreased degree of muscle damage.

The urea-cycle amino acid ornithine is used in the biosynthesis of putrescine by decarboxylation, as well as in the biosynthesis of glutamate by transamination. Spermidine supplementation increased plasma ornithine levels in the control rats, decreased urea excretion, and did not change plasma glutamate levels. These findings suggest that exogenous spermidine diminishes endogenous polyamine synthesis, decreases the entry of ornithine into the urea cycle, and does not affect the transamination of ornithine to glutamate. Similar results were also seen in trauma rats, with the exception of an increase in plasma glutamate levels. Muscle and brain ornithine levels are not affected by spermidine supplementation. Ornithine accumulates in muscle and liver with feeding of putrescine in chicks.[22]

TAA and individual amino acid levels in CSF differ significantly from those in plasma. Glutamine comprises 48% and 12% of TAA in CSF and plasma, respectively. Specific membrane transport systems in the blood-brain barrier may be the reason for higher glutamine levels in CSF. GLN is the only amino acid for which the CSF to plasma ratio is closer to 1 (0.98), demonstrating enhanced uptake of GLN from the plasma to CSF against a concentration gradient.

Control rats fed the 0.05% spermidine-supplemented diet showed (1) a trend for decreasing TAA levels, (2) a significant decrease in all three BCAA levels in plasma and muscle, suggesting an increase in their utilization for protein synthesis, (3) a significant decrease in plasma tryptophan, methionine, lysine, arginine, taurine, tyrosine, citrulline, and EAA levels, and (4) an increase (38%, $P < .05$) in the plasma ornithine level and no change in muscle ornithine. Liver ornithine was increased and muscle ornithine levels did not change in 0.2% spermidine-supplemented chicks.[23]

The profound effects of trauma on plasma amino acid metabolism seen in the basal diet–fed rats were absent in the spermidine-supplemented rats, indicating less muscle protein catabolism. Depletion of plasma GLN levels due to trauma was 33% in basal diet–fed rats, whereas it was only 11% in spermidine-supplemented rats. The plasma arginine to ornithine ratio was significantly increased to 2.22 ± 0.18 from 1.43 ± 0.16 ($P < .005$) mainly due to decreased ornithine levels in spermidine-supplemented trauma rats. CSF, muscle, and brain amino acid levels in spermidine-supplemented rats were not affected by trauma.

Dietary spermidine at higher levels may have greater potential to promote growth and nutritional efficacy with more amelioration of trauma effects. Further studies evaluating the efficacy and dose-dependency of dietary spermidine following injury or stress appear warranted. Measurement of protein turnover and muscle protein mass would allow an exploration of the underlying mechanisms.

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