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Original Communications

Simultaneous Infusion of Glutamine and Branched-Chain Amino Acids (BCAA) to Septic Rats Does Not Have More Favorable Effect on Protein Synthesis in Muscle, Liver, and Small Intestine Than Separate Infusions

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ABSTRACT. *Background:* Glutamine and branched-chain amino acids (BCAA; valine, leucine, and isoleucine) are used as nutrition supplements in the treatment of proteocatabolic illness. We hypothesized that simultaneous administration of BCAA and glutamine affects protein metabolism more significantly than separate administration. In the present study, we evaluated their effect on protein synthesis in skeletal muscle, liver, and jejunum of septic rats. *Methods:* Twenty-four hours after induction of sepsis by subcutaneous injection of turpentine, the rats were infused for 6 hours with 5 mL of 1.75% glutamine, 1.75% BCAA, 1.75% glutamine + BCAA, or saline solution. The control group consisted of intact rats infused with saline. Protein synthesis was measured at the end of infusion by a "flooding method" with [3,4,5-^{3}H]phenylalanine. *Results:* In turpentine-treated animals, we observed a decrease in glutamine concentration in blood plasma and skeletal muscle, a decrease in BCAA concentration in liver and jejunum, and a decrease in protein synthesis in all tissues. Glutamine or glutamine + BCAA infusion increased glutamine concentration in plasma and muscle and stimulated protein synthesis in the liver. The BCAA infusion enhanced concentrations of BCAA in plasma and tissues, but the effect of BCAA on protein synthesis was insignificant. Synergistic effect of simultaneous infusion of glutamine and BCAA on protein synthesis was not observed. *Conclusions:* We conclude that glutamine infusion to rats with septic injury may significantly improve impaired protein synthesis in the liver and that there is no synergistic effect of glutamine and BCAA infusion on protein synthesis in skeletal muscle, liver, and jejunum. (*Journal of Parenteral and Enteral Nutrition* **30:**467–473, 2006)

Severe illness such as sepsis, trauma, and burn injury is frequently associated with protein wasting caused by complex action of cytokines, glucocorticoids, acidosis, and anorexia. Treatment of this metabolic derangement is difficult, and various nutrients, hormones, and drugs have been studied. Branched-chain amino acids (BCAA; valine, leucine, and isoleucine) and glutamine belong among substances used frequently as nutrition supplements in the treatment of severe illness.

The BCAA are essential amino acids that serve as an essential substrate and important regulator in synthesis of body proteins and represent the major nitrogen source for glutamine and alanine synthesis in muscle.[1] Observation of activated oxidation of BCAA in proteocatabolic states and their unique metabolic properties were rational arguments that led to the use of BCAA-enriched solutions in patients with hepatic encephalopathy, sepsis, renal failure, and trauma.[2,3] In addition, recent studies showed the unique specificity of leucine in signaling to stimulate protein synthesis in skeletal muscle.[4] Unfortunately, the clinical data evaluating the effect of BCAA-enriched solutions are controversial, and the utility of BCAA in treatment of proteocatabolic illness remains an open problem.[5,6]

Glutamine, besides its role in acid-base homeostasis and in gluconeogenesis, acts as a "nitrogen shuttle" among organs, as an important fuel for rapidly dividing cells such as enterocytes and cells of the immune system, and as a precursor for the synthesis of nucleotides. A number of researchers have found that glutamine can be a potent enhancer of the heat stress response and that glutamine could be used to enhance heat shock protein expression and attenuate injury in the critically ill.[7,8] Several studies have reported improvements in clinical outcome and in nitrogen balance when glutamine itself, glutamine-containing dipeptides, or α-ketoglutarate has been given to critically ill patients.[5,9–11] However, the mechanism by which glutamine administration affects protein balance is not yet completely clear.[12]

Considering that BCAA are an important precursor of glutamine synthesis in skeletal muscle[13] and that glutamine administration inhibits leucine oxidation,[14] it may be suggested that simultaneous administration of BCAA and glutamine may affect protein metabolism more significantly than separate administration. However, we did not find any study assessing this practi-

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cally important question of therapeutic approach. Therefore, the aim of the present study was to compare the effect of the separate and simultaneous administration of glutamine and BCAA on protein synthesis in septic injury. We studied the response of skeletal muscle, liver, and jejunum (ie, tissues that have the most important role in whole-body protein and amino acid metabolism).

MATERIALS AND METHODS

Animals

Male Wistar rats (BioTest, Konarovice, CR) weighing approximately 250 g (2 months old) were housed in standardized cages in quarters with controlled temperature and a 12-hour light-dark cycle, and received Velaz-Altromin 1320 laboratory chow (Velaz, Prague, CR) and drinking water *ad libitum*. All procedures involving animals were performed according to guidelines set by the Institutional Animal Use and Care Committee of Charles University.

Materials

L-[3,4,5-^{3}H]Phenylalanine was purchased from American Radiolabeled Chemical, Inc (St. Louis, MO). Turpentine oil, amino acids, Folin-Ciocalteu phenol reagent, and albumin were purchased from Sigma Chemical (St. Louis, MO). The remaining chemicals were obtained from Lachema (Brno, Czech Republic).

Experimental Design

A polyethylene cannula was inserted into the jugular vein under light diethyl ether narcosis 24 h before the beginning of the experiment to exclude the effect of stress from surgery. At the same session, animals were injected subcutaneously with saline (control animals, n = 6) or turpentine oil at a dose of 0.5 mL/100 g BW (septic rats, n = 28). Thereafter, the rats fasted for 24 hours. The next day, conscious unrestrained rats were infused intravenously for 6 hours with 5 mL of saline (n = 6 in control group, n = 7 in septic group), 1.75% glutamine solution (n = 7), 1.75% BCAA solution (33.3% valine, 42.6% of leucine, and 24.1% of isoleucine; n = 7), or 1.75% glutamine + BCAA solution (glutamine and BCAA solutions mixed in ratio 1:1). The rate of infusion was based on our previous experiments.[14] Ten minutes before the killing, the rats were injected with a flooding dose of L-[3,4,5-^{3}H] phenylalanine (50 μCi/100 g BW) combined with unlabeled D-phenylalanine (150 μmol/100 g BW).[15] The rats were killed by exsanguination *via* the abdominal aorta. Afterwards, the gastrocnemius muscle, liver, and jejunum were quickly removed and immediately frozen in liquid nitrogen.

Tissue Protein Synthesis and Protein Content

The samples for measurement of protein synthesis were processed as described elsewhere.[16] The fraction of protein mass renewed each day (k_s, % per day) was calculated according the formula derived by McNurlan et al[17]:

$$k_s = (S_b \times 100)/(t \times S_a)$$

where S_b and S_a are the specific radioactivities (dpm/nanomole) of protein-bound phenylalanine and tissue-free phenylalanine in the acid-soluble fraction of tissue homogenates, respectively, and t is the time (days) between injection of isotope and immersion of tissue into the liquid nitrogen. The value of 274 μmol phenylalanine/g protein was used for calculation of protein-bound phenylalanine specific activity.[18] Protein content was measured according to Lowry et al.[19]

Other Techniques

Amino acid concentrations in deproteinized samples of blood plasma or tissues were determined with high-performance liquid chromatography (Aliance, 2695, Waters, Milford, MA, 2475) after derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Waters, Milford, MA). The radioactivity of the samples was measured with a liquid scintillation radioactivity counter LS 6000 (Beckman Instruments, Fullerton, CA).

Statistical Analysis

Results are expressed as the mean ± SE. The effect of sepsis was evaluated using F test and t-test for independent samples (comparison of saline-infused control and saline-infused septic rats). The effects of glutamine and BCAA solution on protein synthesis in septic rats were evaluated using ANOVA and Bonferroni tests (comparison among saline and amino acid infused septic rats). Statistical software NCSS 2001 (NCSS, Kaysville, UT) was used for the analysis. Differences were considered significant at $p < .05$.

RESULTS

There were no significant changes in body weight and glycemia either between control and septic animals or among septic animals infused by tested solutions (data not shown). It was demonstrated in several studies that turpentine treatment has no significant effect on biochemical markers of tissue injury in blood plasma.[20] The effect of separate or simultaneous infusions of glutamine and BCAA on amino acid concentrations in blood plasma and tissues is demonstrated in Tables I–IV. Because phenylalanine administration affected concentration of tyrosine in plasma and liver (probably due to phenylalanine metabolism in liver), we subtracted tyrosine and phenylalanine from the sum of amino acid concentrations (in addition to subtraction of infused glutamine and BCAA) in calculation of the effect of glutamine or BCAA infusion on aminoacidemia.

In turpentine oil–treated animals, a significant decrease in a number of amino acid concentrations was observed in plasma, skeletal muscle, and jejunum. In blood plasma (Table I), a significant decrease in aminoacidemia was caused by decreased concentrations of serine, glycine, glutamine, histidine, threonine, cystine, ornithine, and lysine, whereas glutamate concentration increased. Both in skeletal muscle and jeju-

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TABLE I
Amino acid concentrations in blood plasma

	Control saline infusion (n = 6)	Sepsis			
		Saline infusion (n = 7)	Amino acid infusion		
			Gln (n = 7)	BCAA (n = 7)	Gln + BCAA (n = 7)
Aspartate	13 ± 2	16 ± 2	18 ± 2	18 ± 3	21 ± 2
Glutamate	154 ± 12	209 ± 20*	262 ± 25	246 ± 17	331 ± 21†‡
Serine	275 ± 4	202 ± 11*	208 ± 5	165 ± 10†§	185 ± 8
Asparagine	56 ± 3	43 ± 5	61 ± 8	38 ± 4	54 ± 8
Glycine	309 ± 6	238 ± 21*	225 ± 18	206 ± 19	226 ± 16
Glutamine	787 ± 28	676 ± 32*	1187 ± 52†	741 ± 49§	1089 ± 33†‡
Histidine	88 ± 2	79 ± 4*	88 ± 2	79 ± 6	89 ± 3
Taurine	218 ± 25	207 ± 14	192 ± 18	246 ± 20	244 ± 23
Citrulline	66 ± 8	55 ± 3	62 ± 4	64 ± 6	66 ± 7
Threonine	227 ± 11	171 ± 10*	178 ± 5	123 ± 8†§	113 ± 8†§
Alanine	442 ± 34	444 ± 51	441 ± 40	413 ± 37	424 ± 52
Proline	124 ± 14	106 ± 12	101 ± 6	88 ± 7	93 ± 6
Tyrosine	324 ± 13	357 ± 26	305 ± 9	293 ± 18†	277 ± 12†
Cystine	90 ± 4	64 ± 7*	61 ± 4	57 ± 2	58 ± 5
Valine	174 ± 8	160 ± 11	160 ± 9	735 ± 41†§	677 ± 39†§
Methionine	50 ± 4	44 ± 3	42 ± 1	35 ± 2	41 ± 3
Ornithine	51 ± 5	35 ± 3*	38 ± 1	37 ± 4	39 ± 4
Isoleucine	85 ± 5	87 ± 7	92 ± 6	298 ± 21†§	266 ± 18†§
Leucine	139 ± 10	133 ± 10	143 ± 8	536 ± 35†§	481 ± 31†§
Lysine	342 ± 14	276 ± 18*	252 ± 13	241 ± 18	226 ± 12
Phenylalanine	944 ± 61	789 ± 56	859 ± 49	903 ± 43	851 ± 42
Derived values					
BCAA	399 ± 22	381 ± 28	395 ± 22	1570 ± 98†§	1424 ± 88†§
Total	4957 ± 106	4391 ± 186*	4975 ± 119	5561 ± 185†	5852 ± 176†§
Total-GVLIPT	2503 ± 88	2188 ± 123*	2229 ± 88	2055 ± 92	2210 ± 148

Mean ± SE in μmol/L. BCAA, branched-chain amino acids; Gln, glutamine; Total-GVLIPT, the sum of amino acid concentrations without glutamine, BCAA (valine, leucine, isoleucine), phenylalanine, and tyrosine.
*The difference between saline-infused control and septic rats (*t*-test, $p < .05$). The effect of Gln, BCAA, or Gln + BCAA infusion in septic rats (Bonferroni test): †$p < .05$, Gln or BCAA or Gln + BCAA *vs* saline; §$p < .05$, BCAA or Gln + BCAA *vs* Gln; ‡$p < .05$, Gln + BCAA *vs* BCAA.

TABLE II
Amino acid concentrations in gastrocnemius muscle

	Control saline infusion (n = 6)	Sepsis			
		Saline infusion (n = 7)	Amino acid infusion		
			Gln (n = 7)	BCAA (n = 7)	Gln + BCAA (n = 7)
Aspartate	262 ± 26	313 ± 46	362 ± 44	310 ± 45	368 ± 36
Glutamate	927 ± 80	1022 ± 123	1271 ± 49	1473 ± 288	1502 ± 211
Serine	772 ± 39	536 ± 27*	573 ± 37	366 ± 19†‡	396 ± 25†‡
Asparagine	219 ± 13	185 ± 9*	230 ± 20	132 ± 5†‡	158 ± 14‡
Glycine	4529 ± 199	2816 ± 282*	3099 ± 326	2613 ± 294	2599 ± 254
Glutamine	2678 ± 104	2191 ± 168*	3127 ± 204†	1915 ± 160‡	2524 ± 95‡§
Histidine	215 ± 11	185 ± 10	205 ± 19	146 ± 8‡	152 ± 12‡
Threonine	475 ± 15	312 ± 14*	376 ± 26	226 ± 12†‡	217 ± 19†‡
Alanine	2758 ± 179	2417 ± 104	2635 ± 111	2509 ± 134	2539 ± 127
Proline	715 ± 32	686 ± 12	698 ± 17	668 ± 38	739 ± 26
Tyrosine	260 ± 11	311 ± 22	257 ± 16	231 ± 14†	242 ± 10†
Valine	206 ± 11	212 ± 12	228 ± 17	878 ± 194†‡	719 ± 42†‡
Methionine	72 ± 2	77 ± 5	80 ± 5	61 ± 4‡	66 ± 5
Ornithine	49 ± 9	29 ± 2*	27 ± 2	26 ± 4	23 ± 2
Isoleucine	107 ± 8	125 ± 8	141 ± 12	418 ± 20†‡	309 ± 20
Leucine	172 ± 12	182 ± 12	204 ± 18	717 ± 214†‡	524 ± 31
Lysine	524 ± 32	326 ± 39*	353 ± 55	257 ± 16	212 ± 21‡
Phenylalanine	1261 ± 96	1146 ± 79	1164 ± 55	1190 ± 30	1121 ± 22
Derived values					
BCAA	484 ± 31	520 ± 32	572 ± 47	2012 ± 529†‡	1553 ± 93†‡
Total	16,200 ± 490	13,071 ± 330*	15,030 ± 746	14,137 ± 839	14,410 ± 404
Total-GVLIPT	11,517 ± 456	8904 ± 265*	9911 ± 624	8788 ± 417	8971 ± 353

Mean ± SE in nmol/g of muscle. BCAA, branched-chain amino acids; Gln, glutamine; Total-GVLIPT, the sum of amino acid concentrations without glutamine, BCAA (valine, leucine, isoleucine), phenylalanine, and tyrosine.
*The difference between saline-infused control and septic rats (*t*-test, $p < .05$). The effect of Gln, BCAA, or Gln + BCAA infusion in septic rats (Bonferroni test): †$p < .05$, Gln or BCAA or Gln + BCAA *vs* saline; ‡$p < .05$, BCAA or Gln + BCAA *vs* Gln; §$p < .05$, Gln + BCAA *vs* BCAA.

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TABLE III
Amino acid concentrations in liver

	Control saline infusion (n = 6)	Sepsis			
		Saline infusion (n = 7)	Amino acid infusion		
			Gln (n = 7)	BCAA (n = 7)	Gln + BCAA (n = 7)
Aspartate	701 ± 27	999 ± 117*	1173 ± 102	950 ± 86	912 ± 66
Glutamate	3176 ± 339	3670 ± 441	4716 ± 262	3692 ± 351	5168 ± 436†‡
Serine	731 ± 58	717 ± 50	754 ± 74	642 ± 52	689 ± 37
Asparagine	102 ± 7	95 ± 7	102 ± 5	85 ± 4	96 ± 7
Glycine	2862 ± 163	2824 ± 291	2509 ± 162	2384 ± 195	2262 ± 97
Glutamine	4606 ± 207	5036 ± 421	6306 ± 345	4830 ± 340§	5359 ± 432
Histidine	569 ± 21	601 ± 35	589 ± 19	535 ± 25	560 ± 87
Threonine	316 ± 12	315 ± 19	304 ± 24	168 ± 11†§	196 ± 16†§
Alanine	3073 ± 216	4499 ± 659	3995 ± 296	3460 ± 376	2931 ± 281†
Proline	3362 ± 210	3533 ± 341	3348 ± 160	3093 ± 261	2898 ± 320
Tyrosine	563 ± 31	547 ± 56	457 ± 26	444 ± 27	430 ± 12
Valine	214 ± 7	168 ± 10*	166 ± 13	630 ± 34†§	664 ± 35†§
Methionine	37 ± 3	38 ± 5	35 ± 2	37 ± 2	42 ± 2
Ornithine	363 ± 35	300 ± 15	369 ± 25	321 ± 23	390 ± 37
Isoleucine	130 ± 4	105 ± 8*	107 ± 9	256 ± 15†§	278 ± 14†§
Leucine	231 ± 7	174 ± 12*	178 ± 14	473 ± 26†§	512 ± 25†§
Lysine	549 ± 26	564 ± 54	495 ± 44	405 ± 60	379 ± 24†
Phenylalanine	556 ± 63	410 ± 43	423 ± 41	471 ± 44	531 ± 29
Derived values					
BCAA	575 ± 17	448 ± 29*	451 ± 36	1360 ± 75†§	1456 ± 72†§
Total	22,140 ± 617	24,596 ± 666*	26,026 ± 807†	22,876 ± 629	24,300 ± 920
Total-GVLIPT	15,840 ± 743	18,156 ± 848	18,389 ± 773	15,772 ± 747	16,525 ± 993

Mean ± SE in nmol/g of liver. BCAA, branched-chain amino acids; Gln, glutamine; Total-GVLIPT, the sum of amino acid concentrations without glutamine, BCAA (valine, leucine, isoleucine), phenylalanine, and tyrosine.
*The difference between saline-infused control and septic rats (*t*-test, $p < .05$). The effect of Gln, BCAA, or Gln + BCAA infusion in septic rats (Bonferroni test): †$p < .05$, Gln or BCAA or Gln + BCAA *vs* saline; §$p < .05$, BCAA or Gln + BCAA *vs* Gln; ‡$p < .05$, Gln + BCAA *vs* BCAA.

num, there was a decrease in serine, asparagine, threonine, and lysine (Tables II and IV). In addition, a significant decrease in glutamine, glycine, and ornithine was found in skeletal muscle, and a decrease in methionine and all 3 BCAA in the jejunum. The main response to turpentine oil treatment in the liver was

TABLE IV
Amino acid concentrations in jejunum

	Control saline infusion (n = 6)	Sepsis			
		Saline infusion (n = 7)	Amino acid infusion		
			Gln (n = 7)	BCAA (n = 7)	Gln + BCAA (n = 7)
Aspartate	1043 ± 46	979 ± 59	1033 ± 42	989 ± 35	951 ± 44
Glutamate	3235 ± 186	2914 ± 185	2967 ± 80	3128 ± 86	3298 ± 89
Serine	967 ± 92	584 ± 56*	570 ± 27	552 ± 63	472 ± 32
Asparagine	367 ± 48	207 ± 42*	203 ± 19	215 ± 38	192 ± 19
Glycine	1792 ± 114	1427 ± 187	1247 ± 75	1275 ± 133	1114 ± 59
Glutamine	962 ± 103	812 ± 116	1087 ± 64	808 ± 47	1000 ± 66
Histidine	202 ± 27	133 ± 23	117 ± 9	135 ± 25	115 ± 13
Threonine	639 ± 65	242 ± 48*	273 ± 42	260 ± 45	138 ± 15
Alanine	2747 ± 239	2495 ± 279	2618 ± 134	2295 ± 173	2209 ± 160
Proline	2080 ± 79	1862 ± 127	1928 ± 47	1784 ± 60	1575 ± 46†
Tyrosine	505 ± 39	405 ± 44	328 ± 18	381 ± 31	321 ± 18
Valine	478 ± 62	262 ± 36*	243 ± 18	887 ± 84‡†	705 ± 28‡†
Methionine	178 ± 25	83 ± 10*	74 ± 6	92 ± 14	75 ± 10
Ornithine	64 ± 8	49 ± 7	44 ± 3	45 ± 8	43 ± 7
Isoleucine	326 ± 47	171 ± 24*	156 ± 14	434 ± 46‡†	329 ± 15‡†
Leucine	491 ± 54	278 ± 42*	260 ± 25	787 ± 86‡†	596 ± 28‡†
Lysine	520 ± 66	323 ± 37*	268 ± 40	339 ± 48	264 ± 30
Phenylalanine	1277 ± 84	918 ± 74*	939 ± 60	1015 ± 42	871 ± 57
Derived values					
BCAA	1296 ± 134	712 ± 103*	659 ± 57	2107 ± 213‡†	1630 ± 63‡†
Total	9304 ± 633	7089 ± 681*	7132 ± 207	8317 ± 282	7125 ± 341
Total-GVLIPT	5265 ± 335	4241 ± 383	4119 ± 174	4007 ± 238	3303 ± 206

Mean ± SE in nmol/g of jejunum. BCAA, branched-chain amino acids; Gln, glutamine; Total-GVLIPT, the sum of amino acid concentrations without glutamine, BCAA (valine, leucine, isoleucine), phenylalanine, and tyrosine.
*The difference between saline-infused control and septic rats (*t*-test, $p < .05$). The effect of Gln, BCAA, or Gln+BCAA infusion in septic rats (Bonferroni test): ‡$p < .05$, Gln or BCAA or Gln + BCAA *vs* saline; †$p < .05$, BCAA or Gln + BCAA *vs* Gln.

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TABLE V
Fractional rate of protein synthesis (%/d) in gastrocnemius muscle, liver, and jejunum

	Control saline infusion (n = 6)	Sepsis			
		Saline infusion (n = 7)	Amino acid infusion		
			Gln (n = 7)	BCAA (n = 7)	Gln + BCAA (n = 7)
Muscle	14.4 ± 1.1	11.3 ± 0.8*	13.7 ± 1.8	10.9 ± 0.7	12.7 ± 0.8
Liver	84.5 ± 6.4	58.5 ± 5.3*	85.2 ± 8.7†	75.2 ± 8.1	87.4 ± 2.7†
Jejunum	114.5 ± 5.7	90.3 ± 7.2*	100.2 ± 11.8	97.7 ± 7.1	86.1 ± 15.9

Mean ± SE.
*The difference between saline-infused control and septic rats (*t*-test, $p < .05$). The effect of Gln, BCAA, or Gln + BCAA infusion in septic rats (Bonferroni test): †$p < .05$, Gln or BCAA or Gln + BCAA *vs* saline.

the decrease in aspartate and BCAA (Table III). The main findings derived from infusions of tested solutions to turpentine-treated animals included an increase in glutamine concentration in blood plasma and skeletal muscle after glutamine infusion and an increase in BCAA in blood plasma and tissues after BCAA infusion.

The data demonstrating the changes in protein synthesis are given in Table V. Turpentine oil treatment induced significant decrease in protein synthesis in all studied tissues. Glutamine or glutamine + BCAA infusion enhanced protein synthesis rate to control values in hepatic tissue. We did not find a significant effect of glutamine, BCAA, or simultaneous infusion of these substances on protein synthesis in skeletal muscle and in jejunum.

DISCUSSION

Subcutaneous injection of turpentine induces necrosis surrounded by an infiltrate of inflammatory cells without detectable injury to other tissues. There is general agreement that this model produces many of the features of the acute-phase response to injury.[20] The marked decrease in protein synthesis in muscle, liver, and jejunum indicates that turpentine injection induces severe metabolic disturbances in the whole body, particularly the development of muscle wasting and decreased production of a wide spectrum of acute-phase proteins involved in response of the body against the injury.

As stimulation of protein synthesis in hepatic tissue was observed in rats that were pair-fed or fed *ad libitum* after turpentine treatment,[20] we suppose that the lack of food may explain the impairment of protein synthesis in hepatic tissue observed in our study. It may be suggested that starvation causes the delay in onset of increased hepatic synthesis of acute-phase proteins (eg, C-reactive protein, α_2-macroglobulin, and fibrinogen), which is stimulated by humoral factors produced by immune cells (eg, by cytokines). This suggestion indicates the importance of early nutrition management in treatment of sepsis.

The most important alteration in amino acid concentrations induced by turpentine is probably decreased concentration of glutamine in blood and skeletal muscle. The decrease is related to increased use of glutamine in several tissues, particularly in immune cells, kidneys, and liver, that exceeds activated synthesis of glutamine in skeletal muscle. Lack of glutamine is considered an important factor in the development of cachexia, impaired wound healing, and immunodeficiency in inflammatory injury.[21] Also important practically is the finding of increased concentration of glutamate in blood probably caused by incomplete oxidation of glutamine. Enhanced glutamate levels in the portal vein after turpentine treatment indicate that the main source of glutamic acid is the gut.[22] This observation may be an argument against suggestions to increase the intake of glutamic acid in stress illness.[23,24]

In inflammatory illness, BCAA are used as the principal energy substrate and precursor for synthesis of alanine and glutamine in skeletal muscle. Enhanced proteolysis and BCAA oxidation in skeletal muscle have been demonstrated in different types of stress illness both in animals and humans.[25–28] The finding in the present study of unchanged levels of BCAA in skeletal muscle and blood indicates that the demands of the body for BCAA are saturated by activated breakdown of muscle protein.

The cause of decreased concentrations of BCAA in liver and jejunum is not clear. It may be related to enhanced catabolism, decreased uptake, or increased release of BCAA to the bloodstream. The suggestion of increased release is supported by observation of increased BCAA concentration in effluent of the perfused hepatic tissue of endotoxemic or TNF-α-treated rats.[29] Decreased uptake or enhanced release of BCAA by visceral tissues may prevent the decrease of BCAA in blood plasma and the preferential use of BCAA by skeletal muscle for energy and glutamine and alanine synthesis. However, the lack of BCAA may participate in impaired protein synthesis in these tissues. We suppose that these findings and speculations may be a further rational argument for recommendation to enhance exogenous delivery of BCAA in inflammatory illness.

Because energy content of infused amino acid solutions was about 1.5 kJ and energy output of the laboratory rat is approximately 800 kJ/kg, <0.8% of daily energy requirements was provided *via* infusion. Therefore, the responses induced by infusion of glutamine or BCAA in our study should be related to the role of these amino acids in intermediary metabolism.

Although in some studies glutamine supplementation failed to affect muscle protein kinetics in critically ill patents,[30] in most studies the favorable effect of amino acid solutions enriched by glutamine on protein

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balance has been demonstrated.[5,9–11] In our experiment, glutamine infusion to turpentine-treated animals induced significant increase in protein synthesis in liver. This finding indicates that increased delivery of glutamine may favorably affect the response of hepatic tissue to inflammatory stimuli. In our recent study, increased oxidation of branched-chain keto acids was observed in isolated rat liver perfused by glutamine-deficient medium.[31] Therefore, it also might be suggested that the glutamine supply might decrease BCAA oxidation and enhance availability of these essential amino acids for needs of extrahepatic tissues.[12] Also clinically important is the enhanced glutamine concentration in skeletal muscle, which may favorably affect BCAA oxidation and proteolysis in this tissue.[14]

BCAA infusion did not significantly improve protein synthesis in turpentine-treated animals, although the deficiency of BCAA has been observed in liver and jejunum and increased concentrations of BCAA were found after BCAA or BCAA + glutamine infusion in all examined tissues. Increased concentrations of BCAA in tissues after their infusion indicate that there is no impairment in transport of BCAA across the cell membrane. A possible explanation of the failure of BCAA to improve protein synthesis in inflammatory illness would be that BCAA metabolism is directed towards catabolic reactions. These may saturate energy requirements and inhibition of anabolic reactions.

The main focus of the present study was to estimate the possibility of a synergistic effect of simultaneous infusion of glutamine and BCAA on protein metabolism in septic injury. The hypothesis was based on (1) the well-known relationships between BCAA catabolism and Gln synthesis; (2) characteristic changes in BCAA and glutamine metabolism in severe illness (ie, increased glutamine use and increased BCAA catabolism associated with enhanced glutamine production); and (3) our recent finding that glutamine administration significantly decreases leucine oxidation.[14] An additional argument supporting our hypothesis may be the deficiency of glutamine in skeletal muscle and of BCAA in visceral tissues observed in this study. Unfortunately, the data evaluating the effect of tested solutions on protein synthesis did not demonstrate a significant difference between separate and simultaneous infusions of BCAA and glutamine.

In conclusion, the results indicate that the response of the tissues to parenteral administration of dietary supplements, particularly glutamine, is not markedly inhibited and that increased delivery of glutamine may favorably affect the response of hepatic tissue to inflammatory stimuli. The study did not provide evidence supporting the hypothesis of a synergistic effect of simultaneous infusion of glutamine and BCAA on protein synthesis in inflammatory injury. However, we suppose that some additional studies using other models of inflammatory illness and evaluating some additional parameters, particularly the effect on proteolysis, should be performed before our hypothesis can be rejected.

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