Enteral glutamine supports hepatic glutathione efflux during inflammation

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The role of dietary glutamine in determining fluxes of glutamine (gln), glutamate (glu^-), alanine (ala), and glutathione (GSH) across the gut and liver was determined before and 1 hour after a sublethal dose of zymosan (0.5 mg/g body weight). Sprague-Dawley rats (350–450g) were maintained on either a liquid elemental diet (ED) providing 13% of amino acids as L-gln (18.4g gln/kg)(GLN+ED) or the ED containing a nitrogen equivalent of nonessential amino acids substitute for gln. After 3 days on the pair-fed dietary regime, balance measurements were performed across the gut and liver of paired animals. Net uptake or release rates were obtained from simultaneously determined blood flows and arteriovenous concentration differences. After a control determination, zymosan was administered intraperitoneally and 1 hr later the same fluxes were redetermined. Pre-zymosan hepatic efflux of glu^- was higher in the Gln+ED group and was apparently coupled to a returning ala flux from the periphery. Gut glu^-, ala, and GSH release were also higher. However pre-zymosan gln fluxes were not higher than in the ED group. Zymosan elevated corticosterone levels and gut and liver uptake consistent with inflammation. In the ED group, glu^-, ala, and GSH fluxes were unchanged post-zymosan, although gln appeared to be more fully oxidized by the gut. In contrast, the GLN + ED animals exhibited enhanced gln fluxes and a three-fold increase in hepatic uptake; while glu^- efflux fell, hepatic GSH flux increased, suggesting a coupling of glutamate or glutamine-derived glutamate-to-GSH efflux. Thus, gln in an elemental diet supported elevated secondary fluxes of ala and glu^-. These secondary fluxes were modulated in response to inflammation, which also accelerated glutamine flux, apparently originating from peripheral stores. Thus, it appears that either the enhanced gln flux or secondary glu^- flux supported accelerated GSH fluxes during inflammation and thereby provided a flexible source of free oxygen radical scavengers.

Keywords: glutamine; inflammation; glutathione; glutamate; interorgan fluxes; elemental diet

Introduction

Glutamine is the most prevalent amino acid in the body, accounting for some 70% of the total skeletal muscle pool.[1] Glutamine serves as the major fuel for the mammalian small intestine[2] and renal proximal tubule.[3] In addition, glutamine supports cellular biosynthetic pathways in general, such as purine and pyrimidine, glucosamine,[4] glutathione,[5,6] and biosynthetic pathways at segregated organ specific sites, such as renal ammoniagenesis,[7] gluconeogenesis,[3] and hepatic ureagenesis.[8] Furthermore, intracellular glutamine correlates with protein synthesis in muscle,[9,10] and alanine, a closely related metabolite, supports hepatic protein synthesis.[11] More recently, the unique roles of glutamine as the major interorgan vehicle for carbon and nitrogen flow and the multilevel regulation governing these flows have been realized.[7] These regulatory processes ensure that adequate glutamine and derived metabolites are delivered to specific organ sites responding to physiological and pathophysiological challenges.

Classification of glutamine as a nonessential amino acid is based on the ability of dietary amino acids to support growth[12] and precedes awareness of glutamine's role in interorgan nutrition.[13] The relatively modest demands of somatic growth, reflecting growth hormone-dependent deflection of glutamine nitrogen from ureagenesis,[7] are readily met by de novo glutamine synthesis. In contrast, the extraordinary acute

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demands of catabolic states[14] overwhelm the glutamine synthetic capacity, resulting in a depletion of body glutamine stores[15] and subsequently limiting functional responses.[16] Under these conditions exogenous glutamine supplements could become critical for optimizing these responses. We tested this hypothesis by comparing animals relying on endogenous glutamine (i.e., fed a glutamine-free diet) with animals receiving exogenous dietary glutamine in their response to an acute inflammatory challenge. Our particular focus is the primary flux of glutamine and the related secondary fluxes of glutamate, alanine, and especially the free oxygen radical scavenger glutathione. The results to follow demonstrate that supplemental enteral glutamine in a defined formula diet does indeed support all four interorgan fluxes at a higher flow rate in response to inflammation, the significance of which will be discussed.

Materials and methods

Experiments were performed on male Sprague-Dawley rats weighing 350–450 g and maintained in individual metabolic cages, on a liquid diet.* The animals were randomly divided into two groups, one fed a glutamine-free elemental diet (ED) and the second fed an elemental diet containing 4.9 g of glutamine per liter (Gln + ED). Replacement of glutamine was by a mixture of nonessential amino acids in ED. Pairs of animals, one from each group, were studied over a 3-day period during which intake and urinary excretion were monitored. Urine collections were obtained over 24 hours using concentrated HCl as a preservative and were analyzed for ammonium, urea, creatinine, and glutamine as previously reported.[17]

Balance studies were performed across the gut and liver on day 4. Net uptake or release rates across these beds were obtained from simultaneously measured plasma flows multiplied by the appropriate arteriovenous plasma concentration differences. Glutathione fluxes were measured in whole blood. Balance measurements were performed under Inactin [sodium 5–ethyl–5–(1–methyl–propyl–2-thio) barbituate, BYK Gulden Konstang, FRG] anesthesia as previously described.[7] Briefly, the animal was placed on a heating pad, temperature maintained at 36° C, and a trachea cannula immediately inserted. An infusion line was placed in the right jugular delivering bromosulphalein (BSP) at the rate of 100 μg/min/100 g body weight (BW) in saline (0.020 mL/min/100g BW). Cannulas were then inserted into the carotid artery (arterial blood), portal and hepatic veins (gut and liver venous blood). Portal venous plasma flow was simultaneously monitored by infusing ^{14}C-labeled paraaminohippurate (^{14}C-glycyl, PAH, New England Nuclear, Boston, MA USA) at the rate of 0.003 μCi/min into a tertiary branch of the mesenteric vein. After allowing 45 minutes for equilibration, three blood samples (0.5 mL each) were simultaneously drawn from the three sites, placed on ice, and an equivalent blood volume from a donor littermate immediately returned. Zymosan (Sigma Chemical Co., St. Louis, MO USA) was then administered (0.5 mg/g BW dissolved in sterile saline) intraperitoneally, and after 1 hr a second set of blood samples were drawn followed by prompt sacrificing of the animal (Inactin overdose plus pneumothorax).

Blood samples were immediately processed for glutathione analysis. Aliquots of blood were added to equal volumes of ice-cold distilled water, mixed, and promptly deproteinized (1:0.2, hemolysate: 40% trichloroacetic acid [TCA]). After mixing, samples were centrifuged at 10,000*g* for 10 min at 0–4° C, and aliquots were used for same day analysis of GSH, gln, glu, and ala by high-performance liquid chromatography (HPLC). Plasma was immediately obtained, deproteinized with ice-cold TCA (1:0.07, plasma:40% TCA) and centrifuged, after which the supernatant was used for glutathione determination by enzymatic analysis[18] and glutamine, glutamate, and alanine analysis by HPLC.[19] Remaining plasma was employed for determination of bromosulphalein[17] and ^{14}C-PAH by liquid scintillation spectrometry.

Corticosterone arterial plasma concentration and gut and liver removal rates were studied in a parallel experiment on rats maintained on Purina rat chow (Ralston Purina, St. Louis, MO USA) and water. A sham control group and zymosan-treated group were prepared as above for balance measurement; after the first set of samples, either vehicle (sterile saline) or zymosan was administered and a second set obtained. Comparisons were made between sham and zymosan groups 1 hr after vehicle or zymosan treatment. Corticosterone concentration was determined by a radioimmunoassay kit (Cambridge Medical Technology, Billerica, MA USA). Removal rates were then calculated exactly as for the metabolite fluxes given below.

Plasma flows and arteriovenous concentration differences were simultaneously determined. For liver flows, hepatic venous flow (HVPF) was obtained from BSP clearance[17] and the portal venous plasma flow (PVPF) from PAH dilution.[17] Hepatic arterial plasma flow (HAPF) was estimated as the difference between HVPF and PVPF. Blood flows were obtained from the respective plasma flows multiplied by the hematocrit factor (1/1-Hct). Fluxes expressed as net uptake or release at the gut or liver were estimated from the respective flow multiplied by arteriovenous or portovenous concentration differences. All results are expressed in nmol/min/100 g BW.

Comparisons between ED and GLN + ED were made before and after zymosan to evaluate the effect of enteral glutamine on the fluxes before and during inflammation. Comparison between pre- and post-zymosan fluxes within a group were employed to distinguish the effect of zymosan. The corticosterone response was utilized to confirm that a general inflammation was occurring at the time balances were obtained. Differences between groups and within groups were judged statistically significant at the $P < 0.05$ percentile employing the unpaired Student *t* test for inter-group and paired test for intra-group comparisons.

*The composition of the liquid diet is as follows: Diet composition (dry basis) (g/kg): carbohydrate, 767; nitrogen, 21.3; fat, 10.3. Vitamins (per kg): A, 9328 IU; D_3 746 IU; E, 56 IU; C, 224 mg; B_1, 5.6 mg; B_2, 6.3 mg; niacin, 75 mg; B_6, 8 mg; B_{12}, 22 μg; biotin, 1.1 mg; pantothenic acid, 27 mg; K_1, 83 μg; choline, 275 mg. Minerals and trace elements (per kg): calcium, 1.87 g; phosphorus, 1.9 g; iodine, 280 μg; iron, 34 mg; magnesium, 746 mg; copper, 4 mg; zinc, 37 mg; manganese, 3.50 mg; selenium, 187 μg; molybdenum, 187 μg; chromium, 62 μg; sodium 1.72 g; potassium, 2.92 g; chloride, 3.06 g; phosphorus, 1.87 g. Essential amino acids (AA) (g/kg): l-isoleucine, 11.8; l-leucine, 23.7; l-valine, 11.8; l-lysine, 7.3; l-methionine, 5.2; l-phenylalanine, 7.4; l-threonine, 5.7; l-tryptophan, 1.8. Nonessential AA (GLN-ED/ED) (g/kg): l-alanine, 7.4/12.0; l-arginine, 10.9/10.9; l-aspartic acid, 10.0/16.2; l-glutamine, 18.4/0.0; glycine, 5.7/9.3; l-histidine, 3.4/5.4; l-proline, 7.0/11.3; l-serine, 4.2/6.8; l-tyrosine, 1.2/1.9. Total AA (g/kg): 143/148.

Results

Both groups maintained body weight, consuming 200 ± 4 kcal/kg/day of the liquid diet, and providing 798 ± 68 μmol glutamine/100g BW to the GLN + ED animals. Of this, 26.8 ± 4.5 μmoles/100g BW were excreted compared with only 1.7 ± 0.5 μmoles/100g BW excreted for animals on the ED diet. Although representing a 16-fold increase in excretion, it constituted only a small fraction (3.4%) of the total consumed, but provides evidence that ingested glutamine did in fact escape the liver to reach the periphery. Surprisingly, urea excretion tended to decrease in the GLN + ED group compared to ED alone, 7262 ± 575 versus 8236 ± 504 μmol/100g BW ($P < 0.10$), suggesting that glutamine nitrogen may have been diverted from ureagenesis. Ammonium excretion was unchanged, 412 ± 26 versus 373 ± 31 μmol/100g BW, indicative of acid base homeostasis, while nitrogen excreted as NH_4^+ increased from 4.7 ± 0.3% to 5.8 ± 0.3% ($P < 0.05$) of the total NH_4^+ plus urea excreted, consistent with a relative shift of glutamine utilization from liver (ureagenesis) to kidneys (ammoniagenesis).

Recently, high portal glutamine loads have been shown to elicit a hepatorenal reflex resulting in acute reduction in glomerular filtration.[20] However, in the present study creatinine excretion increased, 4.79 ± 0.28 versus 4.25 ± 0.33 mg/100g BW ($P < 0.05$), suggesting that renal perfusion was maintained or possibly increased.

The effects of exogenous glutamine on arterial plasma glutamine, glutamate, and alanine levels are presented in *Table 1*. Surprisingly, in view of increased glutamine excretion in pre-zymosan animals, the glutamine diet did not elevate plasma glutamine concentration at the time of the study, in contrast to a 27% rise in alanine concentration. Thus, the enteral glutamine load appears to have been absorbed and redistributed at this time. Following inflammatory stress, arterial glutamine and alanine concentrations were 14% and 63% higher in the GLN + ED group than in the ED group. In the absence of exogenous glutamine, zymosan had no effect on arterial glutamine concentration, while alanine concentration fell (18%). In rats fed dietary glutamine, zymosan elevated arterial glutamine concentration (17%) and maintained alanine concentration, demonstrating a greater responsiveness of the two interorgan carriers. On the other hand, arterial glutamate concentration decreased 32% in the GLN + ED group compared with the ED control.

Zymosan elicited a large corticosterone response, as seen in the 70% arterial concentration increase compared with sham-treated time controls. This increase was even more impressive in light of the increased uptake by the gut, 80 ± 11 to 192 ± 31 ng/100g BW/min ($P < 0.05$), and liver, 384 ± 79 to 622 ± 86 ng/100g BW/min ($P < 0.05$). Removal by both sites combined increased from 464 to 814 ng/min/100g BW, in large part reflecting the increased load, but also resulting from enhanced fractional extraction (from 23% to 36% for gut and 64% to 75% for liver). These results are consistent with zymosan provoking an acute general inflammatory response and with glucocorticoid, a known accelerator of interorgan glutamine fluxes,[21] exhibiting enhanced uptake at sites where glutamine utilization is under glucocorticoid control.[22]

The effect of the glutamine load and zymosan on gut glutamine utilization presented in *Tables 2 and 3*. The GLN + ED animals tended to remove more glutamine than the ED group although the fractional extraction rates (pre-zymosan 24% and 21% respectively, and post-zymosan 30% and 29%) were similar. Thus, the tendency for greater uptake of glutamine reflects a load dependency and is also consistent with increased glutamate and alanine release *(Table 3)*. Further, the fact that the gut glutamine uptake exceeded release in the GLN + ED group indicates the studies were clearly undertaken in a post-absorptive state. Zymosan tended to increase glutamine uptake (32% in GLN + ED group, 38% in ED group) by a load-independent mechanism reflected in the rise in fractional extraction from 21% to 29% (ED group) and 24% to 30% (GLN + ED group). In the ED group, glutamine utilization after zymosan *(Table 3)* apparently occurred via the oxidative pathway because alanine release did not increase. In contrast, the GLN + ED group showed a significant alanine release before and after zymosan and a striking reversal in glutamate handling from release (pre-zymosan) to net uptake (post-zymosan) *(Table 3, $P < 0.05$)*. Interestingly, the glutamine-loaded group demonstrated a significant release of glutathione (339 ± 125 nmol/min/100g BW) compared with the ED group after zymosan.

Hepatic handling of glutamine and related metab-

Table 1 Arterial plasma concentrations: effect of dietary glutamine and zymosan

	Glutamine		Glutamate		Alanine	
	Pre	Post	Pre	Post	Pre	Post
ED	398 ± 17	419 ± 25	123 ± 15	135 ± 13	309 ± 9	253[a] ± 31
GLN + ED	408 ± 26	477[a] ± 32	89 ± 9	92 ± 7	393 ± 32	413 ± 25
P	NS	<0.05	NS	<0.05	<0.05	<0.01

Results are means ± SEM in nmol/ml from six rats per group. Pre-zymosan arterial samples were drawn just prior to zymosan administration; post-zymosan arterial samples were drawn after 1 hr.
[a]Significantly different from pre-zymosan values at $P < 0.05$ by paired analysis.

Table 2 Gut glutamine uptake: effect of dietary glutamine and zymosan

	Pre-zymosan				Post-zymosan			
	[A-V][a]	PPF[b]	Load[c]	Uptake[c]	[A-V]	PPF	Load	Uptake
ED	85 ±18	2.5 ±0.6	978 ±196	201 ±56	120 ±15	2.3 ±0.6	945 ±332	278 ±62
GLN+ED	97 ±18	3.0 ±0.5	1238 ±216	300 ±72	142 ±13	2.9 ±0.5	1311 ±169	396 ±66
P	NS	NS	<0.10	<0.10	NS	NS	<0.10	<0.10

Results are means ± SEM from six rats per group.
[a]Arteriovenous concentration in nmol/ml.
[b]Portal plasma flow in mL/min/100g BW.
[c]Arterial load and uptake in nmol/min/100g BW.

Table 3 Gut metabolite release: Effect of dietary glutamine and zymosan

	Pre-zymosan			Post-zymosan		
	Glu[a]	Ala	GSH	Glu	Ala	GSH
ED	9 ±23	70 ±66	−12 ±152	20 ±20	30 ±71	7 ±62
GLN+ED	82 ±66	114 ±31	339 ±247	−85 ±29	169 ±100	339 ±125
P	<0.10	<0.05	<0.10	<0.05	<0.05	<0.05

Results are means ± SEM from six rats per group.
[a]Release in nmol/min/100g BW.
Minus sign represents net uptake.

Table 4 Hepatic glutamine uptake: effect of dietary glutamine and zymosan

	Pre-zymosan			Post-zymosan		
	HV[a]	PV+A[b]	Uptake[c]	HV	PV+A	Uptake
ED	1682 ±356	2030 ±394	348 ±82	1413 ±332	1604 ±419	175 ±106
GLN+ED	1988 ±354	2219 ±369	229 ±49	1628 ±278	2242 ±296	691 ±95
P	NS	<0.05	NS	<0.05	NS	<0.05

Results are means ± SEM in nmol/min/100g BW from six rats per group.
[a]HV represents hepatic venous glutamine load.
[b]PV and A are combined portal venous and arterial glutamine loads.
[c]Uptake is the difference between delivered and released glutamine.

olites is presented in *Tables 4 and 5*. In the pre-zymosan period, glutamine removal was similar in both groups, fractional extraction being less than 10% of the delivered load and therefore showing no activation of the powerful glutamine removal mechanism evident with high portal ammonium loads.[23] Noteworthy, hepatic venous plasma flow (HVPF) tended to be higher in the GLN+ED group (6.4 ± 1.0 versus 5.4 ± 1.1 ml/min/100g BW, $P < 0.10$). Both alanine uptake and glutamate release were higher in the GLN+ED group, consistent with accelerated secondary fluxes. Note that arterial glutamate concentration failed to rise in this group *(Table 1)*, despite the large hepatic and gut glutamate efflux *(Table 3)*. This indicates an enhanced peripheral uptake coupled to peripheral alanine release, because hepatic alanine uptake (793 nmoles/min) greatly exceeded gut alanine release (114 nmoles/min, *Table 3)*.

Zymosan did not alter either hepatic glutamine uptake or release of secondary metabolites in the ED group. In contrast, the GLN+ED group responded with enhanced glutamine removal and alterations in the other fluxes as well. Glutamine uptake increased 3.5 fold, resulting primarily from a rise in fractional extraction from 6% to 18%. Delivered glutamine load rose as a result of increased arterial concentration, attributable to accelerated peripheral release and a higher HVPF (6.2 ± 1.1 versus 4.0 ± 0.6 ml/min/100g

Table 5 Hepatic metabolite release: effect of dietary glutamine and zymosan

	Pre-zymosan			Post-zymosan		
	Glu	Ala	GSH	Glu	Ala	GSH
ED	386 ±102	−475 ±101	161 ±95	363 ±62	−469 ±86	158 ±309
GLN+ED	808 ±178	−793 ±241	469 ±631	305 ±86	−990 ±147	1027 ±321
P	<0.05	NS	<0.05	NS	<0.05	<0.05

Results are means ± SEM in nmol/min/100g BW from six rats per group. Minus sign indicates net uptake.

BW, GLN+ED versus ED, $P < 0.05$). Alanine uptake remained higher in the GLN+ED group than in the ED group (990 versus 469 nmol/min), a difference sustained largely from peripheral rather than gut alanine release (169 versus 30 nmol/min, GLN+ED versus ED group, *Table 3*). In addition, zymosan depressed hepatic glutamate efflux in the GLN+ED group from 808 to 305 nmol/min/100g BW ($P < 0.05$), a rate not different from the ED group. On the other hand, glutathione release was higher in the GLN+ED group (1027 ± 321 nmol/min) versus the ED group (158 ± 309 nmol/min) ($P < 0.05$). Measurement of the plasma glutathione (GSH) flux revealed a small hepatic efflux (14 ± 3.5 nmol/min/100g BW) in the ED group after zymosan and a larger efflux (86 ± 5 nmol/min/100g BW, $P < 0.025$) in the GLN+ED group.

Discussion

Glutamine homeostasis requires that the rate of glutamine removal from blood at major organ sites be matched by glutamine addition to the blood at counterbalancing sites, a condition that can normally be demonstrated in the post-absorptive anesthetized rat.[7] Indeed, the major uptake sites, gut and liver, are balanced by release from the lung and muscle.[7] Within limits of error, measured uptake is balanced by release and averages 820 nmol/min/100g BW, approximating net whole body requirements. Whole body turnover estimated from ^{14}C-glutamine disappearance rates gives a unidirectional breakdown rate of 1497 ± 150 nmol/min/100g BW (unpublished observation). Thus the difference between the whole body net uptake and unidirectional breakdown rates is a measure of whole body glutamine synthesis and approximates 677 nmol/min/100g BW. In the present study, the gut and liver removed a combined total of approximately 539 nmol/min/100g BW in animals fed glutamine-free (ED) and glutamine-containing (GLN+ED) elemental diets. This value is in agreement with those previously published.[17,24] Zymosan did not alter enterohepatic glutamine removal in the ED group. In contrast, after zymosan these two sites increased their removal to 1015 nmol/min/100g BW in the GLN+ED group, a rate that alone would essentially outstrip the endogenous synthesis if an exogenous source was not provided. Converting the enteral uptake to an average minute rate provides 548 nmol/min/100g BW or approximately the incremental consumption by the splanchnic bed during an acute inflammatory response. This suggests that by charging peripheral reserves or supplying readily interconvertible metabolites, the enteral glutamine load provides a means for delivering nutrients at critical sites under conditions in which endogenous supplies are insufficient or nonresponsive.

Our data are consistent with prior (to the inflammatory stress) absorption of the enteral glutamine, increase in peripheral glutamine or metabolite reservoirs, and then maintenance of secondary fluxes of glutamate and alanine. Previously, studies of enteral glutamine loading have estimated that in humans approximately 50% of the load reaches the periphery.[25] In our study, sufficient glutamine escaped the liver to exceed the plasma threshold for renal glutamine absorption, consistent with ample loads to the periphery where glutamine can be accumulated, and/or converted to a readily reversible form and subsequently released under hormonal regulation.[26] In humans[27] jejunal glutamine perfusions have been shown to acutely increase systemic glutamine and glutamate concentrations in a dose-dependent fashion and to raise alanine concentration, but to a lesser degree; the enhanced alanine production was attributed to increased de novo synthesis.[27] In our studies, dietary glutamine did not elevate either arterial glutamine or glutamate concentrations, in contrast to increased arterial alanine concentration. Because hepatic alanine uptake greatly exceeded gut alanine release, alanine flux must be originating from peripheral sites. The large hepatic glutamate efflux measured at this time and directed to the periphery suggested peripheral glutamate removal coupled to alanine release.[28] Note that a shunting of nitrogen from ureagenesis to glutamate efflux would also explain the tendency for urea excretion to fall. Overall, dietary glutamine appears to set in motion secondary fluxes that effectively bypass the ureagenic sink and provide the body with an additional defense mechanism.

Responsiveness to inflammation was greatly enhanced in terms of interorgan fluxes in the glutamine-fed group. Both glutamine and alanine fluxes from the periphery were accelerated, in contrast to the group devoid of dietary glutamine. Indeed, arterial alanine levels even fell in the ED group. Because alanine

release from peripheral reservoirs reflects intracellular precursor availability,[7] this finding is consistent with a peripheral supply limited response. Glucocorticoids are known to mobilize both glutamine and alanine from peripheral sites but to require chronic administration.[21] In the present study, zymosan was shown to acutely elevate arterial corticosterone concentration, presumably through interleukin-1 and the pituitary-adrenal axis[30]. Despite the elevated corticosterone, glutamine flux increased only in the glutamine-fed group. This suggests that the acute response was dependent on peripheral reserves rather than de novo synthesis; presumably the failure to acutely elevate glutamine flux in the ED group resulted from their limited peripheral reserves. Lacking the enhanced glutamine reserve in the ED group, the small intestine responded with increased fractional extraction and apparently more complete oxidation of the available glutamine, because alanine release did not increase. Despite an increased load, the GLN+ED group also demonstrated a rise in fractional extraction of glutamine by the gut, but unlike the ED group, more alanine was released, a difference apparently reflecting the greater glutamine availability.[7] Thus the acute zymosan-elicited effects on interorgan fluxes were limited to the glutamine loaded group, although effects on cellular utilization and metabolic pathways were apparent in both groups.

The importance of dietary glutamine appears related to secondary fluxes as much as to glutamine during the acute response to inflammation. Glutamine, as the primary flux, may provide fuel for the gut[16] and liver[7] and is associated with increased muscle protein synthesis.[10] However, of greater significance in the acute phase are the secondary fluxes responding to the inflammatory challenge only in the GLN+ED group. Most notable is the rise in both gut and hepatic glutathione efflux *(Tables 3 and 5)*. Glutathione synthesis occurs in both the small intestine[6] and liver[5,6] and requires glutamate as a precursor.[6] Although glutamate is not generally considered rate limiting, under certain conditions glutamine-derived glutamate[31] or glutamate[32] may become rate limiting. Note also that peripheral inflammation depresses hepatic glutathione levels[33] and most likely accentuates the demand for glutamate. Because glutamate is not extracted by the liver, it must be produced de novo or be derived from extracted glutamine *(Table 4)*. In the GLN+ED group following zymosan, gut glutamate release reversed to a net uptake coinciding with sustained glutathione release *(Table 3)*. At the same time, hepatic glutamate efflux fell, suggesting a major shift to the enhanced glutathione synthesis at this organ *(Table 5)*. Consistent with this, Hong et al.[34] demonstrated that glutamine supplementation ameliorated acetaminophen toxicity and prevented hepatic glutathione depletion. Further studies are warranted to determine the role of glutamine/glutamate in supporting glutathione efflux, as well as the organ destination of this large efflux occurring during inflammation.

In glutamine-fed animals, alanine flux was also accelerated post-zymosan. Unlike the pre-zymosan flow, which may have been coupled to the glutamate efflux, that occurring during the post-zymosan period appears the result of peripheral de novo synthesis possibly secondary to glucocorticoid action. Accelerated alanine fluxes to the liver may provide a precursor for gluconeogenesis[7] or perhaps play a role in modulating protein synthesis[11] and cellular reparation.[35] In the present study, the alanine load to the liver increased, suggesting that if higher cellular levels ensued, polyribosomal aggregation and protein synthesis may have been fostered.[11] Thus, secondary fluxes of important interorgan nutrients appeared in the glutamine-fed animals, and these were not evident in animals devoid of dietary glutamine.

Administering exogenous glutamine intravenously would enhance the primary glutamine flow but may not provide the critical integrated secondary fluxes that appear with enteral glutamine administration. Infusing exogenous glutamine at systemic sites provokes an exaggerated hepatic glutamine removal that may even result in the reduction of arterial glutamine concentration.[7,23] Consequently, regulating arterial levels and avoiding accelerated ureagenesis becomes a major concern with parenteral glutamine administration. Further complicating the intravenous administration of glutamine is the interorgan reflex resulting in depressed renal perfusion[20]; this reflex occurs in response to elevated portal glutamine concentration and results in an acute reduction of renal blood flow.[26] Thus, the intravenous administration route satisfies the requirement for an immediate glutamine load, but neglects crucial regulatory mechanisms poised for maintaining homeostasis and for integrating interorgan fluxes. In contrast, the dietary enteral glutamine load studied here (18.4 g/kg diet, dry basis) appeared to be easily integrated, perhaps under hormonal control, into the balanced interorgan pattern of primary and secondary fluxes. Thus dietary glutamine administration may offer a rational approach for the management and possibly pretreatment of patients subjected to catabolic and inflammatory stresses.

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