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Effect of Glutamine Supplementation on Protein Metabolism and Glutathione in Tumor-Bearing Rats*

SHOGO YOSHIDA, MD; ATSUSHI KAIBARA, MD; KOKUSHI YAMASAKI, MD; NOBUYA ISHIBASHI, MD; TOSHIHIRO NOAKE, MD; AND TERUO KAKEGAWA MD, FACS

1st Department of Surgery, Kurume University, School of Medicine, Fukuoka, Japan

ABSTRACT. *Background:* Since tumor-bearing rats are deficient in glutamine, we investigated whether (1) glutamine and glutathione deficiency occur in tumor-bearing rats, (2) glutamine supplementation caused an increase of glutathione levels in host tissues and tumor, (3) glutamine enhances protein synthesis in host tissues, and (4) glutamine stimulated the tumor to synthesize protein and DNA. *Methods:* Male Donryu rats were randomized into four groups: (1) non–tumor-bearing rat (NTB) + standard total parenteral nutrition (STPN); (2) NTB + glutamine-supplemented TPN (GTPN); (3) tumor-bearing rat (TB)+STPN; (4) TB+GTPN. On day 0 AH109A rat hepatoma cells were subcutaneously injected into the backs of rats to induce tumor. The animals were maintained on TPN for 6 days from day 10 through day 15. On day 15, 1-^{14}C-leucine was given by a 5-hour continuous infusion (2.0 μCi/h per rat) to determine the fractional synthesis rate and endogenous leucine production. The levels of glutamine and glutathione were measured by HPLC. The tumor DNA synthesis was estimated by bromodeoxyuridine labeling index. *Results:* Tumor development led to a significant weight loss, but this weight loss was significantly lessened by glutamine supplementation because of an increase in muscle protein synthesis. Glutamine did not enhance tumor weight, protein, and DNA synthesis in the tumor. Tumor development caused a significant reduction of glutathione in the muscle, jejunum, and liver, but supplemented glutamine increased the levels of glutathione in the jejunum. *Conclusion:* Glutamine supplementation is beneficial in preventing deficiencies of glutamine and glutathione and in improving protein metabolism in tumor-bearing rats. *(Journal of Parenteral and Enteral Nutrition* **19:**492–497, 1995)

The main site of glutamine synthesis is skeletal muscle, in which glutamine constitutes more than 60% of the free intracellular amino acids.[1] The production of glutamine is increased in trauma and sepsis,[2,3] leading to glutamine depletion in the muscle.[4] Glutamine released from skeletal muscle is used by cells in the intestine and by the immune cells because glutamine is a specific nutrient for these cells.[5,6] We previously reported that supplemental glutamine improved protein synthesis of gut mucosa and muscle in septic rats.[7] Supplementation of glutamine counteracts the depletion of glutamine due to sepsis and increased protein synthesis in muscle, because the level of glutamine in muscle is correlated with muscle protein synthesis.[8]

There is an interest in giving glutamine to a tumor-bearing host, because tumor growth is associated with a flux of glutamine shifting away from the host toward tumor. This trend may contribute to the development of cancer cachexia due to glutamine deficiency in the host tissues. A concern about administering glutamine to cancer patients with cachexia is that it would be utilized by the rapidly proliferating cells, including the malignant cells,[9] with the potential enhancement of tumor growth. Chen et al[10] demonstrated that glutamine derived from skeletal muscle was mainly trapped in the tumor. However, Austgen et al[11] reported that supplemental glutamine did not enhance the tumor content DNA. Klimberg et al[12] previously demonstrated that an elemental diet rich in glutamine elevated the concentration of muscle glutamine and maintained muscle glutamine efflux in fibrosarcoma-bearing rats *vs* rats fed an elemental diet free of glutamine.

Another important role of glutamine is a precursor of glutathione.[13] Glutathione depletion occurs in malnutrition and trauma in association with glutamine depletion.[14,15] Hong et al[16] showed that the administration of glutamine prevented reduction of hepatic glutathione in rats with administered acetaminophen. Similarly, Austgen et al[11] reported that supplemented glutamine increased the jejunal glutathione levels in methylcholanthrene-induced fibrosarcoma bearing rats.

The objectives of this study were to determine whether (1) glutamine and glutathione deficiency occurs in tumor-bearing rats, (2) supplemental glutamine elevated glutamine and glutathione levels and enhance protein synthesis in host tissues and tumor, and (3) supplemented glutamine increases in synthesis of DNA in the tumor.

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Correspondence and reprint requests: Shogo Yoshida, MD, 1st Department of Surgery, Kurume University, School of Medicine, 67 Asahi-machi, Kurume-shi, Fukuoka, Japan 830.
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MATERIALS AND METHODS

Animal Preparation

Male Donryu rats weighing 130 to 140 g were purchased from the Shizuoka Animal Facility Center (Shizuoka, Japan). Rats were housed in the animal care facility at the Kurume University and were fed rat food (CE-2, Clea Japan Inc, Tokyo, Japan) for 1 week with 12-hour light and dark cycles until the experimental protocol was begun.

Tumor Cell Culture and Implantation

Frozen AH109A rat ascites hepatoma cells were cultured in flasks containing RPMI-1640 medium (GIBCO Life Technologies, Inc, Grand Island, NY) for 2 weeks. AH109A cells (5×10^7) were injected into the rats intraperitoneally. One week later, the cells that had proliferated in the abdominal cavity were collected and injected into the abdominal cavity of other rats. This procedure was repeated four times after which the cells were harvested and were injected subcutaneously into the backs of the experimental rats to induce the tumor.

Experimental Design

Male Donryu rats (n = 46, body weight: 150 to 170 g) were randomly divided into 2 groups, tumor-bearing (TB) and non–tumor-bearing (NTB). On day 0, AH-109A rat ascites hepatoma cells (2×10^6) were inoculated into the back of TB rat subcutaneously (n = 30) (Fig. 1). The animals were then returned to the metabolic cages and given access to a stock diet and water *ad libitum* for 10 days.

On day 10, the animals were catheterized into the jugular vein under pentobarbital anesthesia (50 mg/kg IM, Abbott Laboratories, North Chicago, IL). A catheter via a spring apparatus was sutured to the animal's back to protect the lines and were connected to swivel (Instech Co, imported by Muromachi Co, Tokyo, Japan) to allow the animal to move without any restrictions in individual metabolic cages (Nalge Co, imported by Tohei Co, Tokyo, Japan). NTB rats (n = 16) entered the study at this point and were also catheterized for TPN. Each group was subdivided into two groups according to the TPN infusate, standard total parenteral nutrition (STPN) and glutamine-supplemented TPN (GTPN), as follows: (1) non–tumor-bearing rat (NTB)+STPN; (2) NTB+GTPN; (3) tumor-bearing rat (TB)+STPN; (4) TB+GTPN. After the cannulation into the jugular vein, TPN was begun. For the 1st day of TPN, 125 kcal/kg per day and 0.75 gN/kg per day were infused. From the 2nd day through the 6th day of TPN, full strength diet (250 kcal/kg per day, 1.5 gN/kg per day) was given.

On day 15, 1-^{14}C-leucine (Dupont NEN product, Boston, MA) was dissolved in each full strength diet (1 μCi/mL diet) and infused to 32 rats out of 46 cannulated rats continuously for 5 hours at the rate of 2 μCi/h per rat. At the end of isotope infusion, animals were killed and muscle, liver, tumor, and plasma were collected. The jejunum (20 cm from Treitz ligament) and colon (10 cm from cecum) were also taken. Mucosa of jejunum and colon was obtained by gently scraping the opened bowel with a glass slide. All samples were frozen in liquid nitrogen immediately after collection and stored at −70°C until assayed.

Bromodeoxyuridine (BrdU, 20 mg/kg, Sigma Chemical Co, St Louis, MO) was injected to the remaining 14 tumor-bearing rats via TPN catheter on day 15 (TB-STPN: n = 7; TB-GTPN: n = 7) to measure BrdU labeling index in the tumor. The rats were killed 1 hour after the injection of BrdU, and tumor was collected for analysis of DNA synthesis.

Diets

The composition of the diets infused is shown in Table I. Ninety percent of total calories was given as glucose (GE-3, Otsuka Pharmaceutical Co, Tokushima, Japan), and 10% as fat (Intralipid, Otsuka Pharmaceutical Co.) to prevent essential fatty acid deficiency. GTPN was prepared by replacing 1.5 g/100 mL diet of standard amino acid solution (Moriprone FR, Morishita Pharmaceutical Co, Osaka, Japan) with the same nitrogen amounts (1.1 g) of alanyl glutamine (Otsuka Pharmaceutical Co) to make GTPN isonitrogenous with STPN. Twenty-five percent of total nitrogen was given as glutamine in GTPN. Adequate amounts of electrolytes, trace minerals, and vitamins were also added to the both diets.

Measurements of Rat Body Weight and Tumor Weight

Rats were weighed on days 0, 10, and 15. Tumor weight was measured on day 15. The two diameters of tumor were measured on day 10. Tumor weight on day 10 was calculated from the tumor size by the following equation:[17]

$$\text{Estimated tumor weight} = (\text{shorter diameter of tumor})^2 \times (\text{longer diameter})/4$$

The weight change during TPN was calculated as follows:

$$\text{Estimated carcass weight (day 10)} = (\text{actual body weight}) - (\text{estimated tumor weight})$$

$$\text{Actual carcass weight (day 15)} = (\text{actual body weight}) - (\text{actual tumor weight})$$

$$\text{Estimated carcass weight change} = (\text{actual carcass weight}) - (\text{estimated carcass weight})$$

$$\text{Actual body weight change} = (\text{body weight on day 15}) - (\text{body weight on day 10})$$

Analytical Procedures

1. *Glutamine concentration.* Each 200 mg of mucosa from jejunum, muscle, and tumor were thawed and homogenized in 3 mL of 4% sulfosalicylic acid (Wako Chemical Co, Osaka, Japan). The supernatant was separated from precipitate by centrifugation at 10,000 rpm for 20 minutes at 5°C. The supernatant was lyophilized overnight to dryness and was reconstituted with 1 mL of H_2O and 1 N NaOH. The free glutamine concentration was measured on a 0.01-mL aliquot of the aqueous layer, which was injected into high performance liquid chromatography (HPLC, Hitachi Inc, Tokyo, Japan), using precolumn derivatization with o-phthaldialdehyde (Sigma Chemical Co).
2. *Fractional synthesis rate.* Procedures used for determining the fractional synthesis rate of various tissues have been previously described.[7] Briefly, 200 mg of mucosa, muscle, liver, and tumor were

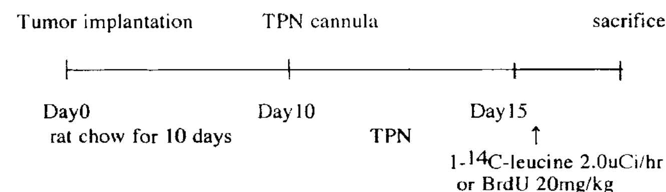

FIG. 1. Experimental protocol. AH109A rat ascites hepatoma cells (2×10^6) were implanted subcutaneously on day 0. On day 10, the rats were catheterized for TPN and maintained on TPN for 5 days. On day 15, 1-^{14}C-leucine dissolved in each diet was given by a 5-hour constant infusion(2 μCi/h per rat). The animals were killed immediately after isotope infusion. Another set of rats (n = 14) received bromodeoxyuridine (BrdU) by IV bolus 1 hour before they were to estimate tumor DNA synthesis.

TABLE I
Composition of standard TPN solution and glutamine-supplemented TPN solution

	STPN	GTPN*
Calories in diet (kcal/mL)	1	1
Glucose (kcal/100 mL diet)	90	90
Fat (kcal/100 mL diet)	10	10
Standard amino acids (g/100 mL diet)	4	2.5
Alanyl-glutamine (g/100 mL diet)	—	1.1
Total nitrogen amount in diet (mg N/100 mL diet)	608	608

*In glutamine-supplemented infusate, 1.5 g/100 mL diet of standard amino acids are replaced with 1.1 g of alanyl glutamine.

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thawed and homogenized in 3 mL of 4% sulfosalicylic acid. To determine the specific activity of protein-bound leucine, the protein precipitate was separated from the supernatant by centrifugation at 10,000 rpm for 20 minutes at 5°C. The precipitate was then washed three times. Eight milliliters of 1 N NaOH was added to the precipitate and sonicated until dissolved. The amount of protein in the aliquot was measured by the modified Lowry method (Sigma Chemical Co). An aliquot of the solution was counted for ^{14}C in a beta counter (Aloka Co, Ltd, Tokyo, Japan).

The supernatant was lyophilized to remove any ketoisocaproate to determine intracellular free leucine specific activity. The dry residue was reconstituted with 1 mL of H_2O and 1 N NaOH. The first aqueous layer was then removed and counted for ^{14}C using a beta counter. The free leucine concentration was measured in a 0.01-mL aliquot of the other aqueous layer, using Hitachi HPLC (Hitachi Inc) by the method of precolumn derivatization with o-phthaldialdehyde.

Methods of calculation. Fractional synthesis rates (ks) were calculated by the formula of Garlick et al,[18] where S_B is the specific activity

$$\frac{S_B}{S_i} = \frac{\lambda_i}{(\lambda_i - k_s)} \cdot \frac{(1 - e^{-k_s t})}{(1 - e^{-\lambda_i t})} - \frac{k_s}{(\lambda_i - k_s)}$$

of protein-bound leucine and S_i is the specific activity of leucine in the intracellular fluid of the muscle, tumor, liver, jejunum, and colon.

3 *Endogenous leucine production.* Four percent sulfosalicylic acid (3 mL) was added to 0.3 mL of plasma. The supernatant was separated from precipitate by centrifugation at 10,000 rpm for 20 minutes. The supernatant was lyophilized overnight to dryness and was reconstituted with 1 mL of H_2O and 1 N NaOH. ^{14}C was counted in a beta counter, and the free leucine concentration was measured by HPLC to determine the plasma specific activity of leucine.

The total leucine production was calculated by Steele equation, where d is isotope infusion rate (dpm/h), and Sp is plasma leucine specific activity (dpm/μmol leucine).

$$\text{Total leucine production} = d/Sp$$

Endogenous leucine production was calculated by subtracting the exogenous leucine infusion rate (μmole leucine/hr) during isotope infusion from the total leucine production.

4 *BrdU-labeling index in the tumor.* The tumor obtained from the rats receiving BrdU was thawed in RPMI-1640 medium and prepared for flowcytometric analysis according to the method of Usami et al.[19] The precipitate was separated from the supernatant by centrifugation at 1000 rpm for 10 minutes, and then the precipitate was washed twice. It was then fixed with 97% absolute ethanol (Wako Chemical Co) overnight and the ethanol was discarded. The precipitate was suspended in phosphate-buffered solution (PBS, Nissui Pharmaceutical Co, Tokyo, Japan) at 4°C for 3 hours. After centrifugation (1200 rpm for 7 minutes), the supernatant was discarded and 4 mL of 0.25% pepsin solution (Nakalai Tesque Inc, Kyoto, Japan) was added, and the precipitate was resuspended. PBS (5 mL) was added and centrifuged at 1000 rpm for 30 seconds. The supernatant was filtered using 50 μm nylon mesh and recentrifuged at 1200 rpm for 7 minutes. The precipitate was washed twice in PBS. The cells were resuspended in 4 N HCl at room temperature for 20 minutes. Anti-BrdU monoclonal antibody conjugated with fluorescein isothiocyanate (Becton-Dickinson, Sunnyvale, CA) was added and the mixture was incubated on ice for 30 minutes. The cells were washed twice in PBS, propidium iodide (10 μg/mL, Sigma Chemical Co) was added and followed by incubation for 15 minutes at 4°C in the dark. The number of BrdU-positive cells were counted and the phase of DNA was measured to determine BrdU-labeling index, using flow cytometry[20] (Otsuka Assay Research Laboratory, Tokushima, Japan).

5 *Glutathione measurement.* Glutathione levels in the liver, jejunum, muscle, and tumor were measured by the method of Neuschwander-Tetri et al.[21] The tissue homogenates were prepared by weighing them and homogenizing them in 25 mmol sodium phosphate/L, pH 6.00 (Wako Chemical Co). To measure total glutathione, 100 μL of homogenates were mixed with 100 μL of 25 mmol dithiothreitol/L (DTT, Wako Chemical Co) and 50 μL 0.1 mol tris(hydroxymethyl)aminomethane (Tris)/L, pH 8.5 (Sigma Chemical Co). After 30 minutes on ice, the protein was precipitated with 2.5% sulfosalicylic acid and centrifuged at 10,000 rpm for 10 minutes. Reduced thiol elimination was done to measure oxidized glutathione, using 2 mmol *N*-ethylmaleimide/L (NEM, Wako Chemical Co). Disulfide was reduced by adding DTT and Tris, and protein was precipitated as described above. The supernatant was injected into HPLC equipped with Hitachi Hibar Lichrosorb column (Hitachi Inc) and Waters 470 fluorescence spectrophotometer (Waters Japan, Tokyo, Japan). o-phthaldialdehyde was used for precolumn derivatization. Oxidized glutathione (GSSX, X = any thiol) was subtracted from total glutathione (GSSX + GSH) to determine reduced glutathione (GSH).

Statistical Analysis

Data are expressed as means ± SEM. Statistical analysis was done by analysis of variance and Fisher's protected least significant difference test was employed to differentiate significant differences among the means, using Macintosh SE/30 (Apple Computers, Inc, Cupertino, CA; Statview 412 supplied by Abacus Concepts, Berkeley, CA). Mean differences were considered statistically significant at $p < .05$.

RESULTS

Body and Tumor Weights

There was no significant difference in body weight during 5 days of TPN between NTB+STPN and NTB+GTPN (NTB+STPN: −5.7 ± 1.5 g *vs* NTB+GTPN: −5.3 ± 1.2, NS) (Table II). A significantly greater loss of body weight was seen with TB+STPN than with NTB+STPN (TB+STPN: −9.4 ± 1.4 g, $p < .05$ *vs* NTB+STPN). This weight loss was prevented with glutamine supplementation in the tumor-bearing rats (TB+GTPN: −5.5 ± 1.2 g, $p < .05$ *vs* TB+STPN). Carcass weight loss in tumor-bearing rats was significantly greater with STPN compared with GTPN (TB+STPN: −13.7 ± 1.5 g *vs* TB+GTPN: −8.5 ± 1.5, $p < .05$). Diet had no effect on tumor weight. Tumor weight on day 15 in the two groups did not differ significantly (TB-STPN: 4.5 ± 0.4 g *vs* TB-GTPN: 4.3 ± 0.5, NS).

Glutamine Concentration

Glutamine levels in the plasma and muscle were decreased 15 days after inoculation in the tumor-bearing rats compared with non–tumor-bearing rats receiving 5 days of standard TPN (plasma, NTB+STPN: 598.3 ± 43.4 μmol/mL *vs* TB+STPN: 419.8 ± 45.9 μmol/mL, $p < .05$; muscle, NTB+STPN: 3.0 ± 0.1 μmol/g tissue *vs* TB+STPN: 2.5 ± 0.1 μmol/g tissue, $p < .05$) (Table III). GTPN improved glutamine levels in plasma and muscle (plasma, TB+GTPN: 672.8 ± 23.8 μmol/mL, $p < .05$ *vs* TB+STPN; muscle, TB+GTPN: 3.1 ± 0.2 μmol/g tissue, $p < .05$ *vs* TB+STPN). No difference was found in the intracellular glutamine levels in the jejunum between STPN and GTPN in non–tumor-bearing rats (NTB+STPN: 1.1 ± 0.2 *vs* NTB+GTPN: 1.1 ± 0.2 μmol/g tissue, NS) and in the jejunum and tumor in the tumor-bearing rats (jejunum, TB+STPN: 1.3 ± 0.2 *vs* TB+GTPN: 1.4 ± 0.3 μmol/g tissue, NS; tumor, TB+STPN: 0.4 ± 0.1 *vs* TB+GTPN: 0.5 ± 0.1 μmol/g tissue, NS).

The fractional synthesis rates (FSR) and whole body protein breakdown rates are summarized in Table IV. Presence of tumor resulted in a significant reduction of muscle FSR with STPN (NTB+STPN: 15.7 ± 1.9%/d *vs* TB+STPN: 10.8 ± 0.7%/d, $p < .05$), but this reduction was improved with glutamine supplementation compared with STPN in the tumor-bearing rats (TB+GTPN: 14.7 ± 0.6%/d, $p < .05$ *vs* TB+STPN).

Neither tumor-bearing nor glutamine supplementation altered the liver FSR; however, there was a significant increase of the intestinal mucosal FSR with glutamine

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TABLE II
Body weight change and tumor weight during 5 days TPN

	NTB+STPN	NTB+GTPN	TB+STPN	TB+GTPN
n	8	8	15	15
BW (day 0)	—	—	198.4 ± 3.1	195.4 ± 3.4
BW (day 10)	257.6 ± 9.8	255.1 ± 9.4	262.0 ± 6.1†	255.1 ± 3.6‡
BW (day 15)	252.4 ± 11.7	249.6 ± 10.9	253.0 ± 4.8	250.8 ± 4.1
BW change (g)	−5.7 ± 1.5	−5.3 ± 1.2	−9.4 ± 1.4	−5.5 ± 1.2*
Carcass weight change	—	—	−13.7 ± 1.5	−8.5 ± 1.5
Estimated tumor weight	—	—	1.4 ± 0.2	1.5 ± 0.2
Tumor weight	—	—	4.5 ± 0.4	4.3 ± 0.5

Estimated tumor weight was calculated by actual diameters of tumor on day 10. Tumor weight was measured at sacrifice on day 15. Body weight (BW) change was calculated from body weight on day 15 subtracted by body weight on day 10. Estimated tumor weight or tumor weight was subtracted from body weight on day 10 and 15, respectively, to calculate carcass weight. Units are grams. *$p < .05$ *vs* TB-STPN (tumor bearing-standard TPN solution). Statistical calculation by ANOVA, using Statview 412.

supplementation in the tumor-bearing rats (jejunum, TB+STPN: 268.5 ± 19.2%/d *vs* TB+GTPN: 333.9 ± 20.2%/d, $p < .05$; colon, TB+STPN: 46.9 ± 4.1%/d *vs* TB+GTPN: 64.0 ± 4.9%/d, $p < .05$). Whole body protein breakdown rate was increased with STPN in the tumor-bearing rats, compared with non–tumor-bearing rats (NTB+STPN: 284.9 ± 57.3 *vs* TB+STPN: 420.9 ± 65.5 μmol Leu/kg per hour, $p < .05$). There was also a significant decrease of whole body protein breakdown rate (WPBR) with glutamine in the tumor-bearing rats (TB+GTPN: 303.2 ± 33.1 μmole Leu/kg per hour, $p < .05$ *vs* TB+STPN).

No differences were found in tumor protein synthesis rate between STPN and GTPN (TB+STPN: 104.5 ± 7.0%/d *vs* TB+GTPN: 106.2 ± 6.7%/d, NS). BrdU-labeling index in the tumor is shown in Table IV. Neither were differences noted in BrdU-labeling index between GTPN and STPN (TB+STPN: 10.1 ± 1.8% gated *vs* TB+GTPN: 10.6 ± 2.3% gated, NS, Table IV).

Glutathione (GSH) levels in the muscle were decreased in the tumor-bearing rats compared with non–tumor-bearing rats with standard TPN solution (NTB+STPN: 0.21 ± 0.01 *vs* TB+STPN: 0.17 ± 0.01 nmol/mg tissue, $p < .05$, Table V). This reduction in the tumor-bearing rats was not prevented by glutamine supplementation (TB+GTPN: 0.18 ± 0.01 nmol/mg tissue, NS *vs* TB+STPN). GSH levels in the liver were reduced in the tumor-bearing rats with standard TPN (NTB+STPN: 9.49 ± 0.77 *vs* TB+STPN: 4.90 ± 0.22 nmol/mg tissue, $p < .05$). GTPN did not enhance GSH levels in the tumor (TB+GTPN: 4.80 ± 0.30 nmol/mg tissue, NS *vs* TB+STPN). In the jejunum, GSH level was significantly decreased in the tumor-bearing rats with standard TPN solution (NTB+STPN: 0.39 ± 0.01 *vs* TB+STPN: 0.14 ± 0.01 nmol/mg tissue, $p < .05$). Glutamine supplementation elevated GSH levels in the jejunum in tumor-bearing rats (TB+GTPN: 0.20 ± 0.02 nmol/mg tissue, $p < .05$ *vs* TB+STPN). There were no differences in tumor GSH levels between the two diets (TB+STPN: 0.29 ± 0.02 *vs* TB+GTPN: 0.28 ± 0.03 nmol/mg tissue, NS).

TABLE III
Glutamine levels in the plasma, host tissues, and tumor

	NTB+STPN	NTB+GTPN	TB+STPN	TB+GTPN
n	8	8	8	8
Plasma GLN	598.3 ± 43.4	790.1 ± 56.4*	419.8 ± 45.9†	672.8 ± 23.8*
Muscle GLN	3.0 ± 0.1	3.0 ± 0.2	2.5 ± 0.1†	3.1 ± 0.2
Jejunum GLN	1.1 ± 0.2	1.1 ± 0.2	1.3 ± 0.2	1.4 ± 0.3
Tumor GLN	—	—	0.4 ± 0.1	0.5 ± 0.1

GLN, glutamine. Units are μmol/ml for the plasma and μmol/g tissue for the muscle, liver, and tumor. †$p < .05$ *vs* NTB-STPN (non–tumor-bearing rats and standard TPN solution). †$p < .05$ *vs* NTB-STPN; *$p < .05$ *vs* TB-STPN (tumor-bearing rats and standard TPN solution). Statistical calculation by ANOVA, using Statview 412.

TABLE IV
Fractional synthesis rate, whole body protein breakdown rate, and BrdU labeling index

	NTB+STPN	NTB+GTPN	TB+STPN	TB+GTPN
n	8	8	8	8
Muscle FSR	15.7 ± 1.9	15.9 ± 1.5	10.8 ± 0.7*	14.7 ± 0.6†
Liver FSR	97.8 ± 17.8	110.3 ± 17.4	120.1 ± 14.5	120.9 ± 19.1
Jejunum FSR	238.4 ± 15.0	308.8 ± 12.8‡	268.5 ± 19.2	333.9 ± 20.2†
Colon FSR	34.9 ± 5.8	45.8 ± 6.3	46.9 ± 4.1	64.0 ± 4.9†
WPBR	284.9 ± 57.3	274.1 ± 73.4	420.9 ± 65.5*	303.2 ± 33.1*
Tumor FSR	—	—	104.5 ± 7.0	106.2 ± 6.7
Tumor BrdU	—	—	10.1 ± 1.8§	10.6 ± 2.3§

FSR, fractional synthesis rate expressed in %/d; WPBR, whole body protein breakdown rate μmol Leu/kg per hour; and BrdU, bromodeoxyuridine labeling index expressed as % gated.
*$p < .05$ *vs* NTB-STPN (non–tumor-bearing rats and standard TPN solution).
†$p < .05$ *vs* TB-STPN (tumor-bearing rats and standard TPN solution).
‡$p < .05$ *vs* NTB-STPN. Statistical calculation by ANOVA and paired *t*-test.
§n = 7.

DISCUSSION

Preliminary experiments showed that subcutaneous implantation of AH109A cells (2×10^6) caused death of the animals around 6 to 8 weeks later with cancer cachexia. In this study, the animals were killed 15 days after inoculation of tumor, because the rate of body weight gain is decreased about 2 weeks after inoculation. A characteristic feature of protein wasting in a tumor-bearing host is impaired protein synthesis in the muscle and increased whole body protein breakdown rate, because amino acids derived from skeletal muscle are used in the tumor, and this results in cancer cachexia.[22,23] In agreement with previous reports, muscle protein synthesis was decreased and whole body protein breakdown was increased compared

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TABLE V
Reduced glutathione (GSH) levels

	NTB+STPN	NTB+GTPN	TB+STPN	TB+GTPN
n	8	8	8	8
Muscle GSH	0.21 ± 0.01	0.20 ± 0.02	0.17 ± 0.01*	0.18 ± 0.01†
Liver GSH	9.49 ± 0.77	10.64 ± 0.61	4.90 ± 0.22*	4.80 ± 0.30†
Jejunum GSH	0.39 ± 0.01	0.40 ± 0.02	0.14 ± 0.01*	0.20 ± 0.02‡
Tumor GSH	—	—	0.29 ± 0.02	0.28 ± 0.03

Units are nmol/mg tissue.
*$p < .05$ *vs* NTB-STPN (non–tumor-bearing rats and standard TPN).
†$p < .05$ *vs* NTB-GTPN (non–tumor-bearing rats and glutamine-supplemented TPN).
‡$p < .05$ *vs* TB-STPN (tumor-bearing rats and standard TPN). Statistical calculation by ANOVA and paired *t*-test.

with non–tumor-bearing rats, suggesting that cancer cachexia was progressing 15 days after inoculation of AH109A cells.

In association with the progress of cancer cachexia, glutamine deficiency in the muscle occurred. However, glutamine levels in the jejunum and tumor were not different from non–tumor-bearing rats. Similarly, glutamine supplementation elevated glutamine levels in the muscle and plasma but not in the jejunum and tumor. This is probably because the site of glutamine pool is skeletal muscle and glutamine derived from muscle is utilized in jejunum and tumor. Because intracellular glutamine levels are regulated by the rate of uptake and utilization of glutamine in jejunum and tumor, glutamine levels in these two sites did not reflect the two parameters mentioned.

Interestingly, glutamine levels in the muscle with not only standard TPN solution but also TPN plus glutamine were lower than with the rats consuming standard rat chow orally (7.70 ± 0.98 μmol/g, adapted from Jepson et al[24]), suggesting that TPN alone causes a depletion of glutamine in the muscle.

A loss of body weight was greater in tumor-bearing rats compared with non–tumor-bearing rats. This weight loss in tumor-bearing rats was prevented by glutamine supplementation. This is because muscle and mucosal protein synthesis was enhanced and whole body protein breakdown was decreased with glutamine supplementation. Jepson et al[24] and Rennie et al[25] reported that glutamine concentration in the muscle was correlated with muscle protein synthesis and that glutamine deficiency results in an increase of muscle protein breakdown, respectively. Muscle protein turnover represents about 50% of whole body protein turnover.[26] The decrease in whole body protein breakdown was found with glutamine supplementation probably due to a decrease in muscle protein breakdown. Thus we conclude that glutamine supplementation attenuates muscle wasting in the tumor-bearing host.

Glutamine is a specific nutrient in the mucosa and glutamine supplementation enhances mucosal growth and DNA and RNA content in the intestine.[27] Our previous report showed that supplementation caused an increase of protein synthesis in ileum and colon in septic rats.[7] Similarly, glutamine enhanced mucosal protein synthesis in jejunum and colon in the tumor-bearing rats.

In contrast to the effect of glutamine on protein synthesis in host tissues, supplemental glutamine did not enhance tumor protein synthesis, DNA synthesis, or tumor weight. Although glutamine is indispensable for tumor growth as an energy source and precursor of DNA,[28] previous reports showed that enteral feeding with glutamine supplementation failed to stimulate tumor growth. Austgen et al[11] demonstrated that glutamine supplementation did not alter DNA content and glutaminase activity in MCA tumor. Collectively, these results suggest that utilization of glutamine in the tumor may be already at the maximum; hence exogenous glutamine is utilized mainly in host tissues, accounting for an increase of muscle and mucosal protein synthesis.

Another important role of glutamine is as a precursor of glutathione. We found that glutamine prevented a reduction of jejunal glutathione levels but had no effect on liver, muscle, and tumor glutathione levels, although tumor bearing caused a decrease of glutathione levels in the muscle, liver, and jejunum. In agreement with our data, Austgen et al[11] reported that glutamine caused an increase of glutathione levels in the jejunum.

An important concern in this study is the relationship between glutathione levels and protein synthesis rate. Keller et al[29] reported that protein synthesis of hepatocytes was not decreased in glutathione-depleted medium. Similarly, there was no difference in hepatic protein synthesis rate in spite of a decrease in glutathione levels in the liver in tumor-bearing rats. In contrast to the impairment of protein synthesis in the muscle due to glutamine deficiency, glutathione levels were not correlated with protein synthesis rate in the muscle. These findings indicated that changes in glutathione levels alone did not change protein synthesis rate in the tissues. This is probably because the tissue glutathione is not utilized as a scavenger of free radicals but as a reservoir for cysteine and glutamate to maintain the protein synthesis rate in the presence of malnutrition.[30]

In summary, glutamine levels in the plasma and muscle decreased in the tumor-bearing rats. These changes were associated with a decrease of protein synthesis rates in muscle and an increase of whole body protein breakdown rate. Glutamine-supplemented TPN lessened the rate of body weight loss, because protein synthesis in the muscle, jejunum, and colon was increased and net proteolysis was decreased with glutamine supplementation, and these benefits occurred without stimulation of tumor growth. Glutamine TPN also prevented a reduction of glutathione in the jejunum but not in the muscle, liver, and tumor.

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