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Response of muscle protein and glutamine kinetics to branched-chain–enriched amino acids in intensive care patients after radical cancer surgery

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Abstract

Objective: Patients with cancer are characterized by decreased muscle protein synthesis and glutamine availability that contribute to an impaired immune response. These abnormalities worsen after surgical stress. We tested the hypothesis that pharmacologic doses of branched-chain amino acids would improve the early metabolic response after major cancer surgery.

Methods: By using a crossover experimental design, we compared the metabolic effects of isonitrogenous solutions of balanced and branched-chain–enriched amino acid mixtures infused at the rate of 82 mg $\cdot$ $h^{-1} \cdot kg^{-1}$ for 3 h in patients with colorectal or cervical cancer on the first and second days after radical surgery combined with intraoperative radiation therapy. The ratios of leucine to total amino acid (grams) in the two mixtures were 0.09 and 0.22, respectively. Muscle protein and glutamine kinetics were determined by using stable isotope of amino acids and the leg arteriovenous balance technique. Glucose and insulin were continuously infused throughout the 2-d study to maintain near euglycemia.

Results: Rates of muscle protein synthesis and degradation were not significantly affected by the balanced amino acid infusion. In contrast, the isonitrogenous, branched-chain–enriched amino acid mixture accelerated muscle protein turnover by stimulating ($P \leq 0.05$) protein synthesis. The rate of muscle glutamine de novo synthesis did not significantly change after infusion of the balanced amino acid mixture but increased ($P \leq 0.05$) by 263 $\pm$ 69% during infusion of the branched-chain–enriched amino acid mixture.

Conclusions: An excess of branched-chain amino acids in the presence of an optimal profile of other essential amino acids acutely increased muscle protein synthesis and glutamine flux from skeletal muscle in cancer patients after surgery.

Keywords: Cancer surgery; Glutamine; Skeletal muscle; Branched-chain amino acids

Introduction

Adaptation of muscle protein metabolism to cancer involves downregulation of the process of protein synthesis [1] and activation of the ubiquitin-dependent proteolytic pathway [2], leading to tissue atrophy and cachexia. In addition, glutamine concentration decreases in plasma and skeletal muscle [3] in part because of increased consump-

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tion of the amino acid by the tumor and immune and splanchnic tissues of the host [4] and because of decreased glutamine synthesis de novo in skeletal muscle [5]. Evidence indicates that glutamine depletion has adverse effects [6], whereas increased glutamine availability in cancer may increase immunoregulation of tumor growth by restoring the function of natural killer cells of the host [7].

Surgical excision of the tumor combined with intraoperative radiation therapy is currently used to treat a variety of locally advanced cancers including those of the colon and uterine cervix [8,9]. This procedure is associated with a significant stress response involving metabolic changes that interrelate with pre-existing alterations. It has been shown that cancer may blunt the metabolic response of muscle to surgical stress [10] that involves stimulation of proteolysis with absolute or relative inhibition of protein synthesis and acceleration of glutamine release into circulation [11]. It is well established that the rate of muscle protein synthesis is acutely regulated by amino acid availability [12]. Evidence suggests that a leucine-enriched amino acid mixture may have greater stimulatory effect on muscle protein synthesis than a balanced solution [13]. This amino acid exhibits the ability to initiate the process of protein synthesis that, in any case, requires an optimal plasma profile of all essential amino acids. In addition to this peculiar stimulatory effect on protein synthesis, an excess of nitrogen derived from leucine and the other branched-chain amino acids, isoleucine and valine, can be directly used as a precursor for glutamine synthesis in skeletal muscle [14]. In addition to the availability of nitrogen from the branched-chain amino acids, an infusion of glucose and insulin may further promote glutamine synthesis by providing the carbon skeleton of the amino acid through activation of the tricarboxylic acid cycle and synthesis of α-ketoglutarate [15].

We studied patients with colorectal or cervical cancer to test the hypothesis that pharmacologic doses of leucine and the other branched-chain amino acids in the presence of an optimal profile of the other essential amino acids may modulate the metabolic stress that follows radical surgery in combination with intraoperative radiation therapy. To this aim, we used a 2-d crossover experimental design to compare the effects of isonitrogenous solutions of balanced and branched-chain–enriched amino acid mixtures on rates of protein synthesis, proteolysis, and glutamine de novo synthesis by stable isotopes of amino acids and the leg arteriovenous balance technique [16,17]. Glucose and insulin were continuously infused throughout the days of the study to maintain near euglycemia.

Materials and methods

Patients

We studied six adult patients (52 ± 4 y of age, three men and three women, body mass index 23 ± 2 kg/m^2) with colorectal (n = 3) or cervical (n = 3) cancer with no major organ system diseases. Informed consent was obtained from all patients before surgery. The experimental protocol was approved by the ethical committee of the Centro di Riferimento Oncologico, Istituto Nazionale Tumori, IRCCS, Aviano, Italy. After a complete course of preoperative external radiotherapy and intravenous chemotherapy, patients underwent a radical hysterectomy or rectosigmoidal resection with pelvic lymphadenectomy and subsequent intraoperative radiation therapy [8,9]. Before surgery, mean hemoglobin level was 128 ± 8 g/L, mean creatinine level was 83 ± 8 μmol/L, and mean albumin level was 43 ± 1 g/L. Two days after surgery, hemoglobin and albumin concentrations decreased ($P < 0.001$) to 112 ± 10 and 33 ± 1 g/L, respectively, whereas creatinine did not change significantly (75 ± 8 μmol/L).

Experimental protocol

After surgery, patients were admitted to the Intensive Care Unit of the Centro di Riferimento Oncologico. Then a continuous intravenous glucose infusion at a rate of 125 mg/h per kilogram of body weight was initiated and continued for the following 48 h. Infusion of regular insulin with the use of a pump was started when plasma glucose level exceeded 6.1 mmol/L, and the infusion was adjusted to maintain near normoglycemia. Adjustments of the insulin dose was based on measurements of whole blood glucose in undiluted arterial blood, performed at 1- to 3-h intervals with the use of a glucose analyzer. In each patient, two leg muscle metabolic studies were performed on the same leg 24 h apart on the first and second days after surgery to evaluate the effects on muscle protein and glutamine metabolism of intravenous infusions of balanced or branched-chain–-enriched amino acid/isonitrogen amino acid mixtures (Tables 1 and 2). To account for time-related changes in muscle metabolism after surgery and for potential interference between two close stable isotope infusions, the balanced amino acid infusion preceded or followed the branched-chain–enriched amino acid infusion. Patients were randomly assigned to one of the two protocols. The morning of the first day after surgery, the first leg muscle metabolic study was performed from 8 AM to 2 PM. Indwelling catheters placed for clinical purposes in femoral and internal jugular veins and in a radial artery were used for isotope infusion and blood sampling. At 8 AM femoral venous blood samples were obtained to measure background phenylalanine enrichment. Then primed continuous infusions of L-[ring-$^2H_5$]phenylalanine (Cambridge Isotope Laboratories, Inc., Andover, MA, USA) were started and maintained constant throughout the experiment (prime dose 1 μmol/kg, infusion rate 0.02 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). After 160 min, three blood samples were taken every 10 min from the radial artery and the femoral vein to determine amino acid enrichments and concentrations. Leg blood flow was measured by plethysmography after every blood sample.

Table 1
Rates of amino acid infusions (mg · kg^{-1} · h^{-1})

Amino acids	Balanced amino acid mixture	Branched-chain–enriched amino acid mixture
Essential		
Lysine	8.4	4.6
Methionine	4.3	2.4
Phenylalanine	4.6	2.5
Threonine	3.3	1.8
Tryptophan	1.2	0.7
Branched-chain amino acids		
Isoleucine	5.7	14.7
Leucine	7.4	18.2
Valine	5.4	13.8
Non-essential		
Alanine	5.8	3.2
Arginine	7.8	4.3
Histidine	2.3	1.3
Proline	9.1	5.0
Serine	4.8	2.7
Cysteine	0.2	0.1
Glycine	11.4	6.3
Total	81.6	81.6

Blood samples were taken at least 5 min after each blood flow measurement. In three of the six patients, at 180 min, a continuous infusion of a balanced amino acid mixture (Freamine III 8.5%, Baxter, Milan, Italy) was initiated and continued for 3 h at the rate of 0.96 mL · kg^{-1} · h^{-1}. The amino acid concentrations reported by the manufacturer (per 100 mL) were 590 mg of isoleucine, 770 mg of leucine, 870 mg of lysine, 450 mg of methionine, 480 mg of phenylalanine, 340 mg of threonine, 130 mg of tryptophan, 560 mg of valine, 600 mg of alanine, 810 mg of arginine, 240 mg of histidine, 500 mg of serine, 18 mg of cysteine, and 1190 mg of glycine. The standard amino acid solution was defined as "balanced" because the relative composition of the essential amino acid was similar to its relative requirement in healthy subjects [18]. Mixed amino acids were infused at the rate of 1.36 mg · kg^{-1} · min^{-1}. The ratio of leucine to total amino acid (grams) was 0.09. In the other three patients, at 180 min, a continuous infusion of a branched-chain–enriched amino acid mixture (Aminoacidi Ramificati, Monico, Italy) containing 1570 mg/dL of leucine, 1285 mg/dL of valine, and 1200 mg/dL of isoleucine was started at the rate of 0.9 mL · kg^{-1} · h^{-1} in combination with Freamine III 8.5% infused at the rate of 0.53 mL · kg^{-1} · h^{-1} and continued for 3 h. Total amino acids were infused at the rate of 1.36 mg · kg^{-1} · min^{-1}. The ratio of leucine to total amino acid (grams) was 0.22. After 160 min of the balanced or branched-chain–enriched amino acid infusions, three blood samples were taken every 10 min from the radial artery and femoral vein to determine amino acid enrichments and concentrations. Leg blood flow was measured by plethysmography after every blood sample as described above. All infusions of labeled and unlabeled amino acids were stopped at 360 min. The morning of the second day after surgery, the second leg muscle metabolic study was performed from 8 AM to 2 PM as described above. In the three patients previously infused with a balanced amino acid mixture, a branched-chain–enriched mixture was started at 180 min. In the other three patients previously treated with the branched-chain–enriched mixture, a balanced amino acid mixture infusion was commenced at 180 min.

Analysis

Concentrations of selected amino acids (phenylalanine, leucine, and glutamine) and isotopic enrichment of L-[ring-$^2H_5$]phenylalanine were measured in plasma samples taken from the radial artery and femoral vein as described previously [16,17]. The stable isotopes L-[1-^{13}C]phenylalanine, L-[1-^{13}C]leucine, and L-[5-^{15}N]glutamine were added to the tubes as internal standards [16,17]. To determine the enrichment of the infused tracer and of the internal standards of free phenylalanine, leucine, and glutamine in plasma, the *t*-butyldimethylsilyl derivatives were prepared as described previously [17]. Tracer/tracee ratios were measured by gas chromatographic mass spectrometric analysis (HP 5985, Hewlett-Packard, Palo Alto, CA, USA) [17].

Table 2
Plasma concentrations of insulin, glucose, and selected amino acids

		Balanced amino acid mixture		BCAA-enriched mixture	
		Basal	Infusion	Basal	Infusion
Insulin (μU/mL)	Artery	16 ± 2	22 ± 4	20 ± 4	24 ± 3
Glucose (mmol/L)	Artery	5.94 ± 0.56	6.05 ± 1.67	6.61 ± 1.67	6.39 ± 0.28
Leucine (μmol/L)	Artery	72 ± 10	182 ± 15†	79 ± 5	424 ± 23*†
	Femoral vein	85 ± 12	175 ± 12†	86 ± 7	382 ± 22*†
Phenylalanine (μmol/L)	Artery	62 ± 6	120 ± 9†	65 ± 4	87 ± 4*†
	Femoral vein	69 ± 7	124 ± 9†	70 ± 5	88 ± 6*†
Glutamine (μmol/L)	Artery	393 ± 30	417 ± 46	422 ± 32	425 ± 37
	Femoral vein	439 ± 30	473 ± 41	462 ± 30	510 ± 42

BCAA, branched-chain amino acid
* $P < 0.05$ branched-chain–enriched versus balanced amino acid infusion.
† $P < 0.05$, infusions versus basal.

Table 3
Leg muscle protein kinetics*

		Phenylalanine Rd to protein synthesis	Phenylalanine Ra from proteolysis	Net balance (Rd − Ra)
Balanced amino acid mixture	Basal	32 ± 8	48 ± 10	−16 ± 3‡
	Infusion	30 ± 6	39 ± 8	−9 ± 4
Branched-chain–enriched amino acid mixture	Basal	21 ± 5	31 ± 5	−10 ± 1‡
	Infusion	43 ± 6†	46 ± 10	−3 ± 7‡

Ra, rate of appearance; Rd, rate of disappearance
Negative values indicate net release.
* Values are nanomoles per minute per 100 mL of leg muscle.
† $P < 0.10$ branched-chain–enriched versus balanced amino acid infusion.
‡ $P < 0.05$ versus 0.

Calculations

Net leg balances for phenylalanine, leucine, and glutamine were calculated from Fick's principle:

$$\text{Net balance} = (C_A - C_v) \cdot BF$$

where C_A and C_V represent plasma amino acid concentrations in the radial artery and femoral vein, respectively, and BF represents leg blood flow. A positive value indicates net uptake, whereas a negative value indicates net release. Skeletal muscle is considered to largely account for amino acid metabolism in the entire leg. In steady-state condition of muscle free amino acid concentrations, amino acid uptake or release across the leg reflects the balance between intracellular production and disposal of that particular amino acid. Thus, net phenylalanine release from leg muscle is a marker of net protein catabolism because this amino acid is not synthesized or hydroxylated in muscle tissue [19]. In contrast, skeletal muscle is the main site of catabolism of the branched-chain amino acids leucine, valine, and isoleucine and of synthesis of glutamine from glutamate. We assumed that amino acids are released from proteolysis in proportion to their relative content in mixed muscle protein [16,17,20]. Thus, the net rates of release from protein catabolism of leucine and glutamine can be calculated from the net rate of phenylalanine release corrected for the molar ratios glutamine/phenylalanine (i.e., 0.92) and leucine/phenylalanine (i.e., 3.10) determined in mixed human muscle protein [16,17,20]. Then the rates of net glutamine synthesis (i.e., differences between rates of synthesis and non-protein utilization of the amino acid) and leucine catabolism can be calculated by subtracting from the total release or uptake of these amino acids the component that is accounted for by protein catabolism:

$$\text{Net glutamine synthesis} = -[\text{net glutamine balance} - (\text{net phenylalanine balance} \times 0.92)]$$

$$\text{Leucine catabolism} = [\text{net leucine balance} - (\text{net phenylalanine balance} \times 3.10)]$$

In our study, estimation of net glutamine synthesis was little influenced by the assumption of a fixed molar glutamine/phenylalanine ratio in muscle protein. Our results showed that absolute values of net glutamine balance were 7 to 90 times greater than those for phenylalanine (Tables 3 and 4). In contrast, estimation of leucine catabolism was strongly influenced by the assumption of a fixed molar leucine/phenylalanine ratio in protein. The absolute values of net leucine balance were similar to those of phenylalanine, except during infusion of the branched-chain–enriched amino acid mixture, when absolute values of net leucine balance were 41 times greater than those of phenylalanine (Tables 3 and 4).

Rates of disappearance of phenylalanine to protein synthesis and appearance from proteolysis were calculated as rates of plasma phenylalanine disposal and appearance [18], respectively:

$$\text{Rd to protein synthesis} = [(C_{A(PHE)} \times E_{A(PHE)} - C_{V(PHE)} \times E_{V(PHE)})/E_{V(PHE)}] \times BF$$

where PHE indicates values of phenylalanine concentrations (C) and enrichments (E), Rd represents rate of disappearance, and Ra represents rate of appearance.

$$\text{Ra from proteolysis} = \text{Rd to protein synthesis} - \text{net phenylalanine balance}$$

Table 4
Leg muscle balance of leucine and glutamine*

		Leucine	Glutamine
Balanced amino acid mixture	Basal	−32 ± 9	−112 ± 29
	Infusion	7 ± 8†	−100 ± 25
Branched-chain–enriched amino acid mixture	Basal	−14 ± 7	−86 ± 19
	Infusion	124 ± 15†‡	−271 ± 51†‡

* Values are nanomoles per minute per 100 mL of leg volume. Negative values indicate net release.
† $P < 0.05$, infusions versus basal.
‡ $P < 0.05$, branched-chain–enriched versus balanced amino acid infusion. All values are significantly ($P < 0.05$) different from 0.

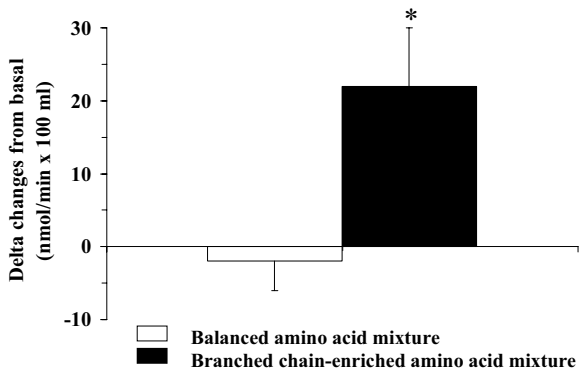

Fig. 1. Delta changes from basal leg muscle phenylalanine rate of disappearance to protein synthesis after infusions of the balanced (white bar) and branched-chain–enriched (black bar) amino acid mixtures. $^{*}P < 0.05$ branched-chain–enriched versus balanced amino acid infusion.

Data presentation and statistics

All data are expressed as mean ± standard error of the mean. Values of leg blood flow, amino acid concentrations, and kinetics obtained under the same experimental conditions at different times after surgery were not significantly different. Thus, results obtained during infusion of the balanced amino acid mixture on days 1 and 2 after surgery were pooled together and compared with the pooled results obtained during branched-chain–enriched amino acid infusion on days 1 and 2 after surgery. Results in the four different experimental conditions (basal state followed by balanced amino acid infusion, basal state followed by branched-chain–enriched amino acid infusion) were compared with Friedman's two-way analysis of variance. Changes from the basal states as mediated by the balanced and branched-chain–enriched amino acid mixtures were compared using Wilcoxon's matched-pair signed-rank test. Correlation studies were assessed by a non-parametric test (Spearman's rank correlation test). $P < 0.05$ was considered statistically significant.

Results

During the experimental period patients were metabolically stable because on days 1 and 2 after surgery the mean ($n = 8$) basal values of the phenylalanine rate of disappearance to protein synthesis (27 ± 4 and 26 ± 9 nmol/min per 100 mL of leg volume, respectively) and rate of appearance to protein degradation (38 ± 4 and 40 ± 11 nmol/min per 100 mL of leg volume, respectively) were not significantly different. Leg blood flow was not significantly different in the basal state and during infusion of the branched-chain–enriched amino acid mixture (2.25 ± 0.27 and 3.25 ± 0.67 ml/min per 100 mL of leg volume, respectively) or balanced amino acid mixture (2.29 ± 0.31 and 1.82 ± 0.23 ml/min per 100 mL of leg volume, respectively). Plasma insulin levels were not significantly different in the basal conditions of the two experimental periods and after infusions of the balanced and branched-chain–enriched amino acid mixtures (Table 2). Changes from basal insulin concentrations were similar during infusions of the balanced (+6 ± 4 μU/mL) and branched-chain–enriched (+4 ± 4 μU/mL) amino acid mixtures. Plasma glucose levels were similar in all experimental periods (Table 2). Table 2 lists values of selected amino acid concentrations in the basal states and during infusions of amino acid mixtures in the radial artery and femoral vein. Arterial phenylalanine concentrations increased significantly by about 95% and 35% during infusions of the balanced and branched-chain–enriched amino acid mixtures, respectively. Arterial leucine concentrations increased significantly by about 150% and 440% during infusions of the balanced and branched-chain–enriched amino acid mixtures, respectively. Arterial glutamine concentrations did not change significantly during infusion of either amino acid mixture.

Table 3 presents the effects of infusions of the two amino acid mixtures on leg muscle protein kinetics. The delta changes from basal of phenylalanine rate of disappearance to protein synthesis were significantly greater after infusion of the branched-chain–enriched amino acid mixture than after infusion of the balanced amino acid mixture (Fig. 1). In the basal states, before infusion of amino acid mixtures, the net balance of phenylalanine was significantly lower than 0, indicating a negative balance of muscle protein. During infusions of the balanced and branched-chain–enriched amino acid mixtures, values of phenylalanine balance were not significantly different from 0. After infusion of the balanced amino acid mixture, leg muscle leucine balance changed from a value that indicated net release to a value that was not significantly different from 0 (Table 4). After infusion of the branched-chain–enriched amino acid mixture, net leucine balance changed significantly from net release to net uptake. Figure 2 shows the calculated values of leucine catabolism. Leucine catabolism did not change

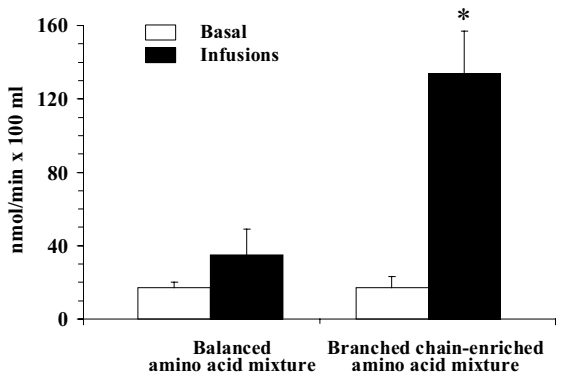

Fig. 2. Rates of leg muscle leucine catabolism before and during infusions of the balanced and branched-chain–enriched amino acid mixtures. $^{*}P <$ 0.05 branched-chain–enriched versus balanced amino acid infusion and basal.

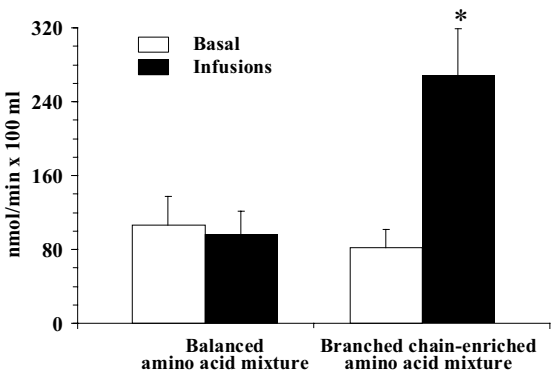

Fig. 3. Rates of leg muscle glutamine synthesis de novo before and during infusions of the balanced and branched-chain–enriched amino acid mixtures. $^{*}P < 0.05$ branched-chain–enriched versus balanced amino acid infusion and basal.

significantly after infusion of the balanced amino acid mixture but the rate of leucine catabolism increased seven to eight times after infusion of the branched-chain–enriched amino acid mixture. Total glutamine release from leg muscle (Table 4) was similar before and during infusion of the balanced amino acid mixture. In contrast, total glutamine release from leg muscle significantly increased after infusion of the branched-chain–enriched amino acid mixture. When we calculated the rate of net glutamine release from de novo synthesis, this figure increased three times after the branched-chain–enriched amino acid infusion, whereas it did not change after the balanced amino acid mixture infusion (Fig. 3). Delta changes from basal leucine catabolism directly correlated with delta changes of glutamine synthesis de novo (Fig. 4).

Discussion

We studied patients with colon or cervical cancer during the first 48 h after complete surgical tumor removal in combination with intraoperative radiation therapy. We compared the effects of isonitrogenous solutions of balanced and branched-chain–enriched amino acid mixtures on rates of protein synthesis, proteolysis, and de novo production of glutamine in skeletal muscle by using a crossover experimental design. The ratios of leucine to total amino acid (grams) in the two mixtures were 0.09 and 0.22, respectively. Glucose and insulin were continuously infused throughout the 2-d study to maintain near euglycemia. We found that, whereas the balanced amino acid mixture did not affect protein kinetics or glutamine production, the isonitrogenous branched-chain–enriched amino acid mixture accelerated muscle protein turnover by stimulating protein synthesis and increased glutamine de novo release from skeletal muscle of patients.

Cancer is characterized by downregulation of the rate of synthesis of skeletal muscle protein [1], leading to increased efflux of amino acids from muscle [21]. In addition, the metabolic response to surgical or accidental trauma includes absolute [22] or relative [23] inhibition of muscle protein synthesis. Major radical surgery in cancer patients therefore may be associated with excessive suppression of skeletal muscle protein synthesis, leading to an increased rate of complicated outcomes and delayed recovery [10]. In our patients, administration of a balanced amino acid mixture did not stimulate the rate of muscle protein synthesis despite the fact that the infusion resulted in a substantial increase in blood concentrations of essential amino acids (Table 2). In contrast, isonitrogenous administration of branched-chain amino acids at pharmacologic doses to increase plasma leucine concentrations four to five times, in the presence of an optimal profile of the other essential amino acids, directly stimulated muscle protein synthesis. This observation confirms numerous reports showing the unique ability of branched-chain amino acids to initiate signal transduction pathways and modulate translation initiation of skeletal muscle protein synthesis [13]. Among the branched-chain amino acids, leucine is the most potent [13].

Immediately after surgery, glucose and insulin infusions were started and maintained throughout the 2-d study to maintain near euglycemia in all patients. Insulin has a well-recognized anabolic effect on muscle protein in the presence of adequate availability of essential amino acids [24], even in patients with severe injury [25] or cancer [26]. We speculate that the branched-chain–mediated stimulation of muscle protein synthesis observed in our study could have been facilitated by the low-grade continuous hyperinsulinemia exhibited by the patients. Nonetheless, the different abilities of the two amino acid mixtures to stimulate muscle protein synthesis were not influenced by insulin because plasma levels of the hormone were similar during the two infusions (Table 2).

In the basal state, before infusions of the amino acid mixtures, the rate of muscle proteolysis was greater than the rate of synthesis. Thus, the patients were in negative

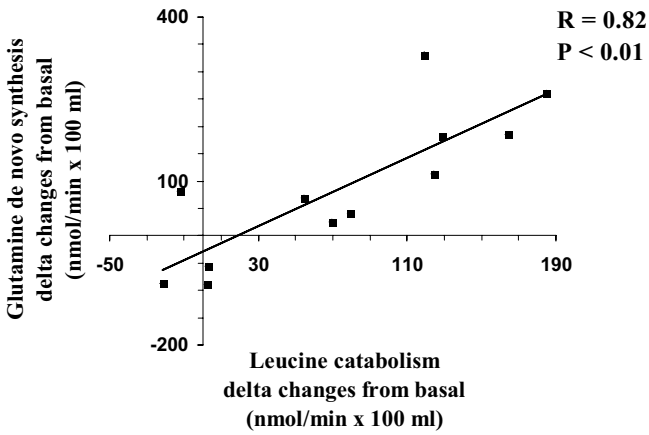

Fig. 4. Relation between changes in rates of leucine catabolism and glutamine de novo synthesis induced by infusions of the balanced and branched-chain–enriched amino acid mixtures.

muscle protein balance despite a continuous infusion of glucose and insulin. During infusions of the balanced and branched-chain–enriched amino acid mixtures, muscle protein balance become neutral because the absolute values of protein degradation were not statistically different, leading to a condition of sparing of muscle protein. Protein balance was substantially neutral even during the infusion of the branched-chain–enriched amino acid solution because stimulation of protein synthesis was matched by a tendency toward an acceleration of proteolysis. Thus, despite the fact that at the molecular level leucine can upregulate protein synthetic pathways [13] and downregulate proteolytic systems [27], in vivo we observed a stimulatory effect of the overall rate of protein turnover with a prevailing increase in protein synthesis over proteolysis. These results are in agreement with most studies performed in vivo in humans using isotopic tracers of amino acids [28]. These studies have demonstrated that in vivo the flux rates of free amino acids to protein deposition and from protein degradation are actually strictly related and that changes of synthesis and degradation in opposite directions are very rare [28].

In this study, the effect of branched-chain amino acid on muscle protein synthesis was paralleled by a stimulation of the rate of net muscle glutamine production de novo. Muscle glutamine production increased by about three times the basal value. Immediate precursors of glutamine synthesis are glutamate and free ammonia, whereas precursors for glutamate synthesis are α-ketoglutarate and the amino nitrogen derived from branched-chain amino acid catabolism [7,15]. In our study, the rate of leucine catabolism increased by seven to eight times basal values after the branched-chain amino acid infusion. Thus, increased glutamate availability from accelerated transamination of the branched-chain amino acids with α-ketoglutarate may have promoted glutamine de novo synthesis [7]. In addition, we speculate that in our study α-ketoglutarate availability was maintained at a high level by simultaneous infusions of insulin and glucose that directly activate pyruvate dehydrogenase, thereby promoting substrate flux through the tricarboxylic acid cycle [7,27].

Anticancer immune defense decreases progressively with tumor growth. In addition, immune function of cancer patients undergoing major surgery could be further impaired by surgical trauma. Glutamine is a key substrate to support the physiologic functions of the immune system. Severe depletion of this amino acid is observed after surgical stress [6,11]. Evidence indicates that glutamine supplementation in intensive care patients may increase survival and decrease rates of infections [29]. Mechanisms leading to glutamine depletion in acute stress may include increased glutamine utilization in the immune cells [11] and impaired synthesis de novo of this amino acid in skeletal muscle [16]. Patients with cancer often exhibit a pre-existing lower glutamine concentration in skeletal muscle [3]. Thus, surgical stress in cancer patients may represent a condition of particularly high risk for glutamine depletion. Metabolic strategies to prevent glutamine depletion may decrease complications from surgery [29] and enhance anticancer therapy as previously shown in animal models [7]. This report has shown that the rate of de novo glutamine synthesis in skeletal muscle can be acutely stimulated in cancer patients after radical surgery by infusing an amino acid mixture enriched with branched-chain amino acids. This approach may directly increase glutamine availability in extramuscular tissue and, possibly, prevent glutamine depletion in skeletal muscle.

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