Glutamine and α-Ketoglutarate Prevent the Decrease in Muscle Free Glutamine Concentration and Influence Protein Synthesis After Total Hip Replacement

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After surgical trauma, protein synthesis, as well as the concentration of free glutamine in muscle, decreases. Total parenteral nutrition (TPN) alone does not prevent the decrease of glutamine in muscle, but TPN supplemented with glutamine or its precursor, α-ketoglutarate, maintains amino acid concentration in muscle and preserves protein synthesis. The aim of this study was to characterize a human trauma model using patients undergoing total hip replacement, and furthermore to investigate whether glutamine or α-ketoglutarate alone without TPN can prevent the postoperative decrease in muscle free glutamine. Metabolically healthy patients undergoing total hip replacement were randomized into three groups. The control group (n = 13) received glucose 2 g/kg body weight (BW) during surgery and the first 24 postoperative hours. The glutamine group (n = 10) received glucose 2 g/kg BW and glutamine 0.28 g/kg BW, and the α-ketoglutarate group (n = 10) received glucose 2 g/kg BW and α-ketoglutarate 0.28 g/kg BW. Muscle biopsies were performed before surgery and 24 hours postoperatively. Free glutamine concentration in muscle decreased from 11.62 ± 0.67 to 9.80 ± 0.36 mmol/kg wet weight in the control group ($P < .01$), whereas it remained unchanged in both the glutamine group and α-ketoglutarate group. Protein synthesis, as reflected by the concentration of total ribosomes, decreased significantly in the control group, but not in glutamine and α-ketoglutarate groups. Polyribosome concentration decreased significantly in both the control and α-ketoglutarate groups. Total hip replacement can be used as a reproducible trauma model, with characteristic changes in the muscle amino acid pattern and protein synthesis 24 hours postoperatively. Glutamine, as well as α-ketoglutarate, attenuated the decrease in free amino acids in muscle tissue after surgical trauma during hypocaloric infusion of glucose.

SURGICAL TRAUMA evokes a metabolic response leading to substrate mobilization from peripheral tissues and characteristic changes in the pattern of free amino acids, as well as in protein synthesis, in skeletal muscle.[1] These changes reflect the degree of muscle protein catabolism, which, if severe, is detrimental to the clinical outcome. To increase our understanding of the underlying mechanisms and possibilities of counteracting severe loss of protein, studies of metabolic events in muscle tissue are necessary. A decline in muscle free glutamine concentration is seen as soon as 12 hours after operative trauma[2] and becomes more pronounced during the following postoperative days.[1,3,4] Protein synthesis, as assessed by ribosome determination, also decreases after elective abdominal surgery in conjunction with the decrease in free glutamine concentration in muscle.[3,5,6] A 20% decrease is seen as early as the first postoperative day, and the decrease is more pronounced on the third day after elective cholecystectomy. Postoperative conventional total parenteral nutrition (TPN), including an amino acid solution not containing glutamine, does not counteract the depletion of free glutamine or the decline of polyribosome concentration in muscle.[1,3]

When glutamine is added to a conventional amino acid solution and given as part of TPN for a 3-day period, the decrease in muscle free glutamine is diminished and the decrease in total ribosome concentration and polyribosome K concentration is counteracted. α-Ketoglutarate, which provides the carbon skeleton of glutamine, exerts the same effect on the postoperative changes in muscle free glutamine and ribosome content.[3,6] It has not been established whether similar effects can be produced by providing either glutamine or α-ketoglutarate as additives to a hypocaloric infusion of glucose alone, in the absence of complete intravenous nutrition. In the present study, patients undergoing hip replacement surgery were studied before surgery and 24 hours postoperatively. Hypocaloric glucose was supplied with and without supplementation with glutamine or α-ketoglutarate. Free amino acids and ribosomes were analyzed in muscle biopsy specimens taken preoperatively and 24 hours postoperatively.

SUBJECTS AND METHODS

Patients (N = 33) scheduled for elective total hip replacement because of advanced osteoarthritis of the hip were randomly allocated to receive either an infusion of glucose 2 g/kg body weight (BW), glucose 2 g/kg BW together with glutamine 0.28 g/kg BW, or glucose 2 g/kg BW together with α-ketoglutarate 0.28 g/kg BW at the start of surgery and during the initial 24 hours after surgery. The amount of glutamine and α-ketoglutarate supplied was equimolar in this regimen. Patients had no history of advanced heart disease, diabetes mellitus, or any other metabolic disease. None of the patients used antiinflammatory drugs regularly. Clinical data on the patients and the operative procedure are listed in Table 1.

The purpose, procedure, and possible risks involved in the study were explained to the patients before obtaining their informed consent. The study protocol was approved by the Ethics Committee of the Karolinska Institute, Stockholm, Sweden.

Anesthesiologic Management

The standard anesthesiologic procedure was epidural anesthesia. After an overnight fast, a morphine derivative, oxycodone (Oxicon; Apoteksbolaget, Stockholm, Sweden), was given intramus-

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Table 1. Clinical Data for the Three Groups (mean ± SEM)

	Control (n = 13)	Glutamine (n = 10)	α-Ketoglutarate (n = 10)
Sex (M:F)	7:6	7:3	3:7
Age (yr)	68 ± 2	68 ± 1	66 ± 2
Weight (kg)	73 ± 4	74 ± 4	72 ± 4
Body mass index ($kg \cdot m^{-2}$)	25.3 ± 1.0	25.5 ± 1.0	24.9 ± 1.3
Duration of surgery (min)	91 ± 7	82 ± 7	94 ± 10
Blood loss (mL)	862 ± 158	795 ± 133	658 ± 142
Blood loss compensation (mL)	208 ± 77	150 ± 81	60 ± 60
Drain loss (mL)	701 ± 182	631 ± 111	514 ± 80
Drain compensation (mL)	109 ± 64	225 ± 75	180 ± 102

cularly in doses of 5 to 10 mg as premedication. A Tuhoy needle was inserted into the extradural space through a lumbar interspace at L3 to L5. The epidural space was identified by loss of resistance. An epidural catheter was inserted, and prilocaine with adrenaline 20 mg/mL (Citanest; Astra, Södertälje, Sweden) was injected in doses of 9 to 18 mL depending on the height, weight, and age of the patient. The spread of anesthesia was assessed by the pin-prick method, and extended from thoracic segment Th2 to Th12. Analgesia was maintained with bupivacaine 5 mg/mL during the operation and bupivacaine 2.5 mg/mL in the postoperative period. The epidural catheter was withdrawn after 6 hours, and pain relief was continued with intramuscular doses of cetobemidone (Ketogan; Lundbeck, Copenhagen, Denmark).

Dextran 70 500 mL (60 mg/L, Macrodex; Kabi Pharmacia, Uppsala, Sweden) was given during surgery and postoperatively as prophylaxis against thromboembolic complications.

Operative Procedure

The operation was performed using a standardized technique with insertion of a Charnley total hip prosthesis (De Puy International, Leeds, England). Perioperative prophylactic antibiotics were given. Blood loss compensation with erythrocyte concentrate was used when the estimated loss reached a level of 33%.[7] Apart from the glucose infusion, patients received acetate Ringer's solution (Kabi Pharmacia) 500 mL before epidural anesthesia to compensate for sympathetic blockade. During the operation and postoperatively, patients received acetate Ringer's solution according to blood loss and circulatory status.

Postoperative blood loss was compensated with packed red blood cells or autotransfusion of drain blood depending on blood loss and hemoglobin values.

Sampling

Blood samples were taken before and 24 hours after surgery for analysis of plasma amino acids, routine blood chemistry, and hormones. Muscle tissue specimens for ribosome and amino acid analysis were obtained by the percutaneous needle biopsy technique from the lateral portion of the quadriceps femur muscle approximately 15 cm above the knee of the contralateral leg. The biopsies were taken after anesthesia, before skin incision, and 24 hours after starting the operation with local anesthesia of the skin. Visible fat and connective tissue were removed from the specimens, which were divided into portions and weighed three times on an automated electrobalance (Can 29; Can Instruments, Cheroots, CA). Muscle specimens were frozen in liquid nitrogen within 3 minutes and stored at −80°C pending analyses. Muscle specimens of 50 to 60 mg wet weight, stored no longer than 3 weeks, were used for ribosome analysis, and specimens of 20 to 30 mg were used for amino acid analyses.

Ribosome Determination

The technique for determination of ribosomal concentration and size distribution in human skeletal muscle tissue has been described in detail elsewhere.[8] For ribosome analysis, biopsy specimens were homogenized in a medium containing ribonuclease inhibitor made from human placenta (Amersham International, Takara, Japan) before being centrifuged at 1,500 × *g* for 10 minutes. Mitochondria, membranes, and nuclei sedimented into the pellets. The supernatant obtained was centrifuged at 202,000 × *g* for 50 minutes in a TLA/100.3 rotor of the TL Tabletop Ultracentrifuge (Beckman Instruments, Palo Alto, CA). The pellet consisting of ribosomes was suspended in 200 μL medium containing a ribonuclease inhibitor, and a portion was removed for spectrophotometric determination at 260 nm. The ribosome suspension was layered onto a linear density gradient of sucrose from 0.4 to 1.5 mol/L. The suspension was ultracentrifuged for 60 minutes at 149,000 × *g* in a swing-out rotor and then pumped through a continuous-flow cuvette. Absorbance at 260 nm was registered automatically, and area under the curve was determined. Peaks corresponding to ribosome subunits, monoribosomes, and polyribosomes were identified, and the percentage of polyribosomes in the total ribosome area was calculated. By multiplying percentage of polyribosomes by total ribosome concentration, polyribosome concentration per milligram wet weight was computed. Ribosome concentration was expressed as optical density units (OD) per milligram wet weight.

Amino Acid Determination

Biopsy specimens of 20 to 30 mg wet weight of muscle were used for amino acid analysis. The samples were homogenized in sulfosalicylic acid, and precipitated proteins were sedimented by centrifugation.[9,10] Free amino acids in the supernatant were separated and quantified by ion-exchange chromatography using an automated amino acid analyzer (Alpha Plus; LKB, Bromma, Sweden) with DC6 ion-exchange resin (Durrum, Interaction, CA) and lithium citrate buffers. Concentration of individual free amino acids in skeletal muscle was expressed as millimoles per kilogram wet weight, and those in plasma were determined and expressed as micromoles per liter of plasma.

Glucose and Hormones

Insulin, C-peptide, glucagon, and cortisol were determined by a radioimmunoassay method.[11-14]

Statistical Evaluation

All values are presented as the mean ± SEM. Wilcoxon's signed-rank test was used to compare observations within groups. One-tailed ANOVA in combination with Sheffe's F test was used to compare observations of clinical data between groups.[15]

RESULTS

After total hip replacement, patients were treated for 24 hours with a hypocaloric infusion of glucose alone or glucose in combination with either glutamine or α-ketoglutarate. Samples were taken before and 24 hours after surgery for analyses of amino acid concentrations in skeletal muscle and plasma, ribosome parameters, and blood hormone levels.

Muscle Amino Acids

In the control group, free glutamine concentration in muscle decreased by 17% ± 5% ($P < .05$) after surgery. In

the glutamine and α-ketoglutarate groups, there was no postoperative change in muscle glutamine (Tables 2, 3, and 4). In all three groups, there was a large scatter of interindividual values for free glutamine concentration in muscle (Fig 1). The decrease induced by surgical trauma in the control group was marginal in patients with a relatively low concentration of muscle free glutamine, whereas some patients with a high concentration of glutamine showed a pronounced decrease. Retention of glutamine in the glutamine group was high in patients with a low preoperative glutamine value. In the α-ketoglutarate group, there was a moderate tendency toward glutamine retention in some patients with a low preoperative value. Basic amino acids (lysine, histidine, and arginine) decreased significantly in the control group by 22% ± 4% ($P < .05$) and in the glutamine group by 24% ± 5% ($P < .05$) and did not change in the α-ketoglutarate group. The sum of branched-chain amino acids remained unchanged in all three groups.

Plasma Amino Acids

In all groups, there was a decrease in total plasma free amino acid concentration ($P < .05$; Tables 5, 6, and 7). Essential and basic amino acids decreased in all three groups ($P < .05$). Branched-chain amino acids decreased significantly in the glutamine and α-ketoglutarate groups ($P < .05$). The decrease in glutamine in the control group was counteracted by including glutamine in the infused solution. α-Ketoglutarate showed no such restoring effect. Phenylalanine increased in all three groups ($P < .01$).

Table 2. Concentration of Intracellular Free Amino Acids (mmol/mg wet weight) in Skeletal Muscle Before and 24 Hours After Total Hip Replacement in the Control Group

Amino Acid	Day 0	Day 1	Postoperative Change (%)
Taurine	10.16 ± 0.98	10.12 ± 0.92	0 ± 5
Aspartate	1.27 ± 0.14	0.96 ± 0.08	−25 ± 8*
Threonine	0.34 ± 0.03	0.34 ± 0.02	−1 ± 7
Serine	0.40 ± 0.03	0.38 ± 0.02	−3 ± 8
Asparagine	0.23 ± 0.02	0.28 ± 0.02	+21 ± 8†
Glutamate	1.93 ± 0.16	1.49 ± 0.12	−23 ± 4*
Glutamine	11.62 ± 0.67	9.80 ± 0.36	−16 ± 6†
Glycine	0.84 ± 0.06	0.98 ± 0.05	+17 ± 7†
Alanine	1.88 ± 0.12	2.26 ± 0.10	+20 ± 5*
Valine	0.20 ± 0.01	0.18 ± 0.01	−5 ± 5
Cysteine	0.28 ± 0.05	0.33 ± 0.07	+16 ± 11
Methionine	0.03 ± 0.00	0.05 ± 0.00	+60 ± 12*
Isoleucine	0.06 ± 0.00	0.07 ± 0.00	+19 ± 9
Leucine	0.12 ± 0.01	0.13 ± 0.01	+12 ± 8
Tyrosine	0.05 ± 0.01	0.07 ± 0.01	+32 ± 11†
Phenylalanine	0.06 ± 0.01	0.09 ± 0.01	+50 ± 9*
Ornithine	0.23 ± 0.02	0.11 ± 0.01	−54 ± 9*
Lysine	0.74 ± 0.05	0.58 ± 0.04	−22 ± 5*
Histidine	0.28 ± 0.02	0.24 ± 0.01	−14 ± 7
Arginine	0.35 ± 0.04	0.29 ± 0.05	−11 ± 9
Essential	1.89 ± 0.13	1.85 ± 0.12	−2 ± 4
Branched-chain	0.38 ± 0.03	0.40 ± 0.02	+4 ± 6
Basic	1.26 ± 0.10	1.02 ± 0.09	−19 ± 4*
Total	30.98 ± 1.82	28.68 ± 1.25	−7 ± 4

NOTE. Mean ± SEM.
*Significantly different from day 0, $P < .01$.
†Significantly different from day 0, $P < .05$.

Table 3. Concentration of Intracellular Free Amino Acids (mmol/mg wet weight) in Skeletal Muscle Before and 24 Hours After Total Hip Replacement in the Glutamine Group

Amino Acid	Day 0	Day 1	Postoperative Change (%)
Taurine	9.32 ± 0.71	9.82 ± 0.38	+5 ± 9
Aspartate	1.18 ± 0.11	0.99 ± 0.09	−16 ± 10
Threonine	0.33 ± 0.03	0.32 ± 0.02	−2 ± 4
Serine	0.44 ± 0.02	0.37 ± 0.03	−16 ± 5†
Asparagine	0.21 ± 0.01	0.26 ± 0.03	+27 ± 9*
Glutamate	2.07 ± 0.18	1.67 ± 0.16	−19 ± 9
Glutamine	10.97 ± 0.61	10.61 ± 0.55	−3 ± 8
Glycine	0.87 ± 0.09	0.96 ± 0.05	+10 ± 8
Alanine	1.78 ± 0.12	2.10 ± 0.08	+18 ± 6†
Valine	0.20 ± 0.01	0.18 ± 0.01	−6 ± 4
Cysteine	0.19 ± 0.03	0.20 ± 0.04	+8 ± 10
Methionine	0.03 ± 0.00	0.04 ± 0.00	+37 ± 8*
Isoleucine	0.06 ± 0.00	0.07 ± 0.00	+1 ± 8
Leucine	0.11 ± 0.01	0.13 ± 0.01	+14 ± 5†
Tyrosine	0.06 ± 0.01	0.07 ± 0.01	+27 ± 7*
Phenylalanine	0.06 ± 0.00	0.08 ± 0.01	+39 ± 7*
Ornithine	0.26 ± 0.03	0.12 ± 0.02	−55 ± 7*
Lysine	0.71 ± 0.06	0.52 ± 0.05	−26 ± 6*
Histidine	0.26 ± 0.02	0.21 ± 0.02	−22 ± 10†
Arginine	0.36 ± 0.03	0.26 ± 0.03	−22 ± 5†
Essential	1.72 ± +0.11	1.66 ± 0.11	−7 ± 2
Branched-chain	0.36 ± 0.02	0.37 ± 0.02	+1 ± 4
Basic	1.26 ± 0.09	0.94 ± 0.10	−25 ± 6*
Total	30.44 ± 1.69	29.52 ± 1.14	−2 ± 7

NOTE. Mean ± SEM.
*Significantly different from day 0, $P < .01$.
†Significantly different from day 0, $P < .05$.

Ribosome Parameters

In the control group, total ribosome concentration decreased from 31.7 ± 1.7 to 27.9 ± 1.4 OD/mg wet weight of muscle ($P < .01$; Table 8). No significant change was observed in the glutamine and α-ketoglutarate groups. The percentage deviation from initial values decreased significantly in the control group ($P < .05$), but not in the glutamine and α-ketoglutarate groups (Fig 2A). The amount of polyribosomes as a percentage of total ribosomes showed no significant changes in any of the groups (Table 8). Polyribosome concentration per milligram wet weight decreased significantly in the control group from 15.9 ± 1.1 to 13.7 ± 1.0 and in the α-ketoglutarate group from 14.8 ± 0.9 to 12.2 ± 0.9 OD/mg wet weight of muscle ($P < .05$; Table 8). No significant changes were observed in the glutamine group. The percentage deviation from initial values confirmed the decrease observed in both the control and α-ketoglutarate groups (Fig 2B).

Glucose and Hormones

Insulin and C-peptide increased significantly in all three groups ($P < .05$), while glucagon remained unchanged. Cortisol increased significantly in the control group ($P < .01$; Table 9).

Table 4. Concentration of Intracellular Free Amino Acids (mmol/mg wet weight) in Skeletal Muscle Before and 24 Hours After Total Hip Replacement in the α-Ketoglutarate Group

Amino Acid	Day 0	Day 1	Postoperative Change (%)
Taurine	10.79 ± 0.99	10.42 ± 0.82	−1 ± 4
Aspartate	1.09 ± 0.15	1.30 ± 0.14	+11 ± 10
Threonine	0.33 ± 0.03	0.35 ± 0.02	+1 ± 8
Serine	0.38 ± 0.03	0.37 ± 0.04	−4 ± 10
Asparagine	0.22 ± 0.02	0.29 ± 0.16	+23 ± 8†
Glutamate	1.96 ± 0.16	1.72 ± 0.22	−14 ± 7†
Glutamine	10.07 ± 0.78	9.20 ± 0.70	−8 ± 5
Glycine	0.75 ± 0.06	0.91 ± 0.04	+19 ± 6†
Alanine	2.00 ± 0.19	2.11 ± 0.15	+3 ± 9
Valine	0.20 ± 0.02	0.21 ± 0.01	−1 ± 7
Cysteine	0.20 ± 0.07	0.15 ± 0.08	+10 ± 20
Methionine	0.03 ± 0.00	0.05 ± 0.01	+46 ± 12†
Isoleucine	0.06 ± 0.01	0.07 ± 0.01	+27 ± 11†
Leucine	0.12 ± 0.01	0.15 ± 0.01	+24 ± 8†
Tyrosine	0.05 ± 0.01	0.07 ± 0.01	+10 ± 13
Phenylalanine	0.07 ± 0.01	0.09 ± 0.01	+19 ± 19
Ornithine	0.22 ± 0.04	0.11 ± 0.02	−46 ± 14†
Lysine	0.76 ± 0.05	0.54 ± 0.06	−19 ± 8†
Histidine	0.27 ± 0.02	0.24 ± 0.04	+4 ± 11
Arginine	0.38 ± 0.02	0.40 ± 0.05	+4 ± 15
Essential	1.85 ± 0.13	1.76 ± 0.11	−2 ± 6
Branched-chain	0.39 ± 0.04	0.45 ± 0.03	+11 ± 7
Basic	1.30 ± 0.10	1.27 ± 0.12	−15 ± 9
Total	29.89 ± 1.72	28.54 ± 1.30	−3 ± 4

NOTE. Mean ± SEM.
*Significantly different from day 0, $P < .01$.
†Significantly different from day 0, $P < .05$.

DISCUSSION

In this study, infusion of glutamine or α-ketoglutarate together with a hypocaloric infusion of glucose reduced the postoperative decrease of free glutamine in muscle and the decrease in protein synthesis associated with surgical trauma. The amino acid pattern in muscle seen otherwise postoperatively is characterized by decreases in muscle free glutamine and basic amino acids and by increases in branched-chain and aromatic amino acids.[9]

After moderate surgical trauma, free glutamine concentration in muscle is diminished to a level of 80% of the preoperative value within 12 hours after surgery and occurs irrespective of gender.[2] The decrease becomes more pronounced during the following days.[2-4] Epidural anesthesia maintained for 24 hours has no influence on the postoperative amino acid pattern, as compared with preoperative values, after hip replacement surgery.[16] Time-limited intraoperative epidural anesthesia has been shown to modulate the muscle amino acid pattern, with characteristic changes in free glutamine 2 and 4 days postoperatively.[17] In the present study, epidural anesthesia was limited to 6 hours. Intravenous or intramuscular analgesia was maintained for the following 18 hours before muscle free amino acid levels were measured the second time. Postoperatively, three groups of patients were given either glucose alone or in combination with glutamine, or an equimolar amount of the glutamine carbon skeleton, α-ketoglutarate. The amount of glucose infused corresponded to a rate of 1.4 mg/kg/min. Administration of exogenous glucose 1.0 mg/kg/min suppresses the endogenous glucose rate at an amount equivalent to the exogenous administration rate. Since an increase in the rate of glucose infusion above the production rate only causes further marginal reductions of endogenous glucose production,[18] an increased amount of glucose was not given.

After the time-limited epidural anesthesia, the decrease in free glutamine in muscle was significant at 24 hours after surgery, but branched-chain amino acids remained unchanged. This is in accord with earlier studies involving

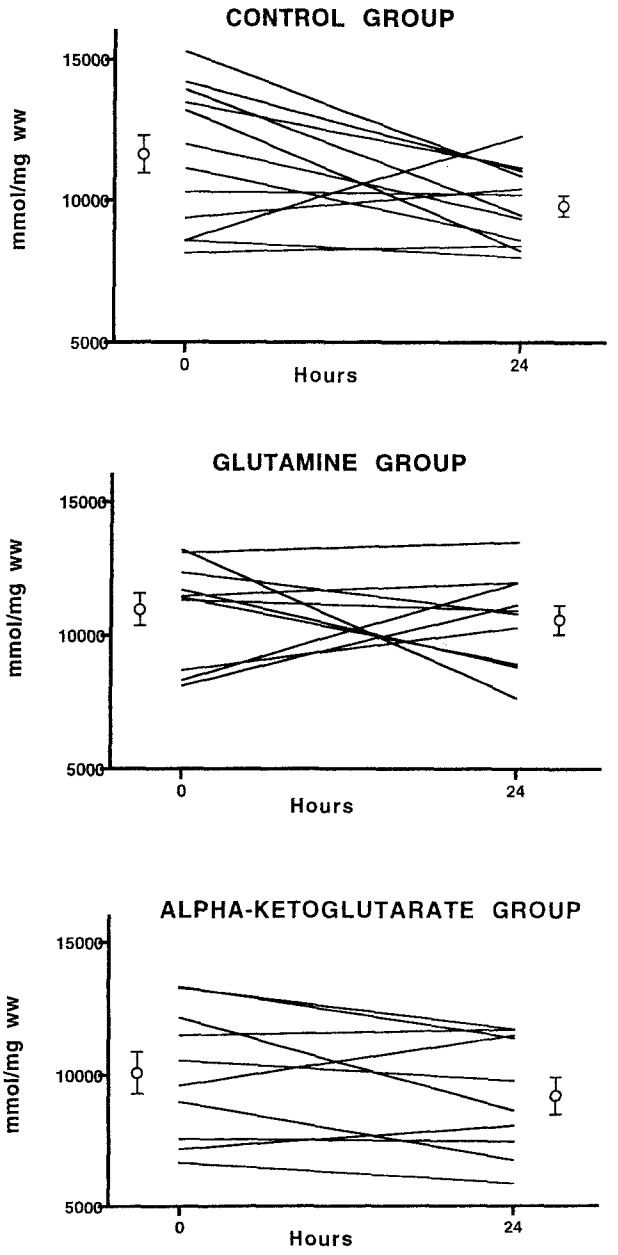

Fig 1. Mean ± SEM and individual values for free glutamine concentrations in muscle after total hip replacement in patients treated with hypocaloric infusions of glucose 2 g/kg BW, glucose 2 g/kg BW + glutamine 0.28 g/kg BW, or glucose 2 g/kg BW + α-ketoglutarate 0.28 g/kg BW. *P* values denote significant differences from paired values 24 hours postsurgery: *$P < .05$. ww, wet weight.

Table 5. Concentration of Free Plasma Amino Acids (μmol/L) Before and 24 Hours After Total Hip Replacement in the Control Group

Amino Acid	Day 0	Day 1	Postoperative Change (%)
Taurine	63.8 ± 4.3	64.9 ± 3.6	+2 ± 8
Aspartate	5.5 ± 0.6	5.6 ± 0.6	+4 ± 7
Threonine	114.4 ± 7.6	85.1 ± 7.5	−26 ± 5*
Serine	198.7 ± 6.5	77.9 ± 4.4	−21 ± 6*
Asparagine	67.2 ± 4.9	53.3 ± 3.1	−21 ± 6*
Glutamate	33.9 ± 4.8	35.2 ± 4.9	+4 ± 11
Glutamine	623.0 ± 34.1	518.4 ± 36.8	−17 ± 5†
Glycine	218.4 ± 15.4	183.5 ± 10.4	−16 ± 4*
Alanine	360.8 ± 22.0	345.1 ± 22.8	−4 ± 6
Valine	226.1 ± 11.3	194.3 ± 14.3	−14 ± 8
Cysteine	132.1 ± 11.3	114.2 ± 10.1	−14 ± 4†
Methionine	25.2 ± 1.7	23.3 ± 1.1	−8 ± 7
Isoleucine	57.0 ± 3.7	49.3 ± 4.6	−16 ± 10
Leucine	121.2 ± 6.6	104.2 ± 7.9	−14 ± 9
Tyrosine	66.1 ± 2.6	64.3 ± 4.2	−3 ± 6
Phenylalanine	71.5 ± 5.7	80.3 ± 3.9	+12 ± 6
Ornithine	67.8 ± 4.6	46.5 ± 5.2	−31 ± 11†
Lysine	185.6 ± 11.4	136.3 ± 11.4	−27 ± 8†
Histidine	85.3 ± 3.6	70.0 ± 5.61	−18 ± 5†
Arginine	90.6 ± 7.7	59.6 ± 6.7	−34 ± 10†
Essential	989.4 ± 38.2	851.2 ± 50.9	−15 ± 6†
Branched-chain	406.2 ± 21.0	347.8 ± 25.8	−14 ± 8
Basic	361.5 ± 20.0	265.8 ± 23.0	−26 ± 7†
Total	2,716.0 ± 87.8	2,311.2 ± 121.9	−15 ± 4†

NOTE. Mean ± SEM.
*Significantly different from day 0, $P < .01$.
†Significantly different from day 0, $P < .05$.

Table 6. Concentration of Free Plasma Amino Acids (μmol/L) Before and 24 Hours After Total Hip Replacement in the Glutamine Group

Amino Acid	Day 0	Day 1	Postoperative Change (%)
Taurine	89.9 ± 4.7	80.7 ± 5.0	−10 ± 7
Aspartate	6.8 ± 0.6	7.1 ± 0.9	+10 ± 10
Threonine	117.5 ± 8.1	80.1 ± 6.4	−32 ± 3*
Serine	121.6 ± 5.1	89.6 ± 6.4	−26 ± 2*
Asparagine	65.1 ± 4.9	50.3 ± 4.8	−23 ± 4*
Glutamate	25.3 ± 3.6	33.1 ± 7.5	+31 ± 25
Glutamine	690.3 ± 17.0	625.0 ± 25.5	−9 ± 4
Glycine	232.0 ± 18.9	185.6 ± 14.6	−20 ± 4*
Alanine	399.2 ± 30.2	388.0 ± 32.7	−3 ± 6
Valine	248.3 ± 8.7	191.6 ± 11.4	−23 ± 4*
Cysteine	134.9 ± 8.5	121.4 ± 7.6	−10 ± 4†
Methionine	24.3 ± 1.6	25.0 ± 1.9	+3 ± 5
Isoleucine	64.3 ± 3.0	39.4 ± 4.8	−39 ± 7*
Leucine	127.9 ± 6.5	103.0 ± 8.8	−19 ± 5*
Tyrosine	67.4 ± 4.8	57.1 ± 3.5	−15 ± 3*
Phenylalanine	59.0 ± 2.7	72.2 ± 3.3	+22 ± 5*
Ornithine	76.5 ± 6.8	40.5 ± 3.8	−47 ± 6*
Lysine	194.4 ± 14.21	133.9 ± 9.2	−33 ± 4*
Histidine	89.1 ± 4.3	71.9 ± 3.7	−19 ± 3*
Arginine	103.9 ± 5.9	70.0 ± 7.2	−36 ± 3*
Essential	1,041.0 ± 38.4	823.7 ± 45.1	−21 ± 3*
Branched-chain	440.5 ± 17.9	334.0 ± 23.1	−24 ± 4*
Basic	390.4 ± 19.5	272.8 ± 16.2	−30 ± 2*
Total	2,940.7 ± 76.7	2,462.9 ± 82.76	−16 ± 2*

NOTE. Mean ± SEM.
*Significantly different from day 0, $P < .01$.
†Significantly different from day 0, $P < .05$.

Table 7. Concentration of Free Plasma Amino Acids (μmol/L) Before and 24 Hours After Total Hip Replacement in the α-Ketoglutarate Group

Amino Acid	Day 0	Day 1	Postoperative Change (%)
Taurine	85.5 ± 11.6	75.7 ± 7.9	−12 ± 16
Aspartate	4.7 ± 1.0	4.1 ± 0.6	−13 ± 16
Threonine	114.4 ± 9.3	76.0 ± 4.8	−34 ± 7*
Serine	116.4 ± 6.4	84.7 ± 3.9	−27 ± 4*
Asparagine	76.0 ± 7.5	52.8 ± 5.0	−27 ± 6*
Glutamate	31.5 ± 5.2	44.3 ± 7.9	+32 ± 16
Glutamine	617.3 ± 31.1	484.6 ± 30.2	−21 ± 4*
Glycine	221.6 ± 27.2	188.3 ± 18.4	−19 ± 6*
Alanine	474.0 ± 61.9	356.4 ± 37.0	−25 ± 9†
Valine	257.7 ± 19.3	206.8 ± 13.7	−20 ± 5*
Cysteine	148.5 ± 15.8	128.8 ± 7.7	−13 ± 7
Methionine	26.1 ± 2.1	23.5 ± 1.7	−10 ± 6
Isoleucine	62.3 ± 6.0	39.9 ± 3.3	−36 ± 8*
Leucine	132.5 ± 11.2	105.4 ± 7.3	−20 ± 6†
Tyrosine	74.7 ± 6.8	61.6 ± 4.2	−18 ± 5†
Phenylalanine	69.2 ± 3.7	83.1 ± 5.1	+20 ± 5*
Ornithine	75.0 ± 2.6	38.8 ± 2.9	−48 ± 3*
Lysine	230.1 ± 11.5	149.1 ± 2.0	−35 ± 5*
Histidine	90.48 ± 3.1	73.9 ± 2.9	−19 ± 4*
Arginine	89.6 ± 7.8	53.1 ± 4.2	−41 ± 7*
Essential	1,115.5 ± 52.2	874.2 ± 27.3	−22 ± 4*
Branched-chain	452.5 ± 35.6	352.1 ± 23.2	−23 ± 5*
Basic	410.5 ± 16.2	276.2 ± 5.3	−33 ± 4*
Total	2,994.9 ± 110.7	2,320.2 ± 70.0	−23 ± 3*

NOTE. Mean ± SEM.
*Significantly different from day 0, $P < .01$.
†Significantly different from day 0, $P < .05$.

postoperative muscle amino acid metabolism, in which the amino acid pattern declines stepwise.[2] Glutamine level decreases continuously, but, on the other hand, the concentration of branched-chain amino acids decreases early on, and then after more than 1 day, an increase to levels above preoperative levels is seen.[19] In the present study, the unchanged levels of branched-chain amino acids may be explained by the fact that the sampling was performed at a point in time when concentrations of branched-chain amino acids were increasing from low early-postoperative levels.

Decreases in concentrations of basic amino acids (lysine, histidine, and arginine), as well as glutamate, were seen without detectable differences between the groups. The decrease in basic amino acids is known to occur concomitantly with the decrease in glutamine during muscle protein catabolism. The decrease in glutamate is always seen immediately after trauma, but in contrast to glutamine, it is restored early afterward.[2,4] These changes may be explained to some extent by an outflow of glutamine from skeletal muscle postoperatively and by an increased use of amino acids with the capacity to contribute the carbon skeleton for de novo synthesis of glutamine. These amino acids are glutamate, histidine, arginine, isoleucine, valine, asparagine, and aspartate.[20] Several factors, such as changes in transmembrane amino acid transport, recruitment from protein breakdown, and transamination reactions, finally determine the concentrations of individual amino acids seen during muscle protein breakdown.

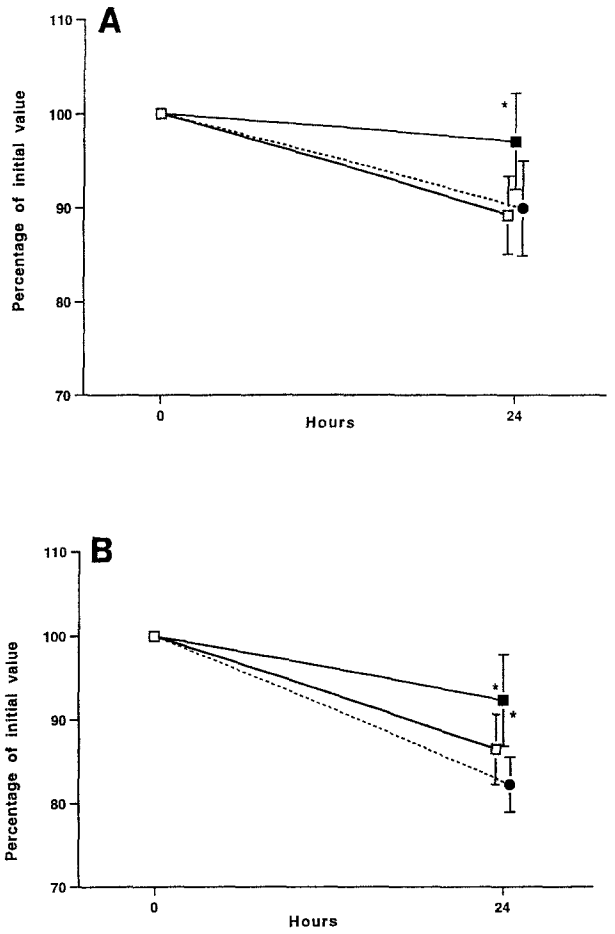

Fig 2. (A) Relative proportion of total ribosomes in skeletal muscle after total hip replacement in patients receiving hypocaloric infusions of glucose 2 g/kg BW (□) during 24 hours of operation and postoperatively, glucose 2 g/kg BW + glutamine 0.28 g/kg BW (■), or glucose 2 g/kg BW + α-ketoglutarate 0.28 g/kg BW (●—●). Preoperative values have been normalized to a value of 100%. Mean ± SEM. *P* values denote significant differences from the paired value at 0 hours: **P* < .05. Note that the scale on the y-axis starts at 70%. (B) Relative proportion of polyribosomes in skeletal muscle after total hip replacement in patients receiving hypocaloric infusions of glucose 2 g/kg BW (□) during 24 hours of surgery and postoperatively, glucose 2 g/kg BW + glutamine 0.28 g/kg BW (■), or glucose 2 g/kg BW + α-ketoglutarate 0.28 g/kg BW (●—●). Preoperative values have been normalized to a value of 100%. Mean ± SEM. *P* values denote significant differences from the paired value at 0 hours: **P* < .05. Note that the scale on the y-axis starts at 70%.

The capacity for protein synthesis at the cellular level is a function of the total ribosome and polyribosome concentration.[21] After hip replacement, the percentage of polyribosomes was unchanged. A decline in the capacity for protein synthesis manifested itself as a decrease in ribosome content. Such was the case in the control group as early as 24 hours after surgery. Whereas the glutamine group retained its initial capacity for protein synthesis, the α-ketoglutarate group showed a decrease, which was significant when polyribosome content was determined. In studies of cholecystectomy patients, a highly reproducible decrease in polyribosome concentration occurs regardless of sex.[22] It may be that the metabolic effect of α-ketoglutarate was delayed as compared with that of glutamine. The fact that α-ketoglutarate was given without an additional nitrogen supply may also have influenced polyribosome content.

It should be noted that the postoperative change in plasma free glutamine was as high in the control group as in the α-ketoglutarate group. Free glutamine content was unchanged in muscle in the glutamine and α-ketoglutarate groups, and a decrease was observed only in the controls. The results indicate a drainage of plasma free glutamine to the splanchnic region. A counteraction was noted on infusion of glutamine, but not with α-ketoglutarate.

The significant increase of insulin and C-peptide in all three groups studied here may be influenced by the afferent neurogenic blockade caused by epidural anesthesia.[23] The increase in cortisol was significant in the glucose group and α-ketoglutarate group, implying a stress effect that may be moderated by infusion of glutamine. Depression of glutamine levels in skeletal muscle is induced by infusion of a combination of the stress hormones, adrenaline, glucagon, and cortisol, in healthy volunteers.[24] The effect is due to a combined action of the hormones and is not reproducible on infusion of a single hormone at any one time.[24]

Hip replacement patients have been used earlier as a trauma model in investigations of the postoperative pattern of muscle free amino acids.[4,16,17,25] In studies reported by Askanazi et al,[4] patients received general anesthesia. On the fourth postoperative day, the muscle amino acid pattern was characterized by an increase in branched-chain amino acids, while nonessential amino acids showed a decline in glutamine as the most marked change. In the study reported by Christensen et al,[16] a comparison was made between patients receiving general anesthesia during surgery and epidural anesthesia during and up to 24 hours after surgery. Sampling was performed on the fourth

Table 8. Total Ribosome Concentration, Percentage of Polyribosomes in the Total Quantity of Ribosomes, and Polyribosome Concentration Before Operation and on the First Postoperative Day

Parameter	Control Group		Glutamine Group		α-Ketoglutarate Group	
	Day 0	Day 1	Day 0	Day 1	Day 0	Day 1
Total ribosome concentration (OD/mg wet weight)	31.7 ± 1.7	27.9 ± 1.4†	30.2 ± 1.3	29.2 ± 1.8	31.6 ± 1.5	28.1 ± 1.4
Percentage of polyribosomes in total ribosomes (%)	50.0 ± 1.4	48.3 ± 1.6	50.4 ± 3.4	48.2 ± 3.1†	47.0 ± 2.3	43.9 ± 1.9
Polyribosome concentration (OD/mg wet weight)	15.9 ± 1.1	13.7 ± 1.0*	15.7 ± 1.4	14.6 ± 1.27	14.8 ± 0.9	12.2 ± 0.9†

NOTE. Mean ± SEM.

*Significantly different from day 0, $P < .01$.

†Significantly different from day 0, $P < .05$.

Table 9. Concentration of Glucose and Hormones Before Operation and on the First Postoperative Day

Parameter	Control Group		Glutamine Group		α-Ketoglutarate Group	
	Day 0	Day 1	Day 0	Day 1	Day 0	Day 1
Glucose (mmol/L)	5.1 ± 0.3	7.6 ± 1.0†	5.4 ± 0.2	6.4 ± 0.2†	5.7 ± 0.2	6.3 ± 0.4
Insulin (μUmL)	8.4 ± 1.1	36.4 ± 7.2*	7.8 ± 1.5	18.1 ± 2.9*	11.0 ± 3.0	19.6 ± 4.7†
C-peptide (ng/mL)	2.3 ± 0.2	5.6 ± 0.8*	1.9 ± 0.2	3.9 ± 0.5*	2.7 ± 0.4	4.4 ± 0.4*
Glucagon (pg/mL)	119.4 ± 37.9	134.1 ± 48.9	71.3 ± 9.6	66.8 ± 7.8	81.8 ± 12.9	65.9 ± 10.0†
Cortisol (nmol/L)	212.3 ± 29.8	580.8 ± 79.2*	297.6 ± 47.0	477.9 ± 49.1	272.1 ± 38.5	461.7 ± 59.4†

NOTE. Mean ± SEM.

*Significantly different from day 0, $P < .01$.

†Significantly different from day 0, $P < .05$.

postoperative day. The postoperative pattern of free amino acids in plasma and muscle was altered and showed significant changes regarding glutamine, valine, and asparagine in the group receiving general anesthesia. In the study reported by Carli and Emery,[17] epidural anesthesia was restricted to perioperative administration. Sampling was performed on the second and fourth postoperative days. The plasma amino acid pattern did not differ significantly regarding branched-chain amino acids, whereas free glutamine in muscle decreased significantly.

Preoperative and postoperative blood loss was greater and more variable in the hip replacement model, as was the time required for surgery, in comparison to other trauma models such as cholecystectomy. However, there were no significant differences with respect to surgical occurrences between the three groups.

α-Ketoglutarate provides the carbon skeleton of glutamine minus two amino groups. After hip replacement, adding amino acids to hypocaloric glucose postoperatively did not influence the amino acid pattern in muscle at 4 days after surgery.[4] α-Ketoglutarate together with TPN counteracts the depletion of free glutamine in muscle and the changes in total ribosomes and polyribosomes after elective abdominal surgery.[6] α-Ketoglutarate is used directly in carbohydrate metabolism[26] and will therefore diminish excretion of nitrogen derived from the amino acid.[6,27] In the present study, α-ketoglutarate was given without an additional nitrogen supply. The provision of α-ketoglutarate attenuated the decrease in free glutamine in muscle.

In previous studies, the capacity for protein synthesis in skeletal muscle, as measured by the ribosome technique, was analyzed in patients undergoing abdominal surgery.[3,5] Cholecystectomy serves as a standardized human trauma model in which characteristic and highly reproducible alterations in amino acid, protein, and nitrogen metabolism are seen.[5,28] To analyze the impact of orthopedic surgery under regional anesthesia on the metabolic changes involving protein and nitrogen metabolism, the previous studies were extended to patients undergoing total hip replacement. To our knowledge, no such studies have been made on skeletal muscle protein synthesis using the ribosome technique in patients undergoing this type of surgery. Samples were taken at 24 hours after surgery to gain information on the response of patients in the early phases of postoperative metabolism. The advanced age of the patients and physical impairment due to partial immobilization may be responsible for the interindividual variation in the results obtained.

In summary, the results obtained show that a hypocaloric glucose infusion, when supplemented with glutamine, improved the nitrogen status of patients within 24 hours after hip replacement. The decline in muscle free glutamine was prevented, as was the decrease in skeletal muscle ribosome concentration. Furthermore, substitution of α-ketoglutarate for glutamine proved to be a possible alternative, as an agent both for sparing muscle free glutamine and for preventing a significant decline in ribosome concentration in skeletal muscle.

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