Ornithine-alpha-ketoglutarate Improves Skeletal Muscle Protein Synthesis as Assessed by Ribosome Analysis and Nitrogen Use After Surgery

JAN WERNERMAN, M.D., FOLKE HAMMARQVIST, M.D., ALEXANDRA VON DER DECKEN, PH.D., and ERIK VINNARS, M.D.

Total parenteral nutrition (TPN) was given for 3 days after elective abdominal surgery. The control group (N = 9) received TPN only and one group of patients (N = 6) received TPN supplemented with ornithine-alpha-ketoglutarate (0.35 g/kg bw/day). Protein synthesis in skeletal muscle was assessed from the total ribosome concentration and the percentage of polyribosomes. In the control group the total concentration of ribosomes decreased after surgery by 23% ($p < 0.05$) and the percentage of polyribosomes decreased by 21% ($p < 0.01$), whereas in the ornithine-alpha-ketoglutarate group both variables remained unaffected. The cumulative urinary urea excretion was significantly larger in the control group than in the ornithine-alpha-ketoglutarate group ($p < 0.05$). The calculated nitrogen balance was negative in the control group on each day of the study ($p < 0.05$), but that of the ornithine-alpha-ketoglutarate group was not statistically different from zero. The results show that postoperative maintenance of muscle protein synthesis and a more effective nitrogen use was achieved by supplementing TPN with ornithine-alpha-ketoglutarate, 0.35 g/kg bw/day.

From the Department of Anesthesiology and Intensive Care, Huddinge University Hospital, Department of Anesthesiology and Intensive Care, St. Göran's Hospital, and Wenner-Gren Institute for Experimental Biology, University of Stockholm, Sweden

IN NONMALNOURISHED ADULT patients, the immediate post-traumatic period is characterized by whole body protein catabolism and a negative nitrogen balance.[1] Nutritional support, including total parenteral nutrition (TPN), counteracts the nitrogen losses and improves the negative nitrogen balance without attaining equilibrium.[2,3] The postoperative nitrogen supply is influenced by increasing the total amount of nitrogen provided[4] or by increasing the proportion of branched chain amino acids given,[5,6] without reversing the negative nitrogen balance to positive values.

After trauma, protein metabolism is influenced differently in individual tissues; skeletal muscle is of special interest because it constitutes the largest protein reservoir in the human body.[7] Protein synthesis in skeletal muscle decreases after surgery,[3,8] whereas the rate of protein breakdown remains unaffected after surgery of a moderate magnitude.[8,9] Nutritional support does not seem to be sufficient to reverse these changes.[3,8]

Supplementation of TPN with ornithine-alpha-ketoglutarate has been shown to maintain nitrogen balance after abdominal surgery.[10] The characteristic postoperative increase in the intracellular concentrations of some essential amino acids in skeletal muscle is also counteracted by ornithine-alpha-ketoglutarate.[11] In this investigation following elective abdominal surgery, a control group of patients received TPN only. Another group, given TPN supplemented with ornithine-alpha-ketoglutarate, showed a lower cumulative urinary urea excretion and a maintenance of skeletal muscle protein synthesis as assessed by the concentration and size distribution of ribosomes compared with the control group.

Materials and Methods

Patients

Fifteen metabolically healthy patients admitted to the hospital for elective cholecystectomy participated in the study. Their voluntary consent was obtained after the study's purpose, procedure, and risks were explained. The study protocol was approved by the Ethics Committee of the Karolinska Institute, Stockholm, Sweden. The characteristics of the patients, along with the operating time and the postoperative blood loss, are given in

This work was supported by grants from the Swedish Medical Council (Project 04210), the TRYGG-HANSA Research Fund, and the ESPEN-Ajinomoto Fellowship 1985.

Reprint requests and correspondence: Jan Wernerman, M.D., Department of Anesthesiology and Intensive Care, Huddinge University Hospital, S-141 85 Huddinge, Stockholm, Sweden.

Submitted for publication: February 23, 1987.

Table 1. Premedication was given in the form of morphine-scopolamine and anesthesia was administered using pentothal, diazepam, fentanyl, pencuronium, and N_2O/O_2. As prophylaxis against postoperative thrombotic complications, 500 mL of dextran 70, 60 mg/mL (Macrodex, Pharmacia Infusion, Uppsala, Sweden), was given. Intercostal blocks using bupivacaine, 5 mg/mL (Marcain, Astra, Södertälje, Sweden), were administered to relieve postoperative pain and ketobemidone was given on request. No patients requiring replacement of blood products were included in the study.

TABLE 1. *Characteristics of Patients and Surgical Procedure (Mean ± SEM)*

	Control Group	Ornithine-alpha-ketoglutarate Group
Sex (female/male)	5/4	4/2
Age (years)	58 ± 4	58 ± 6
Height (cm)	171 ± 4	168 ± 4
Weight (kg)	70 ± 4	69 ± 6
Operating time (min)	86 ± 15	102 ± 12
Peroperative blood loss (mL)	210 ± 30	180 ± 40

Nutritional Support

The patients were given intravenous fluids, 35 mL/kg bw/day. During the surgical procedure, 25 mg/mL of glucose (Rehydrex, Pharmacia Infusion) was infused. TPN included 0.2 g N/kg bw/day given as a balanced amino acid solution (Vamin-Glukos, KabiVitrum, Stockholm, Sweden) and 135 kJ/kg bw/day was supplied as equal parts of fat (Intralipid, KabiVitrum) and carbohydrate (Glukos, 100 mg/mL, Pharmacia Infusion). The nutrition was supplemented with electrolytes, trace elements (Addex-Kalcium Spår, Pharmacia Infusion), and vitamins (Vitalipid Adult and Soluvit, KabiVitrum). On the day of operation, half the nutritional regimen was administered.

The patients were randomized into two groups. The control group (N = 9) followed the TPN regimen previously described and the ornithine-alpha-ketoglutarate group (N = 6) was given the TPN regimen supplemented with ornithine-alpha-ketoglutarate, 0.35 g/kg bw/day (Ornicetil, Laboratoires Jaques Logeais, Paris, France). This amount of ornithine-alpha-ketoglutarate contains 0.01 g/kg bw/day of nitrogen, which was included in the calculation of the nitrogen balance but was disregarded as a nitrogen source with regard to the comparability of the two groups. The caloric content of 0.35 g of ornithine-alpha-ketoglutarate is approximately 5 kJ, which was regarded as neglectable as an energy source in the calculations.

Ribosome Analysis

The method for analyzing the ribosomes from skeletal muscle has been described in detail previously.[12] Muscle biopsy specimens of 50 mg wet weight were taken by the percutaneous needle technique after local anesthesia of the skin.[13] The specimens were frozen in liquid nitrogen within 2 minutes and then stored at −80 C for less than 2 weeks before analysis. The biopsy specimens were homogenized in a medium containing an RNase inhibitor. After centrifugation at 1500 × g for 10 minutes, the pellet was saved for the determination of DNA. The supernatant was ultracentrifuged at 102,000 × g for 2 hours in a 40.2 rotor (Beckman Instruments, Palo Alto, CA). The ribosome pellet obtained was suspended in medium. A portion of the suspension was layered onto a linear density gradient between 0.4 and 1.5 M sucrose. After centrifugation in a SM 50.1 rotor (Beckman Instruments) for 60 minutes at 149,000 × g, the gradient was pumped through a continuous flow cuvette and the absorbency was monitored at 260 nm. The area under the absorbency curve was determined. The total ribosome concentration was determined from the absorbency at 260 nm. The concentration was expressed as optical density (OD) units per milligram of tissue DNA. The DNA content was analyzed by a fluorometric method[14] using salmon DNA as a standard.

Urea Excretion and Nitrogen Balance

Urine was collected in 24-hour portions and assayed for urea.[15] The serum concentration of urea was determined daily and it varied less than 2 mmol/L; hence, the total urea pool of the patients was considered constant since there was no change in their body weight during the study. The nitrogen balance was calculated using the approximation suggested by MacKenzie,[16] estimating the nonurea urinary nitrogen losses to be 1.8 g/24 hours and the nonurinary nitrogen losses to be 1.7 g/24 hours.

Statistics

All values are given as mean ± SEM. Student's t-test for paired samples was used to compare different observations, and a two-way analysis of variance was used to compare the two different groups.[17]

Results

Skeletal muscle biopsies were performed immediately before the operation and on the morning of the third day after surgery. The ribosome fraction was isolated from the biopsy specimen and the concentration and size distribution of the ribosomes was determined. The percentage of polyribosomes was determined from the ribo-

TABLE 2. *Total Ribosome Concentration, Percentage of Polyribosomes, and Concentration of Polyribosomes in Skeletal Muscle of Patients Undergoing Elective Abdominal Surgery (Mean ± SEM)*

	Control Group	Ornithine-alpha-ketoglutarate Group
Total ribosome concentration (OD/mg DNA)		
Before surgery	49.2 ± 5.6	40.0 ± 4.7
After surgery	37.5 ± 4.4*	42.5 ± 3.5†
Percentage of polyribosomes		
Before surgery	50.6 ± 1.5	52.3 ± 2.7
After surgery	39.0 ± 3.3‡	53.2 ± 1.8§
Polyribosome concentration (OD/mg DNA)		
Before surgery	23.4 ± 2.9	20.9 ± 2.6
After surgery	14.5 ± 2.5‖	22.6 ± 1.9§

*$p < 0.01$, ‡$p < 0.05$, ‖$p < 0.001$ compared with preoperative values; †$p < 0.05$, §$p < 0.01$ compared with control group.

some profile, where the area under the curve is proportional to the amount of ribosome particles of different mass. The percentage of polyribosomes out in the total number of ribosomes decreased by 21% on the third day after surgery compared with the initial values in the control group ($p < 0.05$). No such decrease was demonstrated in the ornithine-alpha-ketoglutarate group (Table 2). The alteration was significantly different between the two groups on the third postoperative day ($p < 0.05$).

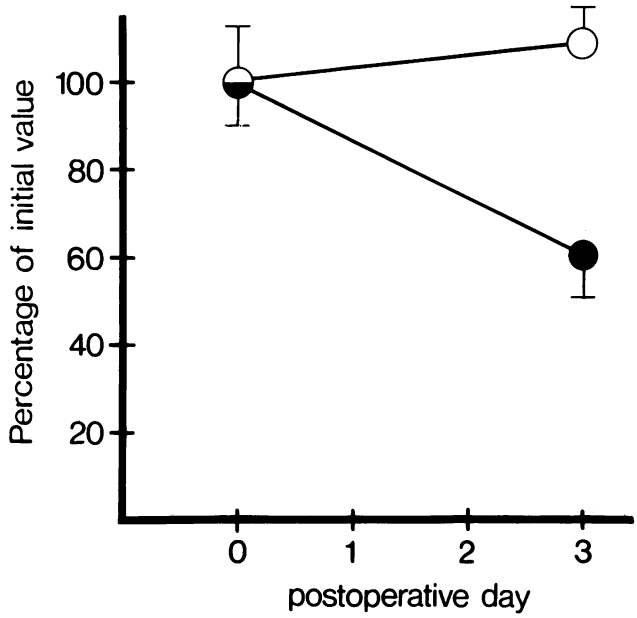

FIG. 1. The polyribosome concentration in skeletal muscle before surgery and on the third postoperative day in patients undergoing elective abdominal surgery. Patients received TPN (N = 9, filled circles) or TPN supplemented with ornithine-alpha-ketoglutarate (N = 6, open circles). The values are expressed as percentage of the original values noted before surgery. In the control group, the polyribosome concentration decreased significantly ($p < 0.001$). There was no postoperative decrease in the ornithine-alpha-ketoglutarate group. On the third postoperative day there was a significant difference between the two groups ($p < 0.01$). Values are given as mean ± SEM.

TABLE 3. *Daily Urinary Urea Excretion and Calculated Nitrogen Balance in the Period Immediately Following Elective Abdominal Surgery (Mean ± SEM)*

	Control Group	Ornithine-alpha-ketoglutarate Group
Urinary urea excretion (mmol/24 hr)		
Postoperative Day 1	278 ± 36	89 ± 28
Day 2	464 ± 46	500 ± 82
Day 3	486 ± 32	404 ± 46
Calculated nitrogen balance (g/24 h)		
Postoperative Day 1	−4.3 ± 1.0	0.9 ± 0.8
Day 2	−2.5 ± 1.3	−3.7 ± 2.3
Day 3	−3.1 ± 0.9	−1.0 ± 1.3

A comparison of the pre- and postoperative conditions in the control group showed a decrease in the total concentration of ribosomes per milligram of DNA by 23% on the third day after the operation ($p < 0.01$; Table 2). In the ornithine-alpha-ketoglutarate group no difference between the values was noted before and after surgery. On the third day following surgery the changes were significantly different between the two groups ($p < 0.05$). The concentration of polyribosomes per milligram of DNA was calculated from the total concentration of ribosomes and the percentage of polyribosomes. In the control group there was a 38% decrease in the polyribosome concentration between the original value and that of the third postoperative day ($p < 0.001$; Table 2). In contrast, the ornithine-alpha-ketoglutarate group showed no change in the polyribosome concentration 3 days after surgery (Fig. 1). On the third day after surgery the difference between the two groups was highly significant ($p < 0.01$).

Urinary urea was analyzed in 24-hour portions (Table 3). There was a significant larger cumulative excretion during the initial 3 days after surgery in the control group compared with the ornithine-alpha-ketoglutarate group ($p < 0.05$). Nitrogen balance was calculated from the urinary excretion of urea as presented in the Materials and Methods section. In the control group the nitrogen balance was negative on each day of the study ($p < 0.05$; Table 3). In the ornithine-alpha-ketoglutarate group the nitrogen balance was not statistically different from zero on any of the first 3 days after surgery. The cumulative nitrogen balance was calculated for the initial 3 postoperative days (Fig. 2). The difference between the two groups became statistically significant on day 3 after surgery ($p < 0.05$).

Discussion

Protein metabolism was evaluated by ribosome analysis. The total concentration of ribosomes is interpreted as a measure of the capacity for protein synthesis.[18,19] A high content of polyribosomes corresponds to a high rate of protein synthesis.[20] Hence, the percentage share of polyribosomes is a measure of the activity of protein synthesis. Especially when several consecutive samples are available, changes in protein synthesis activity can be studied over a prolonged period.[12,18,21] The reproducibility of total ribosome concentration per milligram of DNA and the percentage of polyribosomes is approximately 6% in both cases when the current technique is used.[22]

Protein synthesis in skeletal muscle was assessed from the total concentration and size distribution of ribosomes. Applied to human muscle tissue, this technique shows a decrease in both variables after elective abdominal surgery, regardless of whether TPN is given in the immediate postoperative period.[3] An isonitrogenous amino acid solution enriched with branched-chain amino acids cannot prevent this decrease.[6] Supplementation of a standard TPN program with ornithine-alpha-ketoglutarate prevented the fall in both the total ribosome concentration and the percentage of polyribosomes. These findings are in accordance with the normalization of the intracellular concentrations of free branched chain and aromatic amino acids seen after surgery.[11] The preserving effect of ornithine-alpha-ketoglutarate on whole-body nitrogen economy, as indicated by the improvement in nitrogen balance, confirmed previous results.[10]

The mechanism for the nitrogen sparing effect of ornithine-alpha-ketoglutarate is not clear. Supplementation of TPN with ornithine has no beneficial effect on nitrogen economy after surgery (E. Vinnars, unpublished results). One hypothesis is that alpha-ketoglutarate, being the corresponding ketoacid to glutamic acid, can counteract the increase seen after trauma in nitrogen efflux from the periphery to the splanchnic area. Ornithine-alpha-ketoglutarate, 0.022 g/kg bw/h, given for 150 minutes to healthy male volunteers after an overnight fast, does not affect the efflux of amino acids from peripheral tissue.[23] The effect on amino acid flux in patients in protein catabolic states remains an open question. To improve nitrogen use and to benefit from the nitrogen sparing effect, ornithine-alpha-ketoglutarate possibly should be given with a nitrogen supply.

The balanced amino acid solutions that are commercially available do not include glutamine, and the content of glutamic acid is limited by its tendency to evoke nausea when given in high concentrations. The alpha-ketoglutarate included as ornithine-alpha-ketoglutarate presumably provides enough carbon skeletons to compensate for a shortage of glutamine/glutamic acid in the commercially available amino acid solutions. In animal experiments, post-traumatically administered glutamine prevents the profound decrease in the intracellular free glutamine concentration in skeletal muscle seen otherwise,[24] and the overall nitrogen economy is improved. Ornithine-alpha-ketoglutarate given to patients undergoing colonic resection does not prevent the decrease in the intracellular concentrations of free glutamine.[11] In animals both the influx of branched chain amino acids and the total intracellular free amino acid nitrogen are correlated to the efflux of amino acid nitrogen from peripheral tissue after surgery. To determine if a shortage of glutamine is the critical point in post-traumatic protein catabolism, it seems reasonable to give glutamine to human subjects also. The pharmaceutical difficulty involved in preparing a stable glutamine solution remains a crucial problem. The dipeptides currently available for research purposes may be another future source in this context.

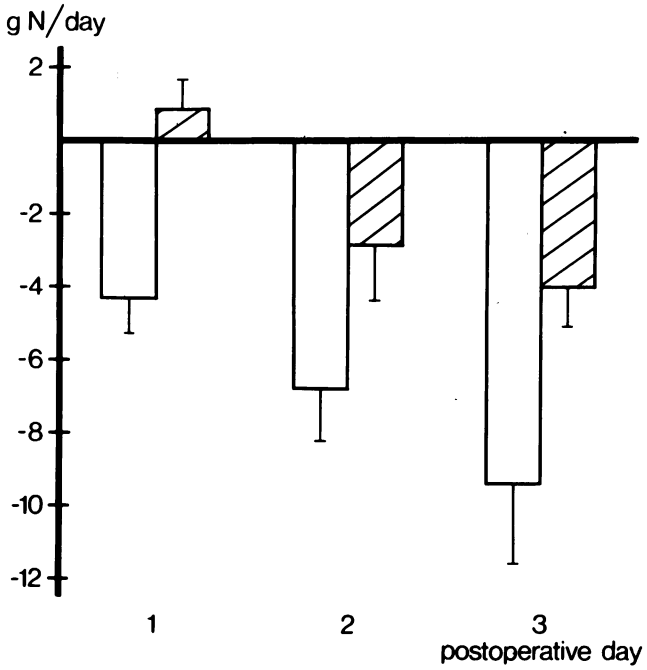

FIG. 2. The cumulative calculated nitrogen balance on days 1–3 immediately after elective abdominal surgery. Patients received TPN (N = 9, open bars) or TPN supplemented with ornithine-alpha-ketoglutarate (N = 6, hatched bars). There was a significant difference between the two groups on each day ($p < 0.05$). Values are given as mean ± SEM.

In conclusion, after elective abdominal surgery, TPN supplemented with ornithine-alpha-ketoglutarate prevented the decrease in skeletal muscle protein synthesis normally observed with TPN only and diminished urinary urea excretion. The findings suggest the use of ornithine-alpha-ketoglutarate as a supplement to nutritional support after major surgery and in septic patients. The

mechanisms underlying the nitrogen sparing effect of ornithine-alpha-ketoglutarate are not clear and require further investigation.

Acknowledgments

The skillful technical assistance of Mrs. C. Hebert and Mrs. S. Sundberg is gratefully acknowledged.

References

1. Kinney JM, Elwyn DH. Protein metabolism and injury. Ann Rev Nutr 1983; 3:433–466.
2. Ewerth S, Allgen L-G, Fürst P, et al. Metabolic effects of four intravenous nutritional regimens after elective surgery. I. Clinical data and biochemistry. Clin Nutr 1983; 1:313–324.
3. Wernerman J, von der Decken A, Vinnars E. Protein synthesis in skeletal muscle in relation to nitrogen balance after abdominal surgery: the effect of total parenteral nutrition. JPEN 1986; 10:578–582.
4. Larsson J, Liljedahl S-O, Schildt B, et al. Metabolic studies in multiple injured patients. Acta Chir Scand 1981; 147:317–324.
5. Desai SP, Bistrian BR, Moldawer LL, Blackburn GL. Whole-body nitrogen and tyrosine metabolism in surgical patients receiving branched-chain amino acid solutions. Arch Surg 1985; 120:1345–1350.
6. Hammarqvist F, Wernerman J, von der Decken A, Vinnars E. The effect of branched chain amino acids (BCAA) on postoperative muscle protein synthesis and nitrogen balance. Clin Nutr 1985; 4(Spec Suppl):68.
7. Young VR. The role of skeletal and cardiac muscle in the regulation of protein metabolism. *In* Munro HN, ed. Mammalian Protein Metabolism, Vol. 4, New York: Academic Press, 1970; 586–674.
8. Rennie MJ, Bennegard K, Eden E, et al. Urinary excretion and efflux from the leg of 3-methylhistidine before and after major surgical operation. Metabolism 1984; 33:250–256.
9. O'Keefe SJD, Moldawer LL, Young VR, Blackburn GL. The influence of intravenous nutrition on protein dynamics following surgery. Metabolism 1981; 30:1150–1158.
10. Leander U, Fürst P, Vesterberg K, Vinnars E. Nitrogen sparing effect of Ornicetil® in the immediate postoperative state: clinical biochemistry and nitrogen balance. Clin Nutr 1985; 4:43–51.
11. Vesterberg K. Leander U, Fürst P, Vinnars E. Nitrogen sparing effect of Ornicetil® in the immediate postoperative period. II. Muscle and plasma amino acids. Clin Nutr, in press.
12. Wernerman J, von der Decken A, Vinnars E. Size distribution of ribosomes in biopsy specimens of human skeletal muscle during starvation. Metabolism 1985; 34:665–669.
13. Bergström J. Muscle electrolytes in man. Scand J Clin Lab Invest 1962; 14(Suppl 168):11–13.
14. Setaro F, Morley CBD. A modified fluorometric method for the determination of microgram quantities of DNA from cell or tissue cultures. Anal Biochem 1976; 71:313–317.
15. Fawcett JK, Scott JF. A rapid and precise method for the determination of urea. J Clin Pathol 1960; 13:156–159.
16. MacKenzie TA, Clark MB, Bistrian BR, et al. A simple method for estimating nitrogen balance in hospitalized patients: a review and supporting data for a previously proposed technique. J Am Coll Nutr 1985; 4:575–581.
17. Snedecor GR, Cochran WG. Statistical Methods, 4th Ed. Ames: The Iowa State University Press, 1967; 91.
18. Wernerman J, von der Decken A, Vinnars E. Polyribosome concentration in human skeletal muscle after starvation and parenteral or enteral refeeding. Metabolism 1986; 35:447–451.
19. Wernerman J, von der Decken A, Vinnars E. The interpretation of ribosome determinations to assess protein synthesis in human skeletal muscle. Infusionstherapie 1986; 13:162–165.
20. Bergen WG. Protein synthesis in animal models. J Animal Sci 1974; 38:1071–1078.
21. Wernerman J, Magnusson K, Ekman L, et al. Comparison of different techniques to measure protein synthesis in skeletal muscle. Clin Nutr 1985; 4(Spec Suppl):0.122.
22. Wernerman J, von der Decken A, Vinnars E. The diurnal pattern of protein synthesis in human skeletal muscle. Clin Nutr 1985; 4:202–205.
23. Eriksson LS, Reihner E, Wahren J. Infusion of ornithine-alpha-ketoglutarate in healthy subjects: effects on protein metabolism. Clin Nutr 1985; 4:73–76.
24. Kapadia CR, Colpoys MF, Jiang ZM, et al. Maintenance of skeletal muscle intracellular glutamine during standard surgical trauma. JPEN 1985; 9:583–589.
25. Johnson DJ, Colpoys M, Smith RJ, et al. Branched chain amino acid uptake and muscle free amino acid concentrations predict postoperative muscle nitrogen balance. Ann Surg 1986; 204:513–523.