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Ketamine anesthesia causes greater muscle catabolism in rabbits than does propofol

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Whereas ketamine and propofol are popular anesthetics in the intensive care unit and trauma/burn surgery, their metabolic effects are not known. Because traumatized patients are under catabolic state and may need multiple operations over the acute period of treatment, the knowledge of metabolic effects of anesthetics should have clinical relevance. We have compared muscle protein kinetics in rabbits under ketamine or propofol anesthesia. Because propofol is given in a fat emulsion (Intralipid), we have also tested the effect of Intralipid in an additional group of animals under ketamine anesthesia by giving the same dose of Intralipid as in the propofol group. In all animals, xylazine was used as a supplemental anesthetic and a balanced amino acid infusion (10% Travasol) was infused at 2 mg · kg^{-1} · min^{-1}. L-[ring-$^{13}C_6$]phenylalanine was infused as a tracer and the arteriovenous balance method was applied to the hindlimb for determination of muscle protein kinetics. Results: the rate of muscle protein breakdown was significantly greater in the ketamine group than in the propofol group and intermediate in the lipid group. These results were consistent with whole body protein breakdown rates reflected by total phenylalanine flux. Rates of muscle protein synthesis were not significantly different among the groups. Consequently, the ketamine group had significantly greater net loss of muscle protein. We conclude that in relation to propofol, ketamine has a greater catabolic effect on muscle protein, which can be attenuated by lipid infusion. Therefore, ketamine is not an optimal anesthetic for catabolic patients. (J. Nutr. Biochem. 8: 133–139, 1997.) © *Elsevier Science Inc. 1997*

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Introduction

Accelerated protein catabolism is a common response to major injuries.[1–3] In some cases, such as severe burns, net protein loss can be extensive and persist for weeks. The continuous loss of protein mass may impair immune function and wound healing, leading to high morbidity and mortality.[4,5] Thus, minimizing the catabolic response becomes an important aspect in the treatment of severely injured patients.

In addition to the primary injury, some therapeutic approaches could cause additional protein catabolism. For example, surgical procedures in of themselves cause a postsurgical catabolic response, even in routine elective cases.[6–8] In the case of patients with major burns, it is common for five or six major operations to be performed over the acute period of treatment (approximately 6 weeks). There is no particular anesthetic that is required for these surgical procedures, yet it is impossible to choose an anesthetic agent based on its metabolic effect, because there have been no studies documenting the metabolic effect of anesthesia. This is particularly important in the case of burn patients, because some surgical procedures, such as escharectomy and skin grafting, are time consuming and therefore the patients are anesthetized for a long period. If an anesthetic agent has catabolic effect on protein itself, this would aggravate the catabolic state.

In this study, we measured the rates of muscle protein synthesis and breakdown, the net amino acid balance and rate of transmembrane amino acid transport in rabbits under

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either ketamine or propofol anesthesia. We have used a phenylalanine tracer and our modified limb arteriovenous balance method.[9] Because propofol contains 10% Intralipid as a solvent, we added a third group in which the rabbits were studied under ketamine anesthesia with 10% Intralipid infusion at the same dose as in the propofol group. In veterinary medicine, ketamine is usually used in combination with a supplemental dose of xylazine for rabbit anesthesia.[10,11] For comparison, we administered the same dose of xylazine in all the animals. Finally, exogenous amino acids were infused into the rabbits of the three groups at a fixed rate to ensure a sufficient availability of amino acids and minimize any potential effect of fasting. This experimental design enabled us to evaluate the metabolic effect of ketamine or propofol anesthesia, and the role of the fat emulsion. Because both ketamine and propofol are very popular in the intensive care unit and in trauma/burn surgery, the knowledge of their relative metabolic effects should have clinical relevance.

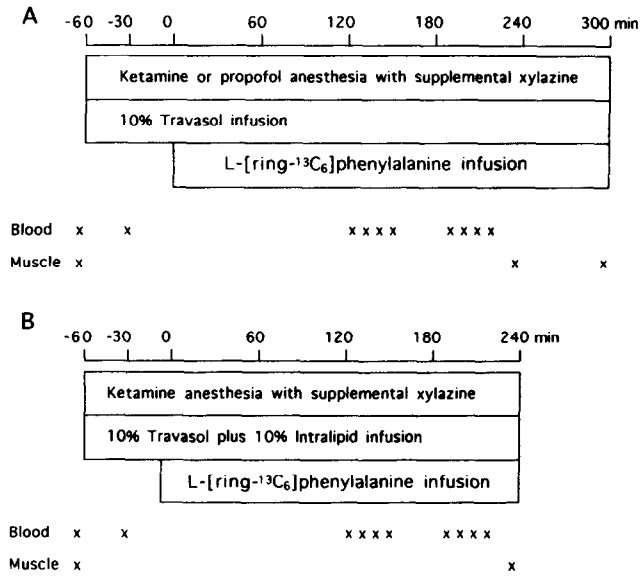

Figure 1 Experimental protocol. A, The ketamine and propofol groups. B, The lipid group. × indicates sampling blood or muscle.

Methods and materials

Animals

Male New Zealand white rabbits (Myrtle's Rabbitry Incorporated, Thompson Station, TN USA), weighing about 4.5 kg, were used for this study. The rabbits were housed in individual cages and consumed Lab Rabbit Chow HF No. 5326 (Purina Mills Inc., St. Louis, MO USA) for weight maintenance. This study was approved by the Animal Care and Use Committee of The University of Texas Medical Branch at Galveston.

Isotopes

L-[ring-$^{13}C_6$]phenylalanine (99% enriched) and L-[ring-$^{2}H_5$]phenylalanine (98% enriched) were purchased from Cambridge Isotope Laboratories (Woburn, MA USA). L-[2-^{15}N]phenylalanine (99% enriched), L-[1-^{13}C]leucine (99% enriched), and L-[1,2-$^{13}C_2$]leucine (99.3% enriched) were purchased from Masstrace, Inc. (Somerville, MA USA). L-[ring-$^{13}C_6$]phenylalanine was dissolved in 0.45% saline as a stock solution and stored at 4°C, which was diluted with 0.9% saline into working solution before each infusion. The other four isotopes were used to prepare three different internal standard solutions for measurement of phenylalanine and leucine concentration or content in the blood and muscle samples. The blood internal standard solution contained L-[ring-$^{2}H_5$]phenylalanine at 29.7 μmol/L and either L-[1-^{13}C] or L-[1,2-$^{13}C_2$]leucine at 69.5 μmol/L. The muscle free pool internal standard solution contained L-[ring-$^{2}H_5$]phenylalanine at 5.97 μmol/L. The muscle protein-bound pool internal standard solution contained L-[2-^{15}N]phenylalanine at 5.17 mmol/L and L-[1,2-$^{13}C_2$]leucine at 13.32 mmol/L.

Experimental design

All the animals were studied after overnight (about 16 hr) food deprivation with free access to water. There were three groups with five each. The rabbits in the ketamine group were given intramuscular ketamine (35 mg/kg) and xylazine (5 mg/kg) as initial anesthesia followed by the continuous intravenous infusion of a solution that contained ketamine 17 g/L and xylazine 0.664 g/L. The rabbits in the propofol group were given intramuscular xylazine (5 mg/kg), subcutaneous midazolam (0.2 mg/kg), and buprenorphine (0.03 mg/kg) as sedative agents. Ten minutes later, the rabbits were given propofol (6.5 mg/kg) through an ear vein for initial anesthesia, followed by continuous intravenous infusion of propofol (10 g/L) and xylazine solution (0.664 g/L). Because propofol inhibits respiration,[12] the rabbits in the propofol group received oxygen through a ventilator (OMNI-Vent; Stein-Gates Medical Equipment, Inc., Topeka, KS USA) to maintain the oxygen saturation between 96% and 100% (monitored from a toe skin surface of the hindlimb using a 3700 Pulse Oximeter, Louisville, CO USA). The rabbits in the lipid group were anesthetized with the same doses of ketamine and xylazine as in the ketamine group and given intravenous 10% Intralipid (Kabi Pharmacia Inc., Clayton, NC USA) to match the doses in the propofol group.

After the initial anesthesia was induced, surgical procedures were performed to insert polyethylene catheters (PE 90; Becton Dickson and Company, Parsippany, NJ USA) in the right femoral artery and vein. The arterial line was used for blood collection and monitoring of heart rate and mean arterial blood pressure; the venous line was used for infusion. A tracheal tube was placed via tracheotomy. The experimental protocol is illustrated in *Figure 1*. After a muscle specimen was obtained through the incision and a blood sample was drawn from the arterial line for background measurements, at −60 min 10% Travasol (Baxter Healcare Corporation, Deerfield, IL USA) was infused at 2 mg · kg^{-1} · min^{-1} (prime, 80 mg/kg) to ensure a sufficient amino acid availability and 0.9% saline was infused to maintain a normal hydration throughout the entire experimental period in all rabbits. In the lipid group, 10% Intralipid was also infused at −60 min and the rate was adjusted to match the propofol group. At −30 min, another arterial blood sample was drawn to determine the possible change of the background enrichment. One hour after the Travasol infusion (0 min in *Figure 1*), the L-[ring-$^{13}C_6$]phenylalanine infusion was started (prime, 6.6 μmol/kg; continuous infusion, 0.15 μmol · kg^{-1} · min^{-1}). During the 120 to 150 min period, four arterial blood samples were collected at 10-min intervals for measurement of arterial phenylalanine enrichment and glucose and lactate concentrations. By 170 min, under additional 1% Lidocaine local anesthesia the femoral artery on the left hindlimb was isolated through an incision on the groin, and a flow probe (model 1RB; Transonics Systems Ins, Inc., Ithaca, NY USA) was placed on the femoral artery and connected to a small animal blood flowmeter (model T106, Transonic Systems, Inc.) for measure-

ment of blood flow rate in the limb. The femoral vein was dissected so that it was visible through the covering fascia and direct puncture for venous blood draw would not change the blood flow rate. During the 190- to 220-min period, four simultaneous arterial and femoral venous blood samples were drawn at 10-min intervals. The blood flow rate in the limb was recorded at the time of each blood sampling. During the 230 to 240 min, a muscle specimen was taken from the biceps femoris, which was required by the A-V model. In the lipid group, these muscle samples were also used for determination of fractional synthesis rate (FSR) of muscle protein. In the ketamine and propofol groups, the isotope infusion was extended for 60 minutes more, then another muscle specimen was taken from the same muscle for determination of FSR. In all the groups, arterial blood was drawn at the end of the isotope infusion for determination of the concentrations of insulin, glycerol, triglyceride, and free fatty acid (FFA). The blood samples were kept in an ice-water bath till the end of the infusion. The tissue samples were immediately frozen in liquid nitrogen, stored in −70°C freezer for later analysis.

The heart rate, mean arterial blood pressure and rectal temperature were maintained stable in each group by adjusting the infusion rates of anesthetic solution and saline and heating lamps. These vital signs were recorded every 30 min. Calibrated Syringe Pumps (Harvard Apparatus; South Natick, MA USA) were used for infusions. For each infusion study, an aliquot of the isotope infusate was analyzed for the exact concentration of the tracer.

Sample analysis

Immediately after the infusion study, the blood samples and the blood internal standard solution were added to the tubes containing sulfosalicylic acid solution.[13] The supernatant was processed to make the N-acetyl, n-propyl ester (NAP) derivatives of the amino acids.[14]

Sixty μL of the muscle free pool internal standard was added to 60 mg of wet muscle, and the sample was homogenized three times in 5% perchloric acid solution at 4°C. Phenylalanine was purified from the pooled supernatant by HPLC,[15] and then the NAP derivative of phenylalanine was made. The muscle precipitate was washed sufficiently and dried at 80°C in an oven as described previously.[15] The weight difference between wet muscle and dry muscle was recorded as muscle water content. To determine the muscle protein FSR, aliquots of the dry protein pellets (equal to 4 mg of wet muscle) were hydrolyzed in 6 N constant boiling HCl. A cation exchange column (Dowex AG 50W-X8, Bio-Rad Laboratories, Richmond, CA USA) was used to purify the amino acids. The samples were prepared for the N-heptafluorobutyryl-n-propyl ester (HFBPr) derivatives of amino acid and used to determine L-[ring-$^{13}C_6$]phenylalanine enrichment in the protein-bound pool.[16] To measure the content of phenylalanine and leucine in muscle protein, three aliquots (about 10 mg each) of the dry protein pellets were taken from each rabbit of the ketamine group. One aliquot served as a background and the muscle protein-bound pool internal standard solution was added to the other two (1.5 μL to 1 mg of dry protein) before hydrolysis. The protein hydrolysate was prepared for the NAP derivative.[14]

The isotopic enrichment in the whole blood, purified phenylalanine from the muscle supernatant, and protein hydrolysate with the internal standard was determined on a Hewlett-Packard 5985 gas chromatograph mass spectrometer (GCMS) (Hewlett-Packard Co., Palo Alto, CA USA) with chemical ionization. Ions were selectively monitored at mass-to-charge ratios of 250, 251, 255, 256 for phenylalanine and 216, 217, and 218 for leucine. The L-[ring-$^{13}C_6$]phenylalanine enrichment in the hydrolysate for measurement of protein-bound enrichment was determined on a GCMS (MD 800, Fisons Instruments, Beverly, MA USA).[16] Isotope enrichment was expressed as tracer/tracee ratio after correction for the contribution of the abundance of isotopomers of lower weight to the apparent enrichment of isotopomers with larger weight, and also a skew correction factor to calculate [ring-$^{13}C_6$]phenylalanine enrichment.[17]

Blood glucose and lactate concentrations were determined on a glucose/L-lactate analyzer (Mode 2300; Yellow Springs Instrument Co., Inc., OH USA). Plasma insulin concentration was measured by radioimmunoassay. Plasma triglyceride, glycerol, and FFA concentrations were measured by enzymatic colorimetric methods using a Triglyceride (GPO-Trinder) kit (Sigma) and a NEFA C kit (Wako, Richmond, VA USA), respectively.

Calculations

The rate of appearance (Ra) of whole body phenylalanine was calculated by the equation $Ra = (F/E_p) - F_{Phe}$, where F is the infusion rate of L-[ring-$^{13}C_6$]phenylalanine, E_p is the phenylalanine enrichment (tracer/tracee ratio) in the arterial blood at isotopic plateau, and F_{Phe} is the infusion rate of unlabeled phenylalanine because of the infusion of 10% Travasol solution.[14]

The calculation of amino acid concentration or content using the internal standard method and the calculation of muscle protein FSR using the direct tracer incorporation method are described in our previous publications.[14,18] Phenylalanine kinetics in the limb was calculated from a three-compartment model.[9] The three pools are arterial pool (pool A), venous pool (pool V), and muscle intracellular free pool (pool M). Further definitions necessary for the description of protein kinetics and amino acid transport are PB (protein breakdown reflected by phenylalanine Ra); PS (protein synthesis reflected by rate of disappearance, i.e., Rd); NB (net balance); F_{in}, rate of amino acid entering the limb via artery; $F_{M,A}$, rate of delivery from pool A to pool M (i.e., inward transport); $F_{V,A}$, rate of delivery direct from pool A to pool V (A-V shunting); $F_{V,M}$, rate of delivery from pool M to pool V (outward transport); Ra_T, total Ra into pool M (i.e., the sum of inward transport and appearance from proteolysis) (see Ref. 9 for the equations).

Leucine net balance in the limb was calculated from the A-V concentration difference and blood flow rate. Because leucine is an essential amino acid, it can not be synthesized in the body, its Ra reflects protein breakdown. However, because leucine can be oxidized in the muscle, its Rd overestimates protein synthesis. Assuming leucine disappearance that is not incorporated into protein = leucine oxidation, the measured value of leucine net balance overestimated net protein balance by the extent of oxidation. In contrast, because phenylalanine is neither synthesized nor degraded in the limb, its net balance represents actual protein net balance. Therefore, the rate of leucine oxidation can be calculated: leucine oxidation rate = leucine net balance − phenylalanine net balance × C, where C is the ratio of leucine content to phenylalanine content in muscle protein.

Statistical analysis

Data are expressed as means ± SE. Differences among the three groups (i.e., ketamine, ketamine + lipid, propofol + lipid) were evaluated using an one-way analysis of variance (ANOVA). Post hoc testing was accomplished using the non-paired Student's t-test combined with Bonferroni's inequalities. A P value less than 0.017 (0.05/3) was considered as statistically significant at 0.05 level. Difference between two groups was test by non-paired t-test and $P < 0.05$ was considered as statistically significant.

Results

The general characteristics of the rabbits are shown in *Table 1*. The body weight in the three groups was not significantly different. All the rabbits maintained stable physiologic and

Table 1 General characteristics of the rabbits

Group	BW kg	Rectal temp. °C	HR beats/min	MAP mmHg	BF mL/min
Ketamine	4.9 ± 0.2	39.7 ± 0.4*	188 ± 4*	71 ± 4	8.9 ± 0.8
Propofol	4.8 ± 0.1	38.3 ± 0.2	156 ± 8	75 ± 2	8.8 ± 1.6
Lipid	4.7 ± 0.1	38.8 ± 0.2	185 ± 10	79 ± 2	9.5 ± 0.6

Data are means ± SE. *$P < 0.05$ versus the propofol group. The values of rectal temperature (temp.), heart rate (HR), and mean arterial pressure (MAP) are all recorded between the 120 to 240 min period. BF, blood flow rate in the femoral artery measured during the 180 to 240 min period; the flow rates have been normalized to 5 kg of body weight (BW).

anesthetic conditions during the isotope infusion, as demonstrated by the relatively constant rectal temperature, heart rate, mean arterial blood pressure, femoral artery blood flow rate, and lack of movement. A small dose (1 mL of 1% Lidocaine) was used for local infiltration when dissecting the femoral vessels by the 170 min of the isotope infusion. The femoral arterial blood flow rates have been normalized to 5 kg of body weight to minimize the variation of the leg size because of the variation in body weight. The ketamine group had significantly ($P < 0.05$) higher rectal temperature and heart rate than the propofol group.

The infusion rates of xylazine in the ketamine group, propofol group, and lipid group were 0.030 ± 0.002, 0.030 ± 0.001, and 0.028 ± 0.002 $mg \cdot kg^{-1} \cdot min^{-1}$ ($P > 0.05$), respectively. The ketamine infusion rates in the ketamine group and lipid group were 0.765 ± 0.029 and 0.724 ± 0.050 $mg \cdot kg^{-1} \cdot min^{-1}$ ($P > 0.05$), respectively. The infusion rate of propofol in the propofol group was 0.53 ± 0.03 $mg \cdot kg^{-1} \cdot min^{-1}$, which delivered 10% Intralipid at 3.2 ± 0.2 $mL \cdot kg^{-1} \cdot h^{-1}$. In the lipid group, the infusion rate of 10% Intralipid was 3.3 ± 0.1 $mL \cdot kg^{-1} \cdot h^{-1}$ ($P > 0.05$).

The measured concentrations of substrates and insulin are presented in *Table 2*. In the propofol and lipid groups, the Intralipid infusion raised plasma triglyceride, glycerol and FFA concentrations several fold. There were no significant differences in muscle free phenylalanine concentrations among the three groups, though the average value was 10 to 20% higher in the ketamine group than in the propofol and lipid groups. The phenylalanine concentrations in the arterial blood followed the same sequence as in the muscle free pool (data presented below). Blood glucose concentration before anesthesia was 3.8 ± 0.3 mmol/L ($n = 6$, drawn from the ear vein). During the isotope infusion there was hyperglycemia in all the groups and there was no significant difference among the groups ($P > 0.05$). The arterial blood lactate concentrations were also not significantly different among the groups ($P > 0.05$). Plasma insulin concentration before the isotope infusion was very low (12 ± 6, 11 ± 6, 15 ± 11 pmol/L in the ketamine, propofol and Lipid group, respectively). After 4- to 5-hr infusions, insulin concentrations were significantly ($P < 0.001$) elevated in all groups, but there were no significant differences among the groups ($P > 0.05$).

In each experiment, 10% Travasol was infused at 2 $mg \cdot kg^{-1} \cdot min^{-1}$, which delivered exogenous unlabeled

Table 2 Substrates and insulin concentrations

	Ketamine	Propofol	Lipid
Triglyceride (mmol/L)	0.4 ± 0.1*†	12.8 ± 0.3	10.6 ± 1.6
Glycerol (mmol/L)	0.2 ± 0.1*†	2.8 ± 0.3	4.1 ± 1.2
Free fatty acids (mg/L)	70 ± 16*†	458 ± 104	590 ± 104
Glucose (mmol/L)	10.2 ± 0.9	14.1 ± 1.2	10.4 ± 0.4
Lactate (mmol/L)	1.2 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
Muscle PHE (mmol/L)	0.16 ± 0.08	0.14 ± 0.06	0.14 ± 0.10
Insulin (pmol/L)	52 ± 18	67 ± 27	74 ± 20

Data are means ± SE. *$P < 0.05$ versus the propofol group; †$P < 0.05$ versus the lipid group. Triglyceride, glycerol, free fatty acid and insulin were measured from the arterial plasma sampled after 4- to 5-hr isotope infusion; glucose and lactate concentrations were the average values of the 120 to 180 and 180- to 240-min periods; Muscle PHE (phenylalanine) was the concentration in the muscle free amino acid pool (including intracellular and interstitial phenylalanine).

phenylalanine at 0.6788 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. The infusion of the Travasol solution caused a slight increase in leucine background enrichment, so the arterial blood samples taken at −30 min were used for leucine background enrichment. Because there was neither [ring-$^{13}C_6$] nor [ring-$^{2}H_5$] phenylalanine in the Travasol solution, its infusion did not change their background enrichment (i.e., zero).

After 2 hr of tracer infusion, the arterial phenylalanine enrichment reached an isotopic plateau which was maintained throughout the infusion period in all rabbits. Whole body phenylalanine Ra was calculated from the plateau enrichment in the arterial blood during the 120- to 240-min period. The ketamine group had the greatest whole body phenylalanine Ra, which was significantly ($P < 0.05$) greater than the propofol group, and the value in the lipid group was intermediate (*Figure 2*).

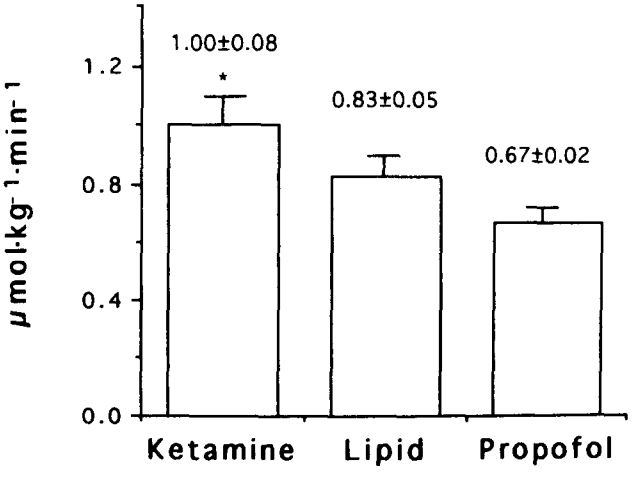

Figure 2 Whole body protein breakdown rates reflected by phenylalanine flux. *$P < 0.05$ versus the propofol group.

Table 3 Phenylalanine concentration in the blood and enrichment in the blood and muscle intracellular free pool

	E_A	E_V	E_M	C_A	C_V
Ketamine					
Mean	0.0865	0.0716	0.0544	0.1022	0.1081
SE	0.0064	0.0054	0.0034	0.0064	0.0065
Propofol					
Mean	0.1092	0.0943	0.0763	0.0920	0.0929
SE	0.0016	0.0034	0.0061	0.0053	0.0058
Lipid					
Mean	0.0970	0.0832	0.0644	0.0933	0.0957
SE	0.0042	0.0047	0.0046	0.0057	0.0063

E_A, E_V, E_M are phenylalanine enrichment (tracer/tracee ratio) in the arterial blood, venous blood and muscle free amino acid pool, respectively; C_A, C_V are phenylalanine concentration (mmol/L) in the arterial and venous blood.

The measured phenylalanine concentration in the blood and enrichment in the blood and in the muscle intracellular free pool are presented in *Table 3*. The data of protein kinetics and phenylalanine transport in the limb are presented in *Table 4*. The ketamine group had the greatest protein breakdown rate in the limb, which was significantly ($P < 0.05$) greater than the propofol group, and the value in the lipid group was intermediate. The protein synthesis rates were not significantly different among the groups, although the average value was the greatest in the ketamine group, lowest in the propofol group and intermediate in the lipid group. The net loss of limb muscle protein was the greatest in the ketamine group, which was significantly ($P < 0.05$) greater than the propofol group, and the value in the lipid group was intermediate. The phenylalanine transport data were not significantly ($P > 0.05$) different among the groups.

The FSR of muscle protein was determined in the three groups. The muscle free phenylalanine enrichments were 0.0551 ± 0.0030, 0.0644 ± 0.0046, and 0.0739 ± 0.0055 in the ketamine, lipid, and propofol group, respectively, which were used as precursor enrichment. The corresponding rates of increase in the protein-bound phenylalanine enrichments were $4.869 \times 10^{-5} \pm 0.475 \times 10^{-5}$/h, $5.632 \times 10^{-5} \pm 0.281 \times 10^{-5}$/h, and $5.870 \times 10^{-5} \pm 0.504 \times 10^{-5}$/h, respectively. The calculated FSRs were 0.089 ± 0.008%/h, 0.089 ± 0.007%/h, and 0.081 ± 0.011%/h ($P > 0.05$) in the ketamine, lipid, and propofol group, respectively.

Table 4 Protein kinetics and phenylalanine transport in the limb

	PS	NB	PB	F_{in}	$F_{M,A}$	$F_{V,A}$	$F_{V,M}$	Ra_T
Ketamine								
Mean	10.4	−3.1*	13.5*	55.0	27.1	27.9	30.2	40.6
SE	1.3	0.3	1.3	6.9	6.7	3.1	6.9	6.9
Propofol								
Mean	7.8	−0.7	8.5	50.0	23.4	26.6	24.1	31.9
SE	0.5	0.4	0.8	10.8	6.2	7.3	6.5	6.8
Lipid								
Mean	9.3	−1.4	10.7	52.4	21.0	31.4	22.4	31.7
SE	1.2	0.6	1.7	2.4	2.4	2.3	2.7	3.4

Unit, μmol·limb^{-1}·h^{-1}. *$P < 0.05$ versus the propofol group. PS is protein synthesis reflected by phenylalanine Rd; PB, protein breakdown reflected by phenylalanine Ra; F_{in}, the inflow of the amino acid via the arterial blood; $F_{M,A}$, the rate of transport of the amino acid from the arterial blood to the muscle intracellular free pool; $F_{V,A}$, the rate of delivery from the artery to the vein; $F_{V,M}$, the rate of transport from the muscle intracellular free pool to the venous blood; Ra_T is the total rate of appearance into the muscle intracellular free pool, which is equal to the sum of inward transport ($F_{M,A}$) plus protein breakdown.

Table 5 Leucine net balance and oxidation rate in the limb

	Leucine Net Balance	Phenylalanine Net Balance × C	Non-incorporaton Leucine Disposal
Ketamine	1.8 ± 0.9	−7.6 ± 0.7	9.3 ± 0.9*
Propofol	3.2 ± 0.8	−1.6 ± 1.0	4.9 ± 0.7
Lipid	4.3 ± 0.8	−3.3 ± 1.5	7.7 ± 1.9

Data are means ± SE. Unit, μmol·limb^{-1}·h^{-1}. *$P < 0.05$ versus the propofol group. Leucine net balance and phenylalanine net balance were calculated from the arteriovenous concentration difference and blood flow rate. C is the ratio of leucine content/phenylalanine content in muscle protein. Non-incorporation leucine disposal is used as an indicator of leucine oxidation.

The content of phenylalanine and leucine in 1 mg of muscle protein was 0.250 ± 0.002 and 0.603 ± 0.009 μmol, respectively. The measured leucine net balance and leucine oxidation rate in the limb are presented in *Table 5*. In the ketamine group, the skeletal muscle oxidized significantly ($P < 0.05$) more leucine than in the propofol group, and the value in the lipid group was intermediate.

Discussion

This study was designed to compare the effect of ketamine versus propofol anesthesia on protein metabolism. The surgical procedures performed in this study (i.e., tracheotomy, right femoral vessels catheterization, and left femoral vessels dissection) could cause stress responses. However, this source of stress cannot explain the metabolic differences among the groups because all the rabbits in the three groups received the same procedures. Thus, our results indicate that ketamine anesthesia is more catabolic than propofol anesthesia.

To compare metabolic effects between anesthetics, it is important to ensure that the rabbits were maintained at a similar depth of anesthesia. In this study we titrated the ketamine or propofol infusion rate to maintain a comparable depth of anesthesia in all rabbits as indicated by stable vital signs (see *Table 1*), lack of movement during the isotope infusion, and requirement of local lidocaine infiltration when dissecting the femoral vessels. A difficult issue in the comparison of these two anesthetics is their opposite effects on respiration. Propofol could depress the ventilatory response to hypoxia in volunteers,[12] suggesting that supplemental oxygen should always be supplied. In contrast, an infusion of ketamine (1 mg/kg) during vaginal deliveries did not change either maternal or infant arterial blood gas values.[19] In comparison with halothane, a continuous infusion of ketamine produced higher arterial oxygen values (Pao_2).[20] Therefore, the rabbits in the propofol group, but not the ketamine groups, received oxygen through an open

ventilator. This procedure prevented hypoxia, as reflected by the monitored oxygen saturation in the skin surface microcirculation. The rabbits in the ketamine and ketamine + lipid groups spontaneously breathed room air because they did not need extra oxygen. The values of arterial lactate concentration (1.0 to 1.2 mmol/L, *Table 2*) indicated aerobic metabolism in all groups. Because ketamine is believed to stimulate sympathetic nervous system activity,[21] whereas propofol is a cardiovascular depressant,[22] the significantly higher rectal temperature and heart rate in the ketamine group in comparison with the propofol group do not necessarily indicate different depth of anesthesia. Under these experimental conditions, ketamine showed a strong catabolic effect on muscle and whole body protein in comparison with propofol. The catabolic effect of ketamine was attenuated, but not totally eliminated, by the infusion of fat emulsion.

The limb A-V balance method demonstrated significantly greater protein breakdown rate and net protein loss in the ketamine group in comparison with the propofol group (*Table 4*). The data of whole body protein breakdown rate (*Figure 2*), as reflected by phenylalanine flux, were consistent with the A-V balance data, supporting a catabolic effect of ketamine anesthesia. The rates of protein synthesis derived from the A-V balance method were not significantly different among the groups, although the average value was the highest in the ketamine group, lowest in the propofol group and intermediate in the lipid group. Because the limb A-V balance data could reflect a minor, but not negligible, contribution from the tissues other than the skeletal muscle (mainly the skin),[23] we used the direct tracer incorporation method to check the muscle protein synthesis rates. The close values of muscle protein FSR in the three groups (0.089, 0.089, and 0.081%/h) confirmed that the muscle protein synthesis rates were not significantly different. A stimulation of protein breakdown, with the absence of a change (or possibly increase) in protein synthesis, appears to be a general stress response, as this is the same response in patients with protein catabolism such as burns.[2]

Our conclusions were supported by the leucine oxidation data. At the whole body level, the rate of leucine oxidation has been used as an indicator of net protein loss.[14] The same rationale applies to the limb A-V system. The significantly greater oxidation rate of leucine in the ketamine group as compared to the propofol group and the intermediate value in the lipid were in agreement with the limb phenylalanine net balance data.

In the lipid group, the values of muscle protein breakdown, net protein balance in the muscle, whole body protein breakdown, and peripheral leucine oxidation were all intermediate, suggesting attenuated protein breakdown rates by the infusion of fat emulsion. Statistical analysis showed that in the lipid group the four catabolic parameters were not significantly different from those in the ketamine and propofol groups. Therefore, the significantly lower protein catabolism in the propofol group was, to some extent, caused by the lipid solvent in the propofol preparation. The protein-sparing effect of fat emulsion has been reported by several investigators.[24–26] The mechanism could involve changes in cellular redox. Tessari et al.[26] found that during the infusion of nicotinic acid, which inhibits the availability of plasma free fatty acids, both leucine flux and oxidation were increased. Because nicotinic acid decreases the hepatic reduced nicotinamide-adenine dinucleotide/nicotinamide-adenine dinucleotide (NADH/NAD) ratio[27] and the plasma β-hydroxybutyrate/acetoacetate ratio,[26] and the activity of the branched chain alpha-ketoacid dehydrogenase complex[28] and the rates of proteolysis[29] have been linked to alternations in the NADH/NAD ratio, they proposed that the changes in cellular redox could account for the observed changes in leucine metabolism.

The rabbits in this study received a fixed dose of Travasol solution, which minimized the possible stress due to the overnight fasting. The infusion of the amino acid solution raised arterial phenylalanine concentrations to twice the value (0.0475 mmol/L) we previously reported in postabsorptive rabbits.[13] There were no significant differences either in the blood and muscle free phenylalanine concentrations (*Tables 2* and *3*) or in the transport parameters (*Table 4*). Thus, the amino acid supply was sufficient in all groups, and the increased rate of muscle protein breakdown in the ketamine group was not due to a deficiency of amino acids. It is well established that ketamine stimulates sympathetic nervous system activity. A body of evidence supports that ketamine produces its sympathomimetic actions primarily by direct stimulation of central nervous system (CNS) structure.[21] However, it is unknown if the catabolic effect of ketamine observed in this study was caused by a direct effect on CNS structures or mediated by counterregulatory hormones.

In general, the animals in all groups in this study had a trend of catabolism as shown by different magnitudes of negative protein balance in the limb. This may indicate an overall catabolic response to general anesthesia as well as surgery, because in the normal conscious human, the infusion of amino acids causes a positive limb phenylalanine balance.[30] Further investigations are necessary to clarify the mechanisms by which the anesthetic agents exert a catabolic effect on protein. Whereas both ketamine and propofol are popular anesthetics in the intensive care unit and trauma/burn surgery, the present study suggest that ketamine is not an optimal anesthetic in catabolic patients because of its catabolic effect on muscle protein. Propofol is less catabolic in comparison with ketamine. This may have to be taken into consideration in the treatment of critically ill patients under severe catabolic stress, especially when prolonged and repeated surgical procedures are required.

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