Hypothermic Anesthesia Attenuates Postoperative Proteolysis

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The catabolic response that commonly occurs after major operation is characterized by net skeletal muscle proteolysis and accelerated nitrogen excretion. This response was absent in patients undergoing cardiac surgical procedures associated with the combination of cardiopulmonary bypass, narcotic anesthesia, neuromuscular blockade, and hypothermia. Forearm nitrogen release was 422 ± 492 nmol/100 ml · min on the first postoperative day, approximately 25% of preoperative values (1677 ± 411, $p < 0.05$). Nitrogen excretion and the degree of negative nitrogen balance were comparable to levels observed in nonstressed, fasting subjects. The potential role of hypothermia, high-dose fentanyl anesthesia, and neuromuscular blockade in modifying the catabolic response to laparotomy and retroperitoneal dissection was further evaluated in animal studies. Six hours after operation, amino acid nitrogen release from the hindquarter was 84% less than control values ($p < 0.05$). Nitrogen excretion and urea production were also reduced compared to normothermic controls. It is concluded that the combination of hypothermia, narcotic anesthesia, and neuromuscular blockade attenuates the catabolic response to injury and thus may be useful in the care of critically ill surgical patients.

MAJOR OPERATIONS, traumatic injury, and sepsis initiate a well-described sequence of metabolic events that results in the dissolution of lean body mass and progressive weight loss. Central to the catabolic response to critical illness is an accelerated loss of body protein, reflected by increased excretion of urinary nitrogen and prolonged negative nitrogen balance.[1,2] This response is associated with increased release of amino acids from skeletal muscle[3–6] and accelerated amino acid uptake in visceral organs.[7,8] As a result of their metabolism by the liver, kidneys, and gastrointestinal tract, the amino acids are converted in large part to urea and ammonia and excreted from the body.

The loss of body proteins is related to the severity and duration of the illness[9]; in injured or septic patients requiring prolonged hospital care, protein deficiencies may delay wound healing,[10] attenuate host responses to infection,[11] and prolong or impair recovery.[12] To offset these catabolic responses, patients receive vigorous enteral or parenteral nutritional support, which stabilizes body weight and attenuates net nitrogen loss.[9] In general, the rate of breakdown of body protein is not greatly altered by feeding these catabolic subjects; however, *net* protein loss is reduced as protein synthesis increases.[13] Even when nutrition is provided throughout the perioperative period, the protein catabolic response to a major operative procedure is not prevented.[6] These observations are consistent with reports that vigorous enteral or parenteral feedings in catabolic hospitalized patients only partially offset the nitrogen loss following catabolic illness.[14,15]

Because of the deleterious effects of protein catabolism in critically ill patients, a major investigative effort has focused on techniques that may minimize this response. Both insulin[16] and growth hormone,[17] when administered to critically ill patients, have been shown to significantly reduce net nitrogen catabolism. More recently, investigators have studied the protein-sparing effects of amino acid solutions supplemented with additional branched chain amino acids.[18] Although initial reports suggested that these specialized solutions minimize protein catab-

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TABLE 1. *Characteristics of Patients Studied (Mean and Range)*

	Forearm Flux Studies (N = 11)	Forearm Flux and Nitrogen Balance Studies (N = 7)
Age (years)	59 (48–69)	61 (52–78)
Sex (M/F)	9/2	3/4
Procedure*	1 MVR 10 CABG	3 MVR 3 AVR 1 Mult VR
Height (cm)	175 (168–180)	173 (154–193)
Weight (kg)	77.8 (57.1–90.0)	74.1 (50.0–106)
Pump time (min)	61 (42–87)	93† (62–135)
Lowest intravascular temperature (C)	29.0 (28.0–30.0)	26.9‡ (25.0–28.6)

* MVR = mitral valve replacement; AVR = aortic valve replacement; CABG = coronary artery bypass graft; Mult = multiple valve replacement.
† $p < 0.02$ by unpaired t-test when compared with other group.
‡ $p < 0.05$ by unpaired t-test when compared with other group.

olism in critically ill patients, clear-cut benefits have not been observed in comparison with standard amino acid solutions.[19] As an alternative approach, the catabolic response following operative procedures on lower extremities or in the lower abdomen can be modified by neurogenic blockade, utilizing epidural anesthesia extended to the high thoracic level.[20] This prevents the usual postoperative increase in blood glucose, cortisol, and stress hormones and attenuates postoperative nitrogen losses.[21] This approach may not affect postoperative metabolic responses in patients requiring operations in the upper abdomen or thorax. Net protein catabolism has also been somewhat reduced in animals following alpha and beta adrenergic blockade[22] or with administration of a prostaglandin synthesis inhibitor.[23]

We recently observed that the expected catabolic response did not develop in a group of patients who underwent major cardiac surgical procedures. The intraoperative management of all of these individuals included cardiopulmonary bypass, hypothermia, high-dose narcotic anesthesia, and neuromuscular blockade. To examine this unexpected response further, studies were performed in laboratory dogs in which surgical stress was standardized, allowing comparison of the protein catabolic response following hypothermic anesthesia with that occurring after narcotic or barbiturate administration. In experimental animals, as well as humans, the combination of hypothermia and anesthesia appears reliably to attenuate posttraumatic proteolysis. This report includes both observations in patients undergoing cardiac surgical procedures and the results of subsequent animal studies.

Materials and Methods

Patient Studies

Patients. Eighteen patients undergoing elective or semielective cardiac surgical procedures were studied. Ten had coronary artery bypass grafting (CABG) for stable angina, and eight had cardiac valve replacement. All patients were clinically stable, and none demonstrated overt signs of congestive heart failure or chronic cardiac decompensation associated with cachexia. All subjects were within 12% of ideal body weight. None of the patients had diabetes mellitus, and all were on weight-maintaining diets containing at least 35% of energy as carbohydrate. All patients received indicated medications, including digitalis preparations, diuretics, nitroglycerine, and beta-blocking agents. The dosage and timing of drug administration were determined by the patients' physicians, and, therefore, drug administration was not standardized in relation to the studies performed.

Experimental protocols. Protocols were approved by the Human Subjects Committee of the Brigham and Women's Hospital. Two groups of patients were studied. In the first group of 11 patients, forearm amino acid and substrate balance were determined. The investigations were performed in the early morning, 1 day before operation and on postoperative days 1, 3, and 5 (POD 1, 3, 5). Ten of these 11 patients underwent CABG procedures, one had a valve replacement, and all were discharged within 10 days of operation (Table 1).

Following review of the data from this study, a second group of seven individuals who did not require beta-blocking agents was evaluated to determine the possible influence of these drugs.[22,24] Subjects in this group were of similar age and weight in comparison with the first group, but the majority had valve replacement procedures (Table 1). In addition to forearm flux studies, nitrogen balance measurements were also carried out in this group.

Operative procedures and perioperative care. All subjects were intubated and ventilated following high-dose narcotic–nitrous anesthesia and muscle relaxation. The total dose of morphine administered to each individual during the operation was 1–2 mg/kg; the fentanyl dose was 50–100 μg/kg. When placed on cardiopulmonary bypass, the blood was cooled to approximately 28 C. The subjects' mean core temperatures fell to 32–34 C, as determined by esophageal temperature probes. The length of time on cardiopulmonary bypass and the lowest blood temperature achieved varied depending on the operation performed; patients undergoing CABG procedures required less time on cardiopulmonary bypass than subjects undergoing valve replacement. As a result, the subjects undergoing valve replacement reached slightly lower core temperatures than those undergoing CABG procedures.

All patients received a crystalloid cardioplegia solution and were rewarmed to 37 C before removal from bypass.

Following the operative procedure, the patients were transferred to the intensive care unit and maintained on a ventilator for the next 18–24 hours. The agents given at the time of operation were not pharmacologically reversed, and small quantities of intravenous narcotics were administered as required to achieve pain control. All patients were extubated the day following operation.

During the initial 24 hours following operation, colloid and packed red cells were administered to correct signs of hypovolemia. Intravenous fluids consisted of 5% dextrose in water infused at rates not exceeding 500 ml/24 h. Arterial and intravenous lines were removed between 24 and 48 hours following operation. On POD 1, the patients were offered a liquid diet, which was gradually advanced to a hospital diet containing 2 g of sodium per day. By 48 hours, all patients were transferred to an intermediate care area where they were managed with oral pain medications and other drugs as required for their specific postoperative care. There were no complications, and all subjects were discharged from the hospital by the tenth postoperative day.

Forearm balance technique. All studies were performed between 6 and 7 A.M. after an overnight fast, as previously described.[25] Under local anesthesia, a catheter was placed in retrograde fashion in an antecubital vein and in a dorsal hand vein for sampling of arterialized venous blood using the hot-hand technique,[26] unless an arterial line was already in place. After waiting at least 30 minutes following catheterization and the placement of a capacitance plethysmograph, a wrist cuff was inflated to exclude blood flow to the hand, and, after at least 2 minutes, blood samples were drawn simultaneously from the arterial or arterialized-venous line and the deep venous catheter. The catheters were then flushed, and forearm blood flow was measured, as previously described.[25] The wrist cuff was then deflated, the circumference of the forearm measured, and the catheters removed.

Nitrogen balance studies. On the day before operation and on POD 1–5, all urine was collected for 24-hour periods from 7 A.M. to 7 A.M. The volume was measured, and the concentration of total nitrogen, urea, and creatinine determined for each 24-hour period. The quantity of all oral intake was also determined during the same time periods. Fixed quantities of foods of known composition were provided, and the calorie and nitrogen intake was calculated from *ad lib.* intake. The quantity of all intravenous fluids was recorded, and the calorie content from this source was included in the intake calculation. The nitrogen content of administered albumin and plasma was included in the nitrogen intake calculation, but not the nitrogen content of administered whole blood. Stool was not collected, but, when stool was passed, an assumed loss of 1.3 g/day was included in the balance calculations.[27] No subjects passed stools before the third postoperative day. Most of the blood and serum draining from the chest tubes was lost on the day of operation, and, therefore, chest tube drainage could not be included in the balance calculation on POD 1. The tubes were removed between 24 and 48 hours after operation.

Control subjects. Forearm substrate and amino acid flux was measured in 13 normal subjects (average age: 29 years, average body weight: 75.7 kg) after a 12-hour overnight fast. The results of these studies have been previously reported in part[25] and are included to provide normal values for forearm substrate exchange.

Animal Studies

Animal preparations. Conditioned male and nonpregnant female mongrel dogs weighing between 20 and 35 kg were studied. They were housed at the Harvard Medical School animal facility in individual kennels, exercised each morning, provided water *ad lib.*, and given a single daily ration of Respond 2000 Dry Dog Chow (Pro-Pet, Syracuse, NY, containing no less than 25% protein by weight). The animals were obtained 5–7 days prior to any studies to allow them to acclimate to the kennel and laboratory conditions, and to be trained to stand quietly in Pavlov slings. All food was removed at 5:00 P.M. on the evening before both basal studies and operation.

Basal studies. All animals in both control and experimental groups were brought to the laboratory 2 days prior to operation, where a biopsy of the vastus lateralis muscle of one leg and arterial blood were obtained under anesthesia, as previously described.[28–30]

Operative procedure.

Control group (N = 6). Following at least 2 days of recovery from the biopsy procedure, the animals were fasted overnight and then taken to the operating room. Anesthesia was induced with sodium pentobarbital (Abbott Labs, N. Chicago, IL; 30 mg/kg, IV), an endotracheal tube was placed, and the animals were allowed to breathe a mixture of room air and oxygen (provided at 5 L/min) throughout the entire operation. A 16-gauge catheter was placed percutaneously into the superior vena cava *via* the external jugular vein. This time was designated as T = 0. An infusion of normal saline was started by constant infusion pump (IVAC, San Diego, CA; 4 ml/h/kg) and continued throughout the 24-hour study period. Keflin (Lilly, Indianapolis, IN; 1 g, IV) was given before and immediately after operation. The urinary bladder was catheterized, and, after the residual urine was discarded, a 24-hour collection was carried out by closed drainage. The abdomen and flanks were shaved and prepped with a povidone iodine solution (Clinipad, Guilford, CT).

Laparotomy was then performed *via* a low midline incision in females and a right paramedian incision in males,

as previously described.[29,30] The retroperitoneum was dissected, exposing the aortic bifurcation and tributaries. Specially prepared catheters were inserted into the internal iliac and deep circumflex iliac arteries. The circumflex vein was cannulated, and the catheter tip positioned in the inferior vena cava below the renal veins. In this way, the blood supply to the hindquarter, which accounts for approximately 50% of total skeletal muscle by weight, was isolated. All catheters were exteriorized through a stab incision in the flank, capped with blunt needles and injection ports (Jelco, Critikon, Tampa, FL), flushed with heparinized saline (5 units/ml), and buried subcutaneously. This allowed easy access to aortic and vena caval blood by percutaneous puncture. After all incisions were closed, the animals were allowed to recover spontaneously from anesthesia. Body temperature was maintained with heating blankets, and extubation was performed when appropriate.

Hypothermia group (N = 6). After placement of a foreleg intravenous cannula, anesthesia was induced with sodium thiopental (5 mg/kg, IV) followed by pancuronium bromide (Organon, West Orange, NJ; 0.25 mg/kg, IV) and fentanyl (Janssen, New Brunswick, NJ; 75 μg/kg, IV over 5 min). Fentanyl was then infused at 0.3–0.6 g/min/kg throughout the period of cooling, operation, and rewarming. An additional dose of pancuronium was given just prior to the start of operation. The animals were then intubated and mechanically ventilated with a Harvard animal ventilator (Harvard Apparatus, Millis, MA) at a tidal volume of 15 ml/kg and a rate of 15 breaths/min. After placing the animals in plastic bags to keep their fur dry, they were immersed in an ice water bath and cooled to a core temperature of approximately 28 C in approximately 132 minutes as determined by an esophageal temperature probe. The animals then underwent the same operative procedure as the previous group, with the start of the procedure designated as T = 0. Following operation, the dogs were rewarmed in a 42 C water bath. All animals were rewarmed to at least 37 C core temperature before the fentanyl drip and mechanical ventilation were discontinued. During rewarming, blood pressure and heart rate were monitored with one of the aortic catheters connected to a pressure transducer. Arterial blood was obtained for blood gas determination. The animals were warm, awake, and in the Pavlov sling before the hindquarter flux studies were performed 6 hours after starting the operative procedure.

Anesthesia controls (N = 3). A third and smaller subset of dogs was studied to determine the effect of high dose fentanyl and neuromuscular blockade on the responses following operation. These animals were anesthetized and paralyzed as described for the group undergoing hypothermia, but they were not cooled prior to operation. Fentanyl anesthesia was continued throughout the operative procedure.

Flux studies. Approximately 5 hours after the start of the experiment, the dogs were awake and placed in Pavlov slings. Para-amino-hippurate (PAH, 0.5% in saline) was then infused at a rate of 0.76 ml/min into the distal aortic catheter with a Harvard pump. After 40 minutes of dye infusion, simultaneous arterial and venous samples were drawn in triplicate at 10-minute intervals for determination of hindquarter flux, as previously described.[30,31] The catheters were then flushed with heparinized saline, and the animals were maintained in a Pavlov sling for the ensuing 18 hours. At T = 24 hours, the flux studies were repeated and the urine collection terminated. A repeat hindlimb muscle biopsy was then performed under sodium thiopental anesthesia, using the leg not previously biopsied. The animals were placed in metabolic cages in order to monitor recovery.

Analytic Methods

Urine. Urine was collected in an acidified refrigerated container, measured into aliquots, and stored frozen at −20 C for later batch analysis. The total nitrogen content was determined by the macro-Kjeldahl method.[31] Urine urea and creatinine were measured on an autoanalyzer (Technicon, Tarrytown, NY).

Blood/plasma. Aliquots of blood and plasma were deproteinized by adding an equal volume of ice-cold 10% perchloric acid and centrifuging at 3000 rpm for 20 minutes at 4 C. A 2 ml aliquot of this supernatant was combined with sodium acetate buffer, adjusted to pH 4.75–4.90 with 5 N potassium hydroxide, brought to a final volume of 4 ml with distilled water, and stored at −20 C for later batch analysis. Glutamine and glutamate were measured by an enzymatic, microfluorometric assay.[32] Other amino acids were measured on a Beckman Model 6300 Amino Acid Analyzer (Beckman Instruments, Palo Alto, CA) or by high performance liquid chromatography (HPLC) after precolumn derivatization with o-phthalaldehyde.[33] The two methods yielded comparable results.

Arterial and venous samples were analyzed for PAH by a spectrophotometric method[29] following deproteinization with 5% trichloroacetic acid. Whole blood and plasma glucose levels were determined in duplicate on a Technicon autoanalyzer. Plasma insulin was determined by radioimmunoassay.[34]

Muscle. Muscle tissue was obtained percutaneously using a Bergstrom needle.[28] The tissue samples were processed according to previously described methods.[29] Free amino acid concentrations per unit of muscle wet-weight were determined using the above-mentioned enzymatic methods for glutamine and glutamate and high performance liquid chromatography for the remainder of the

amino acids.[33] Concentrations in intracellular water were then determined using measured plasma and muscle chloride values and the Nernst equation.[28]

Calculations

Forearm blood flow was determined as the mean of 3–5 determinations. In human studies, the volume of the forearm was calculated from measurements of circumference, and flow was expressed per 100 ml forearm volume. In the dog, hindquarter blood flow was measured by PAH dye dilution and calculated as previously described.[29,30] Blood flow was divided by body weight to correct for variations in animal size.

Amino acid exchange was calculated as the product of blood flow and whole blood arteriovenous concentration differences. In the dog studies, three sets of samples were drawn at both the 6- and 24-hour time points. Flux was calculated for each set, and the mean of the three values was determined. The concentrations of total amino acid nitrogen and branched chain amino acids was calculated by multiplying the value for each amino acid by the number of nitrogens contained and summing these values. Flux data were derived in a similar fashion.

Urea production was calculated in the dog by correcting the total urea nitrogen excretion over 24 hours for any change in plasma urea nitrogen, assuming that urea is distributed in total body water (approximately 58.4% of body weight in the dog).[35] These values were expressed per hour and corrected for animal weight.

Statistical Analysis

Statistical calculations were performed using a standard statistical package (Minitab, Pennsylvania State University, State College, PA, 1983, or AppleStat, Prentice-Hall, Englewood Cliffs, NJ, 1984). Results are expressed as mean ± SEM. Paired and unpaired Student t-tests were used when appropriate, and regression analyses were performed using the method of least squares. Because of the small sample size in the group of animals receiving fentanyl anesthesia without hypothermia, most statistical comparisons were only performed between the other groups. Forearm studies were not performed in all patients at all time points. Therefore, the rank sum test was utilized for the comparison of these data. Differences for all tests were considered significant at the $p < 0.05$ level.

Results

Patients

Nitrogen excretion, intake, and balance. Urinary nitrogen excretion ranged from 5.3 to 13.5 g/day during the preoperative period; the quantity of nitrogen excreted was primarily related to body size and the amount of dietary protein consumed. Urinary nitrogen excretion decreased approximately 30% on POD 1 and then rose with time to return to preoperative levels by POD 5 (Table 2). Blood urea nitrogen level remained stable throughout hospitalization. Nitrogen intake fell following operation. On POD 1, intake averaged 5–6 g/24 hours, with approximately one third of this quantity provided by albumin administration. (This quantity averaged 2 g nitrogen/patient on POD 1). Only one individual received plasma (equivalent to 1 g of nitrogen) on POD 2, and none was administered thereafter. Dietary protein intake gradually increased and returned to normal levels by POD 5.

TABLE 2. *Nitrogen Intake, Excretion, and Balance in Patients (g/day · m²) (Mean ± SEM)*

	Nitrogen Intake	Nitrogen Loss	Nitrogen Balance
Preoperative	5.6 ± 0.4	6.0 ± 0.6	−0.4 ± 0.5
POD 1	3.1 ± 0.7*	4.2 ± 0.5*	−1.2 ± 0.8
POD 2	2.0 ± 0.8*	4.4 ± 0.7	−2.4 ± 0.9
POD 3	3.8 ± 0.9	5.2 ± 0.2	−1.4 ± 0.8
POD 4	4.5 ± 0.6	5.3 ± 0.6	−0.8 ± 0.5
POD 5	6.1 ± 0.8	5.8 ± 0.2	+0.3 ± 0.9

* $p < 0.05$ when compared with preoperative value by paired t-test corrected for multiple comparisons.

Nitrogen balance was negative the first 4 days after operation and returned to balance by POD 5. The average cumulative nitrogen loss was 5.9 ± 1.9 g/m² over 5 days (10.8 g/person over 5 days). If the nitrogen contained in the infused albumin and/or plasma was excluded from the nitrogen balance calculation, cumulative losses averaged 13 g/person over 5 days (the protein equivalent of a lean 12 oz. steak). For all days studied, nitrogen balance was closely related to nitrogen intake ($r^2 = 0.77$).

Forearm flux studies. Arterial whole blood amino acid levels were slightly higher than control in 13 individuals studied in the preoperative period. Following operation, arterial amino acid concentrations fell approximately 25% (Table 3). This was accounted for by a significant ($p < 0.05$) decrease in glutamine and BCAA (Table 3) and also a decrease in threonine, serine, asparagine, proline,

TABLE 3. *Arterial Whole Blood Amino Acid Concentrations in Patients Undergoing Cardiac Operations (Mean ± SEM)*

Day	N	Total Nitrogen (μmol/L)	Alanine (μmol/L)	Glutamine (μmol/L)	Total BCAA (μmol/L)
Preoperative	13	4994 ± 150	341 ± 22	594 ± 35	449 ± 25
POD 1	9	3795 ± 127*	314 ± 21	471 ± 32*	312 ± 27*
POD 3	8	4175 ± 884*	264 ± 13	482 ± 42	444 ± 33
POD 5	10	4479 ± 106	284 ± 16	523 ± 33	482 ± 24
Controls	13	4238 ± 96†	253 ± 13†	520 ± 15	325 ± 19†

* $p < 0.05$ when compared with preoperative values by rank sum test.
† $p < 0.05$ different from preoperative controls by unpaired t-test.

TABLE 4. *Summary of Cardiac Surgical Patients (Mean ± SEM)*

	Preoperative	POD 1	POD 3	POD 5	Controls
Forearm blood flow (ml/100 ml • min)	3.80 ± 0.39	3.37 ± 0.36	3.12 ± 0.57	2.77 ± 0.30	3.00 ± 0.31
Whole blood amino acid nitrogen flux (nmol/100 ml • min)	−1677 ± 411	−422 ± 492*	−739 ± 357	−1027 ± 236	−1202 ± 321
Glutamine flux (nmol/100 ml • min)	−362 ± 95	−141 ± 79*	−322 ± 135	−193 ± 111	−135 ± 126
Alanine flux (nmol/100 ml • min)	−233 ± 45	−150 ± 63*	−154 ± 45	−172 ± 26	−146 ± 23
Total BCAA flux (nmol/100 ml • min)	−165 ± 60	−37 ± 57*	−42 ± 83	−128 ± 37	−186 ± 58
Arterial blood glucose (mg/dl)	105 ± 12	137 ± 15	116 ± 9	110 ± 6	93 ± 2
A–V glucose (mg/dl)	0 ± 1	−3 ± 2	−1 ± 2	3 ± 2	3 ± 1†
Insulin (μU/ml)	24 ± 5	41 ± 6	45 ± 21	22 ± 6	12 ± 1†

* $p < 0.02$ when compared to preoperative value by rank sum test.

† $p < 0.05$ different from preoperative values by unpaired t-test.

citrulline, ornithine, histidine, and arginine (data not shown). These levels gradually returned to normal by POD 5.

Forearm blood flow was within the normal range (approximately 2–4 ml/100 ml • min) in the preoperative period and did not change throughout the study (Table 4). Forearm amino acid flux was −1677 ± 411 nmol/100ml • min, similar to rates of skeletal muscle amino acid release observed in normals. Following operation, forearm amino acid release decreased to −422 ± 492, about one fourth of the preoperative release rate, and then gradually increased with time to return to the preoperative level of amino acid efflux. The decrease in forearm amino acid exchange was related to a generalized decrease in the release of all amino acids measured and could not be specifically related to a fall in alanine, glutamine, or BCAA release.

Blood glucose and insulin tended to rise following operation, but the responses were not consistent and, thus, the observed changes did not achieve significance. Forearm glucose uptake was negligible and could not be distinguished from zero at any of the time points.

TABLE 5. *Hindquarter Blood Flow and Core Temperature (Mean ± SEM)*

	Normothermic Controls		Hypothermia
	Barbiturate (N = 6)	Fentanyl (N = 3)	Fentanyl (N = 6)
Weight (kg)	26.9 ± 2.2	22.5 ± 0.2	25.2 ± 1.4
6-hour blood flow (ml/min • kg)	32.4 ± 6.6	22.2 ± 4.5	21.7 ± 5.0
24 hour blood flow (ml/min • kg)	52.7 ± 9.8	*	46.7 ± 3.2
Mean core temperature (C)			
T = 0 hour	38.9 ± 0.2	39.3 ± 0.1	27.6 ± 0.5
T = 2 hours	—	—	27.5 ± 0.5
T = 6 hours	38.8 ± 0.2	38.9 ± 0.1	37.0 ± 0.2

* Insufficient data.

Animal Studies

General. Animal weight was comparable in all groups (Table 5). At 6 hours after operation, blood flow to the hindquarter tended to be lowest in the hypothermia group, but this difference was not significant. There was little difference in blood flow between groups at 24 hours. All animals survived the operative procedure and the 24-hour study period. Intraoperative hemorrhage was minimal in all animals studied. There were no apparent long-term complications in the dogs undergoing the period of hypothermia.

Surface cooling required an average of 2.2 ± 0.3 hours. The operation lasted 2 hours, and rewarming required 3.7 ± 0.1 hours. The mean temperature of the hypothermic group was 27.6 ± 0.5 C, and this temperature was maintained throughout the operation without further intervention (Table 5). Associated with the hypothermia was mild bradycardia (60 ± 3 beats/minute *vs.* 100–120 beats/minute in euthermic animals). Blood oxygen partial pressure was slightly elevated and pH was normal in all animals.

Nitrogen excretion. During the 24-hour study period, total urinary nitrogen excretion tended to be lower in the animals that received perioperative hypothermia (Table 6), although the difference was not statistically significant. Urea nitrogen excretion also tended to be reduced in the

TABLE 6. *24-Hour Urine Volume and Nitrogen Excretion in Animals (Mean ± SEM)*

	Normothermic Controls		Hypothermia
	Barbiturate	Fentanyl	Fentanyl
Urine volume (ml/kg)	43.1 ± 8.3	43.5 ± 4.6	60.5 ± 5.9
Urea nitrogen (g/kg)	0.41 ± 0.02	0.37 ± 0.01	0.34 ± 0.03
Urea nitrogen production (mg/h kg)	15.0 ± 0.8*	10.9 ± 0.4	11.4 ± 1.3
Creatinine (g/kg)	0.039 ± 0.002	0.033 ± 0.001	0.027 ± 0.003*
Total nitrogen (g/kg)	0.50 ± 0.02	0.47 ± 0.01	0.43 ± 0.04

* $p < 0.05$ when compared to other two groups by analysis of variance.

hypothermia group. However, urea nitrogen production was significantly reduced in animals receiving fentanyl anesthesia and neuromuscular blockade ($p < 0.05$), irrespective of body temperature. Creatinine excretion over the 24-hour study period was significantly diminished in the hypothermic animals.

Whole blood amino acid concentrations. Total blood amino acid nitrogen concentration fell by 6 hours after operation in all groups (Table 7). By 24 hours the normothermic group had again reached preoperative levels, but the hypothermia group continued to lag and was significantly less than control. These changes were reflected in the concentrations of two of the major nitrogen-carrying amino acids in whole blood, glutamine, and alanine. While glutamine fell to very low levels at 6 hours, it returned to preoperative levels by 24 hours. Alanine, however, remained low at this time point, accounting for a portion of the delay in return of total nitrogen to normal (Table 7). The fentanyl-treated animals demonstrated responses that were intermediate in comparison with the other two groups.

Hindquarter flux. Total amino acid nitrogen release from hindquarter skeletal muscle was markedly reduced at 6 hours following initiation of operation in the hypothermia group compared to controls (Table 8). This difference was accounted for, in large part, by the reduced efflux of glutamine and alanine. Arginine, histidine, and glycine efflux were also significantly reduced (data not shown). Total BCAA flux changed from net muscle release in the control group to balance in the hypothermia group. The fentanyl-treated animals appeared to be intermediate to the other two groups. At 24 hours, all groups had similar rates of nitrogen release, although wide variations were observed in the control data (Table 8). Glutamine, alanine, and total BCAA flux were comparable in all groups at this time point.

Muscle glutamine concentrations. Intracellular glutamine fell significantly in response to surgical stress in the control group (21.18 ± 2.64 mmol/L to 16.65 ± 3.20, $p < 0.05$). Glutamine concentrations also tended to decrease in the hypothermia group, but to a lesser degree (21.36 ± 1.11 to 17.84 ± 2.25, N.S.). The overall change in glutamine concentration over 24 hours was not different between the two groups. The fentanyl-treated animals responded in a similar manner.

TABLE 7. *Whole Blood Amino Acid Concentrations (Mean ± SEM)*

		Normothermic Controls		
		Barbiturate	Fentanyl	Hypothermia
Total amino acid nitrogen (mmol/L)	Pre	4.62 ± 0.28	4.18 ± 0.20	4.17 ± 0.06
	6 h	3.73 ± 0.10*	3.47 ± 0.16	3.27 ± 0.14*
	24 h	4.69 ± 0.22	—	3.93 ± 0.11
Glutamine (μmol/L)	Pre	712 ± 42	626 ± 10	630 ± 28
	6 h	542 ± 46*	630 ± 51	379 ± 19*
	24 h	735 ± 54	725 ± 53	637 ± 33
Alanine (μmol/L)	Pre	415 ± 35	304 ± 34	369 ± 20
	6 h	247 ± 14*	273 ± 21	307 ± 17
	24 h	312 ± 43	—	228 ± 18*

* $p < 0.05$ when compared with preoperative values by paired t-tests.

Discussion

Injury, infection, and major operations are associated with increased net protein catabolism and total body nitrogen loss.[2,13] Coincident with the net breakdown of body protein, there is increased amino acid release from skeletal muscle[3-6] and a fall in the skeletal muscle free amino acid pool.[36] In particular, the two amino acids glutamine and alanine are released from muscle and appear to function in the transportation of nitrogen to visceral organs. This translocation of amino acids from skeletal muscle to visceral organs and the utilization of these compounds for gluconeogenesis, for synthesis of acute phase proteins, and as oxidizible fuels leads to accelerated urea production and the loss of nitrogen from the body.

In this study, patients undergoing major cardiac operations in the setting of total cardiopulmonary bypass and moderate hypothermia failed to develop the characteristic protein catabolic responses usually observed in

TABLE 8. *Hindquarter Flux Data (μmol/min · kg)*

	6 Hours			24 Hours	
	Normothermic Controls				
	Barbiturate	Fentanyl	Hypothermic Fentanyl	Normothermic Barbiturate	Hypothermic Fentanyl
Total nitrogen	−16.67 ± 4.10	−6.0 ± 1.1	−2.70 ± 0.50*	−4.51 ± 9.08	−4.95 ± 1.14
Glutamine	−2.35 ± 0.93	−0.61 ± 0.28	−0.51 ± 0.19	−1.80 ± 0.58	−0.77 ± 0.26
Alanine	−1.95 ± 0.49	−0.87 ± 0.31	−0.62 ± 0.15*	−0.77 ± 1.04	−0.93 ± 0.18
Total BCAA	−1.01 ± 0.26	−0.20 ± 0.90	+0.13 ± 0.07*	+0.34 ± 1.14	−0.34 ± 0.18

* $p < 0.05$ when compared to barbiturate controls by unpaired t-test.

TABLE 9. *Comparison of Nitrogen Balance Following Various Operations and Fasting*

Operation	Reference	Food Intake	Average 3-Day Cumulative Nitrogen Balance (g/patient)		
			In	Out	Balance
Cardiac surgery	Present study	Oral *ad lib.*	16.5	25.7	−9.3
Hysterectomy	21	Oral *ad lib.*	14	31	−17
Laparotomy, gastrectomy, or colectomy	38	IV glucose only	0	45.1	−45.1
		IV protein only	41.8	62.2	−20.4
		IV glucose + protein	39	59.8	−20.8
Esophagectomy	6	IV nutrition by central vein	60	84.3	−24.3
Fasting obese subjects	37	Fasting	0	26	−26

postoperative patients. Three-day cumulative nitrogen loss was approximately 26 g, an amount similar to that observed in unstressed fasting subjects[37] and much less than that reported following most other major surgical procedures (Table 9). Nutrient intake was not kept constant during these observations, and nitrogen and calorie intake increased gradually to normal by POD 5. The first 3-day cumulative calorie intake ranged from 1800–2100 kcal/3 days, comparable to the intake in patients undergoing hysterectomy.[21] The cardiac surgical patients excreted less nitrogen, however, in spite of their more extensive operative procedure.

Nitrogen balance is more difficult to compare between patient groups because of the differences in nitrogen and calorie intake. However, most patients reported in the literature received more, not less, nutritional support than those undergoing cardiac operations. This would be expected to reduce the negative nitrogen balance. In spite of this fact, 3-day cumulative nitrogen balance was less in the cardiac surgical patients than in patients undergoing other operative procedures, even in comparison with patients maintained on fixed hypercaloric intravenous feedings. Similar findings following cardiac surgical procedures have been reported by others.[39]

The conclusion that protein catabolism and nitrogen loss was attenuated in postoperative cardiac surgical patients was supported by determinations of forearm amino acid balance. Total amino acid release fell to approximately 25% of preoperative values on POD 1 and approached 50% of preoperative values by POD 3. Preoperative forearm total amino acid nitrogen flux was similar to that measured in normal controls, although the two groups were not matched for age. Thus, it is unlikely that the postoperative decrease in muscle amino acid efflux was a reflection of increased basal protein catabolism in these patients. The marked difference in forearm amino acid nitrogen flux between patients undergoing cardiac operations and those having other major operative procedures is illustrated in Table 10. The diminution in amino acid efflux observed in cardiac surgical patients is comparable only to the response in nonstressed fasting humans.[37]

Although the clinical observations described in the report suggest diminished protein catabolism in the cardiac surgery patients, comparing data from patients undergoing different operations may not be totally appropriate, since the catabolic stimulus may not be the same. Therefore, in order to standardize the operative stress, we studied the effect of a similar anesthetic technique, with or without hypothermia, in an animal model. Using laboratory dogs, a standardized laparotomy and retroperitoneal dissection was utilized both as a reproducible operative stress and a means of catheter implantation.[29,30] The catheters were then utilized to measure amino acid flux across the hindquarter. Earlier studies using this model have shown a consistently high rate of amino acid release from skeletal

TABLE 10. *Comparison of Forearm Amino Acid Flux in Surgical Patients and Normal Fasted Subjects*

Condition	Reference	Amino Acid Exchange*		
		Control	Postinjury	% Change
Cardiac surgical patients	Present study	−1667 ± 441	−422 ± 492	−75
Esophagectomy receiving IV feedings	6	−284 ± 79	−1546 ± 444	+444
Patients undergoing laparotomy—upper abdominal operations	40	−38 ± 12	−97 ± 22	+155
Trauma patients	5	−581 ± 197	−3843 ± 1383	+561
Obese fasted subjects	37	−749 ± 152	−515 ± 108†	−31

* All values are in nmol/100 ml forearm tissue · min except for laparotomy patients, in whom measurements were made on the whole leg (μmol/min).

† Following a 72-hour fast.

muscle by 6 hours after operation.[30,41] In addition, there is a high rate of nitrogen excretion (approximately 13 g) in the first 24 hours after surgery, and negative nitrogen balance persists for approximately 3 days.[42] Skeletal muscle glutamine concentrations decrease as occurs in humans following operation.

Total amino acid nitrogen release from hindquarter skeletal muscle was reduced to approximately 20% of the control value in animals given hypothermic anesthesia. Animals that received fentanyl and neuromuscular blockade exhibited a decreased nitrogen release rate that was intermediate but statistically indistinguishable from the hypothermic group. The release rate of the major nitrogen-carrying amino acids, glutamine and alanine, appeared to be reduced by hypothermia, although only alanine was significantly different compared to controls. Total BCAA flux was negative at 6 hours in the control groups and slightly positive or in balance in the hypothermic anesthesia group. Previous work with this model suggested that BCAA uptake by skeletal muscle was related to an increase in whole blood BCAA concentrations.[41] However, under conditions of hypothermia, net uptake occurred while BCAA levels were lower than either 6-hour control or preoperative levels.

These findings, coupled with the very low levels of total body nitrogen efflux measured in both the human and animal studies, indicate that the combination of narcotic anesthesia, neuromuscular blockade, and hypothermia prevents the catabolic response that normally develops after major operative procedures. All flux measurements were made following anesthesia and hypothermia, but at a time when the subjects or experimental animals were euthermic and awake. The persistent decrease in amino acid efflux at this time suggests that the "signal" for skeletal muscle proteolysis occurred at the time of the surgical procedure and that this stimulus was prevented or blocked by the anesthetic–hypothermic technique. The benefits of this perioperative blockade appear to be long-lasting, suggesting that catabolic processes are initiated by the operative procedure and not triggered by usual postoperative stress.

The mechanism for the decrease in nitrogen catabolism is unknown but presumably is related to the combination of high-dose fentanyl, muscle paralysis, and hypothermia. Fentanyl and paralysis may be responsible for some of the effects observed, since the response of the euthermic fentanyl-treated animals appeared to be intermediate. It is known that fentanyl, in high doses, can block the usual catecholamine and cortisol elaboration associated with intubation and operation,[43] possibly explaining its action. Previous studies have demonstrated that the intravenous administration of approximately 0.75 mg/kg of morphine to burn patients reduced oxygen consumption, core temperature, and urinary catecholamine excretion.[44] Catecholamine blockade in this animal model decreases nitrogen efflux[22] similar to the response observed in the fentanyl-treated animals, further suggesting that this may be the mechanism of the effect.

In this study, hypothermia appears to have resulted in an additional decrease in the postoperative catabolic response. The effect of hypothermia could have resulted from several mechanisms. First, cooling may have directly reduced muscle metabolism to such a degree that hormonal and/or inflammatory mediators (lymphokines, prostaglandins, or associated factors) could not exert their effects. Secondly, the systemic response to cooling may have included reduced elaboration of catabolic hormones and inflammatory mediators.[45,46] Definition of the precise roles and interactions between anesthesia, neuromuscular blockade, and hypothermia will require further study.

It has generally been accepted that the stress of operation, injury, or infection is accompanied by a protein catabolic response. This accelerated proteolysis, which is associated with muscle wasting and negative nitrogen balance, has generally been resistant to pharmacologic and nutritional manipulation. Thus, it has not been possible to evaluate the potential adaptive role that skeletal muscle proteolysis plays in the host response to stress. The observations presented in this report demonstrate techniques for controlling net skeletal muscle breakdown following operation and thus establish methods for evaluating the contribution that nitrogen breakdown makes to the responses of the host to infection and wound healing. In this study, neither patients undergoing cardiac surgical procedures nor animals undergoing laparotomy demonstrated deleterious effects when accelerated skeletal muscle net breakdown and increased amino acid release were prevented. Institution of more vigorous nutritional support in this setting might favor even more marked protein retention and anabolism. This, in turn, could promote more rapid postoperative recovery and improve survival.

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Discussion

DR. BASIL A. PRUITT, JR. (Fort Sam Houston, Texas): This is another nice study by Dr. Wilmore's group, in which they further dissect the metabolic consequences of injury and operative trauma.

The response of nitrogen wasting comes as no great surprise, but it is interesting to note that changes in nitrogen balance correlated with branched chain amino acid flux. It does seem as if that effect was blurred in terms of net change in nitrogen balance over the entire 24-hour period in the experimental animals. I wonder whether in both the human and animal studies the nitrogen released simply represents the participation of nitrogen metabolism in the net reduction of total body metabolic activity that one would anticipate with cooling. I would therefore ask the authors what they consider the Q10 effect to have contributed to this. Alternatively, I wonder whether the reduced nitrogen loss represents a change related to impaired peripheral blood flow in the cardiac patients, since their total cardiac output may have been decreased for the first several days after surgery.

As Dr. Wilmore has previously reported, the catecholamines appear to be the mediators of postinjury hypermetabolism, and I wonder whether the authors measured catecholamine levels in these studies. We have found that glucocorticoids and glucagon also influence the postinjury metabolic response, and I ask whether you measured alterations in those hormones following injury.

My last question has to do with just what narcotic was used in these patients. Earlier studies at our Institute by Dr. Wilmore demonstrated