Anesthesiology
69:319-326, 1988

Anesthesia with Halothane and Nitrous Oxide Alters Protein and Amino Acid Metabolism in Dogs

Fritz F. Horber, M.D., Sebastian Krayer, M.D.,† Kai Rehder, M.D.,‡ Morey W. Haymond, M.D.§*

General anesthesia in combination with surgery is known to result in negative nitrogen balance. To determine whether general anesthesia without concomitant surgery decreases whole body protein synthesis and/or increases whole body protein breakdown, two groups of dogs were studied: Group 1 (n = 6) in the conscious state and Group 2 (n = 8) during general anesthesia employing halothane (1.5 MAC) in 50% nitrous oxide and oxygen. Changes in protein metabolism were estimated by isotope dilution techniques employing simultaneous infusions of [4,5^{3}H]leucine and α-[1-^{14}C]-ketoisocaproate (KIC). Total leucine carbon flux was unchanged or slightly increased in the anesthetized animals when compared to the conscious controls, indicating only a slight increase in the rate of proteolysis. However, leucine oxidation was increased ($P < 0.001$) by more than 80% in the anesthetized animals when compared with their conscious controls, whereas whole body nonoxidative leucine disappearance, an indicator of whole body protein synthesis, was decreased. The ratio of leucine oxidation to the nonoxidative rate of leucine disappearance, which provides an index of the catabolism of at least one essential amino acid in the postabsorptive state, was more than twofold increased ($P < 0.001$) in the anesthetized animals regardless of the tracer employed. These studies suggest that the administration of anesthesia alone, without concomitant surgery, is associated with a decreased rate of whole body protein synthesis and increased leucine oxidation, resulting in increased leucine and protein catabolism, which may be underlying or initiating some of the protein wasting known to occur in patients undergoing surgery. (Key words: Anesthetics, gases: nitrous oxide. Anesthetics, volatile: halothane. Dogs. Metabolism: α-ketoisocaproate; amino acids; leucine; protein.)

IN ISOLATED PERFUSED rat liver, halothane inhibits protein synthesis in a dose-dependent manner.[1] However, halothane in concentrations used clinically results in only a small, reversible decrease of liver and lung protein synthesis *in vitro*.[1-3] During general anesthesia in association with surgery in humans, plasma and muscle concentrations of the branched chain amino acids (leucine, isoleucine, and valine) increase,[4,5] and negative nitrogen balance is observed.[6] These *in vivo* changes are consistent with an increase in whole body protein breakdown, a decrease in whole body protein synthesis, or a combination of these two processes. However, no measurements of whole body protein synthesis or protein breakdown have been made to determine the effects of general anesthesia alone in the absence of concomitant surgery on whole body protein metabolism.

In the postabsorptive (fasting) state the only source of the essential amino acid leucine for protein synthesis and leucine oxidation is leucine derived from the breakdown of endogenous protein. To obtain *in vivo* estimates of whole body protein metabolism, isotope dilution techniques using labeled leucine have been employed.[7-11] By infusing [1-^{14}C]leucine or its alpha-ketoacid, alpha-ketoisocaproate (KIC), in the postabsorptive state, the rates of appearance, disappearance, and oxidation of leucine can be measured to estimate whole body rates of protein synthesis and proteolysis under steady state conditions.[7-11]

To determine whether general anesthesia decreases whole body protein synthesis and/or increases whole body protein breakdown as measured *in vivo*, we studied two groups of dogs: group 1 in the conscious state and group 2 during general anesthesia employing halothane and nitrous oxide.

Materials and Methods

ISOTOPES

L-[1-^{14}C]Leucine (57 mCi/mmol) and L-[4,5-^{3}H]-leucine (120 mCi/mmol) were obtained from Amersham (Arlington Heights, IL); α-[1-^{14}C]KIC was enzymatically prepared from L-[1-^{14}C]leucine as previously described[12] and stored at −70° C until used.

ASSAYS

Plasma leucine and KIC concentrations and specific activities (SA) were determined by high performance liquid chromatography (HPLC) and liquid scintillation spectrometry.[13] The coefficient of variation for KIC and leucine concentrations (SA) was 1.2% (1.3%) and 0.6% (1.1%), respectively.

* Research Fellow, Endocrine Research Unit, Mayo Clinic and Mayo Foundation.

† Research Associate in Anesthesiology, Mayo Clinic and Mayo Foundation.

‡ Professor of Anesthesiology and Physiology, Mayo Medical School.

§ Professor of Pediatrics, Mayo Medical School.

Received from the Departments of Medicine and Pediatrics, Endocrine Research Unit, Department of Anesthesiology, Mayo Clinic and Foundation, Rochester, Minnesota. Accepted for publication March 30, 1988. Supported by US Public Health Service Grant Nos. DK26989 and HL21584 and by the Mayo Foundation. F. F. Horber was supported by the Swiss National Foundation for Scientific Research.

Address reprint requests to Dr. Haymond: Mayo Clinic, Endocrine Research Unit, 5-164 West Joseph Building, Rochester, Minnesota 55905.

TABLE 1. Characteristics of Conscious and Anesthetized Dogs

	Conscious Dogs (Group 1)	Anesthetized Dogs (Group 2)	*P* Group 1 *vs.* Group 2
N	6	8	
Body weight (kg)	16.9 ± 0.8	15.8 ± 0.6	NS
Respiratory frequency (min⁻ffff⁻¹)*	22 ± 3	17 ± 1	NS
Halothane (%)†	—	1.25 ± 0.02	—
Pulse (min^{-1})	80 ± 3	115 ± 6	<0.001
Blood pressure (mmHg)	194 ± 8/111 ± 6	134 ± 4/92 ± 2	<0.02
Temperature (° C)	38.3 ± 0.2	38.0 ± 0.1	NS
*p*H	7.32 ± 0.01	7.34 ± 0.01	NS
Pa_{O_2} (mmHg)	96 ± 2‡	269 ± 6§	<0.0001
Pa_{CO_2} (mmHg)	39 ± 1	36 ± 2	NS
Base excess (mEq/l)	−4.5 ± 0.4	4.6 ± 0.4	NS

Values are reported as the mean values (±SE) calculated from the average of the individual values for each dog during the steady state period (180–240 min).

* Group 1, spontaneous rate; Group 2, ventilatory setting.

† Halothane is expressed as % of the expired gas.

‡ FI_{O_2} 0.21.

§ FI_{O_2} 0.5.

Plasma glucose concentrations were determined using the glucose oxidation method (Glucose Analyzer 2, Beckman Instruments, Irvine, CA). Plasma amino acid concentrations were determined using a Beckman 119CL amino acid analyzer.[14] Plasma D-β-hydroxybutyrate, acetoacetate, lactate, pyruvate, and free fatty acids were determined by microfluorometric enzymatic techniques.[15–17] Plasma insulin was measured by radioimmunoassay.[18]

The ^3H and ^{14}C radioactivity in KIC and leucine and $^{14}CO_2$ were determined using a Beckman LS9800 Series liquid scintillation counter using dual counting mode, which corrects the radioactivity for both quench and the spillover of ^{14}C radioactivity into the ^3H energy spectrum.

EXPERIMENTAL DESIGN

Fourteen healthy mongrel dogs, maintained on commercially available dog chow (Purina Dog Chow, Ralston-Purina, St. Louis, MO), were studied in the postabsorptive state after an 18-h fast. Group 1 was studied in the conscious state (standing), whereas group 2 was studied during general anesthesia (supine) (table 1). The dogs were not studied in a formal random order. In Group 1 (n = 6), a catheter was surgically inserted into the femoral artery 7 days prior to the study using Brevital® (10–15 mg/kg body weight) induction followed by halothane anesthesia. The arterial catheter was filled with heparin, knotted, coiled, and placed sterilely in a subcutaneous pouch. To prevent infection all six dogs received a single dose of ampicillin (1 g) following catheter placement. Prior to the study day all dogs were eating their normal amount of food. On the study day the dogs were placed in a sling and were breathing room air (FI_{O_2} = 0.21). Two additional catheters were inserted on the study day before starting the isotope infusion: one in a hind limb saphenous vein for blood sampling from the inferior vena cava and the other in a cephalic vein for infusion of the radioisotopes. Femoral artery pressure (strain gauge) and rectal temperature (thermistor) were continuously monitored. Arterial blood gases were measured 15 min before and 120 and 210 min after starting the infusions of radioisotopes (table 1).

In group 2 (n = 8) anesthesia was induced at ~0800 h on the morning of the study with 4% halothane in 50% nitrous oxide and oxygen (FI_{O_2} = 0.5). Anesthesia was maintained with an end-expiratory halothane concentration of 1.25% (>1.5 MAC) throughout the study (Beckman LB-2). After induction of anesthesia the dogs were positioned supine, their tracheas were intubated with a cuffed endotracheal tube, and their lungs mechanically ventilated (Harvard ventilator). Minute-ventilation was adjusted to achieve a Pa_{CO_2} ranging between 32 and 40 mmHg (table 1). After induction of general anesthesia three catheters were inserted: one into the cephalic vein for infusion of the radioisotopes, one into the right heart or the inferior vena cava for venous blood sampling, and one into the femoral artery for blood sampling and for measurement of blood pressure (table 1). The venous catheters were kept patent with an infusion of normal saline in both groups.

Arterial blood gases were determined repeatedly and corrected for body temperature. If the base excess was less than −5.5 mEq/l before completing 120 min of tracer infusion, 10 ml of 8.4% Na bicarbonate was slowly infused. To minimize development of atelectasis and hence pulmonary right-to-left shunting, the dogs' lungs were hyperinflated to an airway pressure of 30 cmH_2O every 30 min. The ECG and blood pressure were monitored throughout the study. To maintain body temperature the dogs were placed on a heated water mattress, and inspiratory gas was humidified and heated to 38° C (table 1).

Between 0800 and 0900 h, constant infusions of [^3H]leucine (220 nCi $kg^{-1} \cdot min^{-1}$) and [^{14}C]KIC (75 nCi $kg^{-1} \cdot min^{-1}$) (each dissolved in 33 ml of 0.9% NaCl) were started and continued for 4 h in both study groups. Arterial and venous blood samples (14 ml) were collected −60 (before induction of general anesthesia, only Group 2), −15, 120, 180, 200, 220, and 240 min after starting the infusions. This blood was replaced with an equal volume of 0.9% NaCl at each sampling time. All blood samples were placed in tubes containing

sodium EDTA, the tubes placed on ice and then centrifuged at 4° C.

Starting at 180 min, 2-min samples of expired gas were collected in previously evacuated 20-l Douglas bags (Warren E. Collins, Inc., Braintree, MA) every 20 min.[19] The rate of expired $^{14}CO_2$ was determined as previously described.[20] In addition, $^{14}CO_2$ SA was determined for each gas sample by slowly aspirating expired gas through a scintillation vial containing 2 ml of 0.5 M hydroxide of hyamine in ethanol using thymolphthalein as a *p*H indicator.[21] Fourteen milliliters of Safety Solve® (Research Products International, Mount Prospect, IL) were added and the ^{14}C radioactivity determined by scintillation spectrometry.

After completion of the study, all dogs were killed to determine the position of the tip of the catheters. This protocol was approved by the Mayo Clinic Animal Care and Use Committee.

Calculations

All calculations were carried out at near isotope and substrate steady state (fig. 1).[11] Two independent estimates of the rate of appearance of unlabeled leucine (Ra) were calculated using a reciprocal pool model for leucine metabolism (fig. 2). Under steady state conditions the Ra of leucine carbon equals the rate of disappearance and is referred to as flux.[11] Assuming steady state conditions (fig. 1), flux was calculated by dividing the infusion rates of [^{3}H]LEUCINE or [^{14}C]KIC by the plasma SA of the transaminated product (reciprocal pool) of the infused isotope (fig. 2; *i.e.*, plasma [^{14}C]-leucine SA and [^{3}H]kic SA).[22]

The apparent rate of leucine carbon oxidation was calculated by dividing the measured expired $^{14}CO_2$ ($dpm \cdot kg^{-1} \cdot min^{-1}$) by the mean steady state plasma [^{14}C]leucine SA (fig. 2) and 0.8 (to correct for CO_2 fixation).[23] Total $^{14}CO_2$ production ($mmol \cdot kg^{-1} \cdot min^{-1}$) was calculated by dividing expired $^{14}CO_2$ ($dpm \times kg^{-1} \cdot min^{-1}$) by the $^{14}CO_2$ SA (dpm/mmol).[23] Estimates of nonoxidative leucine disappearance, an indicator of the rate of leucine entering proteins,[24] was calculated by subtracting the rate of leucine oxidation from the estimated total leucine carbon flux derived from either radiotracer.

Mean plasma steady state leucine and KIC concentrations and specific activities (table 2), respiratory frequency, end-expiratory halothane concentrations, pulse rate and arterial blood pressure, body temperature, and arterial blood gas tensions (table 1) are reported as mean values calculated from the average of the individual values for each dog during the steady state period (180–240 min). The values obtained at the different time points were not statistically different from each other using ANOVA.

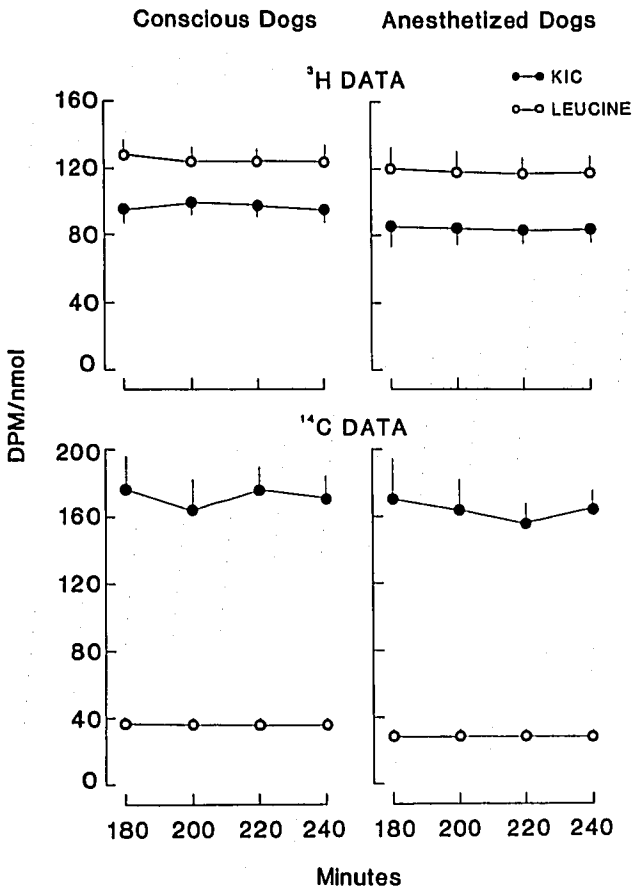

FIG. 1. ^{3}H and ^{14}C specific activities of plasma leucine and α-keto-isocaproate (KIC) in conscious (n = 6) and anesthetized (n = 8) dogs over the last 60 min of a 240-min infusion of [^{3}H]leucine and [^{14}C]KIC (mean ± SE).

Statistical Analysis

All results are expressed as mean ± SE. Values from different time points were meaned and analyzed first using ANOVA. Thereafter, statistical analysis between groups was carried out by using two-tailed paired and unpaired Student's *t* tests where appropriate.

Results

The arterial blood pressure was significantly higher ($P < 0.02$) in the conscious than in the anesthetized dogs, whereas the mean pulse rate was lower ($P < 0.001$) (table 1). Because the lungs of the anesthetized dogs were ventilated with 50% oxygen, the higher Pa_{O_2} is not unexpected (table 1). Body weight, respiratory frequency, body temperature, *p*H, Pa_{CO_2} and base excess were similar in the two groups (table 1).

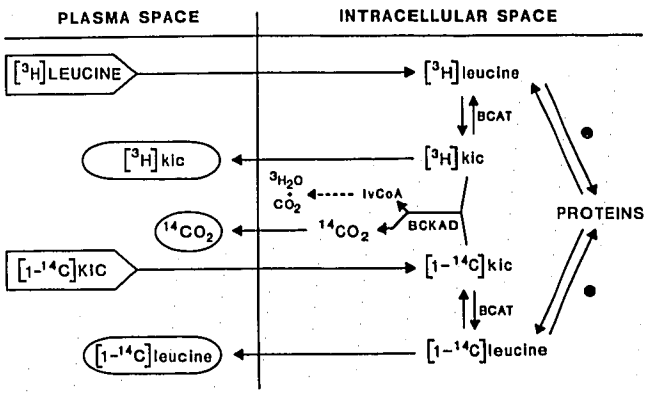

FIG. 2. In the intracellular space the infused tracers [^{3}H]LEUCINE and [^{14}C]α-KETOISOCAPROATE (KIC) are diluted by unlabeled leucine and KIC and rapidly transaminated (not rate-limiting) to [^{3}H]kic and [^{14}C]leucine, respectively, by branched chain amino transaminase (BCAT). The plasma specific activities of [^{3}H]kic and [^{14}C]-leucine are presumed to reflect the intracellular specific activities of [^{3}H]- and [^{14}C]leucine and are used to calculate leucine flux (reciprocal pool model).[22] Therefore, plasma [^{14}C]leucine and not [^{14}C]KIC reflects the intracellular precursor pool for leucine oxidation. Because the BCKAD irreversibly decarboxylates the carbon in position 1 from KIC to form isovaleryl-CoA (Iv-CoA), the only source of expired $^{14}CO_2$ gas is that derived from [^{14}C]kic. Because the rate of leucine oxidation cannot be calculated using the ^{3}H label, the nonoxidative rate of leucine disappearance (●, an estimate of whole body protein synthesis[24]) is calculated by subtracting the rate of leucine oxidation from the total leucine carbon flux derived from both the ^{3}H and ^{14}C radiotracers.

After 180–240 min of nitrous oxide–halothane anesthesia, mean plasma leucine (109 ± 9 *vs.* 154 ± 10 μM, $P < 0.001$) and KIC concentrations (17 ± 1 *vs.* 24 ± 3 μM, $P < 0.01$) increased by about 40%, whereas in conscious dogs no significant changes in mean plasma concentrations of either substrate were observed (table 2). During the final hour of study the SA of [^{3}H]- and [^{14}C]leucine and -KIC (fig. 1), the SA of expired $^{14}CO_2$, and the rate of $^{14}CO_2$ expired were constant, indicating steady state conditions.

$^{14}CO_2$ production (25.3 ± 2.9 *vs.* 38.3 ± 2.5 dpm × 10^3 $kg^{-1} \cdot min^{-1}$, $P < 0.01$) and the $^{14}CO_2$ SA (8.1 ± 1.1 *vs.* 15.5 ± 1.4 dpm × 10^4/mmol, $P < 0.002$) were more than 50% greater in the anesthetized animals when compared to the conscious controls (table 2), whereas the total CO_2 production rate was approximately 25% less in the anesthetized animals.

Total leucine carbon flux was greater using the ^{14}C reciprocal pool SA in the anesthetized group when compared to their controls ($P < 0.05$), whereas the ^{3}H data showed only a nonsignificant increase in the anesthetized animals (table 3). Estimates of leucine oxidation (calculated using the [^{14}C]leucine SA) were greater by more than 80% in the anesthetized animals when compared to their conscious controls ($P < 0.001$) (table 3). When estimates of leucine oxidation were subtracted from the estimates of total leucine carbon flux, anesthetized animals had a decrease in the nonoxidative rate of leucine disappearance, which was significant for the ^{3}H data ($P < 0.05$) (table 3).

The ratio of leucine oxidation divided by the nonoxidative leucine disappearance (NOLD), an indicator of whole body leucine catabolism, was greater by more than 100% in anesthetized dogs when compared to the conscious controls using either tracer ($P < 0.001$) (table 3).

To demonstrate the effect of induction of anesthesia on plasma substrate concentrations, venous plasma concentrations were used because in Group 2 dogs the arterial catheter was only inserted after induction of anesthesia. Plasma glucose, lactate, pyruvate, and alanine concentrations were significantly increased 30 min after induction of general anesthesia (table 4), whereas plasma free fatty acid concentrations were significantly lower (table 4). In addition, total amino acid concentrations were significantly greater 30 min after induction of general anesthesia. This increase was primarily attributable to an increase in nonessential amino acids (in particular alanine) because the essential amino acids

TABLE 2. Infusion Rates, Specific Radioactivities, and $^{14}CO_2$ Production in Conscious and Anesthetized Dogs

	Conscious Dogs (Group 1)	Anesthetized Dogs (Group 2)	*P* Value Group 1 *vs.* Group 2
Infusion rates (dpm × $10^3 \cdot kg^{-1} \cdot min^{-1}$)			
[^{3}H] Leucine	396 ± 20	363 ± 18	NS
α [^{14}C]KIC	160 ± 9	137 ± 5	<0.05
Specific radioactivities (dpm/nmol, mean of 180–240)*			
[^{3}H] Leucine	126 ± 6	119 ± 10	NS
[^{14}C] Leucine	37.6 ± 2.7	29.1 ± 1.9	<0.02
[^{3}H]KIC	98.5 ± 4.4	86.8 ± 7.8	NS
[^{14}C]KIC	174 ± 11	165 ± 15	NS
Concentrations (μM)*			
Leucine			
−15 min	107 ± 6	109 ± 9†	NS
180–240 min	121 ± 4	154 ± 10†	<0.02
KIC			
−15 min	14 ± 1	17 ± 1‡	NS
180–240 min	16 ± 1	24 ± 3‡	<0.05
Expired CO_2			
$^{14}CO_2$ production (dpm × $10^3 \cdot kg^{-1} \cdot min^{-1}$)	25.3 ± 2.9	38.3 ± 2.5	<0.01
$^{14}Co_2$ SA (dpm × 10^4/mmol)	8.1 ± 1.1	15.5 ± 1.4	<0.002
Total CO_2 production ($mmol \cdot kg^{-1} \cdot min^{-1}$)	0.32 ± 0.023	0.24 ± 0.013	<0.01

Values are given as mean ± SE.
* Time points are expressed in minutes after starting the infusions of the radioisotopes.
† $P < 0.001$ (comparison within group 2).
‡ $P < 0.01$ (comparison within group 2).

TABLE 3. Effect of Anesthesia on Reciprocal Pool Estimates of Leucine Metabolism in Dogs

	^{3}H Data		^{14}C Data	
	Conscious	Anesthetized	Conscious	Anesthetized
Total leucine carbon flux	4.04 ± 0.12	4.30 ± 0.19	4.27 ± 0.10‡	4.81 ± 0.19*§
Leucine oxidation	—	—	0.88 ± 0.12	1.66 ± 0.09†
NOLD**	3.19 ± 0.14	2.64 ± 0.19*	3.47 ± 0.18‡	3.15 ± 0.12§
Leucine oxidation/NOLD	0.28 ± 0.04	0.67 ± 0.18†	0.26 ± 0.05‡	0.54 ± 0.04†¶

Values are given as mean ± SE and expressed as $\mu mol \cdot kg^{-1} \cdot min^{-1}$.
* $P < 0.05$; conscious *versus* anesthetized.
† $P < 0.001$; conscious *versus* anesthetized.
‡ $P < 0.05$; ^{3}H *versus* ^{14}C data within each group.
§ $P < 0.001$; ^{3}H *versus* ^{14}C data within each group.
¶ $P < 0.01$; ^{3}H *versus* ^{14}C data within each group.
** NOLD = Non-oxidative leucine disappearance.

(and in particular the branched chain amino acids) remained unchanged during the induction phase of general anesthesia (table 4).

The increased plasma concentrations of glucose, lactate, pyruvate, and total and nonessential amino acids persisted throughout the study in anesthetized dogs (table 5), whereas arterial plasma lactate concentrations decreased significantly in the conscious animals ($P < 0.001$, table 5) during the same period. The plasma concentrations of the essential amino acids remained unchanged in conscious animals throughout the study, whereas in the anesthetized dogs the concentration of these amino acids and in particular the branched chain amino acids increased by more than 25% ($P < 0.05$, table 5). Arterial plasma concentrations of free fatty acids and total ketone bodies were lower in the anesthetized dogs than in the conscious controls during the 4-h study period (table 5). Both plasma free fatty acids (130 ± 20 *vs.* 230 ± 10 μM, $P \leq 0.05$) and total ketone body (45 ± 6 *vs.* 64 ± 8 μM, $P < 0.01$) concentrations increased during the 4-h study period only in the anesthetized dogs but still remained significantly lower ($P < 0.05$) than those observed in the conscious animals.

Plasma insulin concentrations were similar in the anesthetized and in the conscious animals throughout the study period (before induction of general anesthesia in Group 1 and Group 2, 5 ± 1 *vs.* 5 ± 1 μU/ml, respectively; after 30 min of study 5 ± 1 *vs.* 5 ± 1 μU/ml; and after 4 h of study 4 ± 1 *vs.* 3 ± 1 μU/ml).

Discussion

The present study demonstrates that during halothane–nitrous oxide anesthesia in nonsurgically traumatized dogs the whole body leucine rate of oxidation is substantially increased and the rate of entry of leucine into body proteins is decreased, whereas whole body leucine rates of appearance are only minimally increased. Because the only source of the essential amino acid leucine in the postabsorptive state is endogenous protein and assuming no intracellular accumulation of leucine or its alpha-ketoacid KIC, the present data suggest that anesthesia with these two agents results in an increased rate in the oxidative loss of at least one essential amino acid and decreased rates of whole body protein synthesis with little or no effect on whole body rates of proteolysis. Thus, at least some of the losses of whole body protein and nitrogen known to occur with surgical trauma may be attributable to the anesthetic agents themselves.

The most striking finding of the present study was the over 80% increase in the rate of leucine oxidation during the 4 h of anesthesia (table 3). Because the isotope infusions and anesthetic agents were administered at essentially the same time, we cannot determine the time course of the onset of this response to the administration of the anesthetic agents in these dogs. Regardless of the mechanism(s), the increased oxidation of leucine represents a significant and irreversible loss of an essential amino acid as a result of the anesthesia.

Several factors might be considered to explain the increased rate of leucine carbon oxidation observed: 1) We previously observed an inverse relationship be-

TABLE 4. Venous Plasma Concentrations of Plasma Substrates 15 Min before and 30 Min after Induction of General Anesthesia in Dogs

	Before (−60 min)*	After (−15 min)*	*P* Value Before *vs.* After
Glucose (mM)	5.00 ± 0.11	6.49 ± 0.28	<0.002
Lactate (mM)	4.91 ± 0.37	6.24 ± 0.83	<0.05
Pyruvate (μM)	140 ± 10	280 ± 30	<0.001
Alanine (mM)	0.47 ± 0.06	1.07 ± 0.06	<0.001
Free fatty acids (μM)	500 ± 30	220 ± 30	<0.001
Ketone bodies (μM)	63 ± 1	68 ± 6	NS
Total amino acids† (mM)	2.00 ± 0.14	2.76 ± 0.14	<0.005
Essential amino acids‡ (mM)	0.63 ± 0.04	0.70 ± 0.04	NS
Nonessential amino acids§ (mM)	1.37 ± 0.11	2.06 ± 0.12	<0.002
Branched chain amino acids (mM)	0.29 ± 0.02	0.30 ± 0.02	NS

Values are given as mean ± SE; n = 7.
* Minutes before start of the isotope infusion.
† Minus tryptophan and glutamine.
‡ Minus tryptophan.
§ Minus glutamine.

TABLE 5. Arterial Plasma Concentrations of Plasma Substrates in Conscious and Anesthetized Dogs

	Conscious (Group 1)	Anesthetized (Group 2)	*P* Value Between Groups
Glucose (mM)			
−15	5.50 ± 0.06	6.55 ± 0.22	<0.003
180–240	5.33 ± 0.06	6.22 ± 0.22	<0.003
Lactate (mM)			
−15	2.29 ± 0.16§	5.88 ± 0.75	<0.002
180–240	1.39 ± 0.11§	6.02 ± 1.93	<0.05
Pyruvate (μM)			
−15	110 ± 20	270 ± 20	<0.001
180–240	110 ± 10	290 ± 50	<0.009
Alanine (mM)			
−15	0.32 ± 0.03	1.12 ± 0.09	<0.001
180–240	0.34 ± 0.02	1.14 ± 0.12	<0.001
Free fatty acids (μM)			
−15	560 ± 130	130 ± 20¶	<0.003
180–240	680 ± 100	230 ± 30¶	<0.001
Total ketone bodies (μM)			
−15	72 ± 9	45 ± 6**	<0.03
180–240	88 ± 6	64 ± 8**	<0.05
Total amino acids* (mM)			
−15	1.62 ± 0.09	2.86 ± 0.19	<0.001
180–240	1.70 ± 0.05	3.18 ± 0.23	<0.001
Branched chain amino acids† (mM)			
−15	0.28 ± 0.02	0.31 ± 0.03**	NS
180–240	0.32 ± 0.01	0.45 ± 0.02**	NS
Essential amino acids‡ (mM)			
−15	0.60 ± 0.05	0.75 ± 0.06¶	NS
180–240	0.67 ± 0.03	1.00 ± 0.08¶	<0.001
Nonessential amino acids (mM)			
−15	1.02 ± 0.06	2.10 ± 0.14	<0.001
180–240	1.04 ± 0.03	2.18 ± 0.17	<0.001

Values are given as mean ± SE; −15 = 15 min before start of isotope infusion.
* Minus tryptophan and glutamine.
† Minus tryptophan.
‡ Minus glutamine.
§ $P < 0.001$; −15 *versus* 180–240 within group.
¶ $P < 0.05$; −15 *versus* 180–240 within group.
** $P < 0.01$; −15 *versus* 180–240 within group.

tween plasma free fatty acid concentrations and leucine flux and oxidation.[25] Therefore, the increase in leucine oxidation and the modest or minor increase in leucine flux may in part be attributable to the decrease in the plasma free fatty acid concentration, presumably induced by halothane.[26–28] 2) The activity of the branched chain alpha-ketoacid dehydrogenase (BCKAD), the rate-limiting enzyme in the catabolism of leucine (and the other branched chain amino acids) and the enzyme responsible for the oxidative decarboxylation of KIC, is regulated by phosphorylation and dephosphorylation.[29,30] Because halothane is known to inhibit the phosphorylation of at least one regulatory enzyme, intracellular triglyceride lipase,[28] it may also inhibit the phosphorylation of the BCKAD and therefore prevent the inactivation of this enzyme complex. Such a proposed mechanism could account for the increase in the rate of leucine oxidation but not the increase in the plasma concentrations of leucine, KIC, and the other amino acids. Were activation of the BCKAD the only process altered by anesthesia, one might have anticipated that the plasma concentrations of leucine, KIC, isoleucine, and valine might be decreased, which was not the case. 3) Increased concentrations of leucine and KIC[4,5] could have increased leucine carbon oxidation by either increasing substrate availability (without change in the activity of the BCKAD) or by specifically increasing the activity of this enzyme complex.[31] Therefore, increased availability of leucine and/or KIC as a result of a sustained rate of whole body proteolysis in the presence of decreased protein synthesis could be primarily responsible for the observed increased rate of leucine oxidation.

The two independent estimates (^{3}H and ^{14}C) of leucine flux (table 3) were similar in the conscious dogs, although the flux determination with the ^{14}C tracer was slightly but significantly greater than that observed with the ^{3}H tracer. However, in the anesthetized group of animals, the difference between the leucine flux measured with ^{14}C and the ^{3}H tracers (table 3) was nearly twice as great (0.54 *vs.* 0.26 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, ^{14}C and ^{3}H data, respectively) as that observed in the conscious animals. Employing the same isotopes, we have previously observed similar discrepancies under conditions of increased leucine oxidation.[23,24,32] On the basis of these previous studies, this discrepancy between ^{14}C and ^{3}H flux is most likely the result of incomplete equilibration of the [^{14}C]KIC into a relatively large intracellular leucine pool (fig. 2).[23,32] As a result, our estimates of leucine flux using the ^{14}C data may be slightly overestimated[23,32] and could account for the significant increase in leucine flux using the ^{14}C data, which was not observed using the ^{3}H data. In contrast, the flux of leucine estimated from the ^{3}H data is not affected by this disequilibrium because of the very small intracellular pool of KIC and because the only source of KIC is that derived from the transamination of intracellular leucine. The leucine flux estimated from the ^{3}H data in both study groups is most likely an underestimate of the true Ra of leucine because of recycling of trace.[33] Therefore, the ranges of flux observed during anesthesia most likely provide minimum (^{3}H data) and maximum (^{14}C data) estimates of the true leucine flux.[23,32,33] In addition, the rate of leucine oxidation during anesthesia may be similarly overestimated as a result of the disequilibrium of the ^{14}C trace between the intracellular leucine and KIC pools but could not account for the large increase in the rate of leucine oxidation observed.[23,32]

Because the mean arterial blood pressure of the anes-

thetized animals was lower (106 mmHg) when compared to the conscious animals (138 mmHg), organ blood flow might have been decreased as well. Therefore, based on the decrease in the nonoxidative rate of leucine disappearance, we would conclude that administration of halothane–nitrous oxide inhibits the rate of whole body protein synthesis by either a direct or an indirect (*e.g.*, hemodynamic) effect. This decreased whole body protein synthesis was observed in the absence of a change in plasma insulin concentrations. To our knowledge, this is the first evidence of an anesthesia-induced decrease in protein synthesis *in vivo* and is a conclusion consistent with all available data regarding the effects of halothane on protein synthesis *in vitro*.[1–3] In perfused rat lung and liver, halothane rapidly inhibits protein synthesis in a dose-dependent manner with about a 10% depression of the protein synthetic rate for each 1% increment in halothane concentrations.[1–3] The present study demonstrates that the extent of the suppression of whole body protein synthesis as determined by isotope dilution techniques is of the same magnitude (table 3; ~10–15%) as that observed in perfused organs exposed to comparable halothane concentrations.[1–3]

The ratio of leucine oxidation to the nonoxidative rate of leucine disappearance in the postabsorptive state provides an index of severity of the irreversible loss of this essential amino acid *versus* its incorporation into body protein, and minimizes the problems associated with isotope disequilibrium discussed above. In the present study, this ratio was increased twofold in the anesthetized when compared to the conscious control dogs, regardless of which isotopic data was used to calculate leucine flux. Therefore, this ratio emphasizes the leucine catabolic events associated with the use of these two anesthetic agents.

It is of interest to note that during induction of anesthesia (between −60 and −15 min) the plasma concentrations of a number of the substrates were acutely altered (table 4). Our results are consistent with previous reports that enflurane as well as thiopentone in combination with nitrous oxide lead to a decrease in the plasma concentrations of free fatty acids during the induction phase of anesthesia.[34,35] *In vitro,* halothane has been reported to inhibit intracellular triglyceride lipase activity, presumably by inhibiting its phosporylation.[26] Whether the changes observed in plasma free fatty acids in the present study are related to such a mechanism remains to be demonstrated. In contrast, plasma glucose concentrations increased during induction of general anesthesia. This was most likely a result of increased rate of glycogenolysis[36,37] because glucose uptake by skeletal muscle and liver is not affected by halothane.[36,38] However, the disposition of intracellular glucose *in vitro* is strikingly altered by halothane in that glycogen synthesis is inhibited and glycolytic activity is increased, resulting in an increase in the production of both lactate and pyruvate.[36] In addition, it has been proposed that halothane decreases gluconeogenesis and increases glycogenolysis and glycolysis.[37,39] Therefore, the acute increase in the plasma concentrations of glucose, lactate, pyruvate, and alanine (a potential gluconeogenic precursor formed from transamination of pyruvate and glutamate) are the result of the combined effects of halothane–nitrous oxide on these various metabolic processes. The 30% increase in the plasma concentration of total amino acids during induction of anesthesia is attributable almost exclusively to an increase in the plasma alanine concentration alone.

In contrast to the acute effects of halothane–nitrous oxide on plasma substrate concentrations (between −60 and −15 min, table 4), 4 h of anesthesia had no additional effect on many of these substrates because the changes in the plasma concentrations of glucose, pyruvate, alanine, free fatty acids, total ketone bodies, and total nonessential amino acids were similar in the two groups. However, the absolute plasma concentrations of these substrates in the anesthetized animals remained significantly different from those observed in the conscious animals throughout the study, indicating a persistent effect of these anesthetic agents on these various metabolic processes. The plasma concentrations of the total essential amino acids and BCAA increased ($P < 0.001$) with time only in the anesthetized animals, a finding consistent with the observed sustained rate of whole body protein breakdown in the presence of a decrease in the rate of protein synthesis discussed above.

In contrast to humans, the supine position is not natural for dogs. Because the awake dogs were standing during the entire study period, whereas the anesthetized dogs were supine, it remains to be established whether this change in posture may have influenced protein and amino acid metabolism.

The initiation of protein wasting in association with surgical trauma must be in part the result of the surgery itself, particularly if it is associated with immobility and some element of deconditioning and disuse atrophy. However, in many patients undergoing elective outpatient surgery for which a convalescent period of recovery is known not to be associated with significant morbidity, fatigue and weakness frequently occur. The present studies demonstrate that the administration of anesthesia alone is associated with significant alterations in the regulation of amino acid and protein metabolism, which may underlie or initiate some of the protein wasting known to occur with surgical trauma and may contribute to physical symptoms associated with the ad-

ministration of anesthesia in the absence of significant surgical intervention.

The authors thank Joan Aikens, Jane Kahl, Joan King, Collette Schmidt, Carine Horber-Feyder, and Jerry Rach for their skillful technical assistance, and Pat Voelker for her excellent secretarial help.

References

1. Flaim KE, Jefferson LS, McGwire JB, Rannels DE: Effect of halothane on synthesis and secretion of liver proteins. Mol Pharmacol 24:277–281, 1983
2. Rannels DE, Roake GM, Watkins CA: Additive effects of pentobarbital and halothane to inhibit synthesis of lung proteins. ANESTHESIOLOGY 57:87–93, 1982
3. Wartell SA, Christopherson R, Watkins CA, Rannels DE: Inhibition of synthesis of lung proteins by halothane. Mol Pharmacol 19:520–524, 1981
4. Fürst P: Catabolic stress on intracellular amino acid pool. Adv Exp Med Biol 167:571–579, 1982
5. Christensen T, Waaben J, Lindeburg T, Vesterberg K, Vinnars E, Kehlet H: Effect of epidural analgesia on muscle amino acid pattern after surgery. Acta Chir Scand 152:407–411, 1986
6. Manku RS: Effect of anesthesia on protein metabolism in patients undergoing hernioplasty. Anesth Analg 49:446–449, 1970
7. Matthews DE, Bier DM, Rennie MJ, Edwards RHT, Halliday D, Millward DJ, Clugston GA: Regulation of leucine metabolism in man: A stable isotope study. Science 214:1129–1131, 1981
8. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM: Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-^{13}C]leucine. Am J Physiol 238:E473–E479, 1980
9. Tessari P, Tsalikian E, Schwenk WF, Nissen SL, Haymond MW: Effects of [^{15}N]leucine infused at low rates on leucine metabolism in humans. Am J Physiol 249:E121–E130, 1985
10. Tsalikian E, Howard C, Gerich JE, Haymond MW: Increased leucine flux in short-term fasted human subjects: Evidence for increased proteolysis. Am J Physiol 247:E323–E327, 1984
11. Golden MHN, Waterlow JC: Total protein synthesis in elderly people: A comparison of results with [^{15}N]glycine and [^{14}C]-leucine. Clin Sci 53:277–288, 1977
12. Rüdiger HW, Langerbeck U, Goedde HW: A simplified method for preparation of ^{14}C-labelled branched-chain alpha-oxo acid. Biochem J 126:445–446, 1972
13. Nissen SL, Van Huysen C, Haymond MW: Measurement of branched chain amino acids and branched chain α-ketoacids in plasma by high-performance liquid chromatography. J Chromatogr 232:170–175, 1982
14. Stacey-Schmidt C, Berg P, Haymond MW: Use of D-glucosaminic acid as an internal standard in single-column accelerated amino acid analysis of physiological fluids. Anal Biochem 123:74–77, 1982
15. Lowry OH, Passonneau JV: A Flexible System of Enzymatic Analysis. New York and London, Academic Press, 1972, pp 146–218
16. Cahill GF Jr, Herrera MG, Morgan AP, Soeldner JS, Steinke J, Levy PL, Reichard GA Jr, Kipnis DM: Hormone–fuel interrelationships during fasting. J Clin Invest 45:1751–1769, 1966
17. Miles J, Glasscock R, Aikens J, Gerich J, Haymond M: A microfluorometric method for the determination of free fatty acids in plasma. J Lipid Res 24:96–99, 1983
18. Herbert V, Lau K-S, Gottlieb CW, Bleicher SJ: Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 25:1375–1384, 1965
19. Nissen S, Haymond MW: Changes in leucine kinetics during meal absorption: Effects of dietary leucine availability. Am J Physiol 250:E695–E701, 1986
20. Beaufrere B, Tessari P, Cattalini M, Miles J, Haymond MW: Apparent decreased oxidation and turnover of leucine during infusion of medium-chain triglycerides. Am J Physiol 249:E175–E182, 1985
21. Fromm H, Hofmann AF: Breath test for altered bile-acid metabolism. Lancet 2:621–625, 1971
22. Schwenk WF, Beaufrere B, Haymond MW: Use of reciprocal pool specific activities to model leucine metabolism in humans. Am J Physiol 249:E646–E650, 1985
23. Rodriguez N, Schwenk WF, Beaufrere B, Miles JM, Haymond MW: Trioctanoin infusion increases in vivo leucine oxidation: A lesson in isotope modeling. Am J Physiol 251:E343–E348, 1986
24. Schwenk WF, Rubanyi E, Haymond MW: Effect of a protein synthetic inhibitor on in vivo estimates of protein synthesis in dogs. Am J Physiol 252:E595–E598, 1987
25. Tessari P, Nissen SL, Miles JM, Haymond MW: Inverse relationship of leucine flux and oxidation to free fatty acid availability in vivo. J Clin Invest 77:575–581, 1986
26. Mäkeläinen A, Vapaatalo H, Nikki P: Halothane-induced lipolysis in vitro in the rat. Acta Anaesthesiol Scand 17:179–183, 1973
27. Bennis J, Smith U: Studies of the dual effects of halothane on the lipolysis of human fat cells. ANESTHESIOLOGY 45:379–384, 1976
28. Prokocimer PG, Mirsky N, Vickery RG, Hoffman BB, Maze M: Halothane inhibits isoproterenol-stimulated lipolysis in isolated rat adipocytes (abstract). ANESTHESIOLOGY 65:A230, 1986
29. Harper AE, Miller RH, Block KP: Branched chain amino acid metabolism. Annu Rev Nutr 4:409–454, 1984
30. Randle PJ, Fatania HR, Lau KS: Regulation of the mitochondrial branched-chain-2-oxo acid dehydrogenase complex of animal tissues by reversible phosphorylation. Mol Aspects Cell Regul 3:1–26, 1984
31. Aftring RP, Block KP, Buse MG: Leucine and isoleucine activate skeletal muscle branched-chain α-keto acid dehydrogenase in vivo. Am J Physiol 250:E599–E604, 1986
32. Schwenk WF, Haymond MW: Effects of leucine, isoleucine, or threonine infusion on leucine metabolism in humans. Am J Physiol 253:E428–E434, 1987
33. Schwenk WF, Tsalikian E, Beaufrere B, Haymond MW: Recycling of an amino acid label with prolonged isotope infusion: Implications for kinetic studies. Am J Physiol 248:E482–E487, 1985
34. Oyama T, Matsuki A, Kudo M: Effects of enflurane (Ethrane) anaesthesia and surgery on carbohydrate and fat metabolism in man. Anaesthesia 27:179–184, 1972
35. Allison SP, Tomlin PJ, Chamberlain MJ: Some effects of anaesthesia and surgery on carbohydrate and fat metabolism. Br J Anaesth 41:588–593, 1969
36. Rosenberg H, Haugaard N, Haugaard ES: Alteration by halothane of glucose and glycogen metabolism in rat skeletal muscle. ANESTHESIOLOGY 46:313–318, 1977
37. Biebuyck JF, Lund P: Effects of halothane and other anesthetic agents on the concentrations of rat liver metabolites in vivo. Mol Pharmacol 10:474–483, 1974
38. Camu F: Carbohydrate intolerance during halothane anesthesia in dogs. Acta Anaesthesiol Belg 24:177–188, 1973
39. Biebuyck JF, Lund P, Krebs HA: The effects of halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) on glycolysis and biosynthetic processes of the isolated perfused rat liver. Biochem J 128:711–720, 1972