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

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#### Abstract

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 $2(\mathrm{n}=8)$ 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 simultancous infusions of $\left[4,5^{3} \mathrm{H}\right]$ leucine and $\alpha-\left[1 .{ }^{14} \mathrm{C}\right]-$ 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 ( $P<\mathbf{0 . 0 0 1}$ ) 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: $\alpha$-ketoisocaproate; amino acids; leucine; protein.)


In isolated perfused rat liver, halothane inhibits protein synthesis in a dose-dependent manner. ' 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 mus-

[^0]cle 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. ${ }^{7-11} \mathrm{By}$ infusing $\left[1{ }^{14} \mathrm{C}\right.$ ]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

$\mathrm{L}-\left[\mathrm{I}-{ }^{14} \mathrm{C}\right]$ Leucine ( $57 \mathrm{mCi} / \mathrm{mmol}$ ) and $\mathrm{L}-\left[4,5-{ }^{3} \mathrm{H}\right]-$ leucine ( $120 \mathrm{mCi} / \mathrm{mmol}$ ) were obtained from Amersham (Arlington Heights, IL); $\alpha-\left[1-{ }^{14} \mathrm{C}\right] \mathrm{KIC}$ was enzymatically prepared from $\mathrm{L}-\left[1-{ }^{14} \mathrm{C}\right]$ leucine as previously described ${ }^{12}$ and stored at $-70^{\circ} \mathrm{C}$ 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.

Table 1. Characteristics of Conscious and Anesthetized Dogs

|  | Conscious Dogs (Group 1) | Anesthetized Dogs (Group 2) | PGroup 1 <br> vs. Group 2 |
| :---: | :---: | :---: | :---: |
| N | 6 | 8 |  |
| Body weight (kg) | $16.9 \pm 0.8$ | $15.8 \pm 0.6$ | NS |
| Respiratory frequency ( $\left.\mathrm{min}^{-} \mathrm{ffff}\right)^{\text {* }}$ | $22 \pm 3$ | $17 \pm 1$ | NS |
| Halothane (\%) $\dagger$ | - | $1.25 \pm 0.02$ | - |
| Pulse ( $\mathrm{min}^{-1}$ ) | $80 \pm 3$ | $115 \pm 6$ | $<0.001$ |
| Blood pressure ( mmHg ) | $194 \pm 8 / 111 \pm 6$ | $134 \pm 4 / 92 \pm 2$ | $<0.02$ |
| Temperature $\left({ }^{\circ} \mathrm{C}\right)$ | $38.3 \pm 0.2$ | $38.0 \pm 0.1$ | NS |
| pH | $7.32 \pm 0.01$ | $7.34 \pm 0.01$ | NS |
| $\mathrm{Pa}_{\mathrm{O}_{2}}(\mathrm{mmHg})$ | $96 \pm 2 \ddagger$ | $269 \pm 6 \S$ | $<0.0001$ |
| $\mathrm{PaCO}_{2}(\mathrm{mmHg})$ | $39 \pm 1$ | $36 \pm 2$ | NS |
| $\begin{gathered} \text { Base excess } \\ (\mathrm{mEq} / \mathrm{l}) \end{gathered}$ | $-4.5 \pm 0.4$ | $4.6 \pm 0.4$ | NS |

Values are reported as the mean values ( $\pm \mathrm{SE}$ ) calculated from the average of the individual values for each dog during the steady state period ( $180-240 \mathrm{~min}$ ).

* Group 1, spontaneous rate; Group 2, ventilatory setting.
$\dagger$ Halothane is expressed as \% of the expired gas.
$\ddagger \mathrm{FI}_{\mathrm{O}} \mathrm{O} 0.21$.
$\S \mathrm{FI}_{\mathrm{O}_{1}} 0.5$.

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 119 CL amino acid analyzer. ${ }^{14}$ Plasma $\mathrm{D}-\beta$-hydroxybutyrate, acetoacetate, lactate, pyruvate, and free fatty acids were determined by microfluorometric enzymatic techniques. ${ }^{15-17}$ Plasma insulin was measured by radioimmunoassay. ${ }^{18}$

The ${ }^{3} \mathrm{H}$ and ${ }^{14} \mathrm{C}$ radioactivity in KIC and leucine and ${ }^{14} \mathrm{CO}_{2}$ were determined using a Beckman LS 9800 Series liquid scintillation counter using dual counting mode, which corrects the radioactivity for both quench and the spillover of ${ }^{14} \mathrm{C}$ radioactivity into the ${ }^{3} \mathrm{H}$ energy spectrum.

## Experimental Design

Fourteen healthy mongrel dogs, maintained on commercially available dog chow (Purina Dog Chow, Ral-ston-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 $1(\mathrm{n}=6)$, a catheter was surgically inserted into the femoral artery 7 days prior to the study using Brevital ${ }^{\circledR}$ ( $10-15 \mathrm{mg} / \mathrm{kg}$ 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 ( $\mathrm{FI}_{\mathrm{O}_{2}}=0.21$ ). 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 $2(\mathrm{n}=8)$ anesthesia was induced at $\sim 0800 \mathrm{~h}$ on the morning of the study with $4 \%$ halothane in $50 \%$ nitrous oxide and oxygen $\left(\mathrm{FI}_{\mathrm{O}_{2}}=0.5\right)$. Anesthesia was maintained with an end-expiratory halothane concentration of $1.25 \%(>1.5 \mathrm{MAC})$ 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 $\mathrm{Pa}_{\mathrm{CO}_{2}}$ 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 $-5.5 \mathrm{mEq} / 1$ before completing 120 min of tracer infusion, 10 ml of $8.4 \% \mathrm{Na}$ 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 $\mathrm{cmH}_{2} \mathrm{O}$ 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 $38^{\circ} \mathrm{C}$ (table 1).

Between 0800 and 0900 h , constant infusions of $\left[{ }^{3} \mathrm{H}\right]$ leucine ( $220 \mathrm{nCi} \mathrm{kg}{ }^{-1} \cdot \min ^{-1}$ ) and $\left[{ }^{14} \mathrm{C}\right] \mathrm{KIC}(75 \mathrm{nCi}$ $\mathrm{kg}^{-1} \cdot \mathrm{~min}^{-1}$ ) (each dissolved in 33 ml of $0.9 \% \mathrm{NaCl}$ ) 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 $0.9 \% \mathrm{NaCl}$ at each sampling time. All blood samples were placed in tubes containing
sodium EDTA, the tubes placed on ice and then centrifuged at $4^{\circ} \mathrm{C}$.

Starting at $180 \mathrm{~min}, 2-\mathrm{min}$ samples of expired gas were collected in previously evacuated 20-1 Douglas bags (Warren E. Collins, Inc., Braintree, MA) every 20 min. ${ }^{19}$ The rate of expired ${ }^{14} \mathrm{CO}_{2}$ was determined as previously described. ${ }^{20}$ In addition, ${ }^{14} \mathrm{CO}_{2} \mathrm{SA}$ 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 $p \mathrm{H}$ indicator. ${ }^{21}$ Fourteen milliliters of Safety Solve ${ }^{\otimes}$ (Research Products International, Mount Prospect, IL) were added and the ${ }^{14} \mathrm{C}$ 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 fux. ${ }^{11}$ Assuming steady state conditions (fig. 1), flux was calculated by dividing the infusion rates of $\left[{ }^{3} \mathrm{H}\right]$ LEUCINE or $\left[{ }^{14} \mathrm{C}\right]$ KIC by the plasma SA of the transaminated product (reciprocal pool) of the infused isotope (fig. 2; i.e., plasma [ $\left.{ }^{14} \mathrm{C}\right]-$ leucine SA and [ $\left.\left.{ }^{3} \mathrm{H}\right] \mathrm{kic} \mathrm{SA}\right) .{ }^{22}$

The apparent rate of leucine carbon oxidation was calculated by dividing the measured expired ${ }^{14} \mathrm{CO}_{2}$ (dpm $\cdot \mathrm{kg}^{-1} \cdot \mathrm{~min}^{-1}$ ) by the mean steady state plasma [ $\left.{ }^{14} \mathrm{C}\right]$ leucine SA (fig. 2) and 0.8 (to correct for $\mathrm{CO}_{2}$ fixation). ${ }^{23}$ Total ${ }^{14} \mathrm{CO}_{2}$ production ( $\mathrm{mmol} \cdot \mathrm{kg}^{-1}$. $\mathrm{min}^{-1}$ ) was calculated by dividing expired ${ }^{14} \mathrm{CO}_{2}(\mathrm{dpm}$ $\times \mathrm{kg}^{-1} \cdot \min ^{-1}$ ) by the ${ }^{14} \mathrm{CO}_{2} \mathrm{SA}(\mathrm{dpm} / \mathrm{mmol}) .{ }^{23}$ 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 ( $180-240 \mathrm{~min}$ ). The values obtained at the different


Fig. $1 .{ }^{3} \mathrm{H}$ and ${ }^{14} \mathrm{C}$ specific activities of plasma leucine and $\alpha$-ketoisocaproate (KIC) in conscious ( $n=6$ ) and anesthetized ( $n=8$ ) dogs over the last 60 min of a $240-\mathrm{min}$ infusion of $\left[{ }^{3} \mathrm{H}\right]$ leucine and [ $\left.{ }^{14} \mathrm{C}\right] \mathrm{KIC}$ (mean $\pm \mathrm{SE}$ ).
time points were not statistically different from each other using ANOVA.

## Statistical Analysis

All results are expressed as mean $\pm$ 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 $\mathrm{Pa}_{\mathrm{O}_{2}}$ is not unexpected (table 1). Body weight, respiratory frequency, body temperature, $\mathrm{pH}, \mathrm{Pa}_{\mathrm{CO}_{2}}$ and base excess were similar in the two groups (table 1).


Fig. 2. In the intracellular space the infused tracers [ $\left.{ }^{3} \mathrm{H}\right]$ LEUCINE and $\left[{ }^{14} \mathrm{C}\right] \alpha-$ KETOISOCAPROATE (KIC) are diluted by unlabeled leucine and KIC and rapidly transaminated (not rate-limiting) to $\left[{ }^{3} \mathrm{H}\right]$ kic and $\left[{ }^{14} \mathrm{C}\right]$ leucine, respectively, by branched chain amino transaminase (BCAT). The plasma specific activities of $\left[{ }^{3} \mathrm{H}\right] \mathrm{kic}$ and $\left[{ }^{14} \mathrm{C}\right]-$ leucine are presumed to reflect the intracellular specific activities of $\left[{ }^{3} \mathrm{H}\right]$ - and $\left[{ }^{14} \mathrm{C}\right]$ leucine and are used to calculate leucine flux (reciprocal pool model)..$^{22}$ Therefore, plasma $\left[{ }^{14} \mathrm{C}\right]$ leucine and not $\left[{ }^{14} \mathrm{C}\right] \mathrm{KIC}$ 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 ${ }^{14} \mathrm{CO}_{2}$ gas is that derived from $\left[{ }^{14} \mathrm{C}\right] \mathrm{kic}$. Because the rate of leucine oxidation cannot be calculated using the ${ }^{3} \mathrm{H}$ label, the nonoxidative rate of leucine disappearance ( $\bullet$, 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 ${ }^{3} \mathrm{H}$ and ${ }^{14} \mathrm{C}$ radiotracers.

After 180-240 min of nitrous oxide-halothane anesthesia, mean plasma leucine ( $109 \pm 9 \mathrm{vs} .154 \pm 10 \mu \mathrm{M}, P$ $<0.001$ ) and KIC concentrations ( $17 \pm 1$ vs. $24 \pm 3 \mu \mathrm{M}$, $P<0.01$ ) 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 $\left[{ }^{3} \mathrm{H}\right]-$ and $\left[{ }^{[4} \mathrm{C}\right]$ leucine and -KIC (fig. 1), the SA of expired ${ }^{14} \mathrm{CO}_{2}$, and the rate of ${ }^{14} \mathrm{CO}_{2}$ expired were constant, indicating steady state conditions.
${ }^{14} \mathrm{CO}_{2}$ production $(25.3 \pm 2.9$ vs. $38.3 \pm 2.5 \mathrm{dpm}$ $\times 10^{3} \mathrm{~kg}^{-1} \cdot \min ^{-1}, P<0.01$ ) and the ${ }^{14} \mathrm{CO}_{2} \mathrm{SA}(8.1$ $\pm 1.1 \mathrm{vs} .15 .5 \pm 1.4 \mathrm{dpm} \times 10^{4} / \mathrm{mmol}, P<0.002$ ) were more than $50 \%$ greater in the anesthetized animals when compared to the conscious controls (table 2), whereas the total $\mathrm{CO}_{2}$ production rate was approximately $25 \%$ less in the anesthetized animals.

Total leucine carbon flux was greater using the ${ }^{14} \mathrm{C}$ reciprocal pool SA in the anesthetized group when compared to their controls ( $P<0.05$ ), whereas the ${ }^{3} \mathrm{H}$ data showed only a nonsignificant increase in the anesthetized animals (table 3). Estimates of leucine oxidation (calculated using the $\left[{ }^{14} \mathrm{C}\right]$ 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 ${ }^{3} \mathrm{H}$ 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 ${ }^{14} \mathbf{C O}_{2}$ Production in Conscious and Anesthetized Dogs

| Production in Conscious and Anesthetized Dogs |  |  |  |
| :---: | :---: | :---: | :---: |
|  |  |  | $\begin{array}{c}P \text { Value } \\ \text { Conscious Dogs } \\ \text { (Group 1) }\end{array}$ |
|  |  | $\begin{array}{c}\text { Aneshetized } \\ \text { (oogs (Group 2) }\end{array}$ |  |
| us Group |  |  |  |
| 2 |  |  |  |$]$

[^1]Table 3. Effect of Anesthesia on Reciprocal Pool Estimates of Leucine Metabolism in Dogs

|  | ${ }^{\text {sH Data }}$ |  |  |  |
| :--- | :---: | :---: | :---: | :---: |
|  | Conscious | Anesshetized | Conscious | Anesthetized |
|  | $4.04 \pm 0.12$ | $4.30 \pm 0.19$ | $4.27 \pm 0.10 \ddagger$ | $4.81 \pm 0.19 * \S$ |
| Leucine oxidation | - | - | $0.88 \pm 0.12$ | $1.66 \pm 0.09 \dagger$ |
| NOLD** | $3.19 \pm 0.14$ | $2.64 \pm 0.19 *$ | $3.47 \pm 0.18 \ddagger$ | $3.15 \pm 0.12 \S$ |
| Leucine oxidation/NOLD | $0.28 \pm 0.04$ | $0.67 \pm 0.18 \dagger$ | $0.26 \pm 0.05 \ddagger$ | $0.54 \pm 0.04 \dagger \pi$ |

Values are given as mean $\pm \mathbf{S E}$ and expressed as $\mu \mathrm{mol} \cdot \mathrm{kg}^{-1} \cdot \min ^{-1}$.

* $P<0.05$; conscious versus anesthetized.
$\dagger P<0.001$; conscious versus anesthetized.
$\ddagger P<0.05$; ${ }^{3} \mathrm{H}$ versus ${ }^{14} \mathrm{C}$ data within each group.
$\S P<0.001 ;{ }^{9} \mathrm{H}$ versus ${ }^{14} \mathrm{C}$ data within each group.
II $P<0.01 ;{ }^{3} \mathrm{H}$ versus ${ }^{14} \mathrm{C}$ 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 $\pm 20$ vs. $230 \pm 10 \mu \mathrm{M}, P \leq 0.05$ ) and total ketone body ( $45 \pm 6$ vs. $64 \pm 8 \mu \mathrm{M}, P<0.01$ ) 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, $5 \pm 1$ vs. $5 \pm 1 \mu \mathrm{U} / \mathrm{ml}$, respectively; after 30 min of study $5 \pm 1 \mathrm{vs}$. $5 \pm 1 \mu \mathrm{U} / \mathrm{ml}$; and after 4 h of study $4 \pm 1$ vs. $3 \pm 1 \mu \mathrm{U} / \mathrm{ml})$.


## Discussion

The present study demonstrates that during halo-thane-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 sug-
gest 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

|  | $\begin{aligned} & \text { Before }(-60 \\ & \min )^{*} \end{aligned}$ | After (-15 min)* | $P$ Value Before us. After |
| :---: | :---: | :---: | :---: |
| Glucose (mm) | $5.00 \pm 0.11$ | $6.49 \pm 0.28$ | <0.002 |
| Lactate (mm) | $4.91 \pm 0.37$ | $6.24 \pm 0.83$ | $<0.05$ |
| Pyruvate ( $\mu \mathrm{m}$ ) | $140 \pm 10$ | $280 \pm 30$ | <0.001 |
| Alanine ( mm ) | $0.47 \pm 0.06$ | $1.07 \pm 0.06$ | <0.001 |
| Free fatty acids ( $\mu \mathrm{M}$ ) | $500 \pm 30$ | $220 \pm 30$ | <0.001 |
| Ketone bodies ( $\mu \mathrm{M}$ ) | $63 \pm 1$ | $68 \pm 6$ | NS |
| Total amino acids $\dagger$ (mm) | $2.00 \pm 0.14$ | $2.76 \pm 0.14$ | $<0.005$ |
| Essential amino acids $\ddagger$ (mM) | $0.63 \pm 0.04$ | $0.70 \pm 0.04$ | NS |
| Nonessential amino acids§ (mm) | $1.37 \pm 0.11$ | $2.06 \pm 0.12$ | <0.002 |
| Branched chain amino acids (mm) | $0.29 \pm 0.02$ | $0.30 \pm 0.02$ | NS |

Values are given as mean $\pm$ SE; $n=7$.

* Minutes before start of the isotope infusion.
$\dagger$ Minus tryptophan and glutamine.
$\ddagger$ 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 <br> Between Groups |
| :---: | :---: | :---: | :---: |
| Glucose (mm) |  |  |  |
| -15 | $5.50 \pm 0.06$ | $6.55 \pm 0.22$ | <0.003 |
| 180-240 | $5.33 \pm 0.06$ | $6.22 \pm 0.22$ | $<0.003$ |
| Lactate (mm) |  |  |  |
| -15 | $2.29 \pm 0.16 \S$ | $5.88 \pm 0.75$ | <0.002 |
| 180-240 | $1.39 \pm 0.11 \S$ | $6.02 \pm 1.93$ | $<0.05$ |
| Pyruvate ( $\mu \mathrm{M}$ ) |  |  |  |
| -15 | $110 \pm 20$ | $270 \pm 20$ | $<0.001$ |
| 180-240 | $110 \pm 10$ | $290 \pm 50$ | $<0.009$ |
| Alanine (mm) |  |  |  |
| -15 | $0.32 \pm 0.03$ | $1.12 \pm 0.09$ | $<0.001$ |
| 180-240 | $0.34 \pm 0.02$ | $1.14 \pm 0.12$ | <0.001 |
| Free fatty acids ( $\mu \mathrm{M}$ ) |  |  |  |
| -15 | $560 \pm 130$ | $130 \pm 20 \pi$ | $<0.003$ |
| 180-240 | $680 \pm 100$ | $230 \pm 30 \pi$ | $<0.001$ |
| Total ketone bodies ( $\mu \mathrm{M}$ ) |  |  |  |
| $-15$ | $72 \pm 9$ | $45 \pm 6{ }^{* *}$ $64 \pm 8 * *$ | $<0.03$ |
| 180-240 | $88 \pm 6$ | $64 \pm 8^{* *}$ | $<0.05$ |
| Total amino acids* (mm) |  |  |  |
| -15 | $1.62 \pm 0.09$ | $2.86 \pm 0.19$ | $<0.001$ |
| 180-240 | $1.70 \pm 0.05$ | $3.18 \pm 0.23$ | $<0.001$ |
| Branched chain amino acids $\dagger$ ( mm ) |  |  |  |
| $-15$ | $0.28 \pm 0.02$ | $0.31 \pm 0.03^{* *}$ | NS |
| 180-240 | $0.32 \pm 0.01$ | $0.45 \pm 0.02 * *$ | NS |
| Essential amino acids $\ddagger$ (mM) |  |  |  |
| -15 | $0.60 \pm 0.05$ | $0.75 \pm 0.06 \pi$ | NS |
| 180-240 | $0.67 \pm 0.03$ | $1.00 \pm 0.08 \pi$ | $<0.001$ |
| Nonessential amino acids (mm) |  |  |  |
| -15 - | $1.02 \pm 0.06$ | $2.10 \pm 0.14$ | $<0.001$ |
| 180-240 | $1.04 \pm 0.03$ | $2.18 \pm 0.17$ | $<0.001$ |

Values are given as mean $\pm \mathrm{SE} ;-15=15 \mathrm{~min}$ before start of isotope infusion.

* Minus tryptophan and glutamine.
$\dagger$ Minus tryptophan.
$\ddagger$ Minus glutamine.
$\$ P<0.001 ;-15$ versus $180-240$ within group.
$\mathbb{T} P<0.05 ;-15$ 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 there-
fore 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 ( ${ }^{3} \mathrm{H}$ and ${ }^{14} \mathrm{C}$ ) of leucine flux (table 3) were similar in the conscious dogs, although the flux determination with the ${ }^{14} \mathrm{C}$ tracer was slightly but significantly greater than that observed with the ${ }^{3} \mathrm{H}$ tracer. However, in the anesthetized group of animals, the difference between the leucine flux measured with ${ }^{14} \mathrm{C}$ and the ${ }^{3} \mathrm{H}$ tracers (table 3) was nearly twice as great ( 0.54 vs. $0.26 \mu \mathrm{~mol} \cdot \mathrm{~kg}^{-1} \cdot \mathrm{~min}^{-1},{ }^{14} \mathrm{C}$ and ${ }^{3} \mathrm{H}$ 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 ${ }^{14} \mathrm{C}$ and ${ }^{3} \mathrm{H}$ flux is most likely the result of incomplete equilibration of the $\left[{ }^{14} \mathrm{C}\right] \mathrm{KIC}$ into a relatively large intracellular leucine pool (fig. 2). ${ }^{23,92}$ As a result, our estimates of leucine flux using the ${ }^{14} \mathrm{C}$ data may be slightly overestimated ${ }^{23,32}$ and could account for the significant increase in leucine flux using the ${ }^{14} \mathrm{C}$ data, which was not observed using the ${ }^{3} \mathrm{H}$ data. In contrast, the flux of leucine estimated from the ${ }^{3} \mathrm{H}$ 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 ${ }^{3} \mathrm{H}$ 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 ( ${ }^{3} \mathrm{H}$ data) and maximum ( ${ }^{14} \mathrm{C}$ 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 ${ }^{14} \mathrm{C}$ 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 anesthe-sia-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; $\sim 10-15 \%$ ) 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.

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[^1]:    Values are given as mean $\pm$ SE.

    * Time points are expressed in minutes after starting the infusions of the radioisotopes.
    $\dagger P<0.001$ (comparison within group 2).
    $\ddagger P<0.01$ (comparison within group 2).

