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Isoflurane and Whole Body Leucine, Glucose, and Fatty Acid Metabolism in Dogs

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Following 4 h of general anesthesia with halothane [1.5 minimum alveolar concentration (MAC)]-nitrous oxide (50% in oxygen), whole body protein synthesis is decreased and the rate of leucine oxidation is increased in dogs. To evaluate the effects of general anesthesia with isoflurane on whole body fuel metabolism and the effects of duration of anesthesia on these processes, eight dogs were studied, once in the conscious state (over 9 h) and again prior to and during isoflurane anesthesia (1.5 MAC) for 3.5 h (n = 8). Three additional dogs were studied in the conscious state and over 5 h of anesthesia. Changes in protein, fatty acid, and glucose metabolism were estimated using isotope dilution techniques, employing simultaneous infusions of L-[1-^{14}C]leucine, [6-^{3}H]glucose and [9,10-^{3}H]palmitate. Ten minutes after the beginning of the administration of isoflurane, total leucine carbon flux, leucine oxidation, and leucine incorporation into proteins decreased ($P < 0.05$), resulting in a slight decrease in the ratio of leucine oxidation to nonoxidative leucine disappearance (LOX/NOLD, $P < 0.05$), an indicator of leucine catabolism. Throughout the 5 h of anesthesia, whole body protein synthesis remained decreased ($P < 0.01$), whereas leucine flux and oxidation increased progressively throughout the remainder of the study, resulting in a more than 80% increase in the ratio of LOX/NOLD. After 10 min of isoflurane anesthesia, both plasma free fatty acid concentrations and palmitate turnover had decreased by more than 70% ($P < 0.001$) and remained suppressed ($P < 0.001$) throughout the remainder of the anesthesia, consistent with decreased lipolysis. Glucose production was increased 10 min ($P < 0.05$) following induction of anesthesia and peripheral glucose utilization was decreased following 3.5 h of isoflurane anesthesia ($P < 0.05$). These data strongly suggest a widespread and immediate metabolic effect of isoflurane anesthesia, which includes peripheral insulin resistance to glucose disposal, decreased lipolysis, and a progressive increase in protein wasting with increasing duration of anesthesia. (Key words: Amino acids: leucine. Anesthetics, volatile: isoflurane. Metabolism: glucose; nonesterified free fatty acids; protein.)

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INHALATIONAL ANESTHETIC AGENTS may have significant effects on the regulation of multiple cellular metabolic processes, resulting in alterations of glucose, fatty acid, and protein metabolism. *In vitro* halothane has been reported to decrease hepatic gluconeogenesis and glycogen synthesis and to increase glycogenolysis and glycolysis.[1-3] *In vivo* plasma glucose concentrations increase during halothane and nitrous oxide anesthesia.[4] In rats anesthetized with pentobarbital,[5] this increase in plasma glucose concentration has been attributed to increased hepatic production and decreased peripheral utilization.[5] Whether halogenated volatile anesthetics exhibit similar effects on glucose turnover during general anesthesia remains to be established.

Both enflurane and halothane decrease plasma concentrations of free fatty acids (FFA) in humans and in dogs.[4,6,7] *In vitro* halothane is thought to inhibit lipolysis by inhibiting the activation of the intracellular triglyceride lipase in a dose-dependent manner.†† However, no data are available to determine whether plasma FFA turnover is altered *in vivo* during anesthesia with isoflurane, halothane, or enflurane.

Whole body leucine oxidation is increased and the estimated rate of entry of leucine into body proteins is decreased in nonsurgically traumatized dogs after 4 h of anesthesia with 1.5 MAC halothane and 50% nitrous oxide–oxygen.[4] Because the only source of the essential amino acid leucine in the postabsorptive state is endogenous protein, these data suggest that general anesthesia with halothane and nitrous oxide results in increased rates of protein oxidation and decreased whole body protein synthesis.[8] 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. However, we could not determine whether these changes were due to halothane alone or to the combined effect of halothane, nitrous oxide, and oxygen.[4] Moreover, because measurements of protein metabolism were made over the fifth hour of general anesthesia, we could not assess the effects of duration of general anesthesia on these metabolic processes.

The present study was designed to determine the effects of general anesthesia with isoflurane alone on whole body

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amino acid (and protein), glucose, and fatty acid metabolism in dogs as a function of duration of general anesthesia.

Materials and Methods

Isotopes and Drugs

L-[1-^{14}C]Leucine (57 mCi/mmol), [6-^{3}H]glucose (>55 mCi/mmol), [9,10-^{3}H]palmitate (>25 mCi/mmol), and sodium [^{14}C]bicarbonate were obtained from ICN (Biomedical Inc, Irvine, California). [6-^{3}H]Glucose and L-[1-^{14}C]leucine were dissolved in 70 ml of sterile 0.9% sodium chloride. Sodium [^{14}C]bicarbonate was dissolved in a similar volume of 0.9% sodium chloride, *p*H 10. Normal serum albumin (USP 25%, Travenol Laboratories) was used for the preparation of the [^{3}H]palmitate infusate.[9] Isoflurane was donated by Anaquest (Madison, Wisconsin).

Analytic Methods

Plasma leucine, and α-ketoisocaproate (KIC) concentrations and specific activities (SA) were determined by high performance liquid chromatography (HPLC) and liquid scintillation spectrometry as previously described.[9] Plasma palmitate concentrations, SA, and the concentrations of linoleate and oleate were determined by a modification[10] of a previously reported HPLC method.[11] Plasma glucose concentrations were measured using the glucose oxidation method (Glucose Analyzer 2, Beckman Instruments, Irvine, California). Plasma glucose SA was determined as previously described[12] except that 0.5 ml of the perchloric acid precipitated samples were transferred to two 5 × 1 cm columns (Isolab Inc, QS-Q, Akron, Ohio) arranged in series containing 3 ml of a 50% aqueous solution of an anion exchange resin (AG 1-X8, formate form, 100–200 mesh) and a cation exchange resin (AG 50W × 80, H + form, 100–200 mesh) to remove radiolabeled KIC (AG1) and leucine (AG50), respectively. The columns were then washed four times with 2-ml aliquots of deionized distilled water. The effluent plus washings were collected in scintillation vials (Research Products International, Mount Prospect, Illinois) and thereafter processed as previously described.[12] Note that over 97% [^{3}H]palmitate radioactivity was removed as a result of sample processing, resulting in a final contribution of remaining [^{3}H]palmitate to the [^{3}H]glucose SA of less than 1%. Plasma D-β-hydroxybutyrate, acetoacetate, lactate, pyruvate, and total FFA concentrations were determined by microfluorometric enzymatic techniques.[13–15] Plasma insulin and glucagon were measured by radioimmunoassay.[16,17] Plasma norepinephrine and epinephrine concentrations were determined by radiometric enzyme assay.[18] Hematocrit was determined using a microcapillary centrifuge (Model MB, IEC, Boston, Massachusetts). End-tidal isoflurane concentrations were determined using an infrared gas analyzer (Beckman LB-2/Sensormedics, Anaheim, California). Arterial blood gas tensions (Pa_{CO_2} and Pa_{O_2}) and *p*H were determined using a *p*H/blood gas analyzer (IL System 1302, Instrumentation Laboratory Inc., Lexington, Massachusetts).

The radioactivity in [^{14}C]KIC, [^{14}C]leucine, [^{3}H]palmitate, $^{14}CO_2$ and [^{3}H]glucose was determined using a Beckman LS9800 Series liquid scintillation counter, which corrects the radioactivity for both quench and the spillover of ^{14}C (^{3}H) radioactivity into the ^{3}H (^{14}C) energy spectrum.

Study Protocol

Experimental Design

Eleven healthy mongrel dogs (14–19 kg, 7 females, 4 males) were maintained on dog chow (Purina) and one can of meat daily (Alpo Petfoods Inc, Allentown, Pennsylvania) for at least 1 week prior to the first study and throughout the 2 months of subsequent studies. All the dogs were studied in the postabsorptive (fasting) state after an 18-h fast. Eight dogs were studied twice in random order, and the two studies were separated by 2–4 weeks. On one occasion they were studied in the conscious state for the entire duration of the protocol (group 1, fig. 1), and on another occasion they and three additional dogs were studied in the conscious state followed by 3.5 h (n

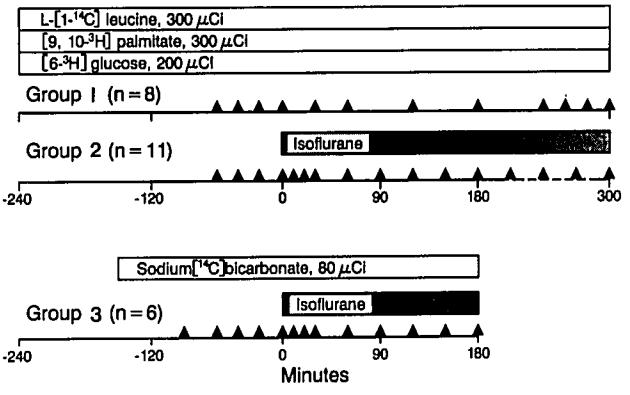

FIG. 1. Dogs of groups 1 (n = 8) and 2 (n = 11 min −240 to +210). In only three of the 11 dogs (broken line) anesthesia was continued to 300 min. All dogs of groups 1 and 2 received iv 300 μCi of L-[1-^{14}C]leucine, 300 μCi of [9,10-^{3}H]palmitate, and 200 μCi [6-^{3}H]glucose from min −240 for the duration of their study. Dogs in group 1 were conscious throughout the study period. After a baseline period in the conscious state (min −60 to 0), dogs in group 2 were anesthetized with isoflurane. Samples of expired gas and blood were sampled as indicated by the arrows (▲). Dogs in group 3 (n = 6) were infused with only Na[^{14}C]bicarbonate. After a baseline period in the conscious state (min −80 to 0), dogs in group 3 were anesthetized for 180 min with isoflurane and only expired air samples were collected as indicated by the arrows (▲).

= 11) of anesthesia with isoflurane (group 2, fig. 1). In the three additional dogs, anesthesia with isoflurane was continued for a total of 5 h. The three additional dogs were studied to permit us to determine whether the effects of isoflurane were sustained over 5 h of anesthesia as we previously observed with halothane and nitrous oxide in oxygen.[4] The effect of isoflurane anesthesia on recovery of $^{14}CO_2$ in expired air during infusion of $NaH^{14}CO_3$ (group 3, fig. 1) was studied 2–3 weeks later in six of the eight dogs studied for 3 h of anesthesia. At least 7 days prior to the first study, female dogs underwent episiotomy (to facilitate bladder catheterization) and a femoral artery catheter was surgically inserted in all dogs during isoflurane anesthesia following methohexital (10–15 mg/kg body weight) induction. The arterial catheter was filled with heparin, knotted, coiled, and placed sterilely in a subcutaneous pouch. All dogs received a single dose of ampicillin (1.25 g) after completion of the surgical procedures. Prior to the study day, all dogs consumed their normal amount of food.

Group 1 (fig. 1): On the day of the study, the subcutaneous arterial catheter was accessed through a cutaneous incision under local anesthesia (2% lidocaine HCl, USP, Invenex, Chagrin Falls, Ohio) and a bladder catheter was inserted while the dogs were standing in a sling. An intravenous (iv) catheter was then inserted into the inferior vena cava *via* a saphenous vein for the infusion of the radioisotopes. ECG, blood pressure (strain gauge), and rectal body temperature (thermistor) were continuously monitored, arterial blood gases were obtained periodically throughout the study, and radioisotopes were infused from min −240 to +300 min (fig. 1).

Group 2 (fig. 1): After a 4-h period of isotope infusion to achieve isotopic equilibration (in the conscious state, minutes −240 to 0, fig. 1), anesthesia was induced with isoflurane (inspired concentration, 4% for about 3 min). Anesthesia was maintained throughout the remainder of the study with an end-tidal isoflurane concentration of 2.2% (1.5 MAC). After induction of anesthesia the dogs were positioned supine, their tracheas were intubated with a cuffed endotracheal tube, and their lungs were mechanically ventilated (Harvard ventilator) with room air containing isoflurane. Minute ventilation was adjusted to result in a Pa_{CO_2} ranging between 35 and 40 mmHg, similar to that observed in the conscious dogs. Arterial blood gas tensions were determined every 30 min. To minimize the development of atelectases, the dogs' lungs were hyperinflated to an airway pressure of 30 cmH_2O every 30 min. To maintain body temperature at about 38° C, the dogs were positioned on a heated water mattress and the inspiratory gas was humidified and heated to 38° C (table 1).

In group 2 anesthesia and isotope infusions were continued to minute 210 in 11 dogs and continued for an additional 90 min in three of these 11 dogs (fig. 1). Before the dogs of group 2 were allowed to awaken, the venous catheters were removed and the arterial catheter washed in Betadine®, filled with heparin, knotted, and in a sterile fashion returned to the subcutaneous pouch. After completion of the study, the dogs received 1.25 g of ampicillin subcutaneously and 150 mg iron dextran (Imferon®, Fisons Corp, Bedford, Massachusetts) iv for 3 days. The dogs were supervised closely until full recovery from anesthesia.

Group 3 (fig. 1): Six dogs were studied in a similar fashion as described above for group 2 with the exception that general anesthesia was induced 160 min after the isotope infusion was initiated and maintained for only 180 min; the only isotope employed was $NaH^{14}CO_3$ (fig. 1). This protocol was approved by the Mayo Clinic Animal Care and Use Committee.

Isotope infusions, blood, and expired air collection: Between 6 A.M. and 7 A.M. on the day of study, constant infusions of L-[1-^{14}C]-leucine (35 $nCi \cdot kg^{-1} \cdot min^{-1}$), [6-^{3}H]glu-

TABLE 1. Characteristics of Dogs before and after General Anesthesia with Isoflurane

	Group 1		Group 2		Group 3	
	Conscious*	Conscious†	Conscious*	Anesthetized‡	Conscious*	Anesthetized‡
N	8 (3M/5F)		11 (4M/7F)		6 (2M/4F)	
Body weight (kg)	15 ± 1		15 ± 1		16 ± 1	
Isoflurane (%)§	—	—	—	2.21 ± 0.02	—	2.20 ± 0.03
Temperature (°C)	38.3 ± 0.1	38.4 ± 0.2	38.3 ± 0.1	38.1 ± 0.1	37.9 ± 0.3	37.8 ± 0.4
*p*H	7.35 ± 0.01	7.34 ± 0.01	7.36 ± 0.01	7.34 ± 0.01	7.36 ± 0.01	7.34 ± 0.01
Pa_{O_2} (mmHg)¶	96 ± 2	93 ± 2	92 ± 2	88 ± 3	92 ± 3	87 ± 3
Pa_{CO_2} (mmHg)	39 ± 1	38 ± 1	39 ± 1	38 ± 1	38 ± 1	39 ± 1
Base excess (mEq/l)	−3.1 ± 0.5	−3.6 ± 0.4	−2.4 ± 0.4	−3.5 ± 0.4	−2.4 ± 0.4	−3.0 ± 0.5
Hematocrit	37 ± 1	34 ± 1**	39 ± 1	34 ± 1**	38 ± 1	33 ± 1**

* Baseline mean (±SE) values were obtained in each group in the conscious state from min −60 to 0 (fig. 1).
† Mean values were obtained in group 1 between 240 and 300 min.
‡ Mean values were obtained during the last hour of isotope infusion.
§ End-tidal isoflurane concentration.
¶ FI_{O_2} 0.21.
** $P < 0.05$ *versus* mean conscious baseline values.

cose (25 nCi · kg^{-1} · min^{-1}) and [9,10-^{3}H]palmitate (35 nCi · kg^{-1} · min^{-1}) (groups 1 and 2) or NaH$^{14}CO_3$ (0.2 μCi and 10 nCi · kg^{-1} · min^{-1}) (group 3) were started and continued for 9 (group 1), 7.5–9.5 (group 2), and 5.7 h (group 3). All tracers were infused using a syringe infusion pump (Razel®, Stamford, Connecticut). Blood samples (10–14 ml) were collected at the time points indicated in figure 1 (total amount of blood withdrawn ~170 ml). The withdrawn blood was replaced with an equal volume of 0.9% NaCl at each sampling time. All blood samples were placed on ice, centrifuged at 4° C, and the plasma stored at −70° C. No blood samples were collected in group 3. Starting at min −60 (groups 1 and 2) and min −80 (group 3) (fig. 1), 2-min samples of expired gas were collected in previously evacuated Douglas bags (Warren E. Collins, Inc., Braintree, Massachusetts) at each of the sampling times as depicted in figure 1. The rate of expired $^{14}CO_2$ and $^{14}CO_2$ SA were determined as previously described.[18–20]

Calculations

Plasma steady state KIC, leucine, palmitate, and glucose concentrations and SA, respiratory frequency, end-tidal isoflurane concentration, pulse rate, arterial blood pressure, body temperature, and arterial blood gas tensions were averaged for each dog during the steady state periods of group 1 (minutes −60 to 0, 0 to 60, 120 to 180, and 240 to 300) and group 2 [minutes −60 to 0, 0 to 60, and 150 to 210, n = 11 (and 240 to 300, n = 3)]. All calculations were carried out at near-isotope and substrate steady state[19] where appropriate. Under steady state conditions the rate of appearance of a substrate (leucine carbon, palmitate, or glucose) equals the rate of disappearance and will be referred to as flux.[20]

The rate of appearance (R_a) of unlabeled leucine was calculated by dividing the infusion rate of L-[1-^{14}C]leucine by the SA of [^{14}C]KIC, the transaminated product of the infused tracer (reciprocal pool model).[20,21] This model assumes that intracellular KIC is solely derived from intracellular leucine and that the plasma SA of [^{14}C]KIC during infusion of [^{14}C]leucine provides a more accurate estimate of intracellular free leucine SA than the plasma [^{14}C]leucine SA. Recent evidence provides strong support for this concept because during combined infusions of [^{3}H]leucine and [1-^{14}C]KIC, the plasma SA of the transaminated product of the infused tracer (reciprocal pool SA, *i.e.*, [^{3}H]KIC and [1-^{14}C]leucine) closely reflected the intracellular leucine SA in all organs investigated (skeletal muscle, diaphragm, heart, and liver) with the exception of kidney, which accounts for less than 1% of whole body protein turnover.[21]

The apparent rate of leucine carbon oxidation was calculated by dividing the measured expired $^{14}CO_2$ (dpm · min^{-1} · kg^{-1}) by the mean steady state plasma [^{14}C]KIC SA.[22] The expired $^{14}CO_2$ was corrected for CO_2 fixation, as measured in dogs in group 3. Estimates of nonoxidative leucine disappearance were calculated by subtracting the rate of leucine oxidation from the estimated total leucine carbon flux.[9] Total CO_2 production (mmol · kg^{-1} · min^{-1}) was calculated by dividing expired $^{14}CO_2$ (dpm · kg^{-1} · min^{-1}) by the $^{14}CO_2$ SA (dpm/mmol).

The rate of systemic glucose flux was determined using the non-steady state equations of Steele[23] as modified by DeBodo *et al.*[24] Because of limited sample availability, results of glucose data are only presented for eight dogs in groups 1 and 2 and through min −60 to +210. The rate of appearance of palmitate was calculated by dividing the [^{3}H]palmitate infusion rate by the [^{3}H]palmitate SA[10,11] and reported as μmol · kg^{-1} · min^{-1} to simplify data presentation.

Statistical Analysis

All results are expressed as mean ± SE. The appropriate method of statistical analysis to determine differences from baseline values over time is controversial.[25] We chose a two-tailed paired Student's *t* test rather than analysis of variance or correction for repeated measures, as has been recently recommended.[25]

Results

Characteristics of dogs: Body temperature, arterial *p*H, Pa_{CO_2}, Pa_{O_2}, and base excess were not different among the study groups and did not change as a result of isoflurane anesthesia. The hematocrit decreased ($P < 0.05$) in all groups, most likely as a result of blood sampling (table 1). Mean arterial blood pressure and pulse rate remained constant in dogs of group 1, indicating that the blood loss did not affect these circulatory parameters (fig. 2). In dogs of groups 2 and 3 mean arterial blood pressure decreased ($P < 0.001$) by about 50% within 10 min following induction of anesthesia, and the pulse rate increased after 30 min ($P < 0.05$, fig. 2).

$^{14}CO_2$ recovery (Group 3): In the conscious state the mean $^{14}CO_2$ recovery in expired gas was 80.8 ± 4.2% (fig. 3) of the infused NaH$^{14}CO_3$. Ten minutes following induction of isoflurane anesthesia, the $^{14}CO_2$ recovery decreased by about 10% ($P < 0.05$). With increasing duration of anesthesia, $^{14}CO_2$ recovery increased progressively reaching a new plateau of 92 ± 3% ($P < 0.05$, conscious *vs.* anesthetized) by 2 h of general anesthesia (fig. 3). In the conscious state the fractional $^{14}CO_2$ recovery was constant and linearly correlated ($r = 0.95$, $P < 0.01$, n = 6) with unlabeled CO_2 production (mmol · kg^{-1} · min^{-1}) and fit the following equation: fractional recovery = 1.95 (kg · min · mmol^{-1}) × CO_2 production + 0.21.

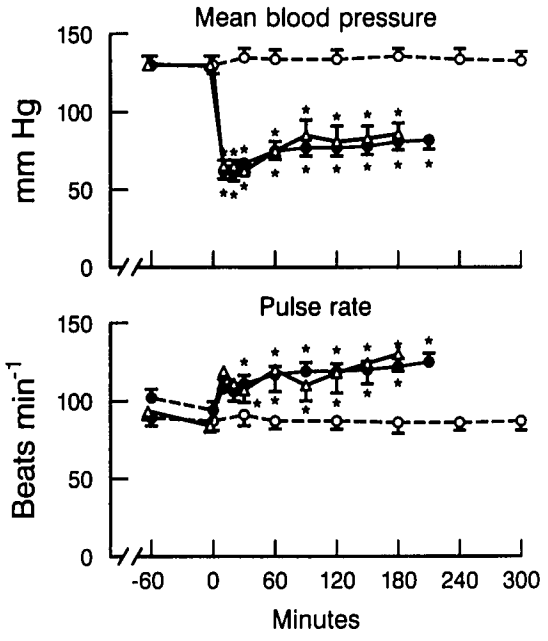

FIG. 2. Mean blood pressure and pulse rate in dogs of group 1 (○ -- ○, conscious throughout the entire study period, −60 to +300 min), group 2 (● -- ●, anesthetized between 0 and 210 min) and group 3 (△ -- △, anesthetized between 0 and 180 min). *$P < 0.05$ *versus* baseline values (min −60 to 0).

Effects of isoflurane anesthesia on whole body leucine metabolism: In conscious animals (group 1) leucine and KIC plasma concentrations and SA, as well as the $^{14}CO_2$ production remained essentially unchanged throughout the period of blood and breath sampling (fig. 4, left panels). In contrast, plasma leucine concentrations in the anesthetized animals (group 2) were increased after 20 min ($P < 0.01$) of anesthesia and continued to increase gradually over the rest of the study (fig. 4, upper right panel), whereas plasma KIC concentrations were decreased 10 min following induction of anesthesia by about 10% ($P < 0.05$) and subsequently increased to values greater than baseline by 210 min of anesthesia ($P < 0.05$, fig. 4, upper right panel). Plasma [^{14}C]leucine and [^{14}C]KIC SA were increased 10 min following induction ($P < 0.01$, fig. 4, right middle panel) to a near-steady state value between 10 and 30 min of anesthesia. Thereafter both the leucine and KIC SA decreased to a new near-steady state following 180 min of anesthesia (fig. 4, right middle panel). $^{14}CO_2$ production decreased 10 min following induction ($P < 0.001$, fig. 4, lower right panel). After 30 min of anesthesia $^{14}CO_2$ production increased progressively to a value higher than baseline (after 60 min, $P < 0.01$) and ultimately reached a value 80% higher than the basal value after 210 min of anesthesia (fig. 4, lower right panel). In the three dogs in which the studies were extended to 300 min, $^{14}CO_2$ production plateaued at a value only slightly higher than that of the 210 min value (data not shown), suggesting that these dogs were approaching a steady state.

Under baseline conditions leucine rate of appearance (leucine flux) was similar in both groups 1 and 2 (table 2). As expected, leucine flux gradually decreased in group 1 (conscious for entire study, table 2) most likely as a result of recycling of tracer (*i.e.*, the release of tracer from the breakdown of proteins radiolabeled over the course

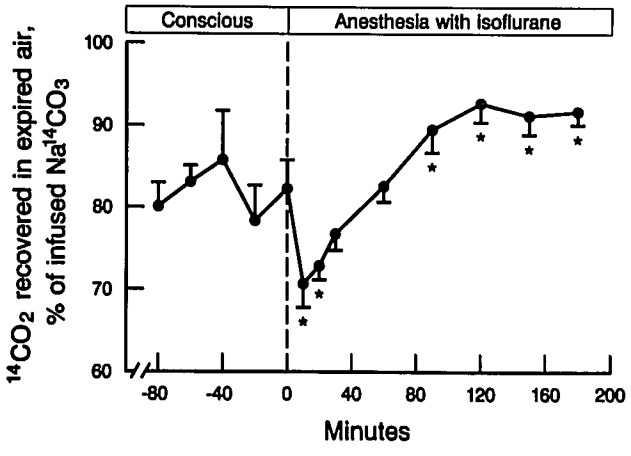

FIG. 3. The effect of isoflurane anesthesia (0 to 180 min) on $^{14}CO_2$ recovery during infusion of Na[^{14}C]bicarbonate (n = 6). *$P < 0.05$ *versus* the mean value for the conscious state (−80 to 0 min).

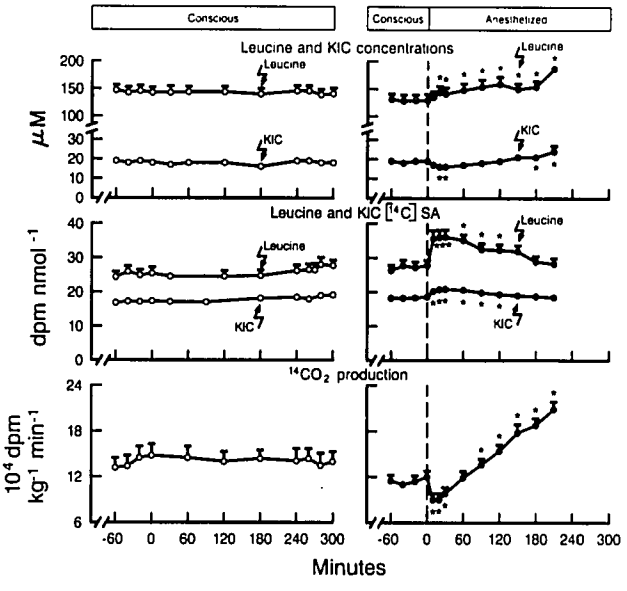

FIG. 4. Plasma leucine and KIC concentrations (upper panel), [^{14}C]leucine and [^{14}C]KIC SA (middle panel), and $^{14}CO_2$ production (lower panel) in dogs of group 1 (conscious throughout the whole study period, left panels) and group 2 (anesthetized with isoflurane from 0 to 210 min, right panels). *$P < 0.05$ *versus* conscious baseline values (min −60 to 0).

TABLE 2. Effects of Anesthesia with Isoflurane on Leucine Rate of Appearance in Conscious and Anesthetized Dogs

	Group 1*		Group 2†	
	Measured	Corrected‡	Measured	Corrected‡
Baseline	4.57 ± 0.16	—	4.51 ± 0.13	—
Hour 1	4.50 ± 0.16	4.57 ± 0.16	3.97 ± 0.11§	4.05 ± 0.11§
Hour 3	4.29 ± 0.08§	4.54 ± 0.08	4.37 ± 0.09	4.65 ± 0.09
Hour 5	4.17 ± 0.10§	4.56 ± 0.10	—	—

Values are mean ± SE and expressed as $\mu mol \cdot kg^{-1} \cdot min^{-1}$. Group 1, n = 8; group 2 baseline through hour 3 (120–180 min), n = 11. Mean values were obtained during each study hour.

* Dogs were studied over 9 h in the conscious state.

† Dogs were first studied in the conscious state (baseline, min −60 to 0, fig. 1) and then during isoflurane anesthesia.

‡ Corrected for recycling of tracer.

§ $P < 0.01$ *versus* baseline values.

of the isotope infusion).[26] Therefore, the actual leucine rate of appearance into the plasma space was progressively underestimated with increasing study duration (group 1 measured values). Assuming that recycling occurred in a linear fashion and was similar in the conscious and anesthetized state, the underestimation of actual leucine flux was estimated to be 0.08 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ per hour of isotope infusion. The measured leucine flux values were corrected appropriately (table 2 corrected values). During the first hour of anesthesia the leucine flux (corrected for recycling) decreased by more than 10%, increased to basal values by 3 h (table 2), and in the three additional dogs, it continued to increase to a value higher ($P < 0.05$) than baseline by hour 5 (4.51 ± 0.13 *vs.* 4.84 ± 0.13 $\mu mol \cdot kg^{-1} \cdot min^{-1}$).

Leucine oxidation (LOX) and nonoxidative leucine disappearance (NOLD) were similar under baseline conditions in groups 1 and 2 (table 3) and remained essentially unchanged throughout the study in group 1. In contrast, after induction of anesthesia (group 2), LOX decreased by about 20% over the first hour and subsequently increased to a value nearly 50% higher than baseline by hour 3 of anesthesia ($P < 0.001$, table 3) and to a value ~ 70% higher in the three dogs studied for 5 h of anesthesia (not shown in table 3). NOLD, an indicator of whole body protein synthesis, decreased following only 10 min of anesthesia and remained suppressed throughout the duration of general anesthesia (table 3).

The ratio of LOX to NOLD, an indicator of whole body leucine catabolism, was constant throughout the entire study in conscious animals (group 1). In anesthetized animals (group 2) this ratio decreased ($P < 0.05$) over the first hour of anesthesia and then gradually increased to a value ~ 40% above baseline ($P < 0.001$) by hour 3 of anesthesia and continued to rise to a value more than 70% above baseline in the three dogs studied for 5 h of anesthesia (not shown in table 3).

The total plasma amino acid concentration was increased 10 min following induction of anesthesia, mainly due to an increase in the nonessential amino acid alanine (fig. 5). Total amino acids, nonessential amino acids, and alanine concentrations reached a plateau after about 1–2 h of anesthesia (fig. 5). Plasma essential amino acids gradually increased throughout the anesthesia period, paralleling the increase in leucine plasma concentrations (fig. 4) and leucine flux (table 2).

Effects of general anesthesia on plasma free fatty acid concentrations and palmitate turnover: In the conscious animals (group 1) plasma concentrations of total FFA, palmitate, linoleate, and oleate increased slightly during the study (fig. 6). In the anesthetized animals (group 2) FFA concentrations decreased by more than 80% within 10 min of induction of anesthesia with isoflurane and then increased slightly throughout the remainder of the study but never reached the values obtained during the conscious baseline period (fig. 6).

The coefficient of variation for replicate analysis of the baseline palmitate flux determination was 15 ± 3%, indicating a relatively high interday variability. Despite the random order of study, the baseline flux of palmitate in groups 1 and 2 was different ($P < 0.05$, fig. 7). In the conscious dogs (group 1) palmitate flux increased progressively throughout the study, most likely due to con-

TABLE 3. Effect of Isoflurance Anesthesia on Leucine Oxidation (LOX) and Nonoxidative Leucine Disappearance (NOLD)

	LOX		NOLD*		LOX/NOLD	
	Group 1 Conscious	Group 2 Anesthetized	Group 1 Conscious	Group 2 Anesthetized	Group 1 Conscious	Group 2 Anesthetized
Baseline†	0.97 ± 0.11	0.78 ± 0.03	3.60 ± 0.16	3.73 ± 0.12	0.27 ± 0.02	0.23 ± 0.01
Hour 1	1.00 ± 0.10	0.64 ± 0.04‡	3.56 ± 0.15	3.42 ± 0.10‡	0.26 ± 0.02	0.19 ± 0.01‡
Hour 3	0.94 ± 0.09	1.12 ± 0.06‡	3.51 ± 0.14	3.53 ± 0.09‡	0.26 ± 0.02	0.32 ± 0.02‡
Hour 5	0.90 ± 0.11	—	3.60 ± 0.15	—	0.25 ± 0.02	—

Values are mean ± SE and expressed as $\mu mol \cdot kg^{-1} \cdot min^{-1}$. Group 1, n = 8; group 2 baseline through hour 3, n = 11. Mean values were obtained during each study hour following the baseline measurements.

* Values corrected for recycling of tracer used for the calculation of NOLD.

† Baseline values in dogs in both groups 1 and 2 were in the conscious state (min −60 to 0, fig. 1).

‡ $P < 0.01$ *versus* baseline values.

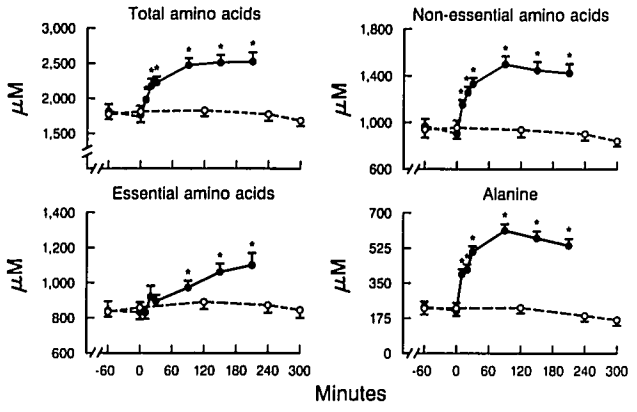

FIG. 5. Plasma concentrations of total, nonessential, and essential amino acids and alanine in dogs of group 1 (conscious throughout the study period; open circles) and group 2 (anesthetized with isoflurane from 0 to 210 min; closed circles). *$P < 0.01$ *versus* baseline values (min −60 to 0). More than 50% of the increase in nonessential amino acids was the result of the increase in plasma concentrations of alanine. Serine, proline, and glycine accounted for 7%, 9%, and 14%, respectively, of the increase in plasma concentrations of nonessential amino acids.

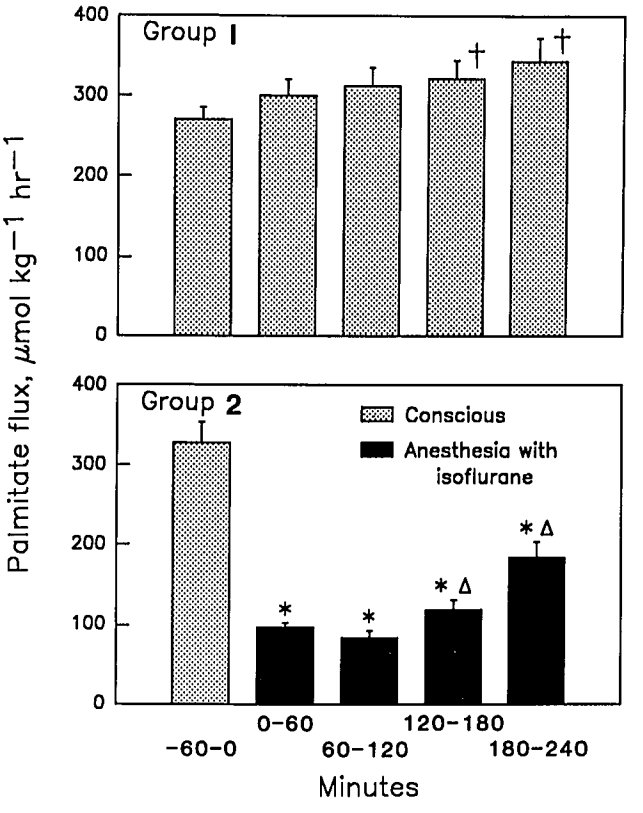

FIG. 7. Effects of isoflurane anesthesia on palmitate flux. †$P < 0.05$ *versus* the mean baseline value in group 1 (conscious throughout the study period). *$P < 0.01$ *versus* the mean baseline value in group 2 (dogs anesthetized with isoflurane from 0 to 210 min). The 180 to 240 min data for group 2 represent data from 180 to 210 min. $^{\Delta}P < 0.05$ *versus* the value obtained during the previous hour of study.

tinued fasting ($P < 0.05$, fig. 7, upper panel). In contrast, palmitate flux decreased by about 70% 10 min after induction of anesthesia in animals of group 2 ($P < 0.001$, fig. 7) and remained suppressed through hour 3 of anesthesia. In the three dogs studied over 5 h palmitate flux increased from hours 3–5 of anesthesia (data not shown). Plasma ketone body concentrations did not change as a result of anesthesia (baseline 0.11 ± 0.01 μmol, after 210 min of isoflurane anesthesia: 0.12 ± 0.01 μmol).

Effects of general anesthesia on glucose metabolism: Plasma glucose, pyruvate, and lactate concentrations increased immediately following induction of anesthesia ($P < 0.01$) and remained elevated throughout the remainder of the study (fig. 8). The basal glucose fluxes (rate of appearance or disappearance) in dogs of groups 1 and 2 were 2.80 ± 0.09 and 3.25 ± 0.13 $mg \cdot kg^{-1} \cdot min^{-1}$, respectively, and demonstrated an interday coefficient of variation of 14 ± 4%. In the absence of anesthesia (group 1) glucose flux remained constant throughout the period of study (table 4). The glucose rate of appearance (R_a) increased within 10 min of induction of anesthesia, whereas its rate of disappearance (R_d) decreased ($P < 0.05$). Subsequently, glucose R_a decreased and R_d increased progressively to similar values (table 4) by 90 min of anesthesia. By 3.5 h glucose flux (both R_a and R_d) was lower ($P < 0.05$) than basal values (table 4). The metabolic clearance rate of glucose, an indicator of the efficiency of glucose transport into cells, was decreased throughout this period of anesthesia ($P < 0.05$, table 4), although we recognize that glucose metabolic clearance rates are not independent of plasma glucose concentrations[27] (table 4).

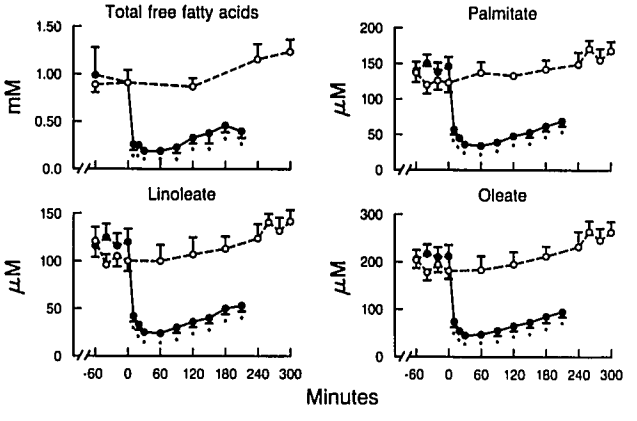

FIG. 6. Plasma concentrations of total FFA, palmitate, linoleate, and oleate in dogs of group 1 (conscious throughout the study period; open circles) and group 2 (anesthetized with isoflurane from 0 to 210 min; closed circles). *$P < 0.01$ *versus* baseline values (min −60 to 0).

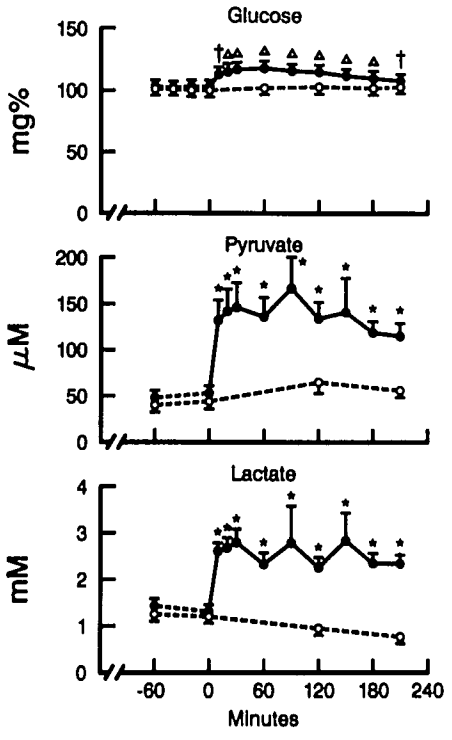

FIG. 8. Plasma concentrations of glucose, pyruvate, and lactate in dogs of group 1 (conscious throughout the study period, open circles) and group 2 (anesthetized with isoflurane from 0 to 210 min, closed circles). †$P < 0.05$, ($^{\Delta}P < 0.01$; *$P < 0.001$) *versus* baseline values (min −60 to 0).

Effects of general anesthesia on plasma hormone concentrations: Plasma insulin concentrations decreased slightly as a consequence of general anesthesia ($P < 0.05$, table 5), whereas plasma glucagon and norepinephrine concentrations remained unchanged in both groups. In contrast, plasma epinephrine concentrations increased by more than 200% immediately following induction of anesthesia in six of the eight dogs, whereas it decreased in two dogs. Only minor struggling and excitation of the dogs was observed following the administration of isoflurane. However, after 3 h of anesthesia, the epinephrine concentrations were again in the normal range in all dogs. No correlation between plasma epinephrine concentrations and glucose, fatty acid, and protein turnover could be detected.

Discussion

We previously demonstrated that during halothane (1.5 MAC)-nitrous oxide (50% in oxygen) anesthesia, leucine oxidation was increased and leucine entry into protein was decreased, suggesting that anesthesia of more than 4 h duration without concomitant surgery induces a protein catabolic state in dogs.[4] The present study was designed to examine the evolution of changes in carbohydrate, fat, and protein metabolism during isoflurane anesthesia. To our knowledge, the present study demonstrates for the first time the rapidity of onset of the effects of isoflurane on *in vivo* endogenous fuel metabolism in dogs and that the magnitude of these changes are in part modulated by the duration of the anesthesia itself. Therefore, experiments designed to examine changes in substrate homeostasis and conducted in the presence of a volatile halogenated anesthetic can be interpreted only when accompanied by appropriate control experiments in awake dogs.

Effects of Isoflurane Anesthesia on Leucine and Protein Metabolism

During the first hour of isoflurane anesthesia, the rates of release of leucine from endogenous protein (leucine R_a), LOX, and NOLD (an indicator of whole body protein synthesis) were all decreased, indicating no increased losses of proteins over this period of time compared with the postabsorptive conscious state. However, a progressive increase in the loss of protein occurred beyond the first hour of anesthesia as indicated by the gradual increase in proteolysis (increased leucine R_a) and the persistent

TABLE 4. Effects of Isoflurance Anesthesia on Glucose Metabolism

	Group	0 min*	10 min	20 min	90 min	150 min	210 min
Glucose (mg/%)	1	100 ± 2	—	—	102 ± 3	—	101 ± 2
	2	102 ± 2	115 ± 5†	118 ± 7‡	116 ± 4‡	113 ± 5‡	112 ± 5‡
Glucose SA (dpm/μg)	1	9.5 ± 0.5	—	—	9.6 ± 0.6	—	10.2 ± 0.7
	2	9.5 ± 1.2	8.9 ± 1.2	8.7 ± 1.0	9.0 ± 1.0	9.6 ± 1.0	10.3 ± 1.2
Rate of appearance	1	2.80 ± 0.09	—	—	2.86 ± 0.17	—	2.78 ± 0.18
($mg \cdot kg^{-1} \cdot min^{-1}$)	2	3.25 ± 0.13	4.55 ± 0.46†	3.47 ± 0.57	3.36 ± 0.18	3.12 ± 0.11	2.85 ± 0.12
Rate of disappearance	1	2.80 ± 0.09	—	—	2.86 ± 0.12	—	2.78 ± 0.18
($mg \cdot kg^{-1} \cdot min^{-1}$)	2	3.25 ± 0.13	2.73 ± 0.26†	2.87 ± 0.17	3.38 ± 0.12	3.20 ± 0.09	2.93 ± 0.11
Metabolic clearance rate	1	2.88 ± 0.07	—	—	2.82 ± 0.12	—	2.72 ± 0.12
($ml \cdot kg^{-1} \cdot min^{-1}$)	2	3.20 ± 0.15	2.53 ± 0.29‡	2.45 ± 0.19‡	2.83 ± 0.17‡	2.76 ± 0.13‡	2.59 ± 0.12‡

Minutes indicate the time after the induction of isoflurane anesthesia in dogs of group 2 and following the baseline period in group 1.
* Baseline values of the dogs of groups 1 (n = 8) and 2 (n = 8) were obtained in the conscious state (min −60 to 0, fig. 1).
† $P < 0.05$ *versus* the baseline value in each group.
‡ $P < 0.01$ *versus* the baseline value in each group.

TABLE 5. Effects of Isoflurance Anesthesia on Insulin, Glucagon, and Catecholamine Plasma Concentrations

	Insulin (μU/ml)		Glucagon (pg/ml)		Norepinephrine (pg/ml)		Epinephrine (pg/ml)	
	Group 1 Conscious	Group 2 Anesthesia	Group 1 Conscious	Group 2 Anesthesia	Group 1 Conscious	Group 2 Anesthesia	Group 1 Conscious	Group 2 Anesthesia
Baseline*	4.4 ± 0.4	4.7 ± 0.7	204 ± 32	220 ± 14	197 ± 31	170 ± 19	128 ± 29	113 ± 34
10 min	—	3.4 ± 0.4†	—	200 ± 15	—	137 ± 32	—	377 ± 129
Hour 3	4.6 ± 0.5	3.1 ± 0.2†	217 ± 45	182 ± 12	162 ± 20	179 ± 29	121 ± 35	175 ± 51

* Baseline values in dogs of groups 1 (n = 8) and 2 (n = 8) were obtained in the conscious state (min −60 to 0, fig. 1).

† $P < 0.05$ *versus* baseline.

suppression in protein synthesis (decreased NOLD). This progressive loss of protein is most dramatically demonstrated by the changes in the ratio of LOX/NOLD (table 3).

The underlying mechanism(s) responsible for these changes in leucine and protein metabolism may not be a direct effect of isoflurane but may be due to the reduced blood pressure (fig. 2) and/or muscle relaxation accompanying isoflurane anesthesia. The reduced blood pressure could be associated with altered tissue perfusion and thus indirectly protein metabolism. Skeletal muscle relaxation, a well-known effect of isoflurane anesthesia,[28] results in a time-dependent increase in protein breakdown *in vitro*.[29,30] In this regard, it is of interest to note that the rate of appearance of leucine paralleled the plasma concentrations of KIC throughout the period of anesthesia (but not plasma leucine during the first hour of anesthesia). This suggests that plasma KIC concentrations might be a better indicator of the free intracellular leucine pool than plasma leucine itself. Regardless of the mechanism, the present and previous (halothane in combination with oxygen and nitrous oxide[4]) studies suggest that the duration of general anesthesia should be minimized to reduce protein losses associated with the anesthesia per se.

We previously observed that whole body protein synthesis was suppressed after 4–5 h of halothane–nitrous oxide anesthesia. This observation is consistent with *in vitro* studies of rat lung[31] and liver[32] and *in vivo* studies of rat albumin production,[33] which demonstrated a dose-dependent inhibition of protein synthesis. The present studies extend our previous finding by demonstrating that whole body protein synthesis decreases abruptly with induction of anesthesia with isoflurane alone and remains suppressed to the same extent throughout the entire 5 h of anesthesia. Because the amino acid, glucose, and FFA results after 4–5 h of anesthesia with isoflurane alone are similar to those previously reported with halothane and 50% nitrous oxide in oxygen,[4] we would conclude that the changes in leucine metabolism observed in this and in our previous study are most likely due to the halogenated anesthetic agents rather than to nitrous oxide or oxygen.

Several possible mechanisms for the suppression of protein synthesis must be considered: 1) isoflurane might inhibit directly protein synthesis, as is thought to occur with halothane[31–33]; 2) because of the abrupt decrease in endogenous protein breakdown, substrate availability for protein synthesis might have been rate-limiting immediately following induction of anesthesia; and 3) changes in protein synthesis could be the result of changes in the plasma concentration of insulin. The abrupt decreased entry of leucine into whole body proteins (protein synthesis) was paralleled by a small but persistent and significant decrease in plasma insulin concentrations. Because insulin is an anabolic hormone, it might be hypothesized that the effects of isoflurane anesthesia on whole body protein synthesis may be in part the result of a decrease in the plasma concentrations of insulin. However, the abrupt decrease in whole body protein synthesis, the absence of an acute increase in the rate of appearance of leucine,[34] and the relatively small change in the plasma insulin concentrations make such a mechanism unlikely. From these data we cannot determine the etiology of the abrupt decrease in whole body protein synthesis. Whether this is a direct effect of isoflurane itself, the result of decreased intracellular availability of leucine for protein synthesis over the induction period of general anesthesia, and/or due to the hypotensive effects of the anesthetic agent remains to be established. Further studies are needed to determine the duration of the effects of anesthesia on protein metabolism following discontinuation of the anesthesia and/or whether the protein catabolic effects of general anesthesia are further accelerated when combined with surgery.

EFFECTS OF ISOFLURANE ANESTHESIA ON FREE FATTY ACID TURNOVER

During anesthesia with halothane or enflurane, plasma concentrations of FFA decrease in humans and dogs.[6,7] Moreover, halothane inhibits the intracellular activation of the triglyceride lipase *in vitro*,†† a key regulating enzyme in adipose tissue lipolysis. The observed decrease in plasma FFA concentrations during halothane–nitrous

oxide anesthesia in our previous study could either be due to a decrease in FFA turnover or an increase in FFA oxidation or both. Using [^{3}H]palmitate in the present study, we demonstrated that the rate of appearance of plasma palmitate (an indicator of lipolysis) was reduced by about 70% within 10 min following induction of anesthesia. These findings are consistent with an isoflurane-mediated inhibition of intracellular triglyceride lipase, as has been observed for halothane *in vitro*.†† Despite the large decrease in both the plasma FFA concentrations and lipolysis, plasma ketone body concentrations did not change during isoflurane anesthesia. This suggests that hepatic β-oxidation was not adversely affected by isoflurane anesthesia or that a higher proportion of plasma FFA was directed to ketogenesis during anesthesia and/or that intrahepatic triglycerides may be serving as the carbon substrate for β-oxidation and ketogenesis during general anesthesia. An alternative possibility is that a significant fraction of ketone body carbon is derived from the increased oxidation of the branched chain amino acids (leucine, fig. 4, table 3; isoleucine, and valine).

Effects of General Anesthesia on Glucose Turnover

The small increase in plasma glucose concentrations observed immediately following induction of anesthesia and the subsequent slight decrease over the remainder of the anesthetic period (table 4, fig. 8) is consistent with previous reports in rats[5,35] and dogs[4] anesthetized with pentobarbital[5] or halothane.[4,35] The rapid initial increase in the plasma glucose, lactate, and pyruvate concentrations was associated with a transient increase in hepatic glucose production (R_a); all of these changes are most likely the result of the increased plasma concentrations of epinephrine, a known activator of glycogenolysis and glycolysis.

Over the course of the study, glucose R_a and R_d decreased to values $\simeq$ 10% below basal by 210 min of anesthesia, consistent with previous reports of decreased glucose turnover in rats during either halothane[35] or pentobarbital anesthesia.[5] The continuous decrease in hepatic glucose production over time might be the result of a progressive depletion of hepatic glycogen stores over the time course of the study[36] and/or a decrease in gluconeogenesis, which is reported to be inhibited by halothane.[2,3] With regards to the decrease in glucose utilization (R_d), whole body glucose utilization decreases in rats with increasing duration of anesthesia.[5,35] Decreased glucose utilization in peripheral tissues, such as brain[37] and postural muscle[5] observed *in vitro* using barbiturate and halothane, may account for the observed decrease in systemic glucose R_d in the present study in dogs and is consistent with previous studies in rats.[5]

In summary, the present studies in dogs provide strong evidence for a widespread and immediate effect of isoflurane on protein synthesis, proteolysis, lipolysis, hepatic glucose production, and peripheral glucose utilization. Whether these results are generally applicable to humans undergoing general anesthesia and the determination of the consequences of these changes in whole body fuel metabolism remain to be elucidated.

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