# Cardiovascular effects of equipotent doses of isoflurane alone and isoflurane plus fentanyl in New Zealand White rabbits (*Oryctolagus cuniculus*)

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**OBJECTIVE**

To determine effects of equipotent concentrations of fentanyl and isoflurane, compared with isoflurane alone, on cardiovascular variables in New Zealand White rabbits (*Oryctolagus cuniculus*).

**ANIMALS**

6 adult female New Zealand White rabbits.

**PROCEDURES**

Rabbits were anesthetized with isoflurane, and lungs were mechanically ventilated. The minimum alveolar concentration (MAC) of isoflurane alone (baseline) and with fentanyl administered IV to achieve 3 targeted plasma concentrations was determined for each rabbit by means of an electrical stimulus. Cardiovascular variables were measured in a separate experiment at 1.3X isoflurane MAC and equipotent doses of isoflurane plus fentanyl at the same 3 targeted plasma concentrations. Blood samples were collected for measurement of blood gas variables and plasma fentanyl concentrations. Treatment effects were evaluated by repeated-measures ANOVA followed by 2-tailed paired *t* tests with sequentially rejective Bonferroni correction.

**RESULTS**

Mean ± SD MAC of isoflurane was 1.95 ± 0.27%. Mean measured plasma fentanyl concentrations of 4.97, 8.93, and 17.19 ng/mL reduced isoflurane MAC by 17%, 37%, and 56%, respectively. Mean measured plasma fentanyl concentrations during cardiovascular measurements were 5.49, 10.26, and 18.40 ng/mL. Compared with baseline measurements, heart rate was significantly lower at all 3 plasma fentanyl concentrations, mean arterial blood pressure and systemic vascular resistance were significantly higher at mean fentanyl concentrations of 10.26 and 18.40 ng/mL, and cardiac output was significantly higher at 18.40 ng of fentanyl/mL.

**CONCLUSIONS AND CLINICAL RELEVANCE**

Administration of fentanyl in isoflurane-anesthetized rabbits resulted in improved mean arterial blood pressure and cardiac output, compared with isoflurane alone. This balanced anesthesia technique may prove useful in the management of clinical cases in this species. (*Am J Vet Res* 2015;76:591–598)

Rabbits are common research subjects and popular household pets, and demand for their veterinary care, including surgical procedures requiring general anesthesia, has increased in the United States in recent years.[1] In 1 study,[2] the mortality rate of rabbits undergoing anesthesia was significantly higher than that of dogs and cats. The exact reason for this is unknown, but 1 proposed mechanism may be hypotension associated with inhalation anesthesia in this species.[3,4]

**ABBREVIATIONS**

| | |
|---|---|
| CO | Cardiac output |
| CVP | Central venous pressure |
| MAC | Minimum alveolar concentration |
| MAP | Mean arterial blood pressure |
| SAP | Systolic arterial blood pressure |
| SVRI | Systemic vascular resistance index |

In some species, μ-opioid receptor agonists, such as fentanyl, reduce the amount of inhalation anesthetic required to prevent movement in response to a noxious stimulus, as evidenced by a reduction in MAC.[5,6] In addition to their analgesic effects, the reduction in inhalation anesthetic dose afforded by opioid receptor agonists is associated with reduced magnitude of cardiovascular depression in some species.[6,7]

Fentanyl has been shown to reduce isoflurane MAC in rabbits[8]; however, to our knowledge, whether this provides cardiovascular benefits has not been investigated. The aim of the study reported here was to determine the effects of equipotent immobilizing doses of isoflurane alone and in combination with fentanyl at 3 targeted plasma concentrations on cardiovascular variables in New Zealand White rabbits

(*Oryctolagus cuniculus*). We hypothesized that combinations of isoflurane and fentanyl would result in significantly less cardiovascular depression than an equipotent concentration of isoflurane alone.

## Materials and Methods

### Animals

Six adult female New Zealand White rabbits (mean ± SD weight, 3.7 ± 0.2 kg) were used in the study. Rabbits were acclimatized for ≥ 2 weeks prior to the study and were group housed in a temperature- and humidity-controlled, 3 × 3-m room with a cycle of 12 hours of light to 12 hours of darkness. Hiding places and toys were provided for enrichment, and the room was spot cleaned once daily and deep cleaned twice weekly. Rabbits were judged to be healthy on the basis of physical examination (LSB). Rabbits were fed a pelleted diet[a] with free access to hay[b] and water; food was not withheld prior to anesthesia. Rabbits were housed individually in 0.6 × 0.6 × 0.4-m stainless steel wire cages[c] overnight following the first experiment (MAC determination) and for 7 days following the second experiment (cardiovascular assessment) to enable observation of appetite, urination, and defecation; assessment for signs of pain; and prevention of access by other rabbits to skin sutures. The study was approved by the university's institutional animal care and use committee.

### Study design

Rabbits were anesthetized for 2 experiments ≥ 2 weeks apart. In the first experiment, MAC was determined in duplicate for each isoflurane-anesthetized rabbit prior to administration of fentanyl (ie, baseline) and at 3 targeted plasma fentanyl concentrations (4, 8, and 16 ng/mL, determined on the basis of results of a previous study[8]). In the second experiment, cardiopulmonary variables were measured at 1.3× individual rabbit's isoflurane MAC at baseline and at the same 3 targeted plasma fentanyl concentrations as in experiment 1.

### Fentanyl infusion scheme

Fentanyl was administered by use of a target-controlled infusion system[d] and syringe pump.[e] On the basis of unpublished pharmacokinetic data obtained previously by our laboratory regarding fentanyl in isoflurane-anesthetized rabbits, this system rapidly loaded the central compartment to the targeted concentration and updated the infusion rate every 10 seconds to maintain a pseudo–steady-state plasma concentration as previously described.[8] For this pharmacokinetic model, volume of the central compartment was 581 mL/kg and clearance was 80.2 mL/kg/min.

### MAC determination

Rabbits were placed in a transparent acrylic chamber (0.4 × 0.2 × 0.25 m) into which isoflurane[f] in oxygen was delivered at a flow rate of 6 L/min via a Bain circuit. Once immobile, rabbits were removed from the chamber and anesthetic delivery was continued via facemask. An endotracheal tube (internal diameter, 3.5 to 4.0 mm) was then placed for anesthetic delivery and connected to a Bain circuit, with the oxygen flow rate at 200 mL/kg/min and an initial isoflurane vaporizer setting of 2.5%. A 22-gauge, 4.4-cm catheter[g] was placed in a lateral saphenous vein for infusion of lactated Ringer's solution[h] (3 mL/kg/h); the same catheter was used for fentanyl[i] infusion. A 22-gauge, 2.5-cm catheter[j] was inserted in an auricular artery for blood sample collection. A multiparameter anesthesia monitor[k] was used to monitor ECG, esophageal temperature, inspired and expired respiratory gases ($Pco_2$ and concentration [% atm] of oxygen and nitrogen), isoflurane concentration, and pulse oximetry data. The monitor was calibrated prior to each experiment against 3 known isoflurane concentrations and a certified thermometer.

Body temperature was maintained between 38.5° and 39.5°C with a warming blanket[l] used as needed. Once instrumentation had been completed, intermittent positive pressure ventilation[m] was instituted to maintain end-tidal $Pco_2$ between 25 and 35 mm Hg. Three pairs of needle electrodes were placed subcutaneously on the ventral surface of the tail for the application of electric stimulus during MAC determination.

Measurements of MAC were performed in duplicate by means of the bracketing method at baseline and at targeted plasma fentanyl concentrations of 4, 8, and 16 ng/mL, administered in ascending order. Fifteen minutes after stable end-tidal isoflurane concentration, end-tidal gas samples were collected in triplicate with a glass syringe. For the purposes of respiratory gas sampling, a catheter was placed through the endotracheal tube adapter into the lumen of the endotracheal tube to its distal third and connected to a 3-way stopcock. Approximately 2 mL of gas was collected at the end of each expiration; collected gas from the first few breaths was expelled to the atmosphere to wash out dead space from the sampling system. Subsequently, each of the 3 samples collected included gas from 5 or 6 breaths. The mean of the 3 samples was calculated and used for analysis.

A supramaximal electrical stimulus[n] (50 Hz, 15 V, and 6.5 milliseconds) was delivered to 1 pair of needles for 1 minute or until gross movement occurred as detected by 2 observers who were not blinded to treatment (LSB and CCT). The end-tidal isoflurane concentration was increased by 10% if movement occurred or decreased by 10% if movement did not occur, and another 15-minute period of stable end-tidal isoflurane concentration was allowed prior to reapplication of the stimulus. This procedure was repeated until 2 successive isoflurane concentrations, one allowing and the other preventing movement, were obtained. The mean of these 2 values was considered the MAC. The position of the stimulus was rotated among the 3 pairs of electrodes for each rabbit throughout the experiment. For determination of plasma fentanyl concentrations, arterial blood samples (0.5 mL) were collected

and centrifuged and plasma was rapidly frozen. This was performed once at baseline and twice for each targeted plasma concentration (4, 8, and 16 ng/mL): the first sample was obtained 15 minutes after starting the fentanyl infusion, and the second was collected at the time of the second MAC determination for each targeted plasma concentration. The mean of these 2 values was considered the measured plasma fentanyl concentration for each targeted concentration. The total volume of blood collected from each rabbit during the MAC determination experiment was 3.5 mL.

## Cardiovascular instrumentation

Anesthetic induction, tracheal intubation, and maintenance of anesthesia with isoflurane were performed as described for the MAC determination experiments. A 22-gauge, 4.4-cm catheter[g] was placed in a saphenous vein for infusion of lactated Ringer's solution[h] at 3 mL/kg/h and fentanyl.[i] After clipping of fur and surgical skin preparation with an aseptic technique, the right jugular vein and carotid artery were surgically exposed through a 2- to 3-cm skin incision. A 22-gauge, 4.4-cm catheter[g] was placed in the carotid artery, and a 14-gauge, 5-cm catheter[j] was placed in the jugular vein and connected to a hemostasis valve[o] to act as an introducer. A 4F, 75-cm thermodilution balloon catheter[p] was passed through the introducer and into the pulmonary artery under fluoroscopic guidance. Rabbits were then positioned in left lateral recumbency, and the lungs were mechanically ventilated[m] to maintain end-tidal $P_{CO_2}$ between 25 and 35 mm Hg. The pulmonary artery catheter was connected to calibrated pressure transducers[q] for recording CVP and pulmonary artery pressure waveforms, which were also used to guide final pulmonary artery catheter positioning. Intended placement of the proximal port of the pulmonary artery catheter was in the right atrium; however, this was not anatomically confirmed in every animal, and its placement in the distal aspect of the cranial vena cava was not ruled out. In addition to CVP and mean pulmonary artery pressure, the pulmonary artery catheter was used to measure mean pulmonary artery occlusion pressure, core body temperature, and CO. Samples of mixed venous blood were also obtained through the pulmonary artery catheter. The carotid artery catheter was connected to a calibrated pressure transducer[q] to measure arterial blood pressures and was used for collection of arterial blood samples.

## Investigation of cardiovascular effects

Following anesthetic induction and instrumentation, end-tidal isoflurane concentration was set to 1.3X MAC determined for the individual rabbit. Following 20 minutes of stable end-tidal isoflurane concentration, measurements were collected. Physiologic acquisition hardware[r] and software[s] continuously monitored and recorded vascular pressures, pulse rate, and ECG. A multiparameter anesthesia monitor,[k] calibrated prior to each experiment, was used to monitor cardiorespiratory variables as described for MAC determination. The CO was determined in triplicate by thermodilution. In brief, 3 mL of iced 5% dextrose in sterile water[h] was injected through the proximal port of the pulmonary artery catheter and CO was determined by a computer.[t] The mean of the 3 measurements was used as CO for data analysis.

Samples of arterial and mixed venous blood (0.3 mL) were collected into heparinized glass tubes[u] for blood gas analysis[v] and into microhematocrit tubes[w] for determination of PCV by microcentrifugation and total protein concentration by refractometry. Additional arterial blood samples (0.5 mL) were obtained at 10 and 20 minutes after starting the fentanyl infusion or changing the targeted fentanyl concentration, to measure fentanyl plasma concentrations as described. After baseline measurements were obtained, fentanyl was administered as for the MAC experiment, with the same targeted plasma concentrations used in ascending order. The end-tidal isoflurane concentration was adjusted to 1.3X the individual rabbit's MAC determined for each corresponding plasma fentanyl concentration. After 20 minutes of equilibration, measurements were repeated for each of the targeted plasma fentanyl concentrations. Total blood volume collected from each rabbit during the cardiovascular investigations was 4.7 mL.

To assess whether anticholinergic administration influenced cardiovascular effects of the tested treatments, following determination of the cardiovascular variables at the highest plasma fentanyl concentration, glycopyrrolate[x] (0.5 mg/kg) was administered IV and heart rate and vascular pressures were recorded for an additional 15 minutes. Because of adverse effects noted in the postoperative period for the first 2 rabbits that received this treatment, glycopyrrolate was not administered to the remaining rabbits.

Cardiac index, stroke index, rate pressure product, SVRI, pulmonary vascular resistance index, left and right ventricular stoke work indices, arterial and mixed-venous oxygen content, oxygen delivery, oxygen consumption, oxygen extraction ratio, alveolar–arterial $P_{O_2}$ difference, and shunt fraction were calculated with standard equations.[9–11] Barometric pressure was obtained from the University of California climate station, and body surface area was determined according to published data.[12]

## Recovery

At the end of the experiments, the fentanyl infusion was discontinued, buprenorphine[y] (0.05 mg/kg) was administered IV, and instruments were removed. After the cardiovascular investigation experiment, digital pressure was applied to the neck for 20 to 30 minutes to achieve hemostasis and the overlying skin was closed with suture.[z] Isoflurane administration was discontinued, and the endotracheal tube was removed when the rabbit was chewing. Meloxicam[aa] (0.5 mg/kg) was administered SC following extubation. The saphenous catheter was

removed when the rabbit began to ambulate. Mentation, appetite, defecation, urination, signs of discomfort, and sites of catheterization, surgical incision, and electrical stimulation were monitored daily for 7 days following both experiments. Rabbits were given additional meloxicam (0.5 mg/kg, SC, q 24 h) if pain was suspected. Skin sutures were removed 7 days after the cardiovascular experiment.

### Plasma fentanyl analysis

Following collection, blood samples were centrifuged for 10 minutes, and plasma was removed and stored at –20°C until analysis. Plasma fentanyl concentrations were determined with liquid chromatography–mass spectrometry as described elsewhere.[13] The lower limit of quantification for the assay was 0.05 ng/mL. Quality control samples were run at 3 concentrations (0.15, 25, and 80 ng/mL), with an accuracy (percentage of nominal concentration) of 113%, 103%, and 101%, respectively, and precision (percentage relative SD) of 12%, 7%, and 4%, respectively. For each targeted plasma concentration, the mean measured plasma concentration was calculated for the purposes of data analysis.

### Statistical analysis

Data are reported as mean ± SD unless otherwise indicated. Mean plasma fentanyl concentrations were compared at each targeted concentration from both experiments by means of a paired *t* test.[bb] In the cardiovascular experiment, the difference between measured and desired end-tidal isoflurane concentrations was expressed as a percentage of the desired concentration (ie, 1.3X isoflurane MAC measured at the targeted plasma fentanyl concentration). Following confirmation that data were consistent with normality (Kolmogorov-Smirnov test), data were analyzed for an effect of fentanyl concentration on the MAC of isoflurane and effect of fentanyl concentration on cardiorespiratory variables by repeated-measures ANOVA.[bb] Where significant effects were detected, comparisons with the baseline value were performed by means of a 2-tailed paired Student *t* test.[bb] Significance was set at $P < 0.05$, and sequentially rejective Bonferroni adjustment was used to account for multiple comparisons.

## Results

Two rabbits received a second dose of meloxicam 24 hours after the MAC experiment because mild swelling and erythema were detected at the sites of electrical stimulation. Following the cardiovascular experiment, one rabbit received 1 additional dose and another received 2 additional doses of meloxicam because of swelling at the incision site and resentment of incisional palpation. The 2 rabbits that received glycopyrrolate had reduced to absent fecal production for 24 hours after the experiment and were syringe fed an herbivore recovery food[cc] twice daily for 2 days after the experiment. By day 3, food consumption and defecation increased and recoveries were considered normal for the remaining period of observation.

### MAC determinations

Baseline isoflurane MAC was 1.95 ± 0.27%. Measured plasma concentrations of fentanyl were 4.97 ± 0.65 ng/mL (targeted concentration, 4 ng/mL), 8.93 ± 0.96 ng/mL (targeted concentration, 8 ng/mL), and 17.19 ± 1.76 ng/mL (targeted concentration, 16 ng/mL). Fentanyl significantly ($P < 0.003$) reduced isoflurane MAC, compared with baseline, by 17 ± 8%, 37 ± 6%, and 56 ± 7% at plasma concentrations of 4.97, 8.93, and 17.19 ng/mL, respectively **(Figure 1)**.

### Effects of fentanyl on cardiopulmonary variables

In the cardiovascular investigations, measured fentanyl concentrations were 5.49 ± 0.19 ng/mL, 10.26 ± 0.81 ng/mL, and 18.40 ± 1.65 ng/mL for the targeted concentrations of 4, 8, and 16 ng/mL, respectively. The measured concentration for each targeted fentanyl concentration did not differ significantly from that observed in the MAC experiments. Measured end-tidal isoflurane concentration varied < 4% from the desired concentration at baseline and at each of the 3 fentanyl concentrations **(Table 1).**

The combination of 1.3X isoflurane MAC plus the lowest mean measured plasma fentanyl concentration (5.49 ng/mL; equipotent to 1.3X isoflurane MAC) had minimal effects on most measured cardiopulmonary variables, compared with baseline measurements; however, significant decreases in heart rate ($P = 0.001$) and rate pressure product ($P = 0.038$) were observed, and stroke index was significantly ($P = 0.002$) increased (Table 1). At the 2 highest mean plasma fentanyl concentrations (10.26 and 18.40 ng/mL, respectively), there were significant increases in

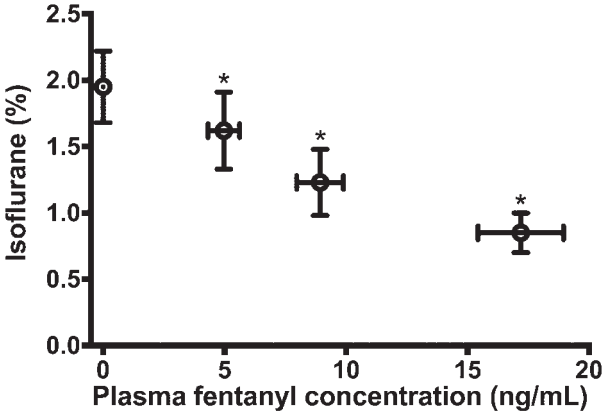


**Figure 1**—Mean ± SD isoflurane MAC before (baseline) and after administration of fentanyl at 3 plasma concentrations in 6 New Zealand White rabbits (*Oryctolagus cuniculus*). After baseline measurements, each rabbit received infusions of fentanyl in ascending order to achieve targeted plasma fentanyl concentrations of 4, 8, and 16 ng/mL, corresponding to mean ± SD measured fentanyl concentrations of 4.97 ± 0.65 ng/mL, 8.93 ± 0.96 ng/mL, and 17.19 ± 1.76 ng/mL, respectively. *Value is significantly ($P < 0.05$, sequentially rejective Bonferroni technique) different from baseline.

**Table 1**—Mean ± SD end-tidal isoflurane concentration and cardiovascular variables at 1.3X MAC of isoflurane (baseline) and equipotent doses of isoflurane plus fentanyl at 3 plasma concentrations in 6 New Zealand White rabbits (*Oryctolagus cuniculus*).

| | | Measured plasma fentanyl concentration (ng/mL) | | |
|---|---|---|---|---|
| **Variable** | **Baseline** | **5.49 ± 0.19** | **10.26 ± 0.81** | **18.40 ± 1.65** |
| End-tidal isoflurane concentration | | | | |
| Measured (%) | 2.57 ± 0.34 | 2.13 ± 0.37* | 1.62 ± 0.31* | 1.12 ± 0.18* |
| Difference (desired vs measured value; %)† | 1 ± 2 | 1 ± 2 | 1 ± 2 | 1 ± 2 |
| Heart rate (beats/min) | 225 ± 15 | 186 ± 12* | 190 ± 10* | 192 ± 13* |
| SAP (mm Hg) | 68 ± 7 | 69 ± 10 | 80 ± 9* | 82 ± 3* |
| MAP (mm Hg) | 40 ± 8 | 42 ± 8 | 50 ± 6* | 56 ± 3* |
| DAP (mm Hg) | 25 ± 9 | 29 ± 8 | 36 ± 6* | 44 ± 2* |
| CVP (mm Hg) | 6 ± 2 | 7 ± 1 | 8 ± 1* | 8 ± 2* |
| MPAP (mm Hg) | 16 ± 1 | 16 ± 1 | 16 ± 2 | 17 ± 2 |
| PAOP (mm Hg) | 9 ± 2 | 10 ± 1 | 10 ± 2 | 10 ± 2 |
| CO (L/min) | 0.47 ± 0.08 | 0.45 ± 0.07 | 0.51 ± 0.07 | 0.52 ± 0.08* |
| Cardiac index (L/min/$m^2$) | 2.0 ± 0.3 | 1.9 ± 0.3 | 2.1 ± 0.3 | 2.2 ± 0.3* |
| Stroke index (mL/beat/kg) | 0.6 ± 0.1 | 0.7 ± 0.1* | 0.7 ± 0.1* | 0.7 ± 0.1* |
| SVRI (dyne•s/$cm^5$/$m^2$) | 1,320 ± 240 | 1,482 ± 357 | 1,607 ± 267* | 1,772 ± 227* |
| PVRI (dyne•s/$cm^5$/$m^2$) | 273 ± 93 | 278 ± 52 | 251 ± 62 | 281 ± 97 |
| RPP (mm Hg•beats/min) | 8,990 ± 1,996 | 7,929 ± 1,970* | 9,571 ± 1,258 | 10,801 ± 1,108 |
| LVSWI (g•m/$m^2$) | 5.4 ± 2.2 | 6.3 ± 1.5 | 8.2 ± 1.8* | 9.3 ± 1.7* |
| RVSWI (g•m/$m^2$) | 2.1 ± 0.5 | 2.3 ± 0.4 | 2.7 ± 0.6 | 2.8 ± 0.6* |

For each rabbit, isoflurane MAC at baseline (ie, before fentanyl administration) and at each of 3 targeted plasma fentanyl concentrations had been previously determined; cardiovascular experiments were performed in rabbits anesthetized with 1.3X MAC of isoflurane and equipotent doses of isoflurane plus fentanyl at each targeted concentration. A stabilization period of 20 minutes at baseline and after each adjustment in plasma fentanyl concentration was allowed before measurements were obtained.

*Within a row, value is significantly ($P < 0.05$) different from value at baseline when adjusted for multiple comparisons (sequentially rejective Bonferroni technique). †Mean desired values for isoflurane concentration were 2.54%, 2.10%, 1.60%, and 1.10% respectively.

DAP = Diastolic arterial blood pressure. LVSWI = Left ventricular stroke work index. MPAP = Mean pulmonary artery pressure. PAOP = Pulmonary artery occlusion pressure. PVRI = Pulmonary vascular resistance index. RPP = Rate pressure product. RVSWI = Right ventricular stroke work index.

**Table 2**—Mean ± SD blood gas and derived cardiorespiratory variables at 1.3X MAC of isoflurane (baseline) and equipotent doses of isoflurane plus fentanyl in the same rabbits as in Table 1.

| | | Measured plasma fentanyl concentration (ng/mL) | | |
|---|---|---|---|---|
| **Variable** | **Baseline** | **5.49 ± 0.19** | **10.26 ± 0.81** | **18.40 ± 1.65** |
| Arterial blood pH | 7.55 ± 0.06 | 7.55 ± 0.07 | 7.54 ± 0.09 | 7.56 ± 0.06 |
| $Pa_{CO_2}$ (mm Hg) | 36.3 ± 2.7 | 38.0 ± 2.0 | 39.8 ± 6.7 | 37.8 ± 5.5 |
| $Pa_{O_2}$ (mm Hg) | 466 ± 37 | 512 ± 20* | 477 ± 30 | 458 ± 34 |
| Arterial hemoglobin (g/dL) | 9.4 ± 0.2 | 9.1 ± 0.2 | 9.2 ± 0.3 | 9.2 ± 0.2 |
| Mixed-venous pH | 7.50 ± 0.05 | 7.49 ± 0.07 | 7.49 ± 0.07 | 7.50 ± 0.05 |
| Mixed-venous $P_{CO_2}$ (mm Hg) | 36 ± 3 | 39 ± 2 | 41 ± 6 | 39 ± 6 |
| Mixed-venous $P_{O_2}$ (mm Hg) | 52 ± 7 | 50 ± 3 | 62 ± 16 | 51 ± 4 |
| Mixed-venous hemoglobin (g/dL) | 9.4 ± 0.6 | 9.2 ± 0.5 | 9.2 ± 0.6 | 9.1 ± 0.5 |
| Arterial lactate (mmol/L) | 1.6 ± 0.8 | 1.8 ± 0.8 | 2.1 ± 0.9 | 2.4 ± 1.0* |
| Arterial oxygen content (mL/dL) | 14.2 ± 0.6 | 13.9 ± 0.7 | 13.9 ± 0.8 | 13.9 ± 0.6 |
| Mixed-venous oxygen content (mL/dL) | 11.5 ± 1.0 | 11.1 ± 0.8 | 11.6 ± 0.8 | 11.1 ± 0.3 |
| Oxygen delivery (mL/min) | 69.6 ± 18.1 | 63.2 ± 11.6 | 70.5 ± 12.5 | 73.0 ± 13.0 |
| Oxygen consumption (mL/min) | 12.4 ± 1.4 | 12.8 ± 1.3 | 11.9 ± 3.8 | 15.1 ± 4.3 |
| Oxygen extraction ratio | 0.19 ± 0.04 | 0.21 ± 0.03 | 0.17 ± 0.04 | 0.20 ± 0.03 |
| Alveolar-arterial $P_{O_2}$ difference (mm Hg) | 181 ± 38 | 136 ± 20* | 173 ± 35 | 198 ± 30 |
| $Q_S/Q_T$ | 0.18 ± 0.06 | 0.13 ± 0.02* | 0.19 ± 0.08 | 0.17 ± 0.03 |
| Total protein (g/dL) | 4.1 ± 0.5 | 3.9 ± 0.3 | 3.9 ± 0.3 | 4.0 ± 0.3 |
| PCV (%) | 28 ± 1 | 27 ± 2 | 27 ± 2 | 28 ± 1 |

$Q_S/Q_T$ = Shunt fraction (venous admixture).
See Table 1 for remainder of key.

SAP ($P < 0.001$ and $P = 0.009$), MAP ($P < 0.001$ and $P = 0.003$), diastolic arterial pressure ($P = 0.007$ and $P = 0.003$), CVP ($P = 0.009$ and $P = 0.004$), stroke index ($P = 0.001$ and $P = 0.003$), SVRI ($P = 0.002$ and $P = 0.003$), and left ventricular stroke work index ($P = 0.002$ and $P = 0.006$) and significant decreases in heart rate ($P$

= 0.002 and $P$ = 0.003), compared with baseline measurements. Additionally, at the highest plasma fentanyl concentration there were significant increases in CO ($P$ = 0.016), cardiac index ($P$ = 0.044), and right ventricular stroke work index ($P$ = 0.013), compared with baseline data. The experimental treatments had minimal effects on blood gas variables **(Table 2)**. Arterial blood lactate concentration at the highest mean plasma fentanyl concentration was significantly ($P$ = 0.044) increased from that at baseline. In the 2 rabbits anesthetized at 1.3X MAC of isoflurane plus the highest concentration of fentanyl, glycopyrrolate (0.5 mg/kg, IV) had no effect on heart rate, SAP, or MAP.

## Discussion

In the present study, we compared cardiovascular variables in rabbits that received isoflurane (1.3X MAC) alone and equipotent doses of isoflurane plus fentanyl at 3 targeted plasma concentrations. Mean ± SD measured concentrations of fentanyl were 5.49 ± 0.19 ng/mL, 10.26 ± 0.81 ng/mL, and 18.40 ± 1.65 ng/mL during investigation of cardiovascular effects. We found a reduced magnitude of cardiovascular depression in rabbits anesthetized with isoflurane plus fentanyl, compared with isoflurane alone, as evidenced by increased SAP and MAP (at mean plasma fentanyl concentrations of 10.26 and 18.40 ng/mL) and CO (at a mean plasma fentanyl concentration of 18.40 ng/mL). These findings are consistent with the reported effects of μ-opioid receptor agonists in cats and dogs under inhalation anesthesia.[6,7] The increase in arterial blood pressures detected in our study was attributable to increases in SVRI as well as in CO, and the changes occurred despite significant decreases in heart rate. Although the CO at 1.3X MAC of isoflurane at a mean fentanyl concentration of 18.40 ng/mL (0.52 L/min or approx 141 mL/kg/min) was significantly higher than the baseline value (ie, the measurement in rabbits anesthetized at 1.3X isoflurane MAC alone), it was much lower than the CO previously described for chronically instrumented awake rabbits (225 mL/kg/min).[14] In the absence of increased preload or decreased afterload, increased stroke index (found at all fentanyl concentrations in our study) suggested that increased myocardial contractility was responsible for the increased CO. This increase in contractility, as well as increased SVRI at higher fentanyl concentrations, is consistent with the effects of a reduction in isoflurane concentration, given that isoflurane has been shown to produce dose-dependent reductions in myocardial contractility and SVRI in mechanically ventilated New Zealand White rabbits.[4] The magnitude of the increase in CO would likely have been greater if heart rate had not decreased, which would also have further improved arterial blood pressures.

In isoflurane-anesthetized dogs, remifentanil administration has been shown to increase SVRI.[15] This is unlikely to be a direct effect of the opioid and more likely a physiologic compensatory response to a reduction in heart rate and, subsequently, CO. Supportive evidence in that study[15] included increases in serum vasopressin concentrations accompanying the reduction in heart rate and increased SVRI. Additionally, in bradycardic dogs anesthetized with isoflurane and fentanyl, normalizing heart rate was associated with a significant reduction of SVRI toward baseline.[6] In the present study, heart rate was significantly reduced at the lowest plasma fentanyl concentration tested (mean, 5.49 ng/mL), where SVRI was unchanged. The heart rate remained at a similar value in rabbits at all 3 plasma fentanyl concentrations, with no reduction in CO. For these reasons, we do not think that a physiologic response to reduced flow explains the increase in SVRI in rabbits observed at higher fentanyl concentrations in this study.

The decrease in heart rate caused by opioids in dogs is believed to be mediated by vagal efferent conduction from the medulla.[16] Use of an anticholinergic agent to prevent bradycardia has been shown to improve CO in dogs anesthetized with enflurane and fentanyl.[6] We attempted to increase heart rate with glycopyrrolate unsuccessfully in 2 rabbits in this study. Because of subsequent profound gastrointestinal effects as well as lack of effect on heart rate, we elected not to administer glycopyrrolate to any other rabbits. The effect of anticholinergic drugs on heart rate is mediated by muscarinic acetylcholine receptor antagonism and resultant decreased parasympathetic tone.[17] Glycopyrrolate was selected rather than atropine for use in our study because rabbits are reported likely to have naturally occurring atropinesterases.[18] The lack of heart rate response to glycopyrrolate in the present study is an interesting finding and suggests that the decrease in heart rate associated with fentanyl administration in isoflurane-anesthetized rabbits may not be mediated by increased parasympathetic tone or that, in the presence of high concentrations of fentanyl, glycopyrrolate at the dose used does not decrease parasympathetic tone enough to increase heart rate. Other mechanisms for the decrease in heart rate could include a decrease in the magnitude of the baroreceptor response subsequent to a high degree of systemic vascular resistance. Alternatively, if sympathetic tone is largely inhibited during anesthesia with isoflurane and fentanyl in rabbits, decreasing parasympathetic tone may not result in increased heart rate. The adverse gastrointestinal effects that developed in the rabbits following glycopyrrolate administration suggested the lack of heart rate response was not attributable to the drug being ineffective as a result of improper manufacturing or handling.

Physiologically, increasing CO to maintain MAP is more likely to maintain tissue perfusion than increasing vascular resistance to maintain MAP, which can adversely affect perfusion.[19] However, compared with other domestic mammals under inhalation anesthesia,[15,20] systemic vascular resistance is substantially lower in rabbits receiving inhalation anesthetics.[4] Therefore, management of inhalation anesthetic–induced hypotension in rabbits by judicious increases

in systemic vascular resistance, in addition to efforts to increase CO, may improve tissue perfusion. Studies examining tissue blood flow under conditions of varying vascular resistance would help to further guide management of inhalation anesthetic–induced hypotension in rabbits.

High doses of fentanyl produce hypoventilation and apnea, necessitating intermittent positive pressure ventilation.[6,21] In addition, hypercapnia increases sympathetic nervous system tone and can alter cardiovascular variables.[22] Both CO and MAP were found to be lower in mechanically ventilated, versus spontaneously breathing, rabbits anesthetized at 4.15% isoflurane (approx 2X MAC).[4] This effect is likely attributable in part to the effect of increasing $Paco_2$ with increasing isoflurane concentrations in spontaneously breathing animals and the negative impact of positive intrathoracic pressures on venous return in mechanically ventilated animals.

In the present study, we used 1.3X the MAC of isoflurane to simulate a surgical plane of anesthesia, as would be likely in a clinical scenario. We achieved this by increasing the end-tidal isoflurane concentration to 1.3X that of the individual rabbit at MAC without adjusting the dose of fentanyl used to achieve each targeted plasma concentration. The targeted plasma fentanyl concentrations were selected as those with the most clinical promise on the basis of results of a previous study[8] in New Zealand White rabbits, which was performed in our laboratory. In that study,[8] plasma fentanyl concentrations of 36 ng/mL were associated with excessive spontaneous movement and rigidity, limiting the clinical benefit of fentanyl doses to achieve that concentration. Interestingly, in that same study,[8] the measured plasma concentrations of fentanyl were approximately half of the targeted values. The source of that discrepancy could not be identified; however, the fact that target concentrations were achieved and slightly exceeded in the present study through the use of a similar pharmacokinetic model suggested an error in drug delivery could have been the source of the earlier discrepancy.

To maintain steady plasma fentanyl concentrations in the present study, target-controlled infusions were used, with treatments administered in ascending order to avoid unintentionally high concentrations during transitions from higher to lower concentrations. Because of the infusion scheme used, we cannot exclude an effect of time under anesthesia on cardiovascular variables; however, the only variable that has been consistently reported to increase with time in anesthetized rabbits is blood lactate concentration,[23] similar to the finding in our study of increased arterial blood lactate concentration at the highest fentanyl concentration. Although there was no significant change in global measurements of oxygen delivery, oxygen consumption, or oxygen extraction ratio, it is possible that local variations in oxygen delivery may have increased lactate production or reduced its clearance.

Buprenorphine was selected for postoperative analgesia because of its high affinity for the μ-opioid receptor, which may partially or fully displace fentanyl from the μ-opioid receptor and thereby reverse the latter drug-induced respiratory depressant and behavioral effects.[24] Additionally, the degree of postoperative pain in both experiments was expected to be mild. This may not be applicable to clinical cases undergoing more invasive and painful procedures. In such cases, the effects of fentanyl in the immediate recovery period should be closely monitored.

The combination of fentanyl and isoflurane appears to hold promise as a balanced anesthesia technique in rabbits. A large, prospective clinical study of the use of fentanyl during inhalation anesthesia is needed in this species to evaluate the effects on short- and long-term recovery and whether this technique alters the morbidity and mortality rates associated with anesthesia in rabbits.

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## Footnotes

a. High Fiber Rabbit Diet No. 5326, PMI Nutrition International, Brentwood, Miss.
b. Oxbow Animal Health, Murdock, Neb.
c. Suburban Surgical, Wheeling, Ill.
d. RUGLOOP I, Demed, Temse, Belgium.
e. Harvard PhD 22/2200, Harvard Apparatus, Hollister, Mass.
f. Abbott Animal Health, Abbott Park, Ill.
g. Intracan Safety Catheter, B Braun Melsungen AG, Melsungen, Germany.
h. Baxter Healthcare, Deerfield, Ill.
i. Fentanyl citrate, Hospira Inc, Lake Forest, Ill.
j. Insyte catheter, Becton-Dickinson, Sandy, Utah.
k. GE Healthcare, Helsinki, Finland.
l. Hotdog, Augustine Temperature Management, Eden Prairie, Minn.
m. Hallowell EMC, Pittsfield, Mass.
n Grass Instrument Co, Quincy, Mass.
o. Check-Flo, Cook Inc, Bloomington, Ind.
p. Thermodilution balloon catheter, Arrow International, Reading, Pa.
q. DTX-Plus, Argon Medical Devices, Plano, Tex.
r. PowerLab, ADInstruments, Dunedin, New Zealand.
s. LabChart, ADInstruments, Dunedin, New Zealand.
t. COM-1, American Edwards Laboratories, Irvine, Calif.
u. Clinitube, Radiometer Medical, Copenhagen, Denmark.
v. i-STAT CG4+ Abbott Point of Care, Abbott Laboratories, Abbott Park, Ill.
w. Fisherbrand, ThermoFisher Scientific, Waltham, Mass.
x. Robinul, West-Ward Pharmaceuticals, Eatontown, NJ.
y. Buprenex injectable, Reckitt Benckiser, Richmond, Va.
z. Ethicon, Somerville, NJ.
aa. Loxicom, Norbrook Laboratories Ltd, Newry, Northern Ireland.
bb. Prism, version 6, GraphPad Software Inc, La Jolla, Calif.
cc. Critical Care, Oxbow Animal Health, Murdock, Neb.

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