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Nitrous oxide improves cardiovascular, respiratory, and thermal stability during prolonged isoflurane anesthesia in juvenile guinea pigs

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Abstract

Anesthesia is frequently used to facilitate physiological monitoring during interventional animal studies. However, its use may induce cardiovascular (central and peripheral), respiratory, and thermoregulatory depression, confounding results in anesthetized animals. Despite the wide utility of guinea pigs as a translational platform, anesthetic protocols remain unstandardized for extended physiological studies in this species. Therefore, optimizing an anesthetic protocol that balances stable anesthesia with intact cardiorespiratory and metabolic function is crucial. To achieve this, 12 age and sex-matched juvenile Dunkin Hartley guinea pigs underwent extended anesthesia (≤150 min) with either (a) isoflurane (ISO: 1.5%), or (b) isoflurane + N_2O (ISO+ N_2O: 0.8% +70%), in this randomized cross-over designed study. Cardiovascular (HR, SBP, peripheral microvascular blood flow), respiratory (respiratory rate, SpO_2), and thermal (T_{re} and T_{sk}) measures were recorded continuously throughout anesthesia. Blood gas measures pre- and post- anesthesia were performed. Incorporation of 70% N_2O allowed for significant reductions in isoflurane (to 0.8%) while maintaining an effective anesthetic depth for prolonged noninvasive physiological examination in guinea pigs. ISO+N_2O maintained heart rate, peripheral blood flow, respiratory rate, and thermoregulatory function at levels closest to those of conscious animals, especially in females; however, it did not fully rescue anesthesia-induced hypotension. These results suggest that for studies requiring prolonged physiological examination (≤150 min) in guinea pigs, 0.8% isoflurane with a 70% N_2O adjuvant provides adequate anesthesia, while minimizing associated cardiorespiratory depression. The preservation of cardiorespiratory status is most marked throughout the first hour of anesthesia.

Abbreviations: ANOVA, analysis of variance; ECG, electrocardiography; HR, heart rate; ISO, Isoflurane; LDF, laser Doppler flowmetry; MAC, minimum alveolar concentration; N_2O, nitrous oxide; NIBP, noninvasive blood pressure; PET, positron emission tomography; PU, perfusion units; RH, relative humidity; RR, respiratory rate; SBP, systolic blood pressure; SpO_2, oxygen saturation; TCO_2, total carbon dioxide; T_{re}, rectal temperature (analogue for core body temperature); T_{sk}, skin temperature.

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1 | INTRODUCTION

Animal models are essential for our understanding of the pathophysiological basis of human health and disease, and provide the means by which novel pharmacological treatments are developed and tested.[1] Anesthesia is utilized to limit movement (e.g., radiological studies), or to mitigate confounding levels of physiological stress induced by handling.[2-4] While murine species are the most commonly anesthetized small animal models,[2,5] guinea pigs are also used in a wide range of research modalities requiring anesthetized monitoring, including pharmacology,[6,7] reproductive and developmental physiology,[8,9] immunology,[10] and toxicology.[11] Despite the wide utility of guinea pigs as a translational platform, a standardized anesthetic protocol remains to be established.

Achieving stable anesthesia without abolishing cardiorespiratory or metabolic function is notoriously difficult, but crucial during physiological studies. Investigators have utilized a wide range of agents and doses to achieve anesthesia in guinea pigs (Table 1).[12] Currently, the favored agents in guinea pigs are ketamine/xylazine and isoflurane.[4,13-15] While injectable agents induce less cardiorespiratory depression in guinea pigs,[16] establishing and maintaining stable vascular access necessary to sustain an appropriate anesthetic plane is difficult to achieve. As such, isoflurane is often preferred due to its ease of titration, steady maintenance, and rapid withdrawal.[13] As the depressive effects of isoflurane are related to anesthetic concentration,[3,17,18] reducing isoflurane concentrations is the best way to improve cardiorespiratory stability, however this comes at the cost of anesthetic depth.[19]

Isoflurane appears to drive cardiovascular depression through combined effects of direct inotropic depression *via* cardiac and vascular ion channels,[7,20] and central autonomic depression, particularly of the nucleus tractus solitarius.[21] Central autonomic depression serves to dampen metabolism, desensitize reflex control of respiration, and widen baroreceptor thresholds.[17,21] Alongside widened baroreceptor thresholds, isoflurane's vascular effects also produce inappropriate vasodilation and impair vasoconstrictory thresholds. Cumulatively, this leads to hypotension and impaired thermoregulation.[19,22-24] Without aggressive extrinsic warming strategies, these vascular effects alongside dampened metabolism can result in hypothermia which in turn further depresses cardiorespiratory function. In larger species, isoflurane's depressive effects on cardiac contractility and vascular control are frequently offset by an increased heart rate,[25] however this does not appear to be the case in small rodents (Table 1). Thus, under typical isoflurane concentrations (~1–2%), physiological responses are blunted which both limits the validity of comparison to conscious cardiorespiratory or metabolic responses and makes it difficult to separate the impact of a given intervention from the impact of anesthesia.

What is already known?

- Comprehensive physiological, pharmacological, and imaging studies frequently use inhalational anesthesia to maintain compliant animal subjects
- Isoflurane anesthesia drives cardiorespiratory depression at concentrations required to safely anesthetize animals.

What this study adds

- Describes an optimized anesthetic protocol for extended minimally invasive physiological monitoring in guinea pigs
- Describes comprehensive central and peripheral cardiovascular function during isoflurane anesthesia.

Clinical Significance

- Increases the translational capacity of the guinea pig as a model for understanding human (patho)physiology.

Anesthetic agents are synergistic in their effects. N_2O is a favored adjuvant to isoflurane due to its low potency and sympathomimetic effects.[26,27] N_2O's addition allows the relative isoflurane concentration to be reduced, ameliorating the adverse cardiorespiratory and thermoregulatory effects of isoflurane, while maintaining the same anesthetic depth.[23,28] Given this, we have chosen to characterize the capacity of isoflurane +nitrous oxide (ISO+N_2O) to achieve optimal anesthetic depth with minimal cardiorespiratory depression, thereby allowing valid physiological comparison between anesthetized and conscious guinea pigs.

We therefore sought to establish: (a) the cardiovascular, respiratory, and thermoregulatory effects of isoflurane compared to ISO+N_2O; and (b) the capacity of N_2O to reduce isoflurane concentrations required to maintain an anesthetic plane in guinea pigs. Additionally, as there is a paucity of data characterizing the sexually dimorphic effects of anesthesia,[29-31] we examined sex-specific effects of isoflurane ± N_2O anesthesia.

2 | MATERIALS AND METHODS

2.1 | Animals

All procedures were approved by the University of Otago, Wellington Animal Ethics Committee and conformed to Health Research Council of New Zealand code of practice for the care and use of animals for

TABLE 1 Cardiovascular, respiratory, and thermal effects of anesthetics commonly used in guinea pigs

Anesthetic Agent	Concentration	Adjuvant	Flow rate L.min⁻¹	Duration	Anesthetic Depth[a]	Physiological Effects					Other
						Heart rate	MABP	TPR	Respiratory rate	Core temp	
Isoflurane	3.0% in 100% O_2[13]	0.04 mg.kg⁻¹ atropine	0.7	55 min	Major surgical procedures	↔	→↓		→↓	→↓	BGlu ↑
	1.5–2.5% in 100% O_2[4]		0.5	65 min	Unstable	→	→		N/A - artificially ventilated		Salivation ↑; PR and QRS interval changes; frequent adjustments to isoflurane required
Isoflurane +nitrous oxide +midazolam	0.55% in 75% N_2O + 1 mg.kg⁻¹ midazolam[57]	0.05 mg.kg⁻¹ atropine; 15 nmol. kg⁻¹ min⁻¹ rocuronium	3	4 hr		↔	↔		N/A - artificially ventilated	↔	
Alfaxalone	5 mg.kg⁻¹ i.m.[58]			30 min	Immobilization /minor procedures	↔↑			↔	↔	
Alfaxalone-alphadolone	9.75 mg.kg⁻¹ h⁻¹ i.v.[14]				Unstable	↔	→		→	→	
Fentanyl	0.32 mg.kg⁻¹ i.m.[59]	2 mg.kg⁻¹ diazepam			Immobilization /minor procedures		↑				HR increased in response to sound stimuli, suggesting light sedation only
Fentanyl/ fluanisone[b] + midazolam[c]	1.25 mL.kg⁻¹ h⁻¹ i.v. maintenance[4]			65 min	Unstable	→	→		N/A - artificially ventilated	→	
Ketamine	100 mg.kg⁻¹ i.m.[59]	2 mg.kg⁻¹ diazepam				→	→				
	75 mg.kg⁻¹ s.c.[13]	15 mg.kg⁻¹ xylazine		100 min	Major surgical procedures	→↓	→↓↔		→	→↓	BGlu ↑
	60 mg.kg⁻¹ i.m.[60]	5 mg.kg⁻¹ xylazine		90 min	Immobilization /minor procedures	→			→		
		5 mg.kg⁻¹ detomidine		120 min	Reflexes present	→			↑↑ (tachypnea)		Gastric reflux, death
		0.5 mg.kg⁻¹ medetomidine		150 min	Immobilization / minor procedures	→			→		
	14.6 mg.kg⁻¹ h⁻¹ i.v.[14]	3.7 mg.kg⁻¹ h⁻¹ xylazine			Major surgical procedures	→↓	→		↔		

(Continues)

TABLE 1 (Continued)

Anesthetic Agent	Concentration	Adjuvant	Flow rate L.min⁻¹	Duration	Anesthetic Depth[a]	Heart rate	MABP	TPR	Respiratory rate	Core temp	Other
						Physiological Effects					
Medetomidine/ midazolam/ fentanyl	0.2/1.0/0.025 mg.kg⁻¹ i.m.[13]			50 min	Major surgical procedures	↔	↔↓		↓	↓↓	BGlu ↑↑
Sodium pentobarbital	24 mg.kg⁻¹ i.p. at 15 min intervals[61]			102 min	Major surgical procedures	↔			N/A - artificially ventilated		No ECG changes
	22 mg.kg⁻¹ i.v.[14]				Major surgical procedures	↔	↓		↓		Near lethal dose required for sufficient anesthesia; tracheal secretions
	6 mg.kg⁻¹ h⁻¹ i.v.[4]	50 µg.kg⁻¹ fentanyl		65 min	Major surgical procedures	↑↔	↔		N/A - artificially ventilated		
	6 mg.kg⁻¹ h⁻¹ i.v.[4]			65 min	Major surgical procedures	↔	↔		N/A - artificially ventilated		
	1.5–3 mg.kg⁻¹ h⁻¹ i.v.[62]			100 min	Major surgical procedures	↔	↔		N/A - artificially ventilated		No ECG changes
Tiletamine-zolazepam	40 mg.kg⁻¹ i.m.[60]	5 mg.kg⁻¹ xylazine		130 min	Major surgical procedures	↓↓			↓		
		5 mg.kg⁻¹ detomidine		220 min	Reflexes present	↓			↑↑ (tachypnea)	↓	Gastric reflux, respiratory difficulties, cyanosis, death
		0.5 mg.kg⁻¹ medetomidine		200 min	Major surgical procedures	↓↓			↓		

Studies which did not explicitly report the effect of anesthesia on cardiovascular, respiratory, or thermal control were excluded, as were studies utilizing anaesthetic agents whose use are no longer supported, such as urethane or available, such as Innovar Vet ® ([0.4 mg.mL⁻¹ fentanyl, 20 mg.mL⁻¹ droperidol]). Maintenance regimens are detailed only, as cardiovascular monitoring is frequently not available during induction. Details regarding induction strategies can be found in individual papers. ↑ indicates increase observed in given parameter in anesthetized animals compared to controls or preanesthetic measurements, or over time during anesthesia; ↓ indicates decrease observed in given parameter in anesthetized animals compared to controls or preanesthetic measurements, or over time during anesthesia; ↔ indicates no difference observed in given parameter in anesthetized animals compared to controls or preanesthetic measurements, or over time during anesthesia. [a]Anesthetic depth is graded, where sufficient evidence exists within published reports to assign a grade, as none/no loss of reflexes, unstable, suitable for immobilization or minor procedures only, or adequate for major surgical procedures.[b]Hypnorm ®; 0.315 mg.mL⁻¹ fentanyl, 10 mg.mL⁻¹ fluanisone.[c]Hypnovel ®; 5 mg.mL–1 midazolam HCl.

scientific purposes. Specific pathogen-free animals were sourced from the University of Otago Wellington Biomedical Research Unit. The study is reported according to the ARRIVE guidelines,[32] and in adherence with the *BJP* guidelines for Design and Analysis, and Animal Experimentation.[33]

Twelve age and sex-matched juvenile Dunkin Hartley guinea pigs (male (n = 6), female (n = 6), mean age: 113d (75–118 d), and mean weight: 678 g (559–800 g)) were included in this randomized crossover study design (Table 2). Group sizes were based on previously published data for similar experiments; we did not conduct sample size calculations for these experiments. Animals were randomized using a computerized method (RAND function in Microsoft Excel) at commencement of study,[34] to isoflurane alone (ISO: 1.5%) or isoflurane + N$_2$O (ISO+ N$_2$O: 0.8% +70%). A 3-day recovery washout period was given between anesthetic regimens.[16,35] Guinea pigs were housed individually in a 12:12 h light and temperature-controlled environment, with *ad libitum* access to standard guinea pig chow (Specialty Feeds, Glen Forrest, Australia) and vitamin C-enriched water. Animals were habituated to conscious experimental manipulations for 4 days prior to initiating the study to minimize the physiological stress response from mechanical restraint and the procedures themselves.

2.2 | Anesthetic protocol

Animals were fasted overnight prior to commencing each protocol. Before initiating the assigned protocols, cardiovascular, respiratory, thermal, and hemodynamic measures were collected in restrained conscious animals. Anesthetic induction was carried out according to a standardized regime of incremental 1–4% isoflurane with supplemental 50% O$_2$ provided at 2L.min^{-1} (Datex-Ohmeda Aestiva 5, GE Healthcare, Chicago, IL). Upon loss of righting reflex and waking body tone, animals were transferred to a nose cone and placed in a prone position. Animals remained freely breathing, with fractional inspired gases (i.e., oxygen, medical air, nitrous oxide, isoflurane) monitored by the anesthetic machine. Monitoring equipment for cardiovascular, respiratory, and thermal measures was placed and animals secured in a thermal wrap maintained at 33°C to ensure a stable external thermal environment for the duration of the protocol.

Anesthesia was then titrated to the maintenance dose over 30 min. For the ISO group, this was accomplished in two steps, whereas three stages were required for ISO+N$_2$O (Figure 1; Table 3). Titration was required as pilot studies identified erratic

respiratory drive and apnea with introduction of N$_2$O at high isoflurane concentrations (Supplemental File 1). Animals were then maintained under their respective maintenance protocol for 150 min. Fluids were not provided pre-, during, or postanesthesia, to ensure a "pure" representation of anesthetic effects on cardiovascular function.

Following discontinuation of anesthesia, all animals were recovered under 100% O$_2$ at 2 L.min^{-1} until spontaneous arousal (5–10 min). Welfare monitoring was conducted during anesthesia (palpebral reflex, pedal withdrawal, respiration, ECG) and daily postanesthesia (weight, behavior, food intake, and fluid balance) to ensure complete recovery prior to commencing the other arm of the anesthetic protocol.

2.3 | Physiological monitoring

2.3.1 | Cardiovascular

Electrocardiography (needle electrode, ADInstruments, Dunedin, New Zealand) was recorded continuously, and systolic blood pressure (Non-Invasive Blood Pressure, ADInstruments) monitored intermittently (5–10 min) via cuff inflation on the forelimb. Microvascular perfusion was continuously measured over proximal (interscapular) and distal (dorsal ear) skin sites using laser Doppler flowmetry (LDF, Probe 457, PeriFlux 5001, Perimed, Jarfalla, Sweden) affixed to depillated skin sites using modified TCO$_2$ fixation rings and double sided tape.

2.3.2 | Respiration

Inspired fractional gas composition, including isoflurane concentration, MAC, F$_I$O$_2$, and F$_I$N$_2$O were monitored using the inbuilt gas analyzer (Datex-Ohmeda Aestiva 5), while respiration rate was monitored using a pressure transducer (finger pulse transducer,
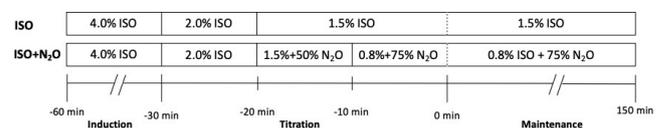
FIGURE 1 Protocol Timeline. "Induction" phase refers to both 8-min induction period, as well as 20-min fitting of monitoring equipment

TABLE 2 Animal Characteristics

| | n | Birth weight | ISOFLURANE | | | ISOFLURANE +NITROUS OXIDE | | |
			Age (days)	Weight (g)	PI (kg.m^3)	Age (days)	Weight (g)	PI (kg.m^3)
Male	6	94.4 ± 9.8	113.2 ± 8.3	725.5 ± 13.5	17.37 ± 0.52	113.2 ± 7.2	740.5 ± 20.4	17.71 ± 0.52
Female	6	94.3 ± 16.6	113.3 ± 8.0	620.5 ± 22.4	17.03 ± 0.4	113.3 ± 5.7	623.5 ± 21.8	17.14 ± 0.58

Values presented as mean ±SEM. Ponderal index (PI) calculated as: PI (kg.m^3) = Weight (kg) / Length (m)^3.

TABLE 3 Anesthetic variables under both isoflurane and isoflurane +nitrous oxide protocols including the induction of anesthesia, titration, and maintenance of anesthesia

	ISOFLURANE					ISOFLURANE +NITROUS OXIDE							
	ISO		MAC	F_IO_2		ISO		MAC	F_IO_2		F_IN_2O		
	Target	Actual	Actual	Target	Actual	Target	Actual	Actual	Target	Actual	Target	Actual	
INDUCT	4.0	3.9 ± 0.0	3.5 ± 0.1	50	51 ± 1	4.0	4.0 ± 0.1	3.4 ± 0.1	50	51 ± 1	0	0.00	
STAGE 1	2.0	2.0 ± 0.2	1.7 ± 0.0	50	51 ± 1	2.0	1.9 ± 0.1	1.7 ± 0.1	50	51 ± 1	0	0.00	
STAGE 2	1.5	1.5 ± .01	1.3 ± 0.1	50	51 ± 1	1.5	1.4 ± 0.1	1.7 ± 0.1	50	46 ± 2	50	50 ± 3	
STAGE 3	N/A	N/A	N/A	N/A	N/A	0.8	0.8 ± 0.0	1.4 ± 0.1	30	30 ± 1	70	69 ± 1	
MNTNCE	1.5	1.5 ± 0.1	1.3 ± 0.1	50	51 ± 1	0.8	0.8 ± 0.0	1.4 ± 0.1	30	30 ± 1	70	70 ± 1	

"Actual" data are inspired gas concentrations recorded for 12 guinea pigs at each stage of experimental protocol. "INDUCT" denotes the final minute of the induction period. Stages 1, 2, and 3 comprised the titration phase. These consisted of two stages in isoflurane and three stages (3 x 10 min) in isoflurane +nitrous oxide. MNTNCE is the duration 0–150 min of consistent anesthetic administration. Values presented as mean ±, and percent inhaled gas. Note: All gas measures are inspired, rather than end tidal, due to the inability of the guinea pig expired breath to exceed expiratory valve pressure of the anesthetic machine, as such it operated as an open system.

ADInstruments) positioned below the diaphragm. Oxygen saturation (SpO_2, ADInstruments) was monitored continuously from the left forelimb.

2.3.3 | Thermal

Core body temperature was recorded using a rectal thermistor inserted 6 cm past the anus (RET-1, ADInstruments). Skin temperature was measured across multiple sites including: left hind foot, rump, interscapular, and right ear (skin temperature probe, ADInstruments). Skin measures were then paired according to proximal (interscapular and rump) and distal (ear, foot) sites.

2.3.4 | Blood gas profile

Blood gas analysis (CG8+, iStat, Abbot Point of Care, Princeton, USA), as a measure of cardiometabolic stability, was measured from microsamples taken from the auricular capillaries of the right ear immediately prior to the induction of anesthesia, and following the termination of anesthesia (+10 min, +2 h, and +24 h).[36]

2.4 | Data analysis

ECG, NIBP, respiration, and core and skin temperatures were sampled using PowerLab (ADInstruments) at a rate of 1 k/s, with an analogue notch filter applied, and recorded in LabChart (ADInstruments). Heart rate and respiration were derived using peak-to-peak analysis. NIBP pulse range was set to 90–420 b.min^{-1}, with a maximal cuff inflation of 200 mmHg. LDF collected alongside central cardiovascular assessments was measured at 32 Hz with a time constant of 0.03 s, assessed in arbitrary perfusion units (PU; equipment calibration checked prior to each recording and calibrated when values ±0.5 outside of calibration norm, or at least monthly), and analyzed using custom software

(PSW2; Perimed). Data were analyzed by a condition-blinded assessor (RPS) and corroborated by a second experienced assessor (RMD), also blinded to experimental group. While recorded continuously, cardiovascular, respiratory, and thermal measures were assessed at 5-min intervals during the titration phase, and 10 min during maintenance of the intervention. At each interval, a 60-s window of representative, artifact-free data were selected for analysis.

2.5 | Statistical methods

Data and statistical analysis comply with the recommendations of *BJP* on experimental design and analysis in pharmacology.[33] Stata/IC 13.0 (StataCorp LP, Texas, USA) and GraphPad Prism 7.04 (GraphPad Software, California, USA) were used for statistical analysis and generation of figures. Data and figures are presented as mean ±95% confidence interval (CI) unless otherwise stated. The number of animals reported is represented as "n." Statistical analysis was only undertaken for comparisons with n ≥ 5. Outliers were included in statistical analyses. The level of statistical significance for all analyses was set at $p \leq 0.05$ using two-tailed comparison. For physical characteristics, differences between groups were analyzed by Student's t-test or ANOVA with sex or treatment as independent variables. Sidak's post hoc analyses were performed whenever the F-statistic reached significance ($p < 0.05$). Repeated measures data (physiological monitoring) were analyzed using multilevel mixed effects linear regression (accounting for the cross-over design) with robust estimate of variance to provide timing-specific standard errors.

2.6 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.

org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[37] and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[38]

3 | RESULTS

3.1 | Animal characteristics

Physical characteristics are presented in Table 2. Males were heavier than females ($p = 0.0002$). There was no change in physical characteristics between regimens, with no significant change in weight between first and second anesthetic treatments (male difference: 15.0 ± 19.6 g, $p = 0.95$; female difference: −3.0 ± 15.6 g, $p = 1.0$).

3.2 | Anesthetic depth

Anesthetic concentrations and inspired fractional gases are presented in Table 3. Within ISO and ISO+N_2O, animals were induced under 3.9 ± 0.1% and 4.0 ± 0.1% isoflurane (corresponding MAC of 3.5 ± 0.1, and 3.4 ± 0.1, respectively). Animals were subsequently titrated to a MAC equivalent to levels known to maintain a consistent anesthetic state (ISO: 1.5 ± 0.1%, MAC: 1.3 ± 0.1; ISO+N_2O: 0.8% +70% (±1.0), MAC: 1.4 (±0.1)). The anesthetic plane was maintained for the duration of testing in all animals.
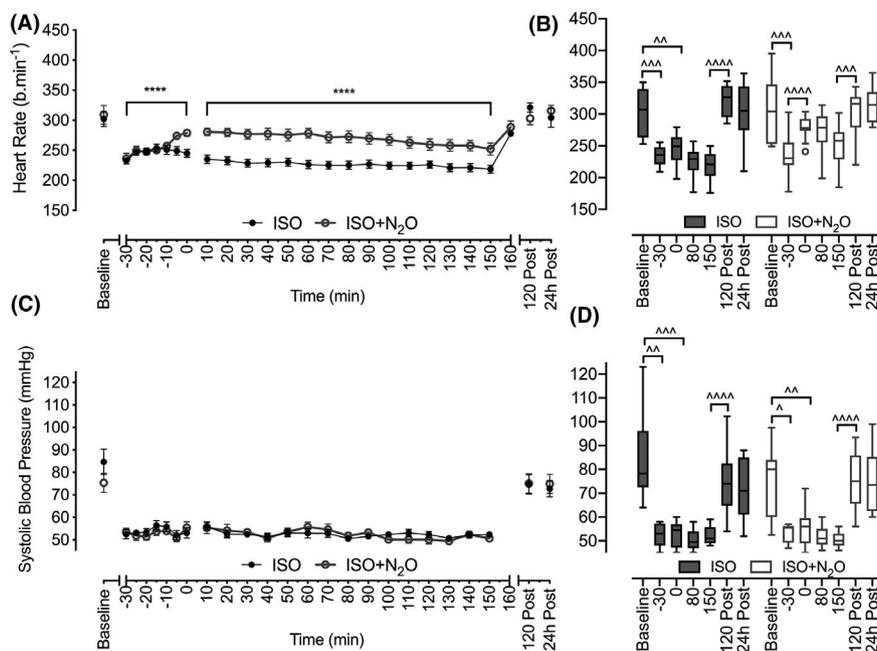
3.3 | Cardiovascular effects

3.3.1 | Heart rate

Conscious baseline heart rate (HR) was comparable between regimens (ISO: 302 ± 29b.min^{-1}, ISO+N_2O: 309 ± 34b.min^{-1}; $p = 0.74$). Immediately following the induction of anesthesia, HR reduced significantly (ISO: 233 ± 11b.min^{-1}, ISO+N_2O: 236±19b.min^{-1}; $p < 0.0001$ for change following induction) with no differences between treatments ($p = 0.65$).

During titration, HR recovered more rapidly under ISO+N_2O than ISO animals (time x treatment: $p < 0.0001$; Figure 2A), such that under ISO+N_2O, HR rose to finish titration significantly higher than that under ISO (mean difference: 34 ± 7b.min^{-1}, $p < 0.0001$), and comparable to conscious levels (ISO+N_2O: 279 ± 11b.min^{-1}, $p = 0.08$; ISO: 245 ± 15b.min^{-1}, $p = 0.001$). During maintenance, anesthetic-induced suppression of HR was less in ISO+N_2O than that in ISO alone ($p < 0.0001$). HR of ISO animals continued to decline slightly from titration to finish maintenance at 219 ± 15b.min^{-1}. Whereas animals within ISO+N_2O, while HR was significantly higher than ISO, it slowly fell during maintenance phase (slope: 0.2b.min^{-1}), to finish at 252 ± 22b.min^{-1}.

Upon cessation of anesthesia, HR rose to 278 ± 11b.min^{-1} and 289 ± 23b.min^{-1} by 10 min post within ISO and ISO+N_2O, respectively. HR reached pretreatment levels by 120 min posttreatment (ISO: 321 ± 18b.min^{-1}, ISO+N_2O 303 ± 24b.min^{-1}; $p = 0.43$) with no difference between treatments ($p = 0.72$), and remained comparable

FIGURE 2 Central cardiovascular response to prolonged anesthesia under ISO and ISO+N_2O. (A) Continuous HR response during titration, and maintenance including comparisons to the waking state; (B) Effects of anesthetic protocols on HR in comparison to the conscious state; (C) Continuous SBP response during titration, and maintenance of anesthesia including comparisons to the waking state; (D) Effects of anesthetic protocols on SBP in comparison to the conscious state. a/c analyzed using multilevel mixed effects linear regression; b/d analyzed using t-test and ANOVA. Data presented as mean ±95% CI. *denotes treatment effect; ^denotes time effect; */^denotes $p = 0.05 – 0.01$; **/^^denotes $p = 0.009–0.001$; ***/^^^denotes $p = 0.0009–<0.0001$.
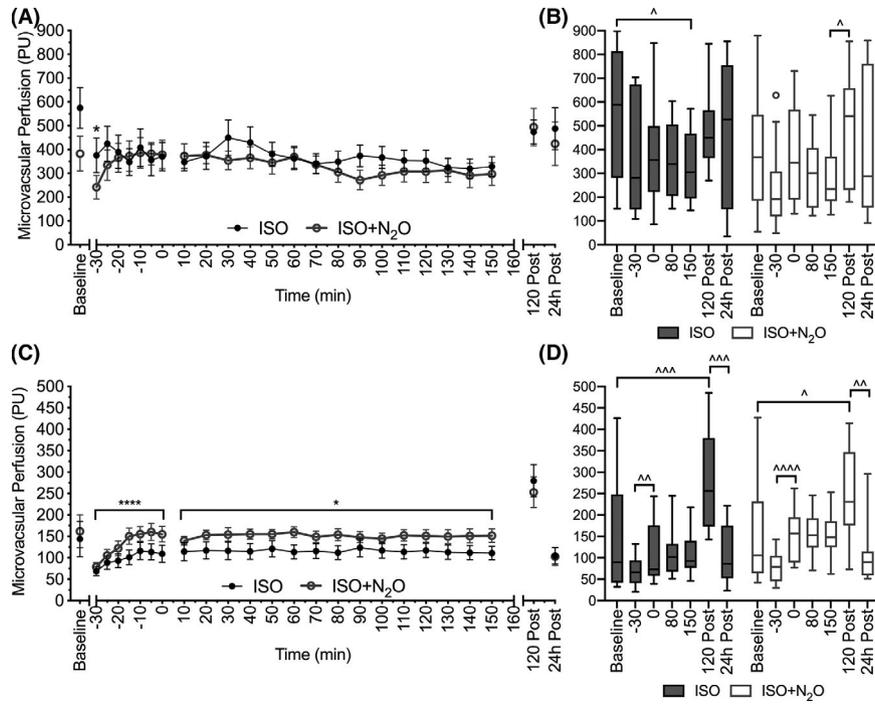
FIGURE 3 Microvascular response to prolonged anesthesia under ISO and ISO+N$_2$O. Stages include conscious baseline, titration, maintenance, and conscious recovery. (A) Distal microvascular perfusion across the duration of anesthesia including titration and maintenance as well as conscious reference measures; (B) Effects of anesthetic protocols on distal microvascular perfusion in comparison to the conscious state; (C) Proximal microvascular perfusion across the duration of anesthesia; and (D) the effects of anesthetic protocols on proximal perfusion. a/c analyzed using multilevel mixed effects linear regression; b/d analyzed using t-test and ANOVA. Data presented as mean ±95% CI. * denotes treatment effect; ^denotes time effect; */^ denotes p = 0.05 – 0.01; **/^^denotes p = 0.009 – 0.001; ***/^^^denotes p = 0.0009 – <0.0001

by 24 h post (ISO: 304 ± 37b.min^{-1}, p = 0.51; ISO+N$_2$O: 316 ± 21b.min^{-1}, p = 0.41).

3.3.2 | Systolic blood pressure

Conscious systolic baseline blood pressure (SBP) was similar between treatment groups (ISO: 85 ± 13 mmHg, ISO+N$_2$O: 75 ± 10 mmHg; p = 0.2). Following induction, SBP fell significantly to 53 ± 3 mmHg (p = 0.001 across both groups), with no effect of treatment (p = 0.83). SBP remained unaffected by anesthetic titration, with ISO and ISO+N$_2$O animals completing at 52 ± 5 mmHg and 51 ± 4 mmHg, respectively (treatment effect: p = 0.55; Figure 2B). SBP did not differ between ISO and ISO+N$_2$O (p = 0.65), but was significantly lower than the conscious state throughout anesthesia (start of maintenance phase: ISO: difference −29 mmHg, p = 0.001, ISO+N$_2$O: difference −20 mmHg, p = 0.002; 150 min (end of maintenance), ISO: difference −32 mmHg, p = 0.0004, ISO+N$_2$O: difference −25 mmHg, p = 0.0001).

Upon cessation of anesthesia, SBP rose to 65 ± 10 mmHg and 75 ± 10 mmHg by 120 min post within ISO and ISO+N$_2$O, respectively. SBP remained stable at 24 h (ISO: 73 ± 8 mmHg; ISO+N$_2$O: 75 ± 9.5 mmHg). There was no difference postanesthesia between treatments, at either 120 min post (treatment: p = 0.95) or 24 hr post (treatment: p = 0.70).

3.3.3 | Microvascular perfusion

Conscious baseline perfusion was not significantly different between treatments (distal perfusion, ISO: 575±193PU, ISO+N$_2$O: 382±163PU, p = 0.10; proximal perfusion, ISO: 144±94PU, ISO+N$_2$O: 162±87PU; p = 0.76). Distal and proximal perfusion reduced significantly during induction (distal: p = 0.02; proximal: p = 0.005). While proximal perfusion was not different between anesthetic treatments (p = 0.64), distal perfusion was significantly different at −30 min (p = 0.03). This difference in distal perfusion was transient, and recovered by −20 min (ISO: 390±155PU, ISO+N$_2$O: 365±136PU; p = 0.79), before titration protocols diverged. No further treatment effects were observed across titration for either proximal or distal perfusion (p = 0.27 and p = 0.13, respectively; Figure 3A & B). However, proximal perfusion significantly increased during anesthesia titration (time effect: p < 0.0001), with ISO and ISO+N$_2$O reaching within 30±86PU and 7±83PU of conscious levels (p = 0.46).

During maintenance phase, proximal perfusion was significantly higher within ISO+N$_2$O animals (ISO+N$_2$O: 155±40PU, ISO: 109±44PU; p = 0.04). This difference persisted for the duration of maintenance (p < 0.0001). Proximal perfusion in ISO+N$_2$O animals was 152±35PU at completion, compared to 111±35PU of ISO animals. Conversely, distal perfusion was similar between treatments at the start of maintenance phase (ISO: 370±131PU, ISO+N$_2$O: 379±132PU; p = 0.93), but significantly lower in ISO+N$_2$O than ISO alone by its end (297±106PU, vs 328±91PU, p = 0.014).
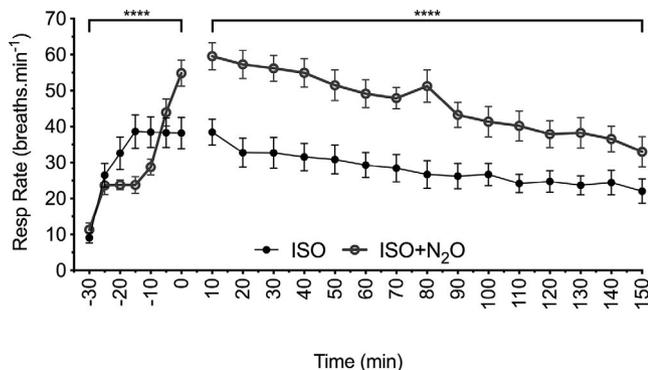
FIGURE 4 Respiratory response to prolonged anesthesia under ISO and ISO+N_2O. Data analyzed using multilevel mixed effects linear regression; Data presented as mean ±95% CI. *** denotes $p = 0.0009 – <0.0001$

Upon cessation of anesthesia, distal perfusion rose back to conscious baseline levels by 120 min post (ISO: 474±115PU, ISO+N_2O: 494±181PU; $p = 0.88$), whereas proximal perfusion exceeded conscious baseline levels at 120 min post (ISO: 280 ± 85PU, ISO + N_2O: 253 ± 82PU; $p < 0.0001$), before settling at 24 h post (ISO: 105 ± 44PU, ISO+N_2O: 103 ± 46PU; $p < 0.0001$). Treatment differences did not persist into postanesthetic recovery (proximal: 120 min post, $p = 0.61$, 24 h post, $p = 0.95$; distal: 120 min post, $p = 0.82$, 24 h post, $p = 0.63$).

3.4 | Respiratory effects

3.4.1 | Respiration

Respiratory rate (RR) changed in a treatment-specific manner across the duration of titration (time x treatment: $p < 0.0001$; Figure 4). Introduction of N_2O at 10 min delayed the rise in RR, allowing RR of ISO animals to rise significantly above ISO+N_2O until 20 min (overall ISO+N_2O difference, 10 min: −8.8 ± 4.6 breaths.min^{-1}, $p = 0.05$; 15 min: −14.9 ± 9.7 breaths.min^{-1}; $p = 0.002$; 20 min: −9.7 ± 9.1breaths.min^{-1}; $p = 0.04$). ISO+N_2O RR then rose significantly above ISO by 30 min as anesthetic dose was further titrated (difference: 16.7 ± 4.7 breaths.min^{-1}, $p < 0.0001$).

During maintenance phase, the RR of ISO+N_2O animals remained significantly greater than that of ISO animals (ISO+N_2O: 54.9 ± 8.1breaths.min^{-1}, ISO: 38.2 ± 9.5breaths.min^{-1}; $p < 0.0001$). This effect persisted throughout the maintenance phase ($p < 0.0001$). At the end of maintenance, RR of ISO+N_2O animals was 33.0 ± 9.2breaths.min^{-1}, compared to 22.0 ± 7.5breaths.min^{-1} of ISO animals.

3.4.2 | Oxygen saturation

SpO$_2$ reduced to 89% and 85% following induction in ISO and ISO+N_2O; it was thereafter maintained at ≥95% throughout titration

and maintenance. There was no effect of treatment protocol or sex on SpO$_2$ at any stage.

3.5 | Thermal effects

3.5.1 | Core body temperature (T$_{re}$)

Conscious baseline T$_{re}$ was similar between groups (ISO: 39.1 ± 0.5°C, ISO+N_2O: 39.1 ± 0.3°C; $p = 0.78$), with a significant reduction following induction of anesthesia in both groups (ISO: 37.2 ± 0.5°C, ISO+N_2O: 37.2 ± 0.4°C; compared to baseline: $p < 0.0001$ for both). T$_{re}$ continued to decline significantly across titration ($p < 0.0001$), with no differences between treatments ($p > 0.99$; Figure 5A).

T$_{re}$ started maintenance similar between ISO and ISO+N_2O (ISO: 35.9 ± 0.4°C, ISO+N_2O: 36.0 ± 0.3°C; $p = 0.59$). T$_{re}$ subsequently rose in ISO+N_2O animals across the first 50 min to plateau at 36.7°C for 30 min, remaining persistently higher than T$_{re}$ for ISO animals for the duration of maintenance ($p < 0.0001$); completing at 36.1 ± 0.5°C. Conversely, T$_{re}$ of ISO animals continued to decline across maintenance, completing at 34.7 ± 0.4°C.

In both ISO and ISO+N_2O, T$_{re}$ rose immediately upon cessation of anesthesia, reaching 35.3 ± 0.4°C and 36.5 ± 0.5°C, respectively, by 10 min postanesthesia; both treatments remaining significantly different ($p = 0.001$). By 120 min post, T$_{re}$ reached conscious baseline levels (ISO+N_2O: 39.3 ± 0.3°C, $p = 0.35$; ISO: 39.5 ± 0.2°C, $p = 0.01$ compared to temperature at conscious baseline). T$_{re}$ of both groups was steady at 24 hr postanesthesia (ISO: 39.4 ± 0.3°C, ISO+N_2O: 39.4 ± 0.2°C).

3.5.2 | Skin temperature (T$_{sk}$)

Conscious baseline T$_{sk}$ was similar between treatments (proximal, ISO: 34.5 ± 1.0°C, ISO+N_2O: 35.1 ± 0.5°C; distal, ISO: 33.4 ± 1.3°C, ISO+N_2O: 33.9 ± 0.8°C; $p = 0.34$). Neither induction, nor titration of anesthesia significantly altered proximal or distal T$_{sk}$ between groups (Proximal T$_{sk}$, induction: time x treatment, $p = 0.39$, titration: $p = 0.84$; Distal T$_{sk}$, induction: $p = 0.81$, titration: $p = 0.92$; Figure 5B & 5C). Collectively, proximal T$_{sk}$ began titration at 34.5 ± 0.3°C and completed at 34.6 ± 0.5°C, while distal T$_{sk}$ began at 33.4 ± 0.5°C, completing at 34.3 ± 0.2°C.

During maintenance phase, proximal and distal T$_{sk}$ of ISO+N_2O animals increased and remained significantly higher than the ISO animals ($p < 0.0001$ for both sites). Proximal T$_{sk}$ of ISO+N_2O animals increased across the first 50 min, to plateau at 35.3°C for 50 min, before declining to complete at 34.9 ± 0.4°C. Whereas, distal T$_{sk}$ of ISO+N_2O animals remained largely unchanged across maintenance, completing at 34.1 ± 0.4°C. T$_{sk}$ of ISO animals continued to decline from the beginning of maintenance to the end, completing with proximal and distal T$_{sk}$ of 33.9 ± 0.3°C and 33.5 ± 0.3°C, respectively.

Upon cessation of anesthesia, T$_{sk}$ of both treatments increased across both skin sites. By 120 min, ISO T$_{sk}$ rebounded to a greater degree than ISO+N_2O at both proximal and distal sites (Proximal ISO: 35.8 ± 0.7°C, ISO+N_2O: 35.0 ± 0.9°C, $p = 0.15$; Distal ISO:
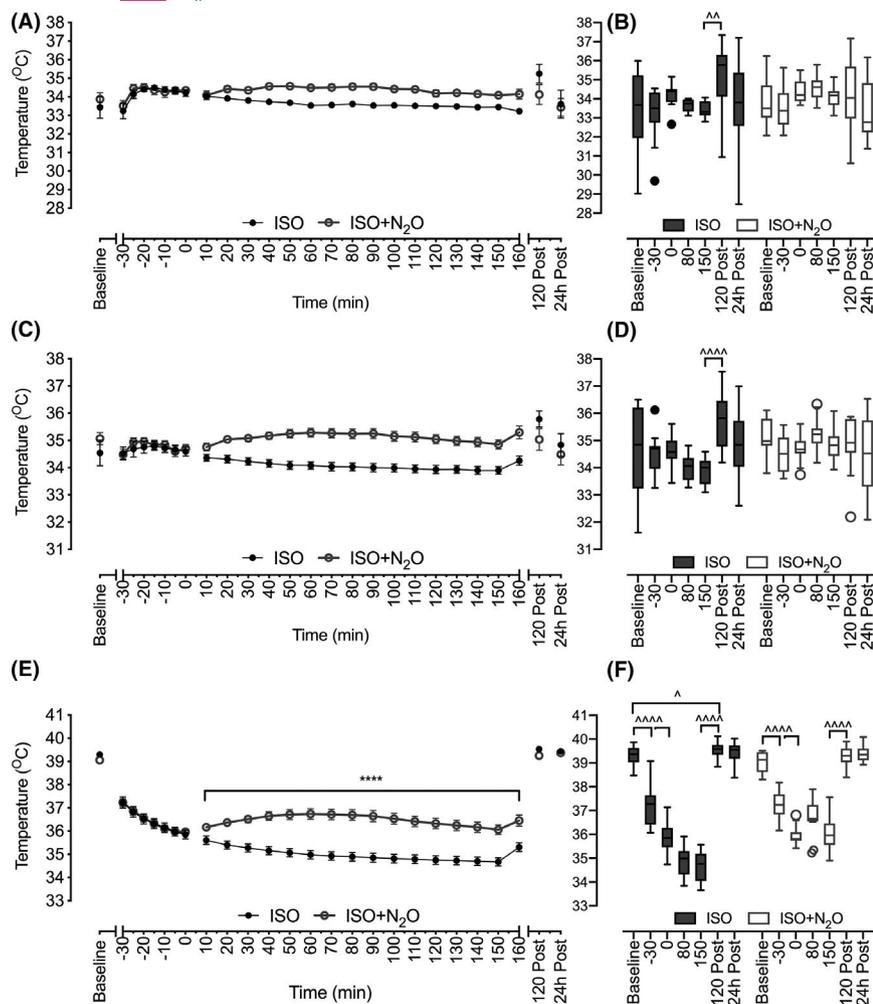
FIGURE 5 Thermoregulatory response to prolonged anesthesia under isoflurane and isoflurane +N_2O. (A) distal T_{sk} (C) proximal T_{sk}, and (E) T_{re} across the duration of anesthesia including titration and maintenance as well as conscious reference measures; (B) distal T_{sk} (D) proximal T_{sk}, and (F) T_{re} comparing the effects of anesthesia to the conscious state. a/c/e analyzed using multilevel mixed effects linear regression; b/d/f analyzed using t-test and ANOVA. Data presented as mean ±95% CI. * denotes treatment effect; ^ denotes time effect; */^ denotes $p = 0.05 – 0.01$; **/^^ denotes $p = 0.009 – 0.001$; ***/^^^ denotes $p = 0.0009 – <0.0001$

35.3 ± 1.13°C, ISO+N_2O: 34.1 ± 1.2°C, $p = 0.14$), significantly higher than conscious baseline (Proximal: $p = 0.06$; Distal: $p = 0.003$). Proximal and distal T_{sk} returned to baseline levels by 24 hr post (Proximal, ISO: 34.8 ± 0.9°C, ISO+N_2O: 34.5 ± 0.9°C; distal, ISO: 33.6 ± 1.7°C, ISO+N_2O: 33.4 ± 1.1°C).

3.5.3 | Ambient temperature

Ambient room temperature and relative humidity were controlled between ISO and ISO+N_2O, and maintained at 23.3 ± 0.6°C 30.4 ± 1.9% (RH), and 23.7 ± 0.6°C 30.6 ± 1.5% (RH), respectively. Bath temperature of water-perfused wrap was maintained at 33°C for the anesthetic duration.

3.6 | Blood gas profile

Blood gas profile was analyzed preinduction, and post-10 min, −120 min, and −24 hr. Results are presented in Table 4. pH was lower (mean difference: 0.04 ± 0.02) following ISO+N_2O than ISO ($p < 0.0001$). By 10 min post, ISO+N_2O blood gas indicated a shift toward acidosis, with pH reducing slightly, from 7.36 ± 0.03 to

7.34 ± 0.03 (in contrast to ISO: 7.38 ± 0.05 to 7.38 ± 0.06), resulting in a net lower pH immediately following ISO+N_2O anesthesia. This was followed by a rise in pH at post 24 hr following both treatments (ISO: +0.10, $p < 0.0001$; N_2O: +0.11, $p < 0.0001$).

The differences in pH were accompanied by differences in base excess (lower following ISO+N_2O, mean difference: −1.79 ± 1.29, $p = 0.006$) and TCO_2 (lower following ISO+N_2O, mean difference: −1.08 ± 0.94 mmol.L^{-1}, $p = 0.02$).

Plasma sodium levels were increased following ISO+N_2O (mean difference: 1.18 ± 0.98 mmol.L^{-1}, $p = 0.02$), with peak levels observed at 120 min postanesthesia, regardless of treatment arm. Other plasma electrolytes (potassium, calcium) and glucose concentrations were not different between groups.

3.7 | Sex effects

3.7.1 | Cardiovascular

Under isoflurane alone, SBP was significantly higher throughout the maintenance phase in males than in females (mean difference: 3.3 ± 2.9 mmHg, $p = 0.02$). This difference was not observed under ISO+N_2O. Conscious baseline HR was significantly different between

TABLE 4 Blood gas profile across conscious measures beginning with conscious baseline, 10 min postanesthesia, 120 min postanesthesia, and 24 hr postanesthesia

	PO$_2$	PCO$_2$	HCO$_3$	TCO$_2$	BE	pH
ISOFLURANE						
BASE (n = 11)	45.5 ± 2.9	32.9 ± 0.8	19.6 ± 0.5	20.7 ± 0.6	−5.5 ± 0.7	7.38 ± 0.02
POST10 (n = 9)	51.0 ± 6.8	34.8 ± 1.2	20.8 ± 1.1	22.0 ± 1.2	−4.2 ± 1.4	7.38 ± 0.02
POST120 (n = 10)	42.5 ± 2.2	31.0 ± 0.9	19.3 ± 0.5	20.3 ± 0.5	−5.4 ± 0.7	7.41 ± 0.01^
POST24HR (n = 10)	37.7 ± 1.7	37.9 ± 1.0^^^	29.1 ± 0.6^^^	30.2 ± 0.7^^^	5.7 ± 0.7^^^	7.49 ± 0.01^^^
ISOFLURANE +NITROUS OXIDE						
BASE (n = 10)	45.2 ± 2.6	35.0 ± 1.5	19.9 ± 0.9	20.6 ± 0.9	−5.3 ± 0.9	7.36 ± 0.01
POST10 (n = 10)	45.4 ± 2.6	33.9 ± 0.9	18.3 ± 0.7**	19.4 ± 0.6**	−7.5 ± 0.8**^	7.34 ± 0.01*
POST120 (n = 9)	45.8 ± 3.0	32.2 ± 1.2^^	18.0 ± 1.0^	19.1 ± 1.0	−7.6 ± 1.4	7.35 ± 0.03*
POST24HR (n = 10)	38.6 ± 2.1^	40.0 ± 1.4^^	28.6 ± 0.9^^^	29.8 ± 0.9^^^	3.3 ± 1.6^^^	7.46 ± 0.01*^^^

Data presented as mean ±SEM. * denotes significant difference from ISO treatment. ^denotes significant difference from baseline (BASE) conscious values. */^ denotes p = 0.05 – 0.01; **/^^ denotes p = 0.009 – 0.001; ***/^^^ denotes p = 0.0009 – <0.0001

sexes (males: 333±35b.min^{-1} vs females: 286±23b.min^{-1}; p = 0.015), with male HR falling to a greater degree over induction (collated male difference: −103±33b.min^{-1}; collated female difference: −47±30b.min^{-1}), so that at the onset of titration, male and female HR were similar (males: 231±19b.min^{-1} vs females 239±10b.min^{-1}; p = 0.958). Differences observed between sexes during titration proceeded into the maintenance phase. Under ISO+N$_2$O, females maintained a stable HR for 80 min before declining (interaction effect: p = 0.018), whereas male HR did not reach a plateau phase. Similarly, under ISO, while male HR declined throughout, female HR plateaued after 30 min of decline (interaction effect: p = 0.013).

Within the peripheral circulation, sex-specific effects occurred immediately following induction of anesthesia, with the fall in proximal perfusion of males approximately two and three times that of females across treatments (ISO+N$_2$O difference, male: −109±267PU, female: −61±160PU; ISO difference, male: −124±345PU, female: −44±181PU). Despite this reduction in perfusion, males within ISO+N$_2$O maintained greater peripheral blood flow throughout maintenance than females (difference: 107±80PU; p = 0.008). Males within ISO+N$_2$O further experienced a significant reduction in distal perfusion throughout maintenance (p < 0.0001), an effect not observed in females (p = 0.38).

3.7.2 | Respiration

When separated by sex, improvements in respiratory function under ISO+N$_2$O were greater in females during titration (30 min ISO vs ISO+N$_2$O: 24.1 ± 5.4breaths.min^{-1}; p < 0.0001) than their male counterparts (30 min ISO vs ISO+N$_2$O: 9.3 ± 13.5breaths.min^{-1}; p = 0.18). This enhanced protective effect of N$_2$O in females was observed throughout maintenance (mean difference: 22.1 ± 1.2breaths.min^{-1}, time x treatment: p = 0.03, compared to 14.4 ± 2.1breaths.min^{-1}, time x treatment p = 0.5; respectively).

3.7.3 | Temperature

While similar during conscious baseline (p = 0.33), following induction, T$_{re}$ of females was 0.7°C lower than males, dropping a total of 2.3°C from baseline (compared to 1.4°C in males) by the end of titration (females: 35.8 ± 0.1°C, males: 36.1 ± 0.3°C; time x sex: p < 0.0001). Furthermore, during maintenance, females in ISO+N$_2$O rebounded from titration levels to plateau at 37.1 ± 0.5°C, from 60 to 90 min; levels greater than immediately postinduction. Whereas males neither rebounded nor sustained a plateau for as long (36.4 ± 0.3°C from 20 to 60 min). T$_{re}$ of both sexes within ISO+N$_2$O declined following their respective plateaus; however, females completed maintenance higher than males at 36.3 ± 0.6°C, compared to 35.8 ± 0.5°C. Females similarly retained a higher T$_{re}$ by the end of ISO anesthesia, despite beginning maintenance at a lower T$_{re}$ (start: females 35.7 ± 0.4°C, males 36.0 ± 0.7°C; end: females 35.0 ± 0.4°C, males 34.4 ± 0.5°C).

Both sexes also separately demonstrated significantly improved T$_{sk}$ with ISO+N$_2$O during maintenance phase (males; p < 0.0001; females: p < 0.0001, for both sites). Interestingly, the magnitude of protection in both distal (females mean difference: 0.8 ± 0.2°C; males mean difference: 0.7 ± 0.3°C) and proximal sites (females mean difference: 1.1 ± 0.2°C; males mean difference: 0.9 ± 0.2°C) was greater in females.

4 | DISCUSSION

This study demonstrated that an inhalational anesthetic regime supplemented with 70% N$_2$O allows for significant reductions in isoflurane concentrations (to 0.8%), while maintaining an effective anesthetic depth for prolonged physiological examination in guinea pigs. We have also established that this balance significantly improves cardiorespiratory and thermoregulatory function in the form of normalized HR, microvascular perfusion, respiration, and

T_{re}. Finally, we have shown significant sex-specific differences in responsiveness of the above measures to N_2O with females displaying significantly greater benefit of the ISO+N_2O gas mix than their male counterparts, with most measured variables trending back toward conscious baseline values.

Isoflurane has been widely acknowledged to depress cardiovascular function, both directly and centrally (*via* the nucleus tractus solitarius).[7,39] While dose reduction to minimize cardiorespiratory depression has been suggested,[21,39] our data do not support this. Isoflurane-induced cardiorespiratory and metabolic depression was marked during anesthetic induction and persisted even after isoflurane concentrations were weaned from 4% to 2.5%. Further titration to 1.5% isoflurane only transiently improved HR and RR, had no impact on SBP, and although proximal perfusion improved compared to levels at 4% isoflurane, both proximal and distal perfusion remained well below conscious baseline levels. Simple dose reduction of isoflurane alone is therefore unlikely to achieve the goal of adequate anesthesia with minimal systemic side effects. Low-dose isoflurane (1.0–1.5%) in guinea pigs and other species does not achieve a reliable plane of anesthesia, especially during noxious or surgical interventions, and is still associated with cardiorespiratory depression.[3,4] A higher dose of isoflurane is therefore required for a surgical plane of anesthesia, although the optimal regimen remains uncertain (Table 1). Additionally, while guinea pigs are an excellent paradigm for human drug development programs,[6] even low-dose isoflurane-related side effects precludes its use during preclinical drug safety screening studies.[4] Thus, it is clear that low-dose isoflurane alone, even at levels suboptimal for anesthesia (~1.25%), produces unacceptable cardiorespiratory and metabolic depression in guinea pigs.

The introduction of 70% N_2O in the current study improved cardiorespiratory stability through reduction of isoflurane concentration to 0.8% while maintaining a stable anesthetic plane. Crucially, MACs were similar between ISO+N_2O and ISO regimens, despite isoflurane concentrations below those previously found to be insufficient for maintaining stable anesthesia.[3,4,40] Others have reported that using 50% N_2O adjunct to isoflurane anesthesia in mice achieved better stabilization of mean arterial pressure and HR beyond isoflurane reductions from 2.0% to 1.5%.[3] While the addition of N_2O stabilizes the cardiorespiratory state, isoflurane and N_2O produce antagonistic effects on descending pain pathways, thereby limiting any additional analgesia by their combination.[41,42] Therefore, it should be noted that 0.8% isoflurane +70% N_2O, used in the current study, is suitable for anesthesia where only minor interventions are required.

As an adjuvant to isoflurane, N_2O improves cardiorespiratory and metabolic stability. N_2O acts to directly counter the cardiovascular and autonomic depressive effects of isoflurane by exciting efferent sympathetic pathways[26,43] and increasing circulating catecholamines.[27] However, the sympathomimetic N_2O effects on vascular smooth muscle cells are blunted by downstream depletion of calcium availability by isoflurane and isoflurane-induced vasodilatation.[22] This may explain why no changes in SBP were observed, contrary to that commonly observed in other models.[27,44,45] Fluid administration could possibly compensate for the

anesthetic-induced cardiovascular reduction, although this would have obfuscated the physiological effects, and venous access in guinea pigs is complicated, requiring either superior vena cava access or subcutaneous bolus.[46]

Isoflurane has also been shown to reduce cerebral oxygen consumption,[47,48] and N_2O attenuates this when used as an adjuvant.[49] In a human study using cerebral PET monitoring, sevoflurane, a halogenated ether with similar properties to isoflurane,[47,49] was shown to reduce the cerebral metabolic rate of oxygen and cerebral blood flow.[49] However, adjuvant N_2O partially attenuated these sevoflurane-induced changes in cerebral metabolism and enabled preservation of cerebral blood flow.[49] While the current study did not assess cerebral oxygenation, we observed significant increases in RR with introduction of N_2O, with a subsequent increase in T_{re}, despite high T_{sk}. Furthermore, when blood gases were assessed, PO_2 was significantly lower than under isoflurane, with similar PCO_2, suggesting that the guinea pigs were not hyperventilating. Additionally, HR is linearly correlated with metabolism in most mammals,[50] with HR significantly higher following the introduction of N_2O in the present study. As such, our results indicate that metabolism of animals under ISO+N_2O was closer to conscious levels than under isoflurane alone. However, a decline in T_{re} following ~60 min suggests that this initial relative preservation of metabolic rate cannot be sustained.

Further to the treatment effect of N_2O, we observed significant sex differences in most physiological variables in response to the different anesthetic regimens. Females treated with ISO+N_2O demonstrated greater cardiorespiratory and metabolic stability than males. Patient sex has long been recognized as an independent factor in anesthetic response, with adequate depth of anesthesia less reliable in females than males.[29] Significant drug metabolism occurs in the liver, with guinea pigs demonstrating similar hepatic drug metabolism pathways to humans,[51] sexual dimorphism may significantly alter this. A number of possible explanations for this have been explored including sex hormone cycle, greater adiposity, and differential drug metabolism.[29,30] As such, while not performed in the current study, controlling for estrous phase may aid in clarifying this difference. Given the similarities in sexually dimorphic fat deposition, guinea pigs may therefore be an ideal paradigm to identify strategies to optimize sex-specific anesthetic regimens.

Establishing an effective balance between anesthesia and physiological depression is extremely important in anesthetized animal models investigating physiological function. Previous studies have sought to use injectable agents (Ketamine/Xylazine,[52] Midazolam-Medetomidine-Fentanyl [13]), or pair inhaled agents (Isoflurane+N_2O,[3,53] Desflurane+N_2O [54]), with varied success. In juvenile male guinea pigs, Schmitz et al observed dramatic reductions in mean arterial pressure, HR, and T_{re} in response to isoflurane (0.7 mL. min^{-1} 100% O_2, 3% isoflurane).[13] When comparing isoflurane at concentrations of 1.5–2.5% to various injectable agents (fentanyl-fluanisone/midazolam, sodium pentobarbitone) in guinea pigs, Mooney et al, observed significantly lower HR and BP under isoflurane anesthesia, excluding its further use from their trial.[4] By using markedly lower isoflurane concentrations, systolic SBP within the present study

were greater than those of Schmitz et al (current study: 53 mmHg, [13] nonsurgical sedation: 43.0 ± 11.2 mmHg), and equivalent to SBP of injectable agents observed by Mooney (~55 mmHg). While SBP in the present study did not differ between groups, pressures were maintained at a more physiological level (53 mmHg) than observed under many other anesthetic regimens. It is interesting to note that the improved cardiovascular function under ISO+N_2O brought HR within comparable ranges of those observed by Mooney.[4] As such, utilizing an isoflurane-N_2O mix minimizes the negative impacts of isoflurane noted by others,[4,15] while maintaining the versatility that makes isoflurane a preferred drug for extended use in various research settings including imaging or biochemical studies.

While this study did achieve a balance between effective cardiorespiratory and metabolic function, this was most apparent within the first hour of anesthetic maintenance. Variables that benefited substantially from ISO+N_2O (HR, RR, T_{re}) retained significantly higher levels than under isoflurane alone. It is likely that the slow decline after the initial response to the ISO+N_2O mix is due to declining T_{re} and thereby metabolism. However, as vasoconstrictory thresholds are inversely proportional to isoflurane dose,[19,55] reducing isoflurane alone should have minimized the impact on T_{re}. Supplementation of N_2O should have further limited this decline of T_{re},[23,53] this was not observed in the current study. While not performed in the current study, administration of warmed saline (s.c. or i.v. would ameliorate this decline in T_{re}.

Additionally, we chose to omit premedication with atropine as is commonly recommended with isoflurane.[16] Atropine is primarily used to reduce bronchosecretion and salivation, but additionally stabilizes cardiovascular function through inhibition of parasympathetic influences on the heart. While others[4] observed a troubling degree of salivation in response to isoflurane induction in absence of atropine premedication, we did not observe excessive salivation. This may be due to fasting (>4 h) prior to anesthesia or our slow rate of induction (0.5% isoflurane/min). We have therefore demonstrated that although commonly used, it is possible to safely administer prolonged anesthesia to guinea pigs without the added physiological confounding of atropine-induced parasympathetic blockade.

Finally, the methodology of the current study cannot be employed for surgical implementation and invasive studies where animals are likely subjected to a higher degree of noxious stimulation. Such studies should still consider the inclusion of N_2O, as cardiovascular stabilization has been repeatedly demonstrated at higher isoflurane/halogenated ether concentrations,[3,44,53] and long-term impacts are minimal.[56] However, studies utilizing the anesthetized animal model for pharmacological interventions should consider any drug interactions that may occur with the inclusion of N_2O.

4.1 | Conclusions

In conclusion, using comprehensive physiological monitoring, we have demonstrated that an anesthetic regimen of 0.8% isoflurane with 70% N_2O returns cardiovascular, respiratory, and thermoregulatory function toward conscious baseline levels, providing a suitable base for extended physiological studies. Adjuvant N_2O enables the reduction of isoflurane concentrations while still maintaining an anesthetized state in juvenile guinea pigs. The current dose of isoflurane with an N_2O adjuvant is beneficial to studies undergoing prolonged examination (~150 min), but appears most effective in the first hour of use.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR'S CONTRIBUTIONS

RMD and MJB involved in conceptualization, project administration, and funding acquisition. RPS, RMD, CLG, and MJB involved in methodology, and review and editing of the manuscript. RPS and RMD involved in validation, investigation, and formal analysis. RPS involved in data curation and writing original draft.

ETHICS APPROVAL STATEMENT

All procedures were approved by the University of Otago, Wellington Animal Ethics Committee (Approval: UOW5-16) and conformed to Health Research Council of New Zealand code of practice for the care and use of animals for scientific purposes.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.