Journal of the American Association for Laboratory Animal Science
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Vol 61, No 5
September 2022
Pages 482–494

Evaluation of Active Warming and Surgical Draping for Perioperative Thermal Support in Laboratory Mice

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Surgical procedures are commonly performed using mice but can have major effects on their core body temperature, including development of hypothermia. In this study, we evaluated active perioperative warming with and without surgical draping with adherent plastic wrap to refine practices, improve animal welfare, and optimize research experiments. Mice were randomized into treatment groups (*n* = 6; 8 CD1 mice per group). Treatments included placement within a small-animal forced-air incubator at 38 °C for 30 min before surgery (Pre), after surgery (Post), or before and after surgery (Both). To explore the effect of surgical draping, one group received incubator warming before and after surgery in addition to surgical draping (Both/Drape), whereas another group received surgical draping only without incubator warming (Control/Drape). The final group of mice received neither warming nor draping (Control). Subcutaneous temperature transponders were placed in all mice. Approximately 5 d after transponder placement, mice were anesthetized with ketamine–xylazine and underwent laparotomy. Subcutaneous body temperatures were collected perioperatively from transponders, and rectal temperatures were taken every minute during surgery. For recovery from anesthesia, mice were placed either in a standard cage on a warm water blanket set to 38 °C (100.4 °F) or in the incubator. Subcutaneous body temperatures were significantly higher in mice prewarmed for 30 min (Pre, Both, Both/Drape) as compared with mice that were not prewarmed. Anesthetic recovery times were significantly longer for mice placed in the incubator (Pre, Post, Both, Both/Drape) than for those that did not receive incubator warming (Control, Control/Drape). Mean intraoperative rectal temperatures of Both/Drape mice tended to be greater than those of mice in the Both group, suggesting a warming benefit of surgical draping. Using a forced air incubator and adherent plastic draping mitigated body temperature loss in mice during both surgery and postoperative recovery.

Abbreviation: HSD, Honestly Significant Difference

DOI: 10.30802/AALAS-JAALAS-21-000036

Thermoregulation is a vital part of normal rodent physiology. Mice, in particular, due to their small body mass and high relative body surface area, are highly susceptible to environmental temperatures.[22] Manipulations for surgical modeling may have additional major effects on mouse maintenance of core body temperature. During anesthesia, dysregulation of body temperature occurs due to suppression of hypothalamic control.[4,30,44] When rodents undergo anesthesia, hypothermia can occur for a variety of reasons, including impairment of cold detection and of central compensatory mechanisms that conserve and produce heat.[22] Hypothermia does not have a single definition but can be described as a decrease in core body temperature below an animal's normal physiologic body temperature range, indicating a failure of compensatory mechanisms to maintain a stable body temperature. Hypothermic events under anesthesia can lead to delayed recovery, increased risk of infection, and tissue injury.[22,28] Mice are at particularly high risk for hypothermia during anesthetic induction[28] and during topical application of disinfectants for skin antisepsis.[9,38]

Anesthesia can be accomplished in rodents by using inhalant or injectable agents. Isoflurane is one of the most commonly used inhalant anesthetics for mice and has dose-dependent vasodilatory effects[8,39] but fewer systemic hemodynamic effects than the common injectable combination of ketamine and xylazine.[25] A desire to avoid the respiratory depression caused by isoflurane[40] may influence preference for an injectable anesthetic regimen, although recent study indicates that both isoflurane and ketamine–xylazine can markedly affect ventilation in C57BL/6 mice.[29] Nevertheless, injectable agents may be preferred in applications when placement of a nose cone or endotracheal tube for inhalant delivery would interfere with animal positioning or when necessary anesthetic equipment for use of inhalant agents is unavailable. Ketamine is a dissociative anesthetic with potent analgesic properties, whereas xylazine is an α_2 adrenoreceptor agonist incapable of producing sleep or loss of righting reflex when used as a sole agent in mice.[14] Physiologic effects of ketamine–xylazine administration in mice include bradycardia, muscle relaxation, catalepsia, and hypnosis.[12] These agents are commonly used in combination due to their paradoxical effects, such as xylazine-induced hypotension and bradycardia compared with the increased myocardial contractility and strong pressor effects of ketamine.[14] Mice have a relatively short recovery time and minimal effects on oxygen partial pressure in blood while unconscious with ketamine-xylazine, although decreased body temperatures remain a consideration regarding the use of these agents in mice.[5,12]

Received: 17 Mar 2021. Revision requested: 05 May 2021. Accepted: 16 Aug 2021.
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The use of preanesthetic warming to offset body heat loss under anesthesia has been evaluated in various animal species and is an established practice in human medicine. In human patients undergoing epidural analgesia and major abdominal surgery, 15 min of active warming with a forced-air blanket set to 40 to 44 °C (104 to 111.2 °F) before and after anesthetic induction prevented postoperative hypothermia.[23] Another study in human patients found that low preoperative body temperatures prior to abdominal surgery were a risk factor for intraoperative hypothermia and that prewarming from a circulating water mattress set to 38 °C (100.4 °F) could reduce hypothermic risk.[26] Previous studies in laboratory rodents have shown that perioperative warming techniques have a positive effect on maintaining body temperature and avoiding hypothermia in animals undergoing anesthesia.[6,35-37,46] In addition, forced-air warming blankets wrapped around standard rodent cages can warm the microenvironment: a standard mouse cage wrapped with a forced-air warming blanket and plastic drape reached a final temperature of 38.6 °C (101.5 °F) at 60 min, which was faster than other methods of warming.[35]

In prior studies, our group has evaluated aspects of passive thermal support during perioperative procedures.[9,38] In the current study, we evaluated active perioperative warming by using a small-animal forced-air incubator, with and without the addition of a surgical drape during surgery, to provide thermal support to laboratory mice undergoing laparotomy. We hypothesized that exposure to a novel forced-air incubator for active perioperative warming, with and without use of a surgical cling wrap drape, would support the body temperature of mice and mitigate body temperature loss during abdominal surgery.

Materials and Methods

Animal housing and husbandry. These studies were approved by the Michigan State University IACUC, and mice were housed in an AAALAC-accredited facility, in accordance with the *Guide for the Care and Use of Laboratory Animals*.[24] Mice (n = 48 [24 female, 24 male]; age, 3 to 6 mo; Crl:CD1[ICR]) were donated from an inhouse breeding colony and housed under a 12:12-h light:dark cycle at a density of 1 to 4 mice per polysulfone microisolation cage (Optimice, Animal Care Systems, Centennial, CO). Housing rooms were maintained at 20 to 22.2 °C (68 to 72 °F), with recorded relative humidity of 39% to 58%. Mice were kept on nonautoclaved aspen chips (Northeastern Products, Warrensburg, NY) and provided with shredded paper and cotton-fiber square enrichment items (Bed-r'Nest, The Andersons, Maumee, OH; Nestlets, Ancare, Bellmore, NY). Wire-lid food hoppers were filled with rodent chow (Teklad Global Diets Irradiated 22/5 Rodent Diet 8940, Envigo, Indianapolis, IN), and mice were provided reverse-osmosis–purified water in bottles; chow and water were available without restriction. Quarterly sentinel samples are tested at a commercial laboratory (IDEXX BioAnalytics); specifically, blood, feces, and oral and fur swabs are submitted for serology, parasitology, or PCR testing. The animals in our study were free of the following pathogens: mouse hepatitis virus, minute virus of mice, mouse parvoviruses, murine encephalomyelitis virus, mouse rotavirus, Sendai virus, pneumonia virus of mice, mouse reovirus, mouse adenoviruses, lymphocytic choriomeningitis virus, ectromelia virus, polyomavirus, mouse cytomegalovirus, mouse thymic virus, *Filobacterium rodentium*, *Mycoplasma pulmonis*, *Encephalitozoon cuniculi*, *Corynebacterium bovis*, murine ectoparasites (*Myocoptes*, *Myobia*, *Radfordia* spp.) and pinworms (*Aspiculuris tetraptera*, *Syphacia obvelata*).

Placement of temperature transponders. Mice were anesthetized briefly with inhaled isoflurane for subcutaneous placement of temperature transponders (IPTT-300, Bio Medic Data Systems, Seaford, DE). These implants transmit real-time body temperature readings to a handheld scanning device (DAS-6007, Bio Medic Data Systems). Anesthesia was induced with isoflurane (3% to 4% in O_2 at 0.4 to 0.6 L/min) in a 0.5-L plastic induction box. Mice were considered to be at an appropriate plane of anesthesia when righting and withdrawal reflexes were absent, as demonstrated by lack of response to toe pinch. Isoflurane was reduced to 1.5% to 3% in 0.4 to 0.6 L/min O_2 for maintenance of anesthesia by using a nose cone for approximately 5 min (SomnoSuite, Kent Scientific, Torrington, CT). All microchip transponders were inserted according to the manufacturer's instructions, and the entry site was closed with tissue adhesive (Vetbond, 3M Animal Care Products, St Paul, MN). Mice recovered from anesthesia in a static cage placed half on a warm circulating-water blanket set to 38 °C (100.4 °F) and were returned to social housing for acclimation prior to surgery.

Animal health scoring. Just prior to surgery, after anesthetic recovery, and at each daily postoperative assessment, mice were scored individually according to an institutional multipoint scoring sheet (Figure 1). Health scoring was performed when mice were in their home cage by personnel conducting surgical procedures; therefore, personnel were not specifically blinded to treatment groups. At our institution, this sheet provides investigators with a practical guide for assessment of animal health and identification of animals that might require veterinary intervention or euthanasia. Indices assessed included body condition score on a 5-point scale,[41] coat appearance, posture, presence or absence of facial expressions that may be associated with discomfort,[27] activity level, response to external stimulus of the placement of a researcher's hand into the housing cage (provoked behavior), respiration, hydration status, and appearance of the incision site. Each item was scored on a scale of 0 (body condition score ≥ 3; no abnormalities) to 3 (severe abnormalities), and scores for all items were tallied. With this scale, an animal's total score ranges from 0 to 27. Animals are removed from study and euthanasia is considered when an animal's total score is 15 or greater or when an animal receives a score of 3 in any 2 categories. These endpoint thresholds are based on experience within our institution.

Incubator testing. Pilot testing of the incubator was conducted to assess the accuracy of manufacturer-suggested temperature settings and to ensure absence of risk of thermal injury to animals while maintained in the incubator. The incubator we used is designed for bird brooding and as an intensive care unit for avian and mammalian species (TLC-30 Eco Parrot Brooder/Intensive Care Unit/Recovery Incubator, Brinsea Products, Titusville, FL; Figure 2). The internal dimensions are 12 in. wide × 12 in. deep × 11 in. high, and warmed air is generated by a heating coil located in the top of the unit. A fan positioned below the coil captures and blows warm air toward the floor where animals can be positioned. The fan and heating coil are not accessible to animals, which can be viewed through a clear polycarbonate door that can be latched securely. The internal temperature is measured by a glass thermometer that is visible through the door, and fresh air passively enters the incubator through a front-mounted vent. The internal temperature is controlled by using a dial on the front of the unit and (according to the manufacturer) has 3 settings of approximately 20, 28, and 38 °C (68, 82.4, and 100.4 °F).

For our study, we set the incubator to the maximum setting of 38 °C (100.4 °F) and compared the reading of the unit's glass thermometer with that of a digital thermometer–hygrometer

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Observations			Description	Score
Body Condition Score (BCS)			BCS > 3 (well-conditioned to over-conditioned)	0
			BCS > 2 and < 3 (mouse is becoming under-conditioned)	1
			BCS > 1 and < 2 (mouse is under-conditioned)	2
			BCS of 1 or less (mouse is emaciated)	3
Physical Appearance: Coat			Shiny, well-groomed	0
			Dull, unkempt (lack of grooming)	1
			Very rough coat (piloerection)	3
Physical Appearance: Posture			Normal	0
			Slightly hunched	1
			Moderate to severely hunched (able to rear up or stretch out)	2
			Severely hunched (unable to rear up to access food and water)	3
Physical Appearance: Facial Expression			Normal, eyes open > 75%, nose & cheeks smooth, ears & whiskers lying naturally	0
			Eyes open 25–49%, slight bulges of nose and/or cheeks, ears slightly pulled back, whiskers pulled back or sticking forward	2
			Eyes open < 25% or closed, severe bulging of nose and/or cheeks, ears tightly pulled back, whiskers flat against face or sticking very forward	3
Behavior: Natural			Normal (interactive in environment)	0
			Slight decrease in activity, less interactive	1
			Abnormal, decreased activity/alertness, isolated	2
			Self-mutilation, either hyperactive or immobile	3
Behavior: Provoked (response to external stimulus)			Normal, i.e., quickly moves away	0
			Slow to move away or exaggerated response	1
			Abnormal, moves away after short period of time	2
			Doesn't move or reacts with excessively exaggerated response	3
Respiration			Normal	0
			Increased respirations	2
			Labored or open mouth breathing	3
Hydration			Normal, no skin tent	0
			Skin tent < 1 but < 3 seconds, eyes bright and moist	2
			Skin tent < 3 seconds, eyes sunken and tacky	3
Study Specific	**Incision Site**	No crusting, redness, or swelling	0	
		Mild serous crusting, redness or swelling	1	
		Moderate serous crusting, redness or swelling; any purulent discharge	2	
		Active bleeding, excessive discharge, open wound	3	

Remove mice from study and consider euthanasia if the points add up to a score $\geq$ 15.

Figure 1. Institutional scoring sheet for health assessment of mice. Animals are removed from study and euthanasia is considered when the total score is ≥ 15 or when an animal receives a score of 3 in any 2 categories.

(Timex TX5170, Maverick Industries, Edison, NJ). The wired remote sensor of the thermometer–hygrometer was placed approximately 2 cm off the floor in the center and at each corner of the incubator, to measure the ambient temperature of the air at animal level when placed within the unit. At 1 h after turning on the unit, the temperature range measured by both the glass

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Figure 2. Small-animal forced-air incubator, front view, with temperature setting dial and manual air vent in the closed position to the left and right, respectively, on the front panel. Animals can be placed in the interior of the incubator by opening the clear, front-facing door..

Table 1. Change in interior temperature (°C) and time to return to starting temperature of forced-air animal incubator after door opening

Door open (s)	Temperature (°C)	Time to return to baseline temperature (37.7 °C)
15	37.2	1 min 20 s
30	26.6–27.5	4 min 59 s
45	26.9–27.6	6 min 46 s
60	27.3–27.5	9 min 43 s

thermometer mounted inside the incubator and the remote sensor probe placed at the center of the incubator's floor space and in each corner was 37.7 to 39.4 °C (99.9 to 102.9 °F). To assess heat loss from the incubator when the door was opened, after the incubator achieved a steady temperature at the 38 °C setting, the door was opened for periods of 15, 30, 45, and 60 s (Table 1). Placing mice in and removing them from the incubator required opening the door for approximately 15 s, and pilot testing indicated only minimal loss of heat from the incubator under these conditions.

At our institution, 40 °C (104 °F) is used as a cut-off temperature for rodent exposures to avoid thermal injury. A previous literature review discusses temperature thresholds for thermal damage to tissues in a variety of species.[45] Cumulative equivalent minutes at 43 °C (CEM_{43}; 109.4 °F) is a commonly used metric for thermal dose assessment and corresponding tissue damage, and CEM_{43} of 21 to 40 min is associated with acute damage to skin function.[45] Therefore, 40 °C is a reasonable cut-off for external air temperature exposure in mice to avoid thermal injury. In subsequent experiments using mice, the fresh-air exchange vent was opened as needed to ensure that the internal temperature of the incubator did not exceed 40 °C.

Warming and draping treatments. After 4 to 5 d of acclimation after transponder placement, mice were randomly allocated into treatment groups (n = 8 per group; 4 male, 4 female) prior to undergoing laparotomy. The experimental unit of study was the individual animal; therefore, each treatment group comprised 8 experimental units. Group size was determined by using a priori power analysis: with 6 treatment groups, a sample size of 8 per group allowed detection of an effect size of 0.6 with 80% statistical power. This effect size corresponded to detecting a difference of 1 °C in mean body temperature between treatment groups at each time point during the surgical procedure. In addition, a sample size of 8 allowed the detection of differences between groups in anesthetic recovery time of 13 min or longer and of differences in postoperative weight changes of 8 g or greater (corresponding to a 20% change from baseline on average). Randomization of mice into treatment groups was conducted by using a publicly accessible online program (Random Team Generator, Random-Lists.com); mice were randomized to surgery day by using the same program. Randomization was performed at the animal level; therefore, as mice were group-housed, mice from different treatment groups shared the same home cages. All procedures involving mice (transponder placement, surgery, and postoperative assessments) were conducted by 2 personnel (KTB, JDV). The 6 experimental groups were as follows: no incubator warming or surgical draping (Control); prewarming in the incubator but without draping during surgery (Pre); warming in the incubator after surgery without draping (Post); warming before and after surgery but no draping (Both); no incubator warming but with draping during surgery (Control/Drape); and warming in the incubator before and after surgery with the addition of draping (Both/Drape). Surgical draping for all rodent groups involved using transparent, adherent plastic wrap (GLAD Press'n Seal, The Clorox Company, Oakland, CA), as previously described.[11]

Surgical procedure. All surgeries were performed in a dedicated procedure room, and no other activities were ongoing in the room during surgery periods. Six surgeries were conducted each day, because this number allowed completion of all surgeries and full anesthetic recovery of all mice within standard working hours. Environmental parameters of the procedure room were recorded at the start of each day on which surgeries were performed (range over surgery days: room temperature, 20.9 to 21.4 °C [69.6 to 70.5 °F]; room relative humidity, 44% to 48%). Before manipulation, mice were evaluated and assigned a baseline health score. Health scores and temperature readings were collected throughout the procedure (Figure 3). In all groups, mice spent a period of 30 min before surgery either in standard caging at room temperature or in the incubator set at 38 °C (100.4 °F; prewarming), depending on their treatment group. A baseline temperature reading was obtained from the subcutaneous transponder at the start of this 30-min period. After 30 min, transponder temperatures were again collected, and mice were weighed on a gram scale, after which they were briefly returned to their respective prewarming condition while anesthetic agents were prepared based on body weight. Mice were anesthetized with ketamine–xylazine (dosage: ketamine, 100 mg/kg IP [Ketathesia, Covetrus, Dublin, OH]; xylazine, 10 mg/kg IP [Anased, Akorn, Lake Forest, IL]). At the time of surgery, body weight across all groups ranged from 25 to 53 g (males, 34 to 50 g; female, 25 to 53 g). Mice were returned to the standard cage or incubator to await anesthetic induction.

Mice were considered anesthetized once a loss of righting reflex was observed; the time from anesthetic administration to loss of the righting reflex and a subcutaneous temperature reading were recorded. Mice were then moved to a designated station for skin preparation, where they were positioned in dorsal recumbency on top of a cloth pad overlying a heated bed (E-Z Heat Surgical Bed Warming System, E-Z Systems, Palmer, PA) set to 38 °C (100.4 °F). A thermometer probe was inserted into the rectum to a depth of 2 cm, as allowed by the design of the probe

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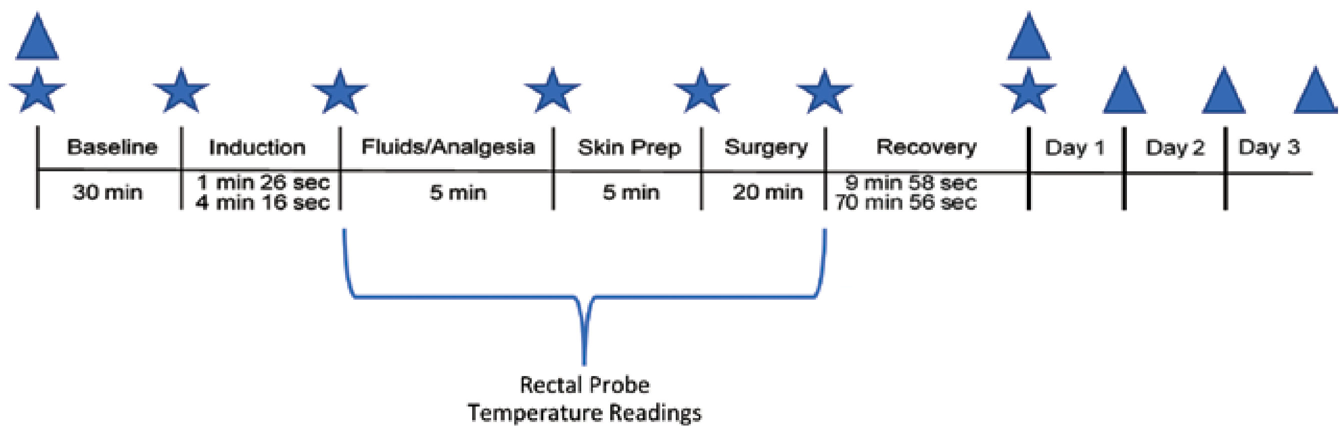

Figure 3. Surgical procedure timeline, including the range for each period of the procedure across all treatment groups. Blue triangles indicate the time points for health scoring; blue stars show the time points at which subcutaneous temperature readings were collected; and the rectal temperatures of anesthetized mice were collected at 1-min intervals during the procedural stages within the blue brackets.

(BIO-BRET3, Bioseb In Vivo Research Instruments, Pinellas Park, FL). Rectal temperature readings were automatically recorded every minute until completion of surgery. Eye lubricant (Artificial Tears Solution, Covetrus, Dublin, OH) was applied to both eyes. Mice received the first dose of meloxicam (2 mg/kg SC; Eloxiject, Covetrus) prior to hair clipping; a second dose of analgesic was given 24 h later. Mice also received 0.5 mL of saline subcutaneously (0.9% Sodium Chloride Injection USP, Braun Medical, Bethlehem, PA). An approximately 3 cm × 3 cm area on the ventral abdomen was clipped. After hair clipping, another subcutaneous temperature reading was obtained. The clipped area was prepped by using 2 consecutive applications of Avagard (61% ethanol and 1% chlorhexidine gluconate; Avagard Surgical Hand Antiseptic, 3M, Saint Paul, MN), as described previously;[9] another subcutaneous temperature reading was taken after skin preparation. Mice were then moved to a designated surgery station and placed on an autoclaved cloth drape overlying a second heated bed (E-Z Heat Surgical Bed Warming System, E-Z Systems) set to 38 °C. When an animal was assigned to a draping treatment group, a surgical drape was placed over the ventrum of the mouse extending from the neck to the tail tip. The drape was gently pressed down to adhere to the prepared surgical site and extended laterally on either side to adhere to the sterile cloth drape placed between the heating bed and animal.

A 2-cm ventral midline incision was made, followed by insertion of hemostats approximately 1 cm into the abdominal cavity. Hemostats were then moved 1 cm cranially, caudally, and laterally to each side of the abdominal cavity to simulate manipulation of abdominal contents during an experimental laparotomy. The abdominal cavity was left open for 10 min to mimic the total time of body cavity exposure during an experimental laparotomy. The peritoneum was closed with simple interrupted sutures (Sharpoint plus Polysyn Undyed Braided Coated Polyconic Acid, Surgical Specialties, Tijuana, Mexico), and skin closure was achieved by using 3 or 4 skin clips (7-mm Reflex Clip, Braintree Scientific, Braintree, MA) placed 2 to 3 mm apart. After skin closure, the rectal probe was removed, and another subcutaneous temperature reading was collected as mice were moved to either a standard cage placed partially on a warm water-circulating blanket set to 38 °C (100.4 °F) or into the incubator for anesthetic recovery.

Postoperative period. Mice were monitored continuously until able to right themselves as a confirmation of anesthetic recovery. The time to righting and a temperature reading were recorded. After the return of righting reflex, animals received a second health score. Once fully recovered, mice were returned to the same caging and social housing conditions in the same room in which they had been housed prior to the procedure. Postoperative checks were performed once daily for 3 consecutive days between 0900 and 1200 and consisted of recording the subcutaneous temperature, body weight, and health score. On postoperative day 3, mice were euthanized by CO_2 inhalation at 30% volume displacement per min, followed by cervical dislocation as a secondary method.

Statistical analysis. As was determined prior to analysis, the criteria for inclusion of mice in statistical analysis were participation in all perioperative portions of the experiment—temperature transponder placement, prewarming condition, laparotomy, anesthetic recovery in postwarming condition, and continued survival for 3 d after surgery. One mouse in the Both/Drape group completed the laparotomy procedure but did not recover from anesthesia; therefore, data from this animal were used for preoperative and surgical statistical analyses only. For all mice, rectal temperature readings were recorded each minute for 30 min during the surgical procedure, giving a total possible number of rectal temperature readings of 1,440; 25 of these readings were omitted from statistical analysis. Specifically, 7 of these potential readings were inadvertently not recorded due to thermometer error (2 readings from a male mouse in the Pre group and 5 from a female mouse in the Both/Drape group); and 18 readings were omitted from analysis as outliers, as further clarified in the Discussion (one reading from a male in the Both group; one from a female in the Both group; 14 from a male in the Control/Drape group, and one from each of 2 female mice in the Both/Drape group). Analyses were conducted by using R statistical programming language.[34] All hypotheses were tested by using a familywise error rate of $\alpha = 0.05$. Because the outcomes were related, experiment-wise type I error was adjusted by using a Bonferroni correction method. A *P*-value of < 0.05 was used to denote a statistically significant effect. In descriptive summary data, means are expressed as mean ± 1 SD.

Preoperative. Initially, one-way ANOVA was used to assess for sex-associated differences in baseline subcutaneous temperature within each treatment group. Next, 2-way ANOVA with interaction was used to assess for sex-associated differences in baseline subcutaneous temperatures by treatment group. Because the interaction between sex and treatment factors was highly insignificant, a 2-way additive model also was used to evaluate baseline subcutaneous temperatures between sexes or treatment groups. One-way ANOVA was used to assess for differences between treatment groups in time to loss of righting reflex after ketamine–xylazine administration.

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One-way and 2-way ANOVA were used to evaluate sex differences in body temperatures within each treatment group during the preoperative acclimation period and sex differences in body temperatures post warming period by treatment group, respectively. A 2-way additive model, used due to the insignificant interaction term, explored the impact of sex and treatment group on body temperature after the 30-min acclimation period, and pairwise comparisons were performed by using the Tukey Honestly Significant Difference (HSD) test.

Surgery. A latent variable-growth model[31] was used to examine the effects of treatment group assignments on intraoperative body temperature (α = 0.05). The model incorporated polynomial functions of time, to include the nonlinear temperature trajectories over time. Mice in the Control group served as a reference group for all comparisons. Therefore, the intraoperative temperature trends of all the remaining groups were assessed as the relative effect of the assigned treatment on temperature as compared with the Control group.

The linear (constant amount of change over time) and quadratic (acceleration or deceleration in the linear rate of change) effects of time on temperature were explored to assess sex-associated differences in temperature trajectories within each treatment group, as done in previous studies.[9,38] In addition, these growth models for each treatment group included the random intercept, random linear time effect, and random quadratic time effect of mice to incorporate mouse-to-mouse variability. Next, the cubic (change in acceleration or deceleration) effect of time on temperature was included to assess treatment differences in temperature trajectories as compared with the Control group.

In the model that considered all treatment groups together, the following random-effects structure was assessed—random intercept, random linear time effect, random quadratic time effect, and correlation between random linear and quadratic time effects for each mouse—to effectively account for mouse-to-mouse variability. The random-effects structure was narrowed by using results from statistically significant likelihood ratio tests.

Recovery. Postoperative recovery times were measured in minutes for each mouse. Welch one-way ANOVA was used to assess sex-associated differences in recovery times within each treatment group. Two-way ANOVA with interaction was chosen to evaluate sex differences in postsurgery recovery times by treatment group. A 2-way additive model and posthoc analysis using the Tukey HSD test were used to compare differences in recovery times between male and female mice overall and between treatment groups.

Postoperative. Across all time points, the distribution of health scores was skewed; therefore, median behavior scores between treatment groups were assessed by using 5 separate tests on correlated outcome variables. The level of significance was adjusted by using the Bonferroni correction method (α = 0.01 for each test), which ensured that the familywise type I error rate did not exceed the threshold of 5%. The Mood median test was performed to assess median health scores across treatment groups. Furthermore, distributions of health scores between treatment groups were assessed by using a one-way permutation test of independence for ordinal data.

Analysis of postoperative subcutaneous temperature readings used a Bonferroni-corrected α value of 0.0125 to account for 4 hypothesis tests on correlated outcomes which bounds familywise type I error rate at 5%. One-way ANOVA was performed on each occasion; and post hoc analysis using the Tukey HSD test was performed for the initial postoperative temperature reading.

Body weight changes from baseline weight were calculated at 24, 48, and 72 h after surgery. A Bonferroni-corrected α value of 0.0167 was used to account for 3 separate tests on correlated outcome variables to bound the familywise type I error rate at 5%. One-way ANOVA and posthoc analysis using the Tukey HSD test were performed.

Results

Preoperative. *Baseline.* No significant differences in baseline subcutaneous temperature were detected between sexes within each treatment group ($P > 0.05$ for all tests) or between treatment groups ($F_{5,36} = 0.520$, $P = 0.759$). The two-way additive model showed no differences in baseline subcutaneous temperatures between sexes ($F_{1,41} = 0.122$, $P = 0.728$) or treatment groups ($F_{5,41} = 1.032$, $P = 0.412$). Time to loss of righting reflex after ketamine–xylazine administration did not differ between treatment groups ($F_{5,41} = 0.376$, $P = 0.862$).

30-min acclimation period. No sex-associated differences in body temperatures were detected during the preoperative acclimation period ($P > 0.05$ for all tests) or the postoperative warming period ($F_{5,36} = 1.022$, $P = 0.419$) within each treatment group. Sex did not have a significant effect on mouse body temperature after the 30-min warming period ($F_{1,41} = 2.393$, $P = 0.130$), but differences between treatment groups were observed ($F_{5,41} = 43.638$, $P = 2.3 \times 10^{-15}$). Groups exposed to the incubator for 30 min (Pre, Both, Both/Drape; mean body temperature, 39.97 ± 0.58 °C [103.95 ± 1.04 °F]) had significantly higher subcutaneous body temperature readings before surgery than did groups maintained in standard cages at room temperature (Control, Post, Control/Drape; mean, 38.0 °C ± 0.32 °C [100.4 ± 0.58 °F]; $P < 0.05$ for all pairwise comparisons with significant differences; 95% CI, 1.69 to 2.25; Figure 4).

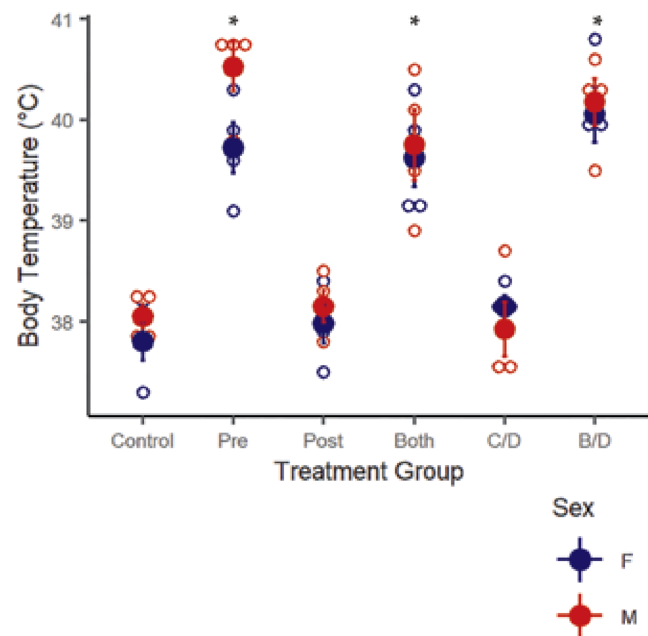

Figure 4. Subcutaneous temperatures (°C, mean ± 1 SD) at end of warming period for different treatment groups shown separately for male and female mice by using line plots. Groups exposed to the incubator before surgery—Pre, Both, and Both/Drape—had significantly (*, $P < 0.05$) higher subcutaneous temperatures than groups maintained in standard cages at room temperature. F, female; M, male; Control, no incubator warming and no draping during surgery; Pre, prewarming in the incubator but no draping during surgery; Post, warming after surgery but no draping during surgery; Both, warming before and after surgery but no draping during surgery; C/D, Control/Drape, no incubator warming but with draping during surgery; B/D, Both/Drape, warming before and after surgery with surgical draping intraoperatively.

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Surgery. Within each treatment group, median body temperature did not differ significantly between male and female mice, as indicated by the overlapping notches in a notched box plot (Figure 5). For all treatment groups, mice had higher temperatures at the start of the anesthetic period and the lowest temperature readings were recorded toward the end of surgery. Furthermore, mice in the Pre, Both, and Both/Drape groups began surgery with higher rectal temperatures (mean, 37.74 ± 0.41 °C [99.93 ± 0.74 °F]) than did the Control, Post, and Control/Drape groups (mean, 36.3 ± 0.63 °C; 95% CI, 1.08 to 1.70), and the variations in temperatures over time differed between treatment groups (Figure 6).

Temperature patterns were not different between male and female mice in each treatment group during the surgical procedure ($P > 0.05$ for all tests). Variation in the individual temperature patterns increased within each treatment group as surgery progressed, particularly in the Post and Both/Drape groups, indicating differences in the individual responses of the mice to anesthesia and exposure of the abdominal cavity for 10 min during surgery.

The linear (constant amount of change over time), quadratic (acceleration or deceleration in the linear rate of change), and cubic (change in acceleration or deceleration) effects of time on temperature were assessed for all treatment groups in comparison with the Control group. Although all treatment groups showed the same general downward trend in temperatures over the course of the procedure, we noted differences in the linear, quadratic, and cubic rates of change between treatment groups. Linear rates of changes were more negative for the Pre, Both, Control/Drape, and Both/Drape groups than for the Control group ($P < 0.05$ for all comparisons), indicating that these groups lost more body heat than did the Control group. The linear rate of change did not differ between the Post and Control groups. The acceleration of the rate of temperature change (quadratic effect) was positive across all treatment groups except the Control group. The Pre, Both, Control/Drape, and Both/Drape groups had greater positive acceleration, indicating that they lost heat more slowly, than did the Control group ($P < 0.05$ for all comparisons). Neither acceleration nor the change in acceleration (cubic effect) differed between the Post and Control groups. However, as compared with the Control group, the Pre, Both, Control/Drape, and Both/Drape groups exhibited a more negative ($P < 0.05$ for all comparisons) change in acceleration (cubic effect), indicating that their acceleration of body temperature change became less positive (that is, slowed down) as time progressed. The mean rectal temperatures of mice in the Both/Drape and the Both groups at end of surgery were: Both, 33.8 ± 0.7 °C (92.8 ± 1.3 °F); Both/Drape, 34.9 ± 0.8 °C (94.8 ± 1.4 °F); $P = 0.1344$; 95% CI, –1.768 to 0.268.

Recovery. Recovery time did not differ between sexes within each treatment group ($P > 0.05$ for all tests). Furthermore, no sex-associated differences in recovery time by treatment group were detected ($F_{5,35} = 1.032$, $P = 0.414$), and anesthetic recovery time did not differ between male and female mice overall ($F_{1,40} = 0.518$, $P = 0.476$). Anesthetic recovery times differed significantly between treatment groups ($F_{5,40} = 6.855$, $P = 1.06 \times 10^{-4}$). With the exception of the Control/Drape group as compared with the Pre group, mice in the Control and Control/Drape groups recovered from anesthesia significantly faster (average, 45.16 min) than did mice in the Pre, Post, Both, and Both/Drape groups (average, 21.4 min; $P < 0.05$ for all pairwise comparisons with significant differences; Table 2 and Figure 7).

Postoperative. ***Health scores.*** Health scores were collected from individual mice immediately before surgery, after anesthetic

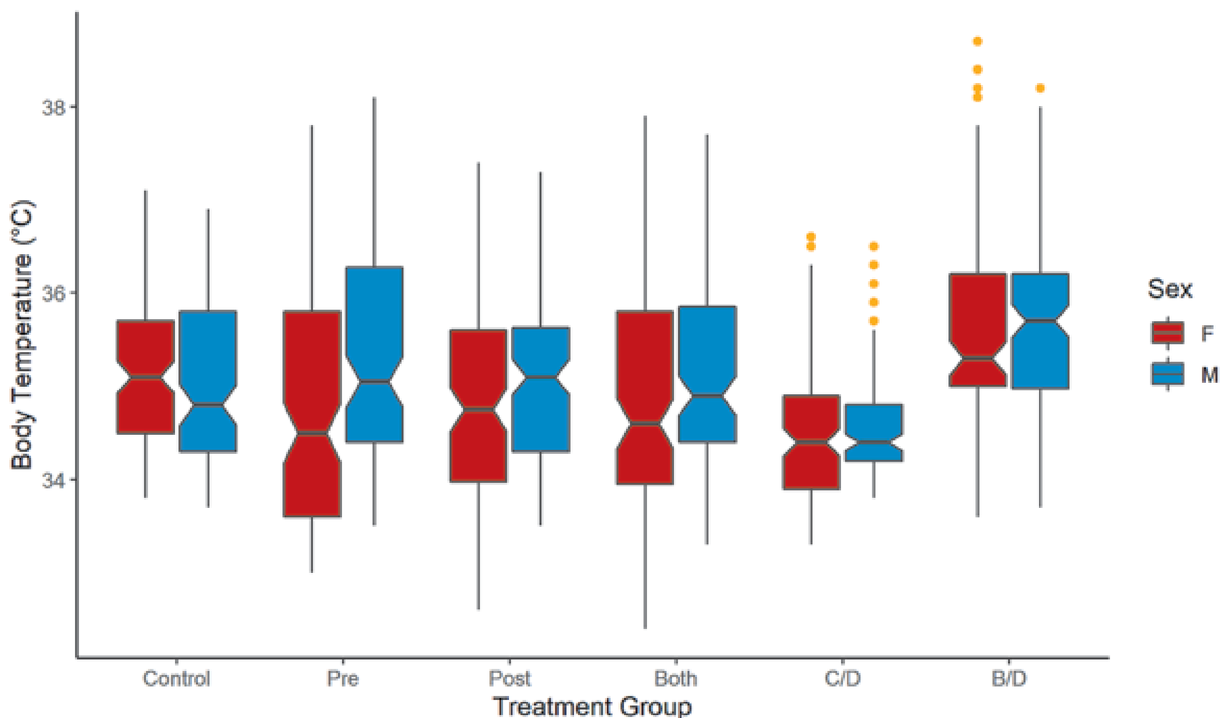

Figure 5. Notched box plots comparing the intraoperative rectal temperatures (°C) of male and female mice within each treatment group. The black line within each box is the median, and each notch displays the 95% CI around the median. Each box indicates the interquartile range, and the whiskers are the minimum and maximum values without outliers. F, female; M, male; Control, no incubator warming and no draping during surgery; Pre, prewarming in the incubator and no draping; Post, warming after surgery with no draping during surgery; Both, warming before and after surgery but with no draping during surgery; C/D, Control/Drape, no incubator warming but with draping during surgery; B/D, Both/Drape, warming before and after surgery and surgical draping intraoperatively.

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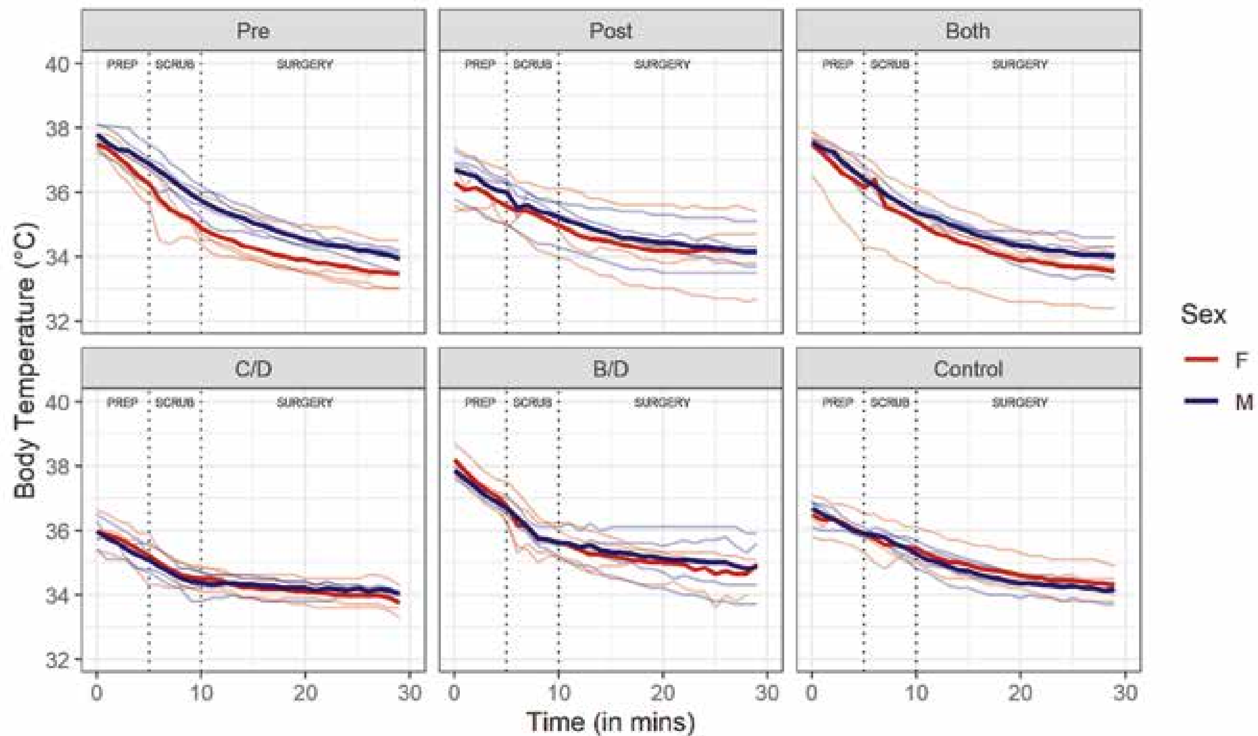

Figure 6. Trajectories of intraoperative rectal temperature (°C) over time (min) for male (blue) and female (red) mice in each treatment group. Thin lines indicate the trajectories of individual mice; the thick line indicates the average temperature of all mice of one sex within a treatment group. Temperature trajectories did not differ significantly between female and male mice within each treatment group. F, female; M, male; Control, no incubator warming and no draping during surgery; Pre, prewarming in the incubator but no draping during surgery; Post, warming after surgery during surgery no draping during surgery; Both, warming before and after surgery but no draping during surgery; C/D, Control/Drape, no incubator warming but draping during surgery; B/D, Both/Drape, warming before and after surgery and draping during surgery.

Table 2. Between-group comparisons of mean anesthetic recovery time

Groups compared	*P*	95% CI (min)
Control and Pre	**0.031**	9.515 to 33.589
Control and Post	**0.013**	12.066 to 35.472
Control and Both	**0.007**	10.134 to 40.712
Control and Both/Drape	**0.001**	18.129 to 43.805
Control and Control/Drape	0.996	−8.278 to 14.882
Control/Drape and Pre	0.098	4.520 to 31.980
Control/Drape and Post	**0.046**	7.029 to 33.904
Control/Drape and Both	**0.025**	5.467 to 38.775
Control/Drape and Both/Drape	**0.004**	12.972 to 42.357
Pre and Post	0.999	−11.617 to 16.050
Pre and Both	0.992	−13.104 to 20.846
Post and Both /Drape	0.759	−5.702 to 24.531
Post and Both	0.999	−15.085 to 18.394
Post and Both /Drape	0.906	−7.608 to 22.004
Post and Both / Drape	0.967	−12.691 to 23.779

n = 8 per group. Mice in the Post, Both, and Both/Drape groups were placed in the incubator during anesthetic recovery; mice in the Pre group were placed in the incubator for 30 min prior to anesthesia but recovered in a standard cage set half on a warm water-circulating blanket set to 38 °C; and mice in the Control and Control/Drape groups were never exposed to the incubator but were recovered in a standard cage set half on a warm water-circulating blanket set to 38 °C (100.4 °F). Bolded values indicate significant differences between groups.

recovery, and at approximately 24, 48, and 72 h after surgery. Health scores across all treatment groups ranged between 0 to 2 at all time points, including the baseline scores for each mouse before surgery and scores at 24, 48, and 72 h after surgery. The medians and distributions of health score values across treatment groups were not statistically different at any time point ($P > 0.05$, for all tests).

Subcutaneous temperatures. Subcutaneous body temperature continued to be monitored after anesthetic recovery and at approximately 24, 48, and 72 h after surgery. Postoperative body temperatures were significantly different ($F_{5,40} = 28.42$, $P = 3.5 \times 10^{-12}$) among treatment groups at the time of anesthetic recovery. Mice in the Post, Both, and Both/Drape groups (mean, 38.77 ± 1.86 °C) demonstrated significantly higher body temperatures at the time of anesthetic recovery than did mice in the Control, Pre, and Control/Drape groups (mean, 33.6 ± 0.85 °C [92.5 ± 1.5 °F]; $P < 0.0125$ for all pairwise comparisons with significant differences; 95% CI, 4.23 to 5.95; Figure 8). Body temperatures were not significantly different between treatment groups at 24 h ($F_{5,35} = 0.42$, $P = 0.83$), 48 h ($F_{5,34} = 0.90$, $P = 0.49$), and 72 h ($F_{5,40} = 1.77$, $P = 0.14$) after surgery.

Body weight change. Mice were weighed before and at approximately 24, 48, and 72 h after surgery. No significant differences in body weight change from baseline weight were found between treatment groups at 24 h ($F_{5,38} = 1.65$, $P = 0.17$) and 48 h ($F_{5,38} = 0.51$, $P = 0.77$) after surgery. At 72 h after surgery, body weight change from baseline differed significantly between treatment groups ($F_{5,38} = 3.62$, $P = 0.01$). Mice in the Control/Drape group had relatively small changes in average body weight over 72 h after surgery (−0.3 g at 24 h, −0.5 g at 48 h, 0.8 g at 72 h). In comparison, larger changes in daily average postoperative body weight occurred in the Both/Drape (−0.7 g at 24 h, −1.0 g at 48 h, −1.7 g at 72 h) and Control (−1.6 g at 24 h, −0.6 g at 48 h, −1.7 g at 72 h) groups. However, posthoc

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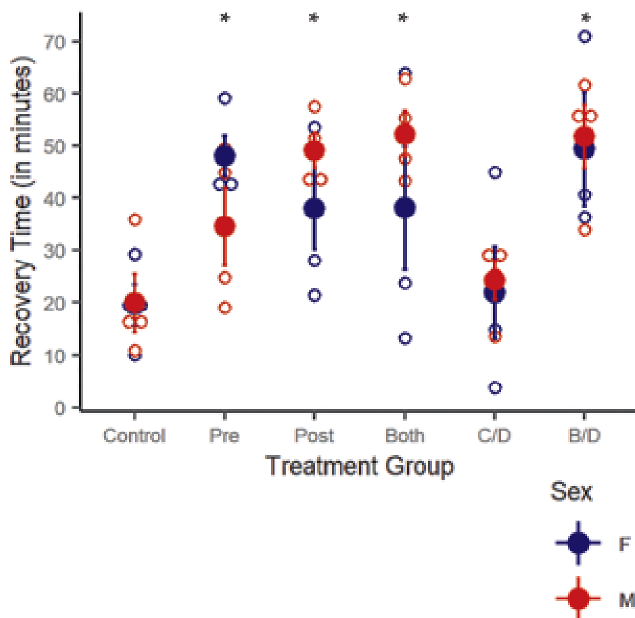

Figure 7. Time (min) to anesthetic recovery of mice in each treatment group. Mice exposed to the incubator required significantly (*, $P < 0.05$) more time to recover from anesthesia than mice recovered on the circulating warm-water blanket in a standard cage. F, female; M, male; Control, no incubator warming and no draping during surgery; Pre, prewarming in the incubator but no draping during surgery; Post, warming after surgery but no draping during surgery; Both, warming before and after surgery but no draping during surgery; C/D, Control/Drape, no incubator warming but draping during surgery; B/D, Both/Drape, warming before and after surgery and draping during surgery.

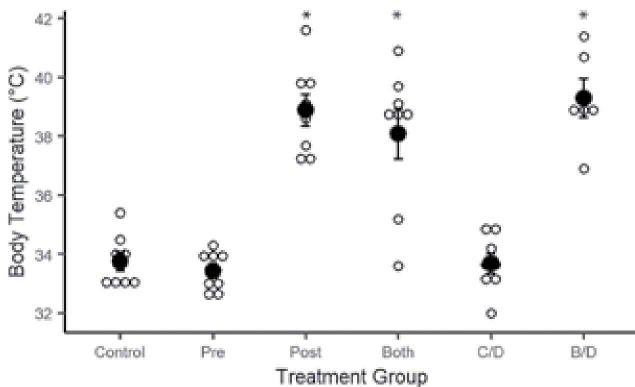

Figure 8. Subcutaneous body temperatures (°C) by treatment group at the time of anesthetic recovery (return of righting reflex). Mice in groups placed in the incubator after surgery—Post, Both, and Both/Drape—had significantly (*, $P < 0.05$) higher body temperatures at the time of anesthetic recovery than mice recovered on the circulating warm-water blanket in a standard cage. Control, no incubator warming and no surgical draping; Pre, prewarming in the incubator but no draping during surgery; Post, warming after surgery but no draping during surgery; Both, warming before and after surgery but no draping during surgery; C/D, Control/Drape, no incubator warming but draping during surgery; B/D, Both/Drape, warming before and after surgery and draping during surgery.

analysis using the Tukey HSD test revealed no statistically significant pairwise contrasts at $\alpha = 0.0167$.

Discussion

Previous work by our research group has focused on refinements for laboratory rodent surgery, particularly on mitigating body heat loss during surgical skin preparation.[9,21,38] Due to their small body-mass–to–surface-area ratio, mice are particularly susceptible to loss of body heat. During surgery in mice, this heat loss can be exacerbated by the application of cool skin preparation agents, inhalation of cold anesthetic gases, and exposure of body cavities to the external environment. In the current study, we compared methods to improve body heat conservation in mice throughout a surgical timeline under injectable anesthesia. Specifically, we investigated the use of a small-animal incubator to provide forced air warming both before and after surgery, with and without the addition of intraoperative draping, in mice undergoing laparotomy. To our knowledge, this report is the first application of a forced-air incubator for presurgical thermal support prior to mouse surgery.

In this study, treatment groups exposed to the incubator for 30 min before surgery (Pre, Both, Both/Drape) had significantly higher subcutaneous temperature readings before surgery than did groups maintained preoperatively in standard cages at room temperature (Control, Post, Control/Drape). This finding was consistent with our hypothesis that using the incubator would provide mice with supplemental heat prior to anesthesia, thereby mitigating heat loss while mice were anesthetized. Similarly, exposure to the incubator after surgery (Post, Both, Both/Drape treatment groups) resulted in significantly higher body temperatures than those of mice recovered in a rodent cage placed half on a warm water blanket set to 38 °C (100.4 °F; Control, Pre, Control/Drape). As previously described in rats, the likely mechanism of body heat conservation through incubator warming is higher skin temperature and, in turn, less transfer of heat from the body core to peripheral tissues.[36] Subcutaneous body temperatures did not differ significantly between treatment groups at 24, 48, and 72 h after surgery, indicating that the warming effects of the incubator were short-term and limited to the perioperative period. This finding is similar to results in rats, in which prewarming provided approximately 15 min of protection from hypothermia under anesthesia, after which time additional warming methods would be required to maintain normothermia.[36] In our current mouse study, the duration of incubator effect may be related to the return of mice to standard rodent housing conditions after anesthetic recovery. Mice are typically housed in facilities with macroenvironmental temperatures of 20 to 26 °C (68 to 78.8 °F), as recommended by the *Guide*,[24] although this temperature range is below their thermoneutral zone, which ranges between 26 to 34 °C (78.8 to 93.2 °F).[19] Under these housing temperatures, mice display a variety of thermoregulatory behaviors, including nest building and huddling.[22] Therefore, such housing environments are likely not conducive to the retention of body heat imparted by exposure to the forced-air incubator described here. Possible mechanisms to help maintain body heat after mice are removed from such a warming device include the use of modified caging systems with built-in heating units or temperature gradients and the provision of additional nesting material.[17,18,20]

During the surgery, all treatment groups showed the same general downward trend in body temperatures over the course of the procedure, as was expected for an anesthetic and surgical event in mice. Differences were detected between treatment groups in the linear (constant amount of change over time), quadratic (acceleration or deceleration in the linear rate of change), and cubic (change in acceleration or deceleration) rates of change. The Pre, Both, Control/Drape and Both/Drape groups lost more heat (linear rate of change) but at a slower rate (quadratic rate of change) than did the Control group. These groups were exposed to the incubator prior to surgery or were

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draped during surgery (or both). These interventions likely account for the differences observed, given that groups that were prewarmed in the incubator had higher subcutaneous temperatures at the start of surgery and then were exposed to room air during surgery, affecting the linear rate of change with initially greater heat loss through radiation type loss when exposed to the cooler room air. The application of an adherent plastic wrap drape provides mice with body temperature insulation during surgery;[11] in the current study, this insulating trait affected the quadratic rate of change, that is, the acceleration of the linear loss of body temperature over time during surgery. Prewarmed mice lost more heat initially during surgery than did mice that were not prewarmed—the loss of the 'excess' heat provided through the prewarming period—but with the provision of a heated surgical bed and a surgical drape in the Both/Drape group, the acceleration of heat loss in prewarmed mice was slower overall. Specifically, the insulating effect of a drape in the Both/Drape group resulted in a slower rate of heat loss as compared with groups that were not draped. The Pre, Both, Control/Drape, and Both/Drape groups experienced a slowing of their acceleration of body temperature change (cubic rate of change) as compared with the Control group, indicating that mice provided incubator warming and surgical draping had a slower acceleration of body temperature loss than mice that did not receive these interventions when undergoing surgery.

From a possible total of 1,440 rectal temperature readings across all treatment groups, 25 were omitted from statistical analysis. Seven readings were not recorded due to equipment malfunction. The remaining 18 were recorded but not included in statistical analysis because they were determined to be outliers. Outlying temperatures were identified during review of raw data when they deviated largely from the immediately flanking readings and therefore did not align with the overall rectal temperature trajectory of the mouse at that stage in the surgical procedure. For example, the outlier removed for the male mouse in the Both group occurred during the skin prep phase of the procedure and was a reading of 33.6 °C (92.5 °F). The reading from this mouse 1 min prior was 37.3 °C (99.1 °F), and the reading 1 min after the aberrant reading was 36.6 °C (97.9 °F), and this mouse's temperature continued to fall steadily for the remainder of the procedure and did not yield multiple consecutive readings of less than 34 °C (93.2 °F) until 17 min later during laparotomy. A likely cause for these outlying temperature readings is error of the rectal probe thermometer. Thermometer errors can occur when the probe is positioned inappropriately (< 2-cm insertion depth) or inadvertently becomes dislodged and contacts nonanimal surfaces in the surgical field.

In all pairwise comparisons but one, mice exposed to the incubator (Pre, Post, Both, and Both/Drape treatment groups) had a significantly longer anesthetic recovery time than did mice that did not receive incubator warming. Placement of an adherent plastic drape during surgery did not have an apparent effect on anesthetic recovery, because recovery time did not differ significantly between the Control and Control/Drape groups. Longer recovery times for mice exposed to the incubator were unexpected; we had hypothesized that mice would recover faster when exposed to the incubator because of improved support of body temperature. Previous studies have reported faster anesthetic recovery times in rats provided with forced-air prewarming,[37] whereas in a study of healthy adult dogs, lower esophageal temperatures at the end of a surgical procedure were associated with longer anesthetic recovery times.[33] Differences in anesthetic recovery time could reflect effects of changes in body temperature on drug metabolism and redistribution. Studies have investigated the effects of hypothermia on drug metabolism, with findings of lower rates of both drug absorption and drug availability; several of these studies in both humans and animals were reviewed by van den Broek et al.[42] Mice in our study that recovered in a room temperature cage in the Control, Pre, and Control/Drape groups had significantly lower temperatures at the time of anesthetic recovery than did mice recovered in the incubator. Of these 3 groups, only mice that were never placed in the incubator—Control and Control/Drape groups—had significantly faster recovery times than mice that recovered in the incubator. Anesthetic recovery times of mice in the Pre group were not significantly different from those of the Post, Both, and Both/Drape groups. This finding indicates that provision of incubator warming at any time point may have increased the availability and absorption of anesthetic agents and, in turn, the time needed for full anesthetic recovery.

The longer anesthetic recovery times for mice recovered in the incubator may represent the influence of slower metabolism as compared with that of mice recovered in a room temperature cage with an underlying heating bed. In one study, C57BL/6NCrl male mice housed for 4 wk at 21 °C (69.8 °F) had energy expenditure that was 3.1 times their basal metabolic rate, whereas those housed as 30 °C (86 °F) had an energy expenditure of only 1.8 times their basal metabolic rate.[13] This pattern is similar to that in humans, who typically can remain in their thermoneutral zone through ambient temperature control and clothing choices and whose typical energy expenditure is 1.6 times their basal metabolic rate.[13] Compared with mice housed at 30 °C, mice housed at 20 to 22 °C (68 to 71.6 °F) demonstrate significantly elevated physiologic parameters, including a doubling of heart rates and oxygen consumption.[16] Exposure to the warmed incubator environment may have a similar effect on temporarily reducing metabolic expenditure and, in turn, slowing the metabolism of anesthetic agents.

Although the incubator-warmed mice in our study did not experience hyperthermia according to subcutaneous and rectal body temperature measurements, the induction of experimental hyperthermia in humans affected blood vessel function and blood flow, resulting in increased vasodilation, cutaneous blood flow, and cardiac output.[43] Visceral blood flow to the gastrointestinal tract and kidneys decreased by 30 to 35% and hepatic blood flow and clearance by 30% when subjects' body temperatures were raised to 41 °C (105.8 °F) for 3 h.[43] A similar decrease in blood flow to the kidneys and liver resulting in decreased drug clearance may have contributed to longer anesthetic recovery times in our Pre, Post, Both, and Both/Drape groups. Ketamine undergoes hepatic biotransformation by action of cytochrome P450 enzymes[10] and is further demethylated to produce the metabolite norketamine, which retains psychoactive properties and anesthetic effects.[10] This enzymatic transformation makes ketamine more water-soluble and thus easier to excrete in urine.[10] Reductions in visceral blood flow may reduce the rate of ketamine hepatic biotransformation and renal excretion in mice exposed to incubator warming. Furthermore, ketamine pharmacokinetics and pharmacodynamics show high interindividual variability, which may have influenced anesthetic recovery times in our current study.

Alternatively, mice that were not exposed to the incubator (Control and Control/Drape groups) may have experienced thermal discomfort, which acted as a noxious stimulus speeding up anesthetic recovery. Postoperative hypothermia can cause thermal discomfort in human patients, resulting in increased shivering, oxygen consumption, and vasoconstriction,[3] whereas prewarming has been associated with increased thermal

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comfort.[2] Additional studies with measurements of metabolic parameters are needed to explore the mechanisms behind the association of perioperative warming with prolonged anesthetic recovery time seen in this study.

Subjective observations made during anesthetic recovery revealed that, although mice exposed to the incubator took longer to recover, their hair coats were less ruffled, and the mice were normotaxic with normal posture (not hunched) once they regained a righting reflex. On recovery, mice exposed to the incubator could be returned to home cages quickly. In contrast, mice recovered in the standard rodent cage remained ataxic and hunched for approximately 15 to 20 min beyond return of the righting reflex and based on investigator experience regarding anesthetic recovery in mice, had to remain on the circulating warm water blanket for this additional period before return to their home cage. Further investigation and objective measurements of recovery differences in mice placed in a forced-air incubator compared with traditional techniques, such as in a cage placed on a warm-water blanket, would be valuable additions to the collective knowledge regarding the effects of forced air warming in rodents.

Postoperative assessments of health and subcutaneous temperature readings 24, 48, and 72 h after surgery showed no difference in median health scores, and all temperature readings were within the normal range for mice. These findings imply no prolonged warming effect of the incubator or draping beyond the perioperative period. In addition, body weight at 24 and 48 h after surgery did not differ significantly among treatment groups. However, at 72 h after surgery, the Control and Both/Drape groups tended toward lower average body weights than those of the Control/Drape group. Across all groups, mice maintained clinically normal health scores, including appropriate body condition scores and hydration status, through 72 h after surgery. We would not anticipate further body weight changes or overall health concerns postoperatively if the experiment had been continued beyond this time point.

Several previous studies have explored the provision of perioperative thermal support, including active warming or forced-air methods, in laboratory animal species. Placement of Sprague–Dawley rats in a recovery cage overlying a 43 °C heating pad for 30 min effectively prevented hypothermia during recovery from a 40-min period of isoflurane anesthesia.[46] However, in another investigation, a forced-air warming system was found to be superior to passive methods of warming (that is, circulating water blanket, infrared heat emitter) for heating the microenvironment of a rodent recovery cage.[35] Prewarming by exposure to an anesthetic induction chamber, warmed to 43 °C (109.4 °F) with forced air, for 20 min before isoflurane anesthesia prevented hypothermic events and increased core body temperatures in Sprague–Dawley rats throughout the subsequent 40-min anesthetic period.[37] Furthermore, placing Sprague–Dawley rats in a forced air heating chamber set to 34.4 ± 1.6 °C (93.9 ± 2.9 °F) for 35 min prior to anesthesia delayed the onset of hypothermia by raising skin temperature, thereby reducing the temperature gradient between core and peripheral body temperatures and decreasing the transfer of heat from the core to the periphery.[36] In C57BL/6J mice anesthetized with ketamine–xylazine for intraperitoneal telemetry implantation, attachment of an air-activated thermal device to the bottom of the recovery cage mitigated body temperature decreases during the first 3 h after surgery.[6] In callimicos (a small NHP species with a large body surface area–to–mass ratio, similar to mice), use of an underbody forced-air warming blanket was superior to passive warming methods for the maintenance of body temperature under isoflurane anesthesia.[7] In contrast to the positive effects of forced-air warming reported in these laboratory species, prewarming for 20 min in an infant incubator set to 43 °C prior to anesthesia in small breed (< 10 kg body weight) pet dogs did not decrease the incidence of perioperative hypothermia compared with dogs that were not prewarmed.[1]

Limitations of this study included use of an institutionally created scoring sheet for animal health scoring. This sheet has not undergone validation testing but instead serves the purpose of a practical tool that can be used by researchers to easily evaluate mice used in a variety of research applications. In this capacity, the sheet has provided consistency for researchers to determine when veterinary intervention or euthanasia may be indicated. In the current study, the scoring sheet allowed consistent clinical assessment across mice, which showed only minimal postoperative changes as compared with baseline scoring. In addition, mice in the prewarming groups had to be removed from the incubator to obtain body weights and administer injections of anesthetic. Mice frequently urinated and defecated in the incubator during the 30 min warming period; therefore, we chose to briefly remove mice from the incubator and weigh them just before anesthetic administration to obtain the most accurate body weights possible. Mice were returned to the incubator after weighing while anesthetic doses were prepared. In total, mice were outside of the incubator for approximately 15 to 20 s for weighing and intraperitoneal injection. Given that mice in prewarming groups had significantly higher subcutaneous body temperatures at the start of surgery than did groups that were not prewarmed, these brief removals from the incubator did not diminish the overall warming effect of the 30-min incubator exposure time. We also noted marked variability in anesthetic recovery times, both within and between treatment groups. Prolonged recovery is common with injectable anesthesia regimens in mice and may be related to physiologic changes such as respiratory depression and hypotension, both of which are documented effects of ketamine–xylazine.[15,32] Additional physiologic monitoring during anesthesia would allow future studies to detect such changes and any correlations with recovery time.

In summary, exposing mice to a novel forced-air animal incubator before and after surgery significantly supported body temperature during the perioperative period. In addition, the provision of incubator warming and surgical draping decreased the rate of body heat loss during surgery and more rapidly stabilized body temperature as compared with mice that did not receive these interventions. These findings indicate that using a small-animal forced-air incubator and surgical draping during rodent surgery are effective at minimizing body heat loss during laparotomy. Furthermore, the use of a surgical drape without incubator warming likewise resulted in mice losing body heat more slowly and experiencing stabilization of body temperature sooner than control mice. Use of the incubator provided these same benefits during surgery, while also resulting in significantly higher body temperatures after animals' placement in the incubator before or after surgery. Surgical draping and a forced-air animal incubator can be used together to maximize body temperature conserving effects, which was the overarching goal of the study. Providing additional thermal support measures to mice during all phases of surgical procedures bolsters ideal metabolic and physiologic functioning, thereby improving animal welfare overall.

Acknowledgments

We thank the laboratory of Dr. Amy Ralston for donating the mice used in this study. We also thank the veterinary team and staff of

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MSU Campus Animal Resources for their assistance with this project. Funding sources included the Michigan State University Office for the Vice President for Research and Innovation, the Boehringer-Ingelheim Veterinary Scholars Program, and fellowship funds from the Michigan State University Graduate School. The authors declare no conflicts of interest in conducting this study and reporting the results. Study data may be accessed by contacting the corresponding author.

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