Journal of the American Association for Laboratory Animal Science
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Vol 00, No 00
Month 2024
Pages 1–9

Evaluation of Thermal Support during Anesthesia Induction on Body Temperature in C57BL/6 and Nude Mice

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Heat supplementation during surgery is a common practice; however, thermal support is not commonly used during anesthesia induction. Mice lose body temperature quickly, and air movement can exacerbate this, potentially putting mice at a thermal deficit before surgery. Whether the method of warming during induction affects overall heat loss during anesthesia is unknown. We hypothesized that the method of heating would affect body temperature (Tb) during anesthesia induction, maintenance, recovery, and once placed back on the rack. Mice (C57BL/6NHsd-6M/6F [C57BL/6]; Hsd:Athymic Nude-Foxn1nu [Nude]; N = 24;12M/12F) were assigned to a treatment in a factorial design: thermal chamber (TC; ambient temperature [Ta] = 28.8 °C); heating pad (HP; induction chamber placed on an electric heating pad;Ta = 28.4 °C); and control (Ctrl; Ta = 21.6 °C). During induction, one mouse at a time was anesthetized with isoflurane over a 3 min period and then maintained under anesthesia for 10 min on a hot water heating pad (33 °C). Then isoflurane was stopped and time to ambulation was recorded. Tb and activity were tracked in the home cage on the rack before and after anesthesia. During induction, Ctrl mice lost significantly more heat (−2.8 °C) than did TC (+0.2 °C) and HP mice (+0.1 °C) but TC and HP were not different. During anesthesia maintenance, Ctrl mice regained 1 °C, but their Tb was still lower than that of the treated groups. Nude mice consistently had a lower Tb than C57BL/6 mice, regardless of treatment or anesthesia phase. C57BL/6 Ctrl mice took longer to ambulate than either HP or TC mice, but the method of heating did not differentially affect Nude mice. In general, C57BL/6 as compared with Nude and females as compared with males were comparatively more active and had higher Tb during certain times of day, regardless of the heating methods. Overall, our findings support the provision of heat during anesthesia induction, regardless of method, to reduce overall Tb loss during a short anesthesia event.

Abbreviations and Acronyms: Ctrl, control; HP, heating pad; LSM, least square mean; RFID, radio-frequency identification; Ta, ambient temperature; Tb, body temperature; TC, thermal chamber

DOI: 10.30802/AALAS-JAALAS-23-000085

Introduction

Anesthesia is required for animals undergoing surgery of any duration. Rodents experience high rates of heat loss during anesthesia due to their small surface area-to-mass ratio.[5,17,23] The normal body temperature of a mouse ranges during rest and activity between 36.5 and 38 °C.[10,17] Small mammals can experience a decrease of 4 to 10 °C in core body temperature in just 15 to 20 min of general anesthesia.[23] For homeotherms, even a 0.5 °C change is considered biologically significant and may affect the outcome and quality of the data being collected in animal research. Hypothermia is known to have negative consequences in humans, including cardiac arrhythmias and ischemia, increased susceptibility to infection, decreased drug metabolism, increased peripheral vascular resistance, prolonged anesthetic recovery time, and increased potential for anesthetic toxicity[7,12,13,23] and can also cause reduced renal blood flow, tremors, and immune suppression in animals.[4,11,25]

Given the importance of maintaining normal body temperature during anesthesia, various thermoregulatory devices have been evaluated with the goal of reducing hypothermia during the maintenance phase of anesthesia, when surgical procedures are typically carried out. Common thermoregulatory devices are heating pads, circulating hot-water blankets, thermogenic gel packs, and reflective foils.[5,21,23] Although heat supplementation is common during the maintenance of anesthesia, the provision of thermal support during anesthesia induction may be deemed unnecessary, especially for short procedures. Thermal support may be viewed as unnecessary during the few minutes of induction because warmth will be provided during the procedure. Recommendations are not available regarding when heat should first be provided to mice undergoing anesthesia. In addition, the air movement needed to introduce the anesthetic gas to the chamber can further exacerbate heat loss, potentially leading to a thermal deficit before starting the procedure. This possibility is likely of even greater concern for nude animals, which lack fur insulation and therefore likely experience a greater degree of heat loss.

The goal of this study was to determine whether different types of heat supplementation during anesthesia induction affect the heat loss over the course of an anesthesia procedure. We hypothesized that the method of heating would affect body temperature during anesthesia induction, maintenance, and recovery (immediately after anesthesia and once the mouse was returned to the rack). We specifically predicted that the

Submitted: 16 Aug 2023. Revision requested: 02 Oct 2023. Accepted: 05 Feb 2024.
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temperatures of mice in a heated ambient chamber would be less affected than those in a chamber heated from below by a thermal pad. We further predicted that mice without heat supplementation during induction would be at a thermal deficit throughout the study. Finally, we predicted that nude mice (without fur) would be affected more than furred mice.

Materials and Methods

Ethical statement. All animal use was approved by Novartis' Institutional Animal Care and Use Committee. Mice were free of a list of common infectious agents at the start of the study as indicated by the vendor (envigo-rodent-health-procedures-screen.pdf). No further health monitoring was conducted for mice on this study.

Study design. A factorial experimental design was used to balance animal numbers across the following factors: 3 heating treatments, 2 strains, and 2 sexes (Figure 1A). A total number of 24 mice were determined a priori via Mead's Resource Equation (23 total DF- 5 model DF = 18 error DF; Mead requires the error term to be between 10 and 20 DF).[8,16] Body temperature was the primary outcome variable (see outcome measures below describing how this was measured). Mice were randomly assigned (random.org; Integer Generator) to one of the following treatments: thermal chamber (ambient temperature [Ta] = 28.8 °C; Compac5; 20.9 × 3.7 × 3.4 in.; VetEquip, Livermore, CA); heating pad (mobile induction chamber placed on top of a heating pad; Ta = 28.4 °C; Kent Scientific RightTemp Jr; 6.0 × 8.0 in., Torrington, CT); or control (mobile induction chamber placed on the table with no heat added; 9.0 × 3.7 × 3.7 in.; Ta = 21.6 °C) (Figure 1B). The thermal chamber and heating pad treatments were preheated for approximately 2.5 h to reach the reported 28 °C temperatures. Each mouse was implanted with an RFID chip (UCT-2112; Unified Information Devices, Lake Villa, IL) that measured its temperature every 20 s during the anesthesia phase of the study. Mice were anesthetized, one at a time, with isoflurane under the assigned treatment (3 min; Induction phase; Figure 1C) and then maintained under anesthesia for 10 min (Maintenance phase) on a circulating hot water pad (T = 33 °C). After 10 min, the anesthesia was turned off, but oxygen was still provided, and the mouse was left on the heating pad to recover. Once the isoflurane was turned off, the time to ambulation was recorded (Recovery phase). Once ambulatory, the mice were returned to cages on the rack and monitored for the next 7 d.

The whole study was carried out over 12 d, beginning with the implantation of the RFID chips (D-4). Baseline data were recorded on D-3 through D-1 in the home cage; the anesthesia event occurred on D0. General activity and body temperature were monitored remotely on the rack (On-rack phase) from RFID implantation until D7, except for the anesthesia phase; during that period, the RFID chip was manually scanned for body temperature.

Experimental procedures. ***Animal care.*** Mice were obtained from Envigo/Harlan (Livermore, CA), were randomly assigned to cages (balanced across experimental factors), and were given 72 h to acclimate to their new environment before RFID chip implantation. A total of 8 cages housed 24 mice, and 3 mice (one assigned to each treatment) were housed together in a single cage. Mice were housed in Allentown Nextgen caging (7.6 × 7.1 × 15.6 in.; 51 air changes per hour; air temperature similar to room temperature reported below) with corncob bedding (1/4 in.; Anderson, Maumee, OH). Two types of mice were studied, C57BL/6NHsd (C57BL/6): 6 males/6 females; and Hsd:Athymic Nude-Foxn1nu (Nude): 6 males/6 females. For enrichment, C57BL/6 mice received one 4 g Bed'r nest (Anderson) and one cotton square (Ancare, Bellmore, NY). Nude mice, due to eye-related issues with the cotton squares, received two 4 g Bed'r nests. Water pouches and food (Teklad Soy protein-free diet 2920) were provided. Lights were on a 14:10 light cycle (on at 0400 and off at 1800). The room was maintained at 20.6 ± 3.2 °C and 48.6 ± 13.7% humidity over the duration of the study.

Implantation of RFID chip (D4). On D-4 (4 d before the study anesthesia event), mice were anesthetized via inhalation of isoflurane at 2.5% in 1 L/min oxygen. The right rear flank of the mouse was shaved and swabbed with 70% ethanol. The chip delivery needle (15.5G) was inserted subcutaneously based on manufacturer recommendations. The RFID chip (UCT-2112; Unified Information Devices) was positioned so that it would not restrict mobility. Skin glue (Vetbond; 3M, Maplewood, MN) was applied to the injection site, and the mouse then recovered from anesthesia. Each RFID chip was used only once.

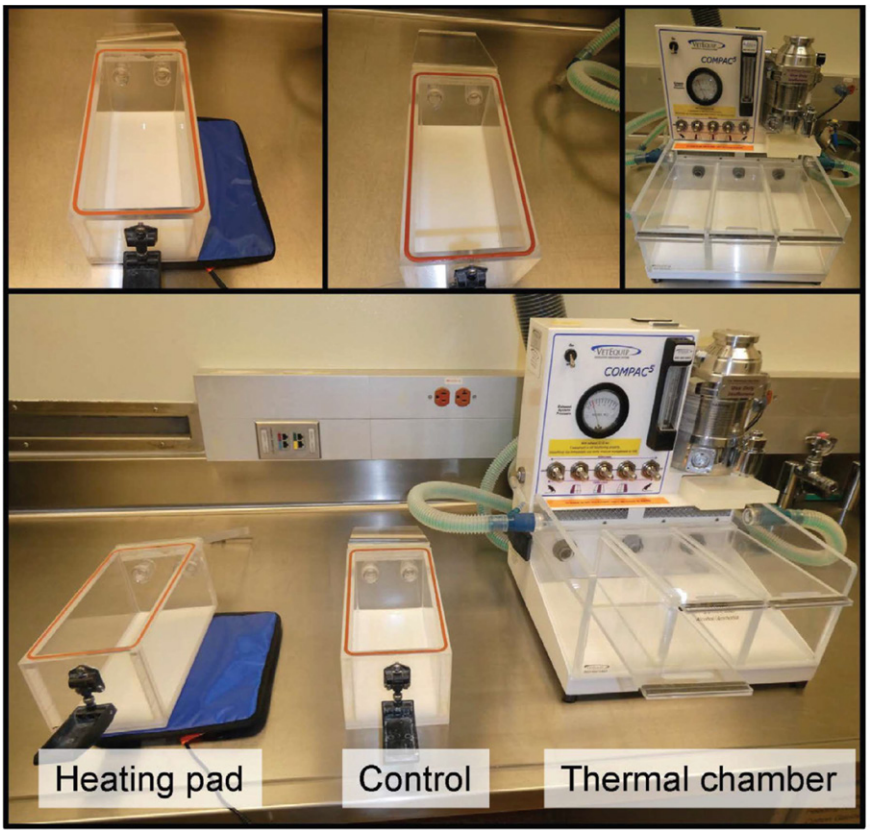
Induction and maintenance phase of anesthesia (D0). Before starting the study, time of day, ambient temperature, and chamber floor surface temperature were recorded. Thermometers (Cole-Parmer Traceable temperature and humidity monitor, EW-90080-06; Vernon Hills, IL) were used for recording ambient temperature and were placed in the chamber until the temperature was stable for at least 1 min. For measuring floor surface temperature, an infrared thermometer (FLIR IR Spot Thermometer TG54; accuracy ± 1.0 °C; Wilsonville, OR) was used to record temperature in all 4 corners and in the center of the chamber. All mice in a single cage were induced one at a time before starting the next cage. Between cages, chambers were verified to have maintained their set temperature. The order of procedures used for mice in a given cage was determined using a random sequence (random.org). The selected mouse was placed inside the anesthesia chamber and the first scan of the RFID chip was taken before starting anesthesia. Anesthesia induction was delivered with 4% isoflurane in combination with oxygen 1 L/min. The mouse in the box was scanned every 20 s for 3 min (Induction phase). The mouse was then moved from the induction box to a circulating hot water pad (Stryker T/pump temp therapy system model TP700C; Portage, MI), where it remained under anesthesia with 2% isoflurane via a nose cone for 10 min (Maintenance phase). During anesthesia maintenance, the mouse was again scanned every 20 s to record temperature. The circulating hot water pad used during anesthesia maintenance was set to the low setting (38 °C). The surface temperature of the hot water pad was confirmed at least once per cage. After 10 min, the anesthesia was turned off while the nose cone remained in place and provided 100% O_2 (1 L/min) during recovery. The time to shuffle or step was recorded using a stopwatch. RFID scanning was not performed during this time. Once the mouse was ambulatory, it was returned to its home cage. After all 3 mice in the cage had been tested, the cage was placed back on the rack.

Outcome measures. Temperature and activity were recorded using the UID Mouse Martix (Unified Information Devices), which is a remote RFID-enabled monitoring system that continuously monitors rodent location, temperature, and activity within the home cage. This system was combined with the Allentown racking system. Activity was measured via a gross movement index that calculates the distance between zone antennae. The activity index provides a gross measurement of distance traveled in inches. Temperature was measured via the temperature programmable microchip (Unified Information Devices UCT-2112; 13-mm-long; 0.102 g in weight; Lake Villa, IL) with a high degree of resolution (± 0.1 °C) and accuracy (± 0.1 °C at 38 °C; measured

A

	Heated chamber	Heated pad	Control
C57BL/6NHsd (C57BL/6)	2 M | 2 F mice	2 M | 2 F mice	2 M | 2 F mice
NudeHsd:Athymic Nude-Foxn1nu (Nude)	2 M | 2 F mice	2 M | 2 F mice	2 M | 2 F mice

B



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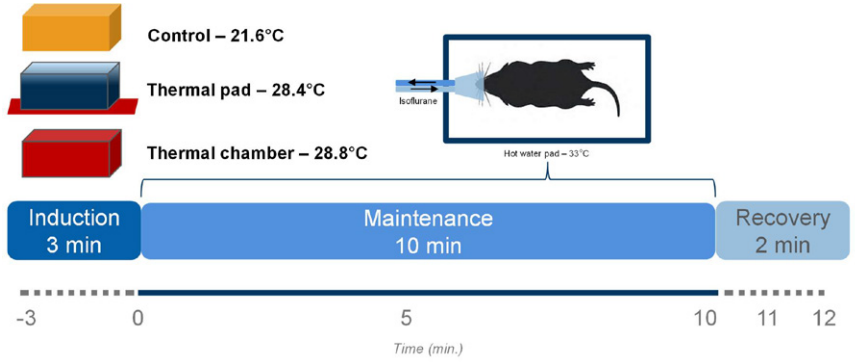

Figure 1. Study design. (A) Experiment treatments studied. (B) Pictures illustrate the 3 thermal treatments used during anesthesia induction. Left: heating pad treatment in which an induction chamber was placed on top of a heating pad; Center: control condition in which heat supplementation was not provided during induction; Right: thermal chamber and anesthesia machine. (C) Timeline for anesthetic event and treatments provided during induction. F, female; M, male; the experimental unit was the mouse. n = 24 mice, determined a priori via Mead's resource equation.

in increments of 0.1 °C). The microchips are calibrated and come with a certificate of analysis. Chips are used only once. All chip specifications can be found at https://www.uidevices.com/product/temperature-programmable-microchip/. The RFID chips were implanted on the flank of the mouse; according to the manufacturer, the chips then provide a 0.3 to 0.5 °C lower temperature than rectal measurements. Because all mice received the same type of chip and location of implant, groups

can be compared, despite the measurements being lower than true core temperature. Thus, when we refer to body temperature (Tb) in this manuscript, we refer to the animal's temperature, read by the RFID chip and not thermal treatment temperatures.

When a cage was on the housing rack, the system passively recorded Tb and activity every 10 min. During anesthesia on D0, Tb from the RFID chip was manually scanned with a wand every 20 s. Mice were returned to the rack once they were fully recovered from anesthesia. Activity and Tb were then recorded for the following week (On-rack activity and Tb). For the on-rack Tb and activity measurements, data were averaged every hour of the day over 3 time intervals (events). Events were created by averaging the data from each hour over a 24-h period. For example, Tb was measured passively by the software every 10 min. Measurements taken between 0100 and 0159 were averaged to create the average Tb for 0100. These 24-hourly averages provided a baseline circadian Tb for each mouse. The same procedure was repeated for the 48 h after anesthesia and for the 144 h thereafter.

Statistical analysis. General linear mixed models in JMP 14 were used to test associations between thermal treatments on Tb and activity. Anesthesia stages (induction, maintenance, and recovery) were analyzed separately. Before testing, all linear model assumptions were confirmed visually including independence of residuals, homogeneity of variance, normality of residuals, and multicollinearity in the data. MouseID, nested within strain, sex, and thermal treatment, was considered random. The fixed independent variables included in the model were strain, sex, thermal treatment, and time point. The following model was initially tested for induction and maintenance phase data:

Dependent variable = MouseID (Strain*Sex*Thermal Treatment) Strain + Sex + Thermal treatment + Time point + Strain*Sex + Strain*Thermal treatment + Sex*Thermal treatment + Thermal treatment*Time point + Strain*Sex*Thermal treatment.

A slightly different model was used for Recovery data. It was not blocked by MouseID, but average temperature from the last 2 min of the maintenance phase was included as a quadratic covariate.

Dependent variable = Strain + Sex + Thermal treatment + Time point + Strain*Sex + Strain*Thermal treatment + Sex*Thermal treatment + Thermal treatment*Time point + 2 min Tb + 2 min Tb*2 min Tb.

On-rack Tb and activity were analyzed similarly to the induction and maintenance phase data. Hour indicates the hour of the day (0 to 23), which provides circadian patterns. Event indicates the average of hourly data used for circadian comparison before and after anesthesia (−48 and 48 h, respectively) and 6 d later (144 h) as follows:

Dependent variable = CageID (Strain*Sex) + Strain + Sex + Thermal treatment + Event + Hour + Strain*Sex + Strain*Thermal treatment + Strain*Event + Strain*Hour + Sex*Thermal treatment + Sex*Event + Sex*Hour + Thermal treatment*Event + Thermal treatment*Hour + Event*Hour + Average Activity.

A sensitivity analysis approach was used for all analyses to determine the final model.[24] If explanatory factors were not significant, they were dropped from the final model. However, terms that were not significant but addressed a specific question (for example, an interaction with treatment) were retained in the model. The Akaike information criterion (AIC) was also used to identify the best model. Significance level was $P < 0.05$. Significant effects were further analyzed post hoc with Tukey-adjusted pairwise comparisons or Bonferroni-corrected test slices. Data are presented as least square mean ± standard error (LSM ± SE).

Full statistical models for each outcome measure are listed in Supplemental Table S1. This provides full transparency of the analysis conducted and what was controlled for in the final model.

Results

Induction phase. A significant thermal treatment by time point interaction affected mouse temperature ($F_{2,174} = 127.4$; $P < 0.0001$; Figure 2A). During the 3 min of induction, nonheated control mice lost more Tb (−2.8 °C) than those that were warmed via either thermal chamber (+0.2 °C) or heated pad (+0.1 °C). The main effect of thermal treatment ($F_{2,11.01} = 10.6$; $P = 0.0027$) indicated that overall, the Tb was not different between the 2 heating methods during induction. Predicted values of Tb at the end of the induction phase (−20 s in Figure 2A or after 3 min) were 37.8, 37.9, and 34.5 °C, respectively, for thermal chamber, heated pad, and control. Nude mice consistently had a lower Tb than C57BL/6 mice ($F_{1,11.01} = 17.0$; $P = 0.0017$; Figure 2B), regardless of heating treatment. LSM (± SE) values were 37.7 °C (0.25) for C57BL/6 mice and 36.2 °C (0.27) for Nude mice. Sex was not

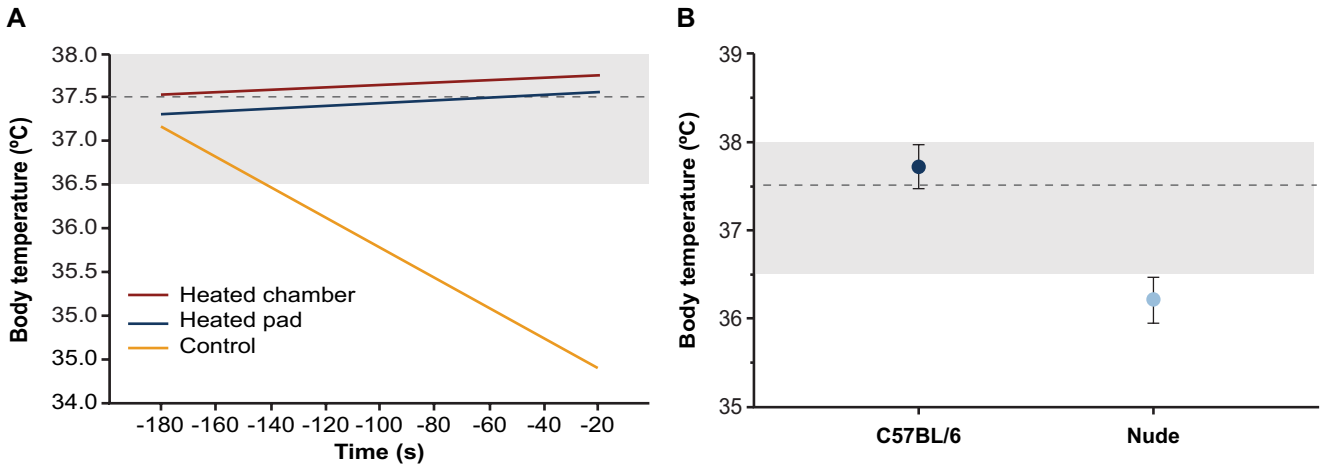

Figure 2. Change in mice body temperature with (A) different heating methods and (B) type of mouse over 3 min of anesthesia induction. The gray overlay on the graph indicates the normal range (36.5 and 38 °C) of mouse body temperature. The dotted line shows the expected body temperature of a normally awake mouse (37.5 °C). Data are presented as the (A) estimated line or (B) least square means ± SE.

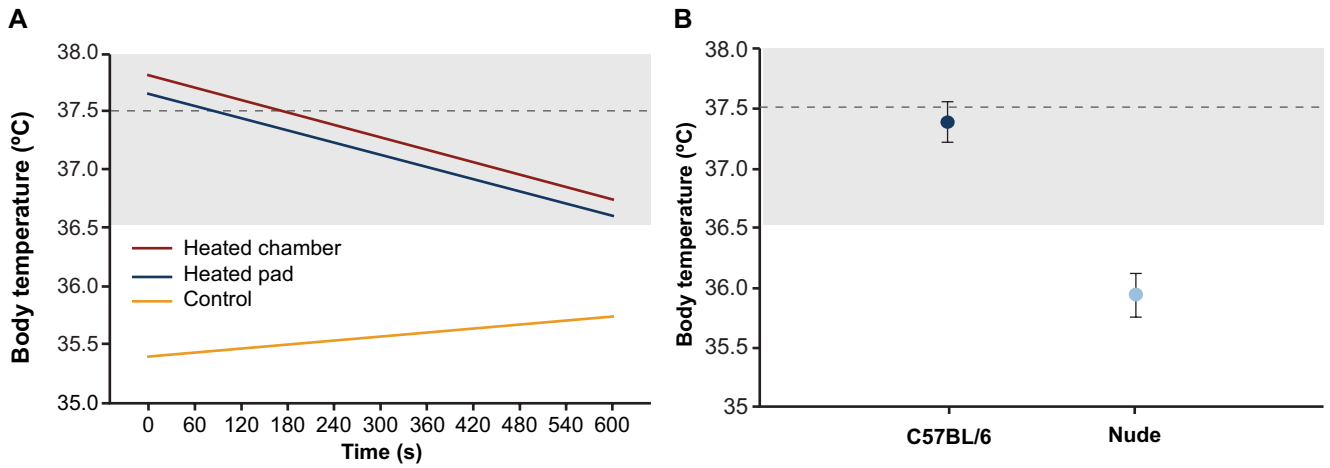

Figure 3. Change in mouse body temperature with (A) different heating methods and (B) type over 10 min of maintenance. The gray overlay on the graph indicates the normal range (36.5 and 38 °C) of mouse body temperature. The dotted line shows the expected body temperature of a normally awake mouse (37.5 °C). Data are presented as the (A) estimated line or (B) least square means ± SE.

associated with difference in Tb during induction ($F_{1,11.02} = 0.05$; $P = 0.8322$) with LSM (± SE) values of 37.0 °C (0.25) and 36.9 °C (0.27), respectively for male and female mice.

Maintenance phase. During the 10 min of anesthesia maintenance, control mice regained approximately +1 °C but their overall Tb was still lower than that of treated mice ($F_{2,682} = 217.9$; $P < 0.0001$; Figure 3A). At the end of the 10 min of anesthesia (600 s in Figure 3A), Tb values predicted by the model were 36.8, 36.6, and 35.7 °C, respectively, for thermal chamber, heated pad, and control. Nude mice (35.9 ± 0.2°C) consistently had a lower Tb than C57BL/6 mice (37.4 ± 0.2 °C), regardless of treatment ($F_{1,11} = 34.2$; $P = 0.0001$; Figure 3B).

The slope of the line (heat loss over the course of the maintenance phase) was different between males and females ($F_{1,682} = 19.01$; $P < 0.001$). Females had a steeper negative slope and lost heat faster than males over the 10 min.

Recovery phase. C57BL/6 mice in the control group took longer to ambulate than did mice warmed in the thermal chamber, but treatment did not affect time to ambulation in the Nude mice ($F_{2,12} = 6.02$; $P = 0.0154$, Figure 4). The LSM values for the heated C57BL/6 mice are below zero due to alterations made by the model when controlling for other variables.

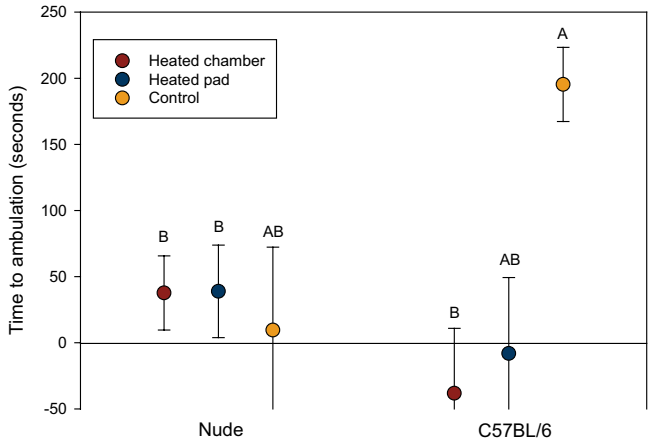

Figure 4. Time(s) to ambulation after the anesthesia had been discontinued (recovery phase). Data are presented as least square means ± SE. The least squares mean values may not reflect raw mean values due to alterations made by the statistical model when controlling for other variables. Different letters above the data points indicate significant post hoc Tukey comparisons.

Sex and thermal treatment showed a significant interaction ($F_{2,12} = 4.68$; $P = 0.0314$) in which control males took significantly longer to ambulate than males kept on the heated pad ($P < 0.05$). Further, a quadratic covariate of temperature (last 2-min average temperature from the maintenance phase) explained a large amount of variability in how quickly mice started to ambulate after anesthesia ($F_{1,12} = 16.08$; $P = 0.0017$).

On-rack activity and temperature. Circadian patterns were evaluated to determine how long changes in activity and Tb lasted after anesthesia. Heating treatments did not generally alter activity with regard to before or after anesthesia (treatment*event; $F_{4,1422} = 0.41$, $P = 0.80$) or general circadian rhythm (treatment * hour; $F_{46,1422} = 1.17$, $P = 0.20$). However, circadian patterns of activity differed at a few points during the 48-h before or after anesthesia (event*hour; $F_{46,1422} = 1.59$, $P = 0.0081$; Figure 5A). Test slices were used to evaluate differences over the 24-h period (α level adjusted to 0.00208 for multiple comparisons) and are indicated with an asterisk in Figure 5. Circadian activity patterns also differed by type of mouse ($F_{23,1422} = 3.61$, $P < 0.0001$; Figure 5B). C57BL/6 mice were more active than Nude mice during the early hours of the day and toward the end of the 24-h period. Females also showed more general activity than did males ($F_{23,1422} = 1.74$, $P = 0.016$; Figure 5C).

In general, Tb and activity showed similar patterns over the course of the 24 h. Treatment did not significantly alter temperature either before or after anesthesia (treatment*event; $F_{4,1421} = 0.77$, $P = 0.54$) or with regard to the general circadian rhythm (treatment*hour; $F_{46,1421} = 0.60$, $P = 0.99$). Further, differences between pre- (−48) and post-anesthesia (48 and 144) time points were also not observed (event*hour; $F_{46,1421} = 1.19$, $P = 0.19$; Figure 5D). C57BL/6 mice, in general, had higher Tb than Nude mice, but this difference depended on the time of day ($F_{23,1421} = 7.3$, $P < 0.0001$ Figure 5E). Similarly, females had higher temperatures than males, but this difference was also specific to the time of day ($F_{23,1421} = 1.8$, $P = 0.02$; Figure 5F).

Discussion

This study demonstrated that the provision of heat during induction, independent of the 2 methods tested, can mitigate heat loss during a short anesthesia event. Specifically, mice that experienced anesthesia induction without heat supplementation were hypothermic throughout anesthesia (induction and maintenance). In contrast to our hypothesis

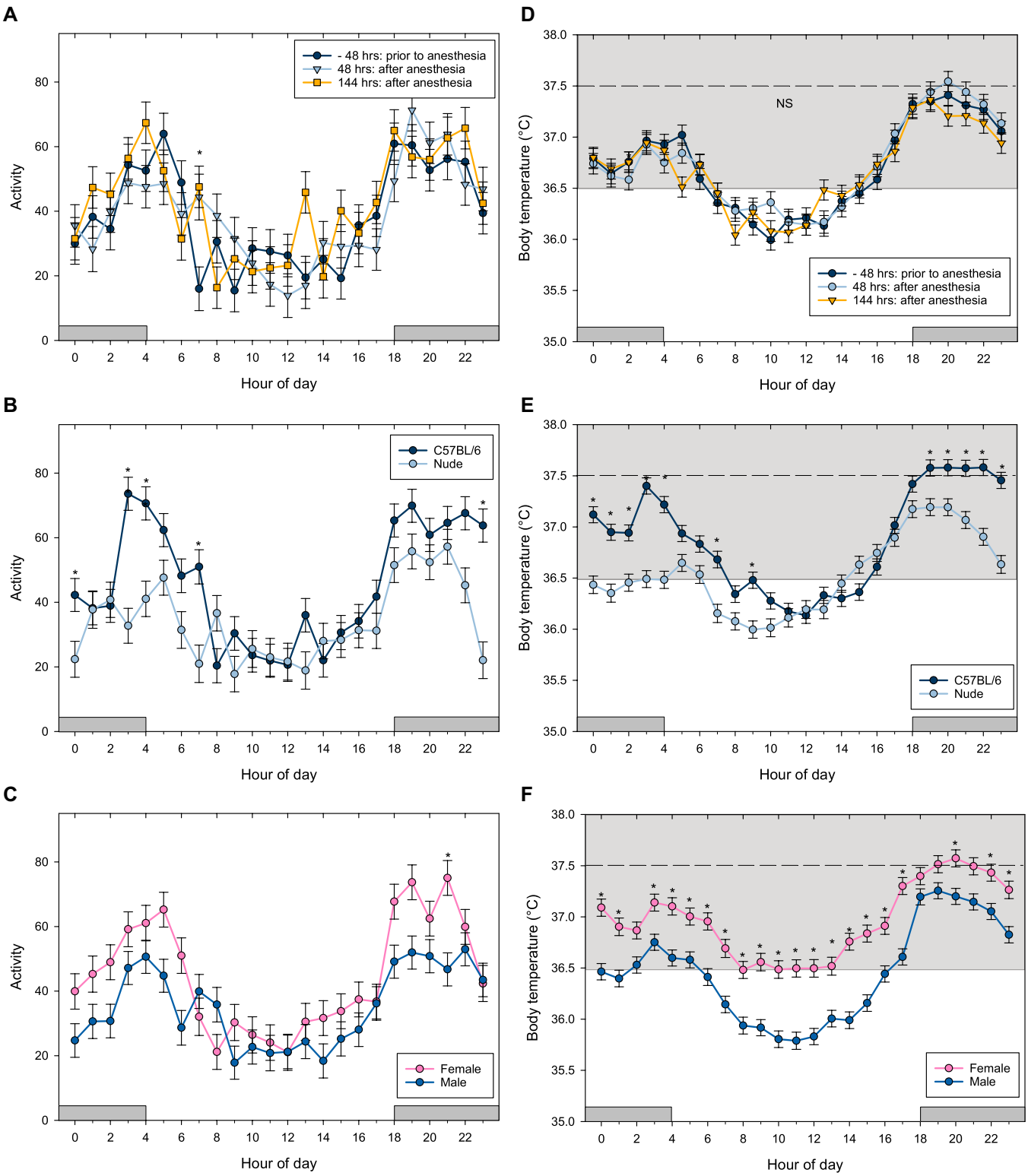

Figure 5. Circadian patterns of temperature and activity measured in the home cage on the rack. (A–C) Circadian activity data and (E–F) temperature data is shown as follows: (A and D) data collected before anesthesia (−48 to −24 h) and after anesthesia (24 to 48 h and 120 to 144 h); (B) strain patterns (all data); and (C) sex-related patterns (all data). Temperature and activity were measured remotely suing the RFID chip. *, Significant post hoc test slice in JMP at that hour. Post hoc comparisons were Bonferroni corrected for the 24 tests. The gray bar at the bottom of the graph indicates when lights were off. (E–F) Data are displayed with the gray overlay on the graph indicating the normal range (36.5 to 38 °C) of mouse body temperature, and the dotted line indicates typical body temperature (37.5 °C). Data are graphed as least square means ± SE. NS indicates that the interaction in the statistical model was not significant.

that Nude mice would be more susceptible to heat loss during induction than C57BL/6 mice, both types of mice appeared to lose heat at a similar rate during induction. However, Nude mice started at a lower body temperature than C57BL/6 mice, which was below what is considered a normal range.[10,17] What is not clear from our references is whether these normal ranges (36.5 to 38 °C) are based on different types of mice or only from furred ones.

In this study, we evaluated 2 different types of induction heating methods to determine if 1) heating during this short interval would affect temperature throughout the anesthesia event and 2) whether specialized equipment was necessary to create this change. Most animal research facilities have mobile induction chambers, but they may not have invested in heated chambers for anesthesia, such as the device used in this study. Therefore, we wanted to see if the same benefits could be achieved by using a more cost-effective option. Our data indicate that mice lose less heat during a 13-min anesthesia event (3-min induction + 10-min maintenance) if induction is conducted using either of the 2 warming methods we tested. When we began, we did not know if the ambient temperature could be increased to a similar level with both the heating pad and the heated chamber. However, with ample time to preheat (approximately 2 h), both methods provided similar ambient temperatures. The heated pad method is easy to implement because these pads are commonly used in the vivarium; however, preheating is essential. Otherwise, the mice are unlikely to benefit from the warmth. The need for preheating may itself be a barrier to appropriate implementation.

Heat loss is common during anesthesia and surgery, and mice are particularly susceptible because of their high surface area-to-mass ratio. In various species including dogs and mice, hypothermia leads to slower recovery from anesthesia.[5,18] Normothermia (or maintaining normal body temperature) is a key aspect of accelerating human recovery from anesthesia[3] and thus is also potentially important to animal welfare after anesthesia. Most of the procedures that require anesthesia are not intended to put the animal at a thermal or energetic disadvantage but instead want the animal to recover quickly from anesthesia. In this study, all mice recovered within 2 min of termination of isoflurane. However, C57BL/6 control mice took longer to ambulate than did heated mice of the same strain. This supports previous findings of a negative correlation between temperature and recovery from anesthesia.[2] However, all of the studies we cited in this manuscript used furred mice (specifically C57BL/6).[2,5,6,14,15,22,23] In our study, Nude mice recovered at a similar rate regardless of heating treatment, even though their core temperature was consistently lower than that of C57BL/6 mice. Furthermore, recovery was described by a quadratic body temperature curve, rather than a straight line, as reported previously.[5] The predicted values of this covariate indicate that mice (either Nude or C57BL/6) with average body temperatures that were below 35.1 °C or above 37.1 °C during the final 2 min of anesthesia required over 100 or more seconds to ambulate. We do not know whether this inconsistency with the literature is due to either a lack of data from Nude mice or an innate difference in thermoregulation and anesthesia recovery of Nude mice.

Small animals, especially prey species, can find human handling aversive, which complicates measurement of Tb because handling can cause stress-induced hyperthermia;[19] therefore, we used a remote method of measuring body temperature that gives a more realistic temperature measurement while maximizing animal welfare. The ability of our approach to measure both activity and temperature is important due to interactions of these 2 measures. We recorded mouse Tb by using an RFID chip that remotely scans the subject at a desired interval. Although we could have used rectal probes during the maintenance portion of the anesthesia event, using this approach during induction and after recovery could have produced questionable data due to handling. New in-cage monitoring technologies are likely to improve data quality while also providing more natural biological rhythms. An added benefit of the RFID system is that 1) it does not require major surgery for device implantation and 2) the size of this device is appropriate for mice, making this technology advantageous for animal welfare.

Previous literature has focused on evaluating heat supplementation during anesthesia maintenance, particularly for longer surgical procedures. One study in rats and mice compared a heating pad, a circulating hot-water blanket, and a control during 60-min of anesthesia. As compared with their own baselines, rats and mice that were on the circulating water blanket respectively showed decreases of 0.11 ± 0.19 °C and 0.46 ± 0.05 °C, respectively, whereas the heating pad was associated with significant increases of 0.96 ± 0.10 °C and 0.94 ± 0.13 °C, respectively. In comparison, body temperatures decreased significantly in rats and mice (4.42 ± 0.60 and 9.90 ± 0.35 °C, respectively) that had no supplemental heat.[23] We found similar results in that the temperatures of the control group fell significantly during the induction phase as compared with the heated groups. However, a distinguishing feature is that we provided heat supplementation during the induction of anesthesia. Proper heating during induction is most important for short-term procedures because the short duration does not allow enough time for conductive heat transfer to warm the animal enough to make a difference in overall heat loss. Furthermore, if a surgery is performed, mice would be likely to lose more heat through the incision and surgical preparation.

Two previous studies tested whether prewarming before induction of anesthesia can help reduce heat loss during the anesthesia and postanesthesia recovery phase. One group[20] prewarmed rats by placing them in a preheated chamber until their core temperature increased by 1% (median 0.4 °C); anesthesia was then induced and the rats were exposed to active warming (using a heating pad) during maintenance phase of anesthesia. The data from this study showed that prewarming of rats was effective in maintaining normothermia during anesthesia until recovery. Another study tested 3 groups of rats, 1) no warming (NW), 2) limited warming (LW; heat pad during anesthesia), and 3) prewarming (PW; warm air exposure before anesthesia, followed by use of a heating pad). In the PW group, core temperature was maintained during anesthesia and recovery. By contrast, the NW group was hypothermic during anesthesia, and LW group showed a decrease in temperature during recovery.[21] Both these studies were similar to ours in that they evaluated the effects of warming prior to anesthesia maintenance, but we noted a few differences. First, the experimental animals used were rats, whereas we used mice. Second, animals were preheated before the induction of anesthesia in contrast to the heat supplementation that we provided during induction. Finally, the duration of the maintenance phase was much longer than that used in our study. Our study provides a practical approach to heat supplementation for nonsurgical procedures in small animals, like mice, that require short anesthesia.

This study compared 2 types of mice, C57BL/6 and Nude. In oncology studies, Nude mice commonly undergo short anesthesia events for tumor cell injections, likely without heat supplementation during induction or the procedure. While our results show that Nude mice generally have a lower body temperature than C57BL/6 mice, they do not appear to experience a higher rate of heat loss (slopes of the heat loss lines are not statistically different between the 2 types of mice). These data indicate that regardless of the type of mouse, providing heat during induction will refine the procedure by reducing overall heat loss and improving animal welfare. However, nude mice may start the procedure at a disadvantage due to their initially lower Tb (mean 35.8 °C at the start of anesthesia induction as

compared with 37.4 °C for C57BL/6) or the overall heat loss between the 2 types of mice might differ if the induction period was longer.

The Nude and C57BL/6 mice received different nesting materials in the home cage. This extrinsic difference could differentially affect these 2 types of mice. Due to concerns about eye lesion development,[1] Nude mice received two 4 g (8 g total) crinkle paper Bed'r Nests while C57BL/6 mice received one 4 g Bed'r Nest and one cotton square (approximately 6.5 g). Previous literature indicates that 6 g of crinkle paper is necessary to make a fully domed nest but does not provide sufficient insulation for a 20 °C ambient temperature.[9] Thus, the 1.5-g difference in bedding might not be biologically significant with regard to heat conservation or thermal comfort between the 2 types of mice. Nest shape was not scored as part of this study but could have indicated the functional significance of the material to heat loss after anesthesia.[10] However, if nesting did have a differential effect, it would have provided more thermal support to the Nude mice, which had lower body Tb than did C57BL/6 mice throughout the study. Nude mice may need more thermal support from nesting material than is provided by a minimum of 8 g, as previously recommended based on a study that used furred mice.[9]

In addition to differences in Tb between C57BL/6 and Nude mice, we also found sex-related differences in Tb. Even though differences between sexes were not a primary question in this study, we knew that the inherent differences in body size between the sexes were likely to affect heat loss.[5,17,23] Furthermore, as regulatory bodies increase their emphasis on sex as a biological variable, we tested for differences related to this intrinsic variable. Females generally had a higher Tb than males, perhaps because they were more active. A study of the effects of nesting material on Tb found a similar relationship.[10] Thus, these differences appear to be consistent across strains and stock.

While this study found some interesting results, the data and conclusions have limitations. First, both heating methods require a considerable time to heat up, which may limit their use during induction. Second, other heating methods (for example, far-infrared heating pads)[17] may be more efficient in promoting the return of mice to a normal Tb. Application of our findings may depend on the heating method used. Third, our measurements of Tb are not equivalent to true core temperature. Although the RFID chips we used were implanted in the same way for all mice and treatments, the values we measured may be lower than true core readings. Finally, we did not conduct surgery; hypothermia may be greater when mice become wet during preparation for surgery and then experience exposure of the body cavity.

In conclusion, providing supplemental heat during anesthesia induction reduces the overall heat loss of mice during a short period of anesthesia (13 min total, including both induction and maintenance). Providing warmth shortened the time to recovery in C57BL/6 mice but not in Nude (nonfurred) mice. Although the rate of heat loss was similar between both types of mice, Nude mice consistently had lower Tb than did C57BL/6 mice. Heating had few effects on Tb or activity once mice were returned to the rack. Overall, our findings support the provision of heat during induction to reduce overall body temperature loss during a short anesthesia event.

Acknowledgments

We thank Sarabjeet Kaur and Ishita Guha Thakurta of Novartis Healthcare, Hyderabad, India for medical writing, funded by Novartis Biomedical Research, in accordance with Good Publication Practice (GPP4) guidelines.

Conflicts of Interest

The author(s) have no conflict(s) of interest to declare.

Funding

The study was sponsored and funded by Novartis. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship of this article, take responsibility for the integrity of the work as a whole, and have given their approval to this version to be published. BNG designed the study, analyzed the data, and edited the manuscript, FS assisted in designing the study and edited the manuscript, CB assisted in designing the study and helped to collect data, IZ ran the study and collected data, and KM helped design the study and edited the manuscript.

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