Use of Air-activated Thermal Devices during Recovery after Surgery in Mice

Corinna N Beale,^{1,*} Michael Y Esmail,¹ Ariel M Aguiar,² Lily Coughlin,³ Anne L Merley,⁴ Tania M Alarcon Falconi,⁵ and Scott E Perkins¹

Laboratory mice (*Mus musculus*) are susceptible to hypothermia, especially during anesthetic events, disease states, and exposure to environmental stressors. Thermal support devices for small mammals are numerous, but often require a power source and may be impractical to use for cages on a rack. Air-activated thermal devices (AATD) are mixtures of chemicals that cause an exothermic reaction. In this study, we examined the environmental effects of AATD on internal cage temperatures without the use of additional equipment as well as the physiologic effects of AATD as postoperative thermal support in mice. For environmental experiments, temperatures measured inside the cage and above the AATD peaked at 35.6 ± 2.5 °C (13.4 °C higher than control cages). We also demonstrated that the amount of heat produced by AATD and its temporal distribution are dependent on cage and rack types. For physiologic experiments, mice were surgically implanted with an intraperitoneal temperature telemetry device in a static cage setting. Recovery times and final body temperature at 5 h postoperatively did not differ significantly between mice with and without AATD. During the first 0 to 3 h after mice returned to their home cages, body temperature dropped markedly in mice without AATD but not in mice with AATD. Based on this result the physiologic results of our study support that AATD can be useful in providing extended thermal support for mice housed in static microisolation cages to help maintain body temperature postsurgically. Environmental results of our studies demonstrated that AATD provide local clinically relevant thermal support for 2.5 to 6 h, depending on cage set-up.

Abbreviation: AATD, air-activated thermal device

DOI: 10.30802/AALAS-JAALAS-17-000077

Providing thermal support to ill, anesthetized, or surgically manipulated animals is an important intervention and is emphasized in the *Guide for the Care and Use of Laboratory Animals*.¹⁹ Due to their high ratio of surface area to body weight,^{16,19} mice and rats are especially susceptible to heat and body-temperature loss. Hypothermia is, therefore, a common side effect in mice and rats after anesthesia,⁶ surgery, infection,^{6,29} lethargy, excessive hair loss (due to shaving, grooming, or alopecia),¹⁵ or becoming wet. Other stressors in the microenvironment can contribute, including decreased ambient temperature, lack of suitable shelter or nest, and increased airflow in IVC. In converse, due to the same large ratio of body surface area to mass, mice rapidly absorb heat, and it is important to ensure that they do not overheat and become injured due to thermal supportive devices.⁴ In addition, surgically manipulated mice are anesthetized, and both injectable and inhalant anesthetics cause core temperature to drop markedly in the absence of thermal support.²⁸ Thus, the *Guide for the Care and Use of Laboratory Animals*¹⁹ emphasizes the use of thermal support during the surgical and postsurgical periods. Given these guidelines, most animal care and use committees require the provision of thermal support during major surgeries. Providing thermal support significantly decreases the drop in temperature that mice otherwise experience owing to surgery.²⁸ Most institutions

mandate that investigators must provide thermal support to mice and rats until they are sternally recumbent. However, making this determination is subjective, and the current experiments were designed to investigate the potential benefit to providing prolonged thermal support to rodents during the first several hours after surgery and their return to housing.

Providing thermal support to a cage of mice in its home rack location is difficult, given that thermal support devices such as warm-water recirculating blankets, microwaveable or thermogenic gel packs, and heating lamps are often too large, require supervision, are costly, or may not provide cooler microclimates to allow mice to escape heat. Given the limitations of current thermoregulatory devices, an effective, reliable, inexpensive method of providing in-cage thermal support is needed. Air-activated thermal devices (AATD), containing exothermic chemical mixtures, have been suggested as a potentially long-lasting thermal support device. Previous studies have explored the use of AATD for supplemental heat for mice.^{7,9,17} Although these studies showed that mice and rats will use the devices, the AATD evaluated necessitated the use of additional equipment or additional space to accommodate a cage with a false floor¹⁷ or para-aramid synthetic fiber pouches.⁹ Instead our institution and neighboring institutions have provided AATD directly onto the outside of a standard IVC or static cage for rodents in need of thermal support.⁷

In the current study, we present environmental and physiologic experiments to demonstrate the effectiveness and efficacy of placing AATD on the outside of IVC and static mouse cages. To determine the efficacy of AATD, we first performed environmental experiments by measuring the temperatures of a cage

Received: 30 Jun 2017. Revision requested: 31 Jul 2017. Accepted: 24 Jan 2018.

¹Division of Laboratory Animal Medicine, Tufts University, Boston, Massachusetts;

²School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania;

³University of Massachusetts–Amherst, Amherst, Massachusetts; ⁴College of Veterinary

Medicine, University of Minnesota, St Paul, Minnesota; and ⁵Department of Civil and Environmental Engineering, Tufts University, Medford, Massachusetts

*Corresponding author. Email: Corinna.Beale@Tufts.edu

with and without a device. For these experiments, we hypothesized that the cage temperature above the area of the AATD would be significantly higher than the same area of other cages without an AATD and other areas of the same cage not near the AATD. We also hypothesized that the temperature effects of the AATD inside an IVC would differ significantly from those inside a static cage set-up. For this reason, we tested both setups separately. After our initial environmental studies confirmed that AATD do raise the temperature inside of the cage in both IVC and static cages, we sought to determine the efficacy of this localized increased temperature area created by the AATD on raising the body temperature of live mice implanted with telemetry devices. For these physiologic experiments, we hypothesized that the use of AATD would increase the mouse's postoperative temperature, decrease the mouse's time to recovery, and increase preference for the warmer areas of the cage.

Materials and Methods

Study materials. Toe warmers (HotHands Toe Warmer, Kobayashi Consumer Products, Dalton, GA) were used for this study. This AATD was chosen because it contains an adhesive side, and the manufacturer states that it provides heat for as long as 8 h. According to the manufacturer, this activated thermal device contains a mixture of iron, water, cellulose, vermiculite, activated carbon, and salt. When exposed to air, the iron oxidizes and produces heat. The salt acts as a catalyst, and the carbon helps to disperse the heat through the warmer. The vermiculite acts as an insulator, which prevents the heat from dissipating too rapidly.^{18,25} The duration of heat provided by AATD is variable and depends on size, material, and manufacturer.^{20,21}

Facility and caging system. All work in the present study was performed at an AAALAC-accredited institution. The Tufts University IACUC approved all animal work, and all rodent health, monitoring, husbandry, and experimental procedures were performed in compliance with the recommendations of the *Guide for the Care and Use of Laboratory Animals*.¹⁹ All rooms for environmental and physiologic testing were maintained within a range of 20 to 22.2 °C and 30% to 70% humidity. The number of room air changes hourly for environmental and physiologic studies was 16 and 6, respectively. Environmental studies were performed in a negative-pressure housing room, and physiologic studies were performed in a positive-pressure housing room. These slight differences between our environmental room conditions and our physiologic room conditions were necessary because the telemetry equipment cannot be removed from its current location; the environmental experiments could not be performed inside the telemetry room due to space and use restrictions.

All cages used in this study were made of polysulfone (7.7 in. × 12.2 in. × 5.9 in.; cage model 9, Thoren Caging Systems, Hazleton, PA). Components of each cage included a wire-bar lid, filled water bottle (16 oz. plastic bottle [Thoren] with stainless steel cap), rodent chow (Teklad 2918 Irradiated Laboratory Animal Diet, Envigo, Indianapolis, IN), filter cage top (Thoren), and 400 cm³ of corncob bedding (catalog no. 7092-7097, Teklad, Madison, WI). All cage components were disinfected prior to use. Each IVC for environmental studies contained a single supply and exhaust hole (0.3 psi supply and 0.25 psi exhaust settings). Rack air supply for IVC consisted of HEPA-filtered (Thoren) room air and a central ventilation system for rack exhaust.

Environmental analysis. To assess the influence of AATD on the microenvironment within the cage, 2 hygrometers (catalog no. 445715, Extech Division, FLIR Commercial Systems, Nashua, NH) were placed inside each cage (Figure 1 B). Each

hygrometer had a detachable wired probe, and a probe was placed in contact with the bedding in zone 1 or zone 3 (Figure 1 B). The 3 zones were established to represent areas of the cage directly above, next to, and opposite the AATD (Figure 2). Because we observed a positive increase in temperature directly above the AATD, and no change in temperature in the area on the opposite side of the cage, further breakdown of cage microclimates did not seem necessary. Hygrometers were angled so that the screen could be read without opening or moving the cage. Hygrometers were unused prior to this study and were previously calibrated by the manufacturer by using an environmental chamber (FLIR Commercial Systems).

Four groups were tested during the environmental studies: IVC cage with AATD (IVC+AATD), control IVC cage without AATD (IVC+Ctrl), static cage with AATD (Static+AATD), and control static cage without AATD (Static+Ctrl; Figure 1 A). In addition, we later tested 2 additional static cages on a plastic surface (compared with the stainless steel surface).

In AATD groups, a single AATD was adhered to the underside of the cage at zone 1 (Figure 1 C). According to the manufacturer's recommendation, the plastic package containing the AATD was torn, the AATD was removed without shaking or rubbing, and the tape was removed to expose the adhesive side. The AATD was then attached to the outside surface of the bottom of the cage at Zone 1. Only one AATD was used per cage, and each AATD was not adjusted or replaced at any time during these experiments.

Temperatures from zones 1 and 3 were measured every 5 min for the first half hour, every 15 min for the second half hour, and then every hour up until 6 h after AATD application. A final temperature reading was taken 24 h after initial activation of the AATD. This study was repeated once daily for 3 d in the same room by using the 4 different cage setups per day. Both IVC and static cages occupied the same area of the room, with the static cages placed on a stainless steel cart (Figure 1 A). An identical hygrometer was placed in the same room on an opposite wall and measured ambient room temperature and humidity. Because nesting material insulates heat,¹² adding a nesting material to the cage might have affected heat dissipation from the AATD to the rest of the cage. To avoid this potential confounding factor, nesting material enrichment was left out of each cage (Figure 1 B).

After a preliminary review of the environmental data, we noted that Static+AATD cages, which were placed on a metal surface, relative lost heat much more rapidly than IVC+AATD cages. To explore this finding further, a fifth group was added. By using an identical setup and recording scheme as described earlier, we placed 4 static cages with AATD (Static+AATD) on a plastic cart (catalog no. 4520-88, Rubbermaid Utility Cart, Ancare, Atlanta, GA) instead of a metal cart.

Animals. We purchased 16 male C57BL/6J mice (age, 10 wk; strain no. 000664; Jax no. 3981839) from Jackson Laboratories (Bar Harbor, ME). Mice were group-housed (4 mice per cage) in static cages as previously described. Because the telemetry room that houses the telemetry devices is on a reverse light cycle, all animals were acclimated to the reverse light cycle room for 7 d prior to the experiment. Mice were provided with a standard free choice rodent chow, acidified tap water, and corncob bedding prior to the start of the experiment.

Physiologic evaluation. Mice were allocated randomly into one of 2 groups, with 8 mice per group. Cages with AATD were equally distributed between the 8 telemetry-pad cages, so that every location in the room was represented by both AATD and control conditions during the 2 d of experiments. Both groups

Figure 1. (A) On each study day, 4 cages were set up in this fashion, with the IVC group placed in the recirculating rack and the static cages placed on a cart. The cart pictured here is stainless steel. (B) Two hygrothermometers are inside each cage. One probe sits directly on the bedding over the area of the AATD (or lack of AATD for control cages), and the other probe sits at the opposite end of the cage. Hygrothermometer screens are angled so that temperature and humidity readings are visible without opening the cage. Arrows indicate the area of the hygrothermometer that detects temperature and humidity. (C) Cage with bedding removed to show the location and placement of an AATD on the bottom of the cage.

underwent surgical implantation of telemetry devices and recovered either with or without an AATD. This study was performed over 2 d by using 16 mice. To conduct the surgery,

Figure 2. Schematic diagram of zones associated with the placement of AATD for environmental and physiologic experiments. Zone 1 is directly over the adhered AATD. Zone 2 is adjacent to the area over the AATD. Zone 3 is in the last third of the cage on the opposite side from the AATD. The × represent the locations of the hygrothermometer probes for environmental studies. The inner red box indicates the AATD under the cage. Front indicates the user-facing side of the cage.

each mouse was weighed and sedated with a combination of ketamine (85 mg/kg) and xylazine (8.5 mg/kg IP). Once mice were unresponsive to a toe pinch, a manual rectal temperature was determined and recorded (TCAT-2DF controller, Physitemp, Clifton, NJ). The controller was calibrated prior to the experiment by using thermoelectric voltages traceable to the National Institute of Standards and Technology and was accurate to an uncertainty of 0.02 °C. Preoperative analgesia was provided by using buprenorphine hydrochloride (0.07 mg/kg SC; Buprenex, Patterson Veterinary Supply, Devens, MA). Ophthalmic ointment was applied to both eyes. Mice were clipped from sternum to pubis. After removal of clipped hair, mice were positioned in dorsal recumbency, and limbs were taped in place. The skin was aseptically prepped for surgery by topically alternating povidone-iodine scrub and 70% isopropyl alcohol swabs 3 times. A circulating water blanket (model TP-500C, T Pump Heat Therapy Unit, Gaymar Industries, Orchard Park, NY) set at 40 °C provided intraoperative thermal support. Surgical scissors were used to make a 1.5-cm ventral midline incision through the skin. The linea alba was identified, and surgical scissors were used to incise the linea and peritoneum to open the abdominal cavity. In each case, a sterilized telemetry device (model TA11ETA-F10, Data Science International, St Paul, MN) was placed inside the peritoneal cavity by using forceps, to ensure that all leads were contained within the peritoneum. The body wall was closed with 5-0 polydioxanone suture (PDS II, Ethicon, Somerville, NJ) in a simple continuous pattern. The skin was closed by using 5-0 nylon suture (Ethilon, Ethicon) in a simple continuous pattern. A set of autoclaved instruments were opened at the beginning of the day and sterilized between surgeries by using a glass-bead sterilizer. A new set of autoclaved instruments was opened for use after 4 animals. Due to the size of the implant compared with the size of the abdominal cavity, possible migration or movement of the telemetry device within the peritoneal cavity was minimal. Consistent positioning of the telemetry device within the abdominal cavity was confirmed at necropsy, during removal of the device at the experimental endpoint. The telemetry devices from day 1 were removed immediately after euthanasia and resterilized for the following day's experiments. Depending on its randomly assigned experimental group, each mouse was singly housed in an IVC with or without an adhered AATD. As previously described for the environmental testing conditions, the AATD was placed on the outside of the cage in zone 1 (Figure 1 C). The cage was placed on top of the corresponding telemetry device-recording pad in a dark, reverse-light-cycle room. Temperature recordings were based on wireless unidirectional radio frequency communication. The first

telemetry temperature recording noted immediately after the mouse was placed onto the telemetry pad after surgery was recorded as that animal's baseline temperature. Subsequent temperatures were collected and recorded every 10 s for 5 h. With the assistance of a red flashlight, mice were observed visually every 15 min, for a total of 19 to 21 total observations per mouse. The activity level and location based on zone (Figure 2) were recorded. A single experimenter was responsible for all observations across this study to allow for consistency. After 5 h of recording, the mice were removed from their cages, and a final rectal temperature was recorded. Rectal temperatures obtained before and after the experiment were compared with the first and last telemetry temperatures measured, to determine consistency between rectal and telemetry recordings. At the conclusion of the experiment, mice were euthanized by isoflurane overdose followed by cervical dislocation and laparotomy (to determine whether the telemetry device had migrated).

Statistical analysis. The effect of AATD on cage temperature was investigated by first conducting an exploratory data analysis for different caging systems. Descriptive statistics for each caging system were stratified by zone and by the presence or absence of an AATD. One-way ANOVA was used to compare between groups (Table 1). Caging systems (IVC, static cage on metal rack, and static cage on plastic rack) were compared by using one-way ANOVA followed by the Tukey posthoc test for multiple comparisons. After statistical analysis confirmed that AATD do raise the temperature in zone 1 in both IVC and static cages, we explored the effects of AATD over time. Repeated-measures ANOVA was conducted to compare the effect of AATD on cage temperature in zone 1, with time as the repeated-measures variable. Because the effect of AATD changes over time, we conducted simple main-effects analysis followed by the Tukey posthoc test for multiple comparisons (Table 2).

By using cumulative observations from all mice at all time points, Student *t* tests were performed to compare the means between AATD and control groups for total time until awake, presurgical temperature, immediate postoperative temperature, and final temperature at 5 h postoperatively (Table 3). Student *t* test analyses were performed in Excel 2010 (Microsoft, Redmond, WA).

The difference in mouse body temperature over time was calculated from the initial temperature of the mouse immediately after being placed on the telemetry pad after surgery. The effect of AATD on the change in postoperative body temperature was explored by using a similar procedure as outlined for the environmental analysis. Descriptive statistics for each caging system were stratified by the presence or absence of an AATD, and means were compared by using one-way ANOVA. The effect of AATD over time was explored by using repeated-measures ANOVA, followed by simple main-effects analysis and the Tukey posthoc test (Table 4). R statistical software (version 3.4.3, R Core Team, 2017)²⁴ was used for all ANOVA statistical analysis.

By using cumulative observations from all mice at all time points during the postoperative period for awake mice, odds ratios and confidence intervals were calculated by dividing the proportion of mice in zone 1 of AATD cages by the proportion of mice in zone 1 of cages without AATD. A *P* value less than 0.05 was considered significant. The odds-ratio test was performed in Microsoft Excel 2010.

Results

Application of an AATD was quick and simple. Any residue remaining on the cage after removal of the AATD was easily cleared by standard cage-washing techniques.

Environmental analysis. Cage temperature in zone 3 remained at an average of approximately 22 °C for all cages tested, regardless of presence or absence of an AATD and the caging system (Figures 3 B and 4 B, Table 1).

Cage temperature in zone 1 was higher for cages that had AATD as compared with the control group (Figure 3 A). IVC cages with AATD had an average temperature of 30.0 ± 5.2 °C as compared with 22.1 ± 0.5 °C for the control cages ($P < 0.001$, Table 1). Static cages with AATD that were on a metal surface had an average temperature of 25.1 ± 1.1 °C compared with 22.4 ± 0.4 °C for the control cages ($P < 0.001$, Table 1). The caging system influenced the amount of heat transferred to the cage by the AATD. The posthoc Tukey test confirmed the AATD IVC cage had on average a higher temperature than cages with AATD and placed on metal ($P < 0.001$). Temperatures did not differ significantly between IVC and static cages on plastic ($P = 0.067$) or between static cages on metal compared with plastic ($P = 0.107$).

A plot of cage temperature over time showed that the effect of AATD changed over time in zone 1 for all caging systems (Figure 4 A). For IVC with AATD, the average temperature increased over time to reach a maximum of 35.6 ± 2.5 °C at 45 min and then decreased steadily to 23.2 ± 0.4 °C at 360 min. For static cages with AATD on a metal surface, the average temperature reached a maximum of 26.1 ± 0.5 °C at 60 min and decreased slightly to 25.0 ± 0.5 °C at 360 min. For static cages with AATD and placed on plastic, the average temperature reached a maximum of 30.2 ± 2.7 °C at 180 min and decreased to 28.4 ± 2.5 °C at 360 min. Comparison of cages with AATD with those in the control group showed that for both IVC and static cages on metal showed that average cage temperature differed significantly ($P < 0.001$) starting at 5 min and continued throughout the monitoring period (Table 2).

Physiologic evaluation. Body temperature was calculated as the difference from time point 0, reflecting the temperature of the mouse immediately after being placed on the telemetry pad postsurgically. There was no statistically significant difference between groups of mice with AATD and without AATD for presurgical and postsurgical body temperatures before placement on an AATD or in a cage without an AATD (Table 3). The average change in postoperative body temperature was higher for mice in cages with AATD as compared with control cages (Figure 5). Average change in body temperature mice was -0.02 ± 1.83 °C in cages with AATD as compared with -3.09 ± 3.50 °C for mice in the control cages ($P < 0.001$). Consistent with results regarding cage temperature, the effect of AATD changed over time (Figure 6). Comparison of mice in cages with and without AATD showed that the difference in average body temperature became statistically significant at 5 min ($P = 0.001$) and persisted until 187 min ($P = 0.049$). Although we observed a difference in cage temperature between AATD and control cages at 240 min, that was not the case for mouse body temperature ($P = 0.104$, Table 4).

Rectal temperatures were recorded immediately before and after mice were placed on telemetry devices. Comparison of these temperatures between methods (rectal compared with telemetry) at these specific time points revealed that the difference in temperature was consistent and linear ($y = 0.6355x + 39.39$; $R^2 = 0.93076$). According to these findings, our telemetry temperature recordings were consistent but not accurate

Table 1. Cage temperatures (°C) by zone and caging system

Zone	Rack	<i>n</i>	AATD	Control	<i>P</i>
1	IVC	56	30.026 ± 5.196	22.090 ± 0.462	<0.001
	Static: metal	42	25.087 ± 1.073	22.374 ± 0.442	<0.001
	Static: plastic	56	27.688 ± 2.797	NA	NA
3	IVC	56	22.587 ± 0.460	22.238 ± 0.394	0.067
	Static: metal	42	22.534 ± 0.568	22.357 ± 0.634	0.3927

NA, not applicable.

Data are given as mean ± 1 SD.

P values were calculated by using one-way ANOVA. A *P* value less than 0.05 was considered significant.

Table 2. AATD and control cage temperature (°C) in zone 1 by caging system at specific times

Rack	Time (min)	AATD	Control	<i>P</i>
IVC	0	22.806 ± 0.530	22.472 ± 0.664	0.365
	5	26.792 ± 2.029	22.347 ± 0.567	<0.001
	240	24.528 ± 1.067	21.667 ± 0.261	<0.001
	300	23.750 ± 0.692	21.736 ± 0.166	<0.001
	360	23.236 ± 0.441	21.694 ± 0.397	<0.001
Static: metal	0	22.500 ± 0.611	22.185 ± 0.263	0.315
	5	23.519 ± 0.251	22.500 ± 0.500	<0.001
	240	25.352 ± 0.516	21.704 ± 0.085	<0.001
	300	25.333 ± 0.494	21.889 ± 0.000	<0.001
	360	25.037 ± 0.516	21.796 ± 0.306	<0.001

Data are given as mean ± 1 SD.

P values were calculated by using Tukey posthoc testing after repeated-measures ANOVA and simple main-effects analysis. A *P* value less than 0.05 was considered significant.

Table 3. Total time until awake and presurgical, immediate postoperative, and final body temperature at 5 h after surgery of mice

	AATD	Control	<i>P</i>
Time until awake (min)	198.9 (31)	204.9 (52.9)	0.80
Presurgical temperature (°C)	38.1 (2.3)	39.0 (1.0)	0.32
Temperature immediately after surgery (°C)	35.1 (1.1)	35.1 (1.7)	0.97
Temperature at 5 h after (°C)	36.8 (1.1)	36.47 (0.8)	0.50

P values were calculated by using Student *t* tests.

compared with the standard of care (measurement of rectal temperature).

Every 15 min, mice were observed visually for ambulation and activity. Time until fully active and ambulatory did not differ significantly between mice with or without AATD (Table 3).

Preference inside the cage. Compared with those housed without AATD, mice fully ambulatory after surgery in cages with AATD spent 48% more time on average in zone 1 (Figure 7) and were 16.3 times more likely to be in zone 1.

Discussion

We hypothesized that an AATD would create a localized area of increased temperature inside the cage. One of the added benefits of using an AATD is that the surface area of the air-activated warmer is smaller than the total surface area of the cage bottom. This feature creates differing microclimates in the cage, allowing the animal to vary its preferred environment as its health condition changes. We were able to prove our hypothesis and thus showed that a temperature increase consistently was

achieved directly above the AATD in both IVC and static microisolation cages. The AATD heat packs were easily applied to the bottom of all cages and proved to be a cost-effective way to provide prolonged thermal support. The advantage of applying AATD directly to cage bottoms is that no additional device or attachment is required, thus making AATD easy to implement in most facilities. The disadvantage of applying AATD directly to cages without using a device is that clinically relevant heating lasted only 2.5 h for IVC and 6 h for static cages, as compared with the 12 h that reportedly was achieved with an external device.¹⁷ Despite the fact that our experiments showed significant elevations in temperature over the AATD, this difference was not clinically significant in terms of practical application beyond 2.5 h for IVC.

According to the product label, the AATD we chose last as long as 8 h; however, this AATD is designed for application in an insulated snow boot with minimal air exposure and circulation. We believe that temperatures peaked much sooner due to an accelerated chemical reaction because we did not use the product in an insulated environment; this lack of insulation

Table 4. Change in postoperative body temperature (°C) of mice in AATD and control cages at specific times.

Time (min)	AATD	Control	P
5	-1.044 (0.385)	-1.725 (0.469)	0.001
187	0.089 (1.400)	-1.897 (2.714)	0.049
188	0.100 (1.396)	-1.844 (2.690)	0.052
240	1.459 (0.756)	0.194 (2.229)	0.104

Data are given as mean (1 SD).

P values were calculated by using Tukey posthoc testing after repeated-measures ANOVA and simple main-effects analysis. A P value less than 0.05 was considered significant.

Figure 3. (A) Boxplots for cage temperature in environmental conditions in zone 1. (B) Boxplots for cage temperature in environmental conditions in zone 3.

also explains why the duration of increased temperature was much shorter. Because IVC are suspended, the airflow around the AATD on the outside of the cage is much higher than for the static cages, where the cages were placed directly onto the plastic or metal cart and the AATD were sandwiched between the cart surface and the cage. The direct contact between the bottom of the AATD and the surface of the cart may have limited air flow and thus delayed the chemical reaction inside the AATD, thereby explaining the prolonged duration of heat provided by the AATD in static caging (Figure 1 A). Putting this information into the context of a lab setting, when housing mice in IVC containing AATD as a supplemental heat source, the AATD would need to be changed after 2.5 h to provide additional supplemental heat. In contrast, when using AATD in a static caging, supplemental heat could be sustained for 6 h. Interestingly, the temperatures inside the cages were slightly higher than room temperature across all experimental conditions. This could potentially be explained by the difference in location of the room temperature hygrometer across the room or possibly that a small amount of heat was created by the hygrometer device itself. While this difference was only a couple degrees it is worth noting.

Figure 4. (A) Cage temperature in environmental conditions over time in zone 1. Individual experiments are shown as dashed lines, and the solid line indicates the mean for all experiments. (B) Cage temperature in environmental conditions over time in zone 3. Individual experiments are shown as dashed lines, and the solid line represents the mean for all experiments.

The environmental studies proved that the temperatures above the AATD were significantly and consistently higher than the control temperatures; however, physiologic studies were required to prove whether the elevation in temperature inside the cage could significantly affect the outcome for postsurgical mice. Our hypothesis that the average time until full recovery would be decreased in groups housed with AATD was disproven, given that there was no significant difference in time until recovery between groups (Table 3). In addition, the final body temperature at 5 h postoperatively did not differ between groups (Table 3). However, body temperature dropped during postoperative recovery after cages were placed back on the housing rack. To correct for the difference between the starting body temperature of individual mice after surgery, we looked at the average change from baseline body temperature (time 0), when mice were first returned to the housing rack after surgery.

Figure 5. Boxplots of change in postoperative body temperature.

Figure 6. Postoperative body temperature change over time compared between mice with and without AATD. ($n = 8$ mice per group). Individual mice are as dashed lines, and the solid line indicates the mean for all mice.

(Figure 6). The temperatures of mice without AATD consistently dropped significantly from baseline within the first 2 h postoperatively. Previous mouse studies did not examine the prolonged postoperative effects of anesthesia and body temperature.^{3,6,16,17,28} Although the drop in body temperature seen was transient, it was consistent and significant in mice without AATD. Several studies in humans have proven that hypothermia secondary to anesthesia can significantly affect physiologic parameters and outcomes, such as causing increased postoperative infections, coagulopathies and arrhythmias which will ultimately confound research results.^{10,22} In addition, hypothermia is known to alter absorption, metabolism, and excretion;²⁷ for pharmacokinetic studies that involve sedation and surgery, this postoperative drop in temperature likely alters the results of drug discovery studies as well. Because this postoperative transient drop in temperature in mice has not been previously documented, further studies examining the specific effects and

Figure 7. Average time mice spent in different cage zones after regaining an ambulatory state.

duration of this phenomenon on parameters such as drug metabolism and other physiologic parameters could be beneficial.

According to our results, AATD appear to have provided significant thermal support to mitigate the postsurgical drop in internal temperature and allowed mice with AATD to maintain increased temperature consistently until the animals had reached temperatures within the physiologic normal range.

Of note, body temperature recordings by telemetry were consistently lower compared with rectal temperature readings. Conversation with the manufacturer of the telemetry devices did not confirm nor deny any dysfunction of the hardware (telemetry device and telemetry recording pad), or software. Because obtaining rectal temperatures is the standard of care, given the difference between these values, we conclude that the telemetry temperatures were inaccurate but nonetheless were consistent; creating a significant and reliable trend between mice that had AATD for thermal support and those that did not.

Of note, one AATD in this experiment appeared to underperform compared with the other 3 in the same group, reaching a maximum temperature of only 26.5 °C, compared with 31.3 °C, 32.6 °C, and 35.8 °C. While this was still within 2 standard deviations of the mean maximum temperature within this group, it is pragmatically lower than the other AATD. The other 9 AATD in our environmental studies appear to have activated consistently based on the limited variation within their group. Further testing is necessary to confirm the exact consistency and failure rate of the AATD which likely varies between brands. After contacting the manufacturer of HotHands Toe Warmer (Kobayashi Consumer Products, LLC, Dalton, GA) the company could not confirm quality control results of their product. One way to confirm product activation would be to perform a manual spot check of the AATD 1 to 2 h post application to confirm if the product has activated appropriately. This would have the added benefit of checking on the animals after return to their housing. If a manual check revealed a lack of perceptible heat from the AATD, the authors would recommend replacing the AATD and performing an additional check an hour later.

Through a combination of physiologic and behavioral changes, conscious healthy mice can maintain their body temperature

across a range of ambient temperatures.³⁰ Mice prefer environmental conditions within their thermoneutral range—29 to 31 °C—where there is no additional energy expenditure beyond their basal metabolic rate.¹⁴ When exposed to temperatures outside of that range, mice attempt to maintain their body temperature through changes in behavior (for example, thermotaxis, fur licking, body extension, huddling, nest building), which are energetically economical. When behavioral changes are insufficient, more energetically expensive autonomic mechanisms (for example, piloerection, blood redistribution, shivering thermogenesis, nonshivering thermogenesis) are engaged.¹³ The effects of anesthetic and surgical manipulations, including high surface-area-to-mass ratio,²³ recumbency and reduced autonomic responses,⁵ vasodilation and heat loss,^{1,2} fluid irrigation, and evaporation, all inhibit the inherent ability to thermoregulate. The fact that in our experiment overall recovery rates did not differ between groups of mice with and without AATD speaks to mice's inherent ability to compensate for hypothermic conditions. The metabolic costs of this process and the potential effects on research involving surgery and anesthesia are worth considering and require additional experimentation to quantify.

Not surprisingly, mice housed with AATD spent more time overall directly above the AATD than in other areas of the cage (Figure 7). This finding indicates that the mice preferred the warmer area of the cage after surgery. Given this preference, providing an AATD could arguably be considered a form of enrichment for improving animal welfare in addition to serving as extended supportive care.

Traditional thermal supportive care in mice includes recirculating warm-water blankets, microwavable or thermogenic gel packs, heating pads, and heating lamps^{6,11,26,28} and is only provided at the start of anesthetic induction and continued until the mouse has regained sternal recumbency. This practice makes practical sense because these devices require direct supervision and significant bench-top space. Our physiologic results confirmed that alterations in temperature continue after the immediate postoperative period of thermal support; therefore providing an external heat source for a prolonged time may benefit surgical recovery and overall success.^{3,8}

The recommended pre- and intraoperative recommended temperature for thermal supportive devices is around 35 °C.⁴ In light of our environmental results, AATD did not achieve 35 °C for a sufficient period of time to condone their use as pre- or intraoperative thermal supportive devices, and we do not recommend this application for AATD. Specific recommendations regarding the postsurgical use of prolonged thermal support (that is, AATD) for mice are currently unavailable in the literature. The results of our physiologic studies suggest that AATD might be used to prolong heat support for operated mice and might thus benefit their overall recovery maintaining core temperature more effectively within 5 h after anesthesia. This advantage might be of particular importance for mice that are anesthetized with injectable anesthesia due to its longer duration of action compared with inhalant anesthesia. Although we did not measure specific physiologic parameters (for example, cortisol levels, hormonal levels, enzymatic activity) during this study, we consider that providing prolonged heat support, such as with AATD, could ultimately lead to more accurate and stronger research results for surgical mouse models.

Acknowledgments

We thank Mark Aronovitz (Director, Mouse Physiology Core, Tufts Medical Center) for his expertise and input on working with telemetry device implants. Thanks also to Larry Breindel (Research Assistant, Tufts University) for his assistance and support with study review.

References

1. Akata T. 2007. General anesthetics and vascular smooth muscle: direct actions of general anesthetics on cellular mechanisms regulating vascular tone. *Anesthesiology* **106**:365–391. <https://doi.org/10.1097/00000542-200702000-00026>.
2. Allweiler SI. 2016. How to improve anesthesia and analgesia in small mammals. *Vet Clin North Am Exot Anim Pract* **19**:361–377. <https://doi.org/10.1016/j.cvex.2016.01.012>.
3. Bagis H, Odaman Mercan H, Dinnyes A. 2004. Exposure to warmer postoperative temperatures reduces hypothermia caused by anaesthesia and significantly increases the implantation rate of transferred embryos in the mouse. *Lab Anim* **38**:50–54. <https://doi.org/10.1258/00236770460734399>.
4. Baran S, Flegel M, Johnson E, Perret-gentil M. [Internet]. 2017. Intraoperative physiological monitoring in rodent surgical research. [Cited 11 June 2018]. Available at: <https://www.alnmag.com/article/2012/10/intraoperative-physiological-monitoring-rodent-surgical-research>
5. Cannon B, Nedergaard J. 2011. Nonshivering thermogenesis and its adequate measurement in metabolic studies. *J Exp Biol* **214**:242–253. <https://doi.org/10.1242/jeb.050989>.
6. Caro AC, Hankenson FC, Marx JO. 2013. Comparison of thermoregulatory devices used during anesthesia of C57BL/6 mice and correlations between body temperature and physiologic parameters. *J Am Assoc Lab Anim Sci* **52**:577–583.
7. Chamberlain P, Kilpatrick J, Scott K, Fox J. 2016. Efficacy of supplemental heat options for supportive care in flooded mouse cages. Abstracts presented at the AALAS National Meeting, Charlotte, North Carolina. 30–3 November 2016. *J Am Assoc Lab Anim Sci* **55**:651–652.
8. Clement JG, Mills P, Brockway B. 1989. Use of telemetry to record body temperature and activity in mice. *J Pharmacol Methods* **21**:129–140. [https://doi.org/10.1016/0160-5402\(89\)90031-4](https://doi.org/10.1016/0160-5402(89)90031-4).
9. Donohue SS. 2010. Handwarmer placed in a para-aramid synthetic fiber pouches for supportive care of mice and juvenile rats. Abstracts presented at the AALAS National Meeting, Atlanta, Georgia. 10–14 October 2010. *J Am Assoc Lab Anim Sci* **49**:683–684.
10. Frank SM, Fleisher LA, Breslow MJ, Higgins MS, Olson KF, Kelly S, Beattie C. 1997. Perioperative maintenance of normothermia reduces the incidence of morbid cardiac events. A randomized clinical trial. *JAMA* **277**:1127–1134. <https://doi.org/10.1001/jama.1997.03540380041029>.
11. Gaertner DJ, Hallman TM, Hankenson FC, Batchelder MA. 2008. Anesthesia and analgesia for laboratory rodents. Chapter 10. p 239–297. In: Fish RE, Brown MJ, Danneman PJ, Karras AZ, editors. *Anesthesia and analgesia for laboratory animals*. London (United Kingdom).
12. Gaskill BN, Gordon CJ, Pajor EA, Lucas JR, Davis JK, Garner JP. 2012. Heat or insulation: Behavioral titration of mouse preference for warmth or access to a nest. *PLoS One* **7**:1–11. <https://doi.org/10.1371/journal.pone.0032799>.
13. Gaskill BN, Gordon CJ, Pajor EA, Lucas JR, Davis JK, Garner JP. 2013. Impact of nesting material on mouse body temperature and physiology. *Physiol Behav* **110–111**:87–95. <https://doi.org/10.1016/j.physbeh.2012.12.018>.
14. Gordon CJ. 1985. Relationship between autonomic and behavioral thermoregulation in the mouse. *Physiol Behav* **34**:687–690. [https://doi.org/10.1016/0031-9384\(85\)90365-8](https://doi.org/10.1016/0031-9384(85)90365-8).
15. Gordon CJ. 2012. Thermal physiology of laboratory mice: defining thermoneutrality. *J Therm Biol* **37**:654–685. <https://doi.org/10.1016/j.jtherbio.2012.08.004>.
16. Gordon CJ. 2017. The mouse thermoregulatory system: its impact on translating biomedical data to humans. *Physiol Behav* **179**:55–66. <https://doi.org/10.1016/j.physbeh.2017.05.026>.
17. Gordon CJ, Puckett ET, Repasky ES, Johnstone AFM. 2017. A device that allows rodents to behaviorally thermoregulate when housed in vivariums. **56**:173–176.
18. Greenberg J. [Internet]. 2014. The chemical reactions that make hand warmers heat up. [Cited 11 June 2018]. Available at: <https://www.wired.com/2014/12/whats-inside-hot-hands/>

19. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
20. **Jackson J.** [Internet]. 2015. What are the best hand warmers? [Cited 11 June 2018]. Available at: <https://www.outsideonline.com/1785906/what-are-best-hand-warmers>
21. **Kobayashi Healthcare Europe.** [Internet]. 2017 Hothands. [Cited 11 June 2018]. Available at: <https://www.kobayashihealthcare.co.uk/HotHands>
22. **Kurz A, Sessler DI, Lenhardt R.** 1996. Perioperative normothermia to reduce the incidence of surgical-wound infection and shorten hospitalization. *N Engl J Med* **334**:1209–1215. <https://doi.org/10.1056/NEJM199605093341901>.
23. **Phillips PK, Heath JE.** 1995. Dependency of surface temperature regulation on body size in terrestrial mammals. *J Therm Biol* **20**:281–289. [https://doi.org/10.1016/0306-4565\(94\)00061-M](https://doi.org/10.1016/0306-4565(94)00061-M).
24. **R Core Team.** [Internet]. 2017. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. [Cited 11 June 2018]. Available at: <https://www.R-project.org/>
25. **Raleigh G, Rivard R, Fabus S.** 2005. Air-activated chemical warming devices: effects of oxygen and pressure. *Undersea Hyperb Med* **32**:445–449.
26. **Rembert MS, Smith JA, Hosgood G.** 2004. A comparison of a forced-air warming system to traditional thermal support for rodent microenvironments. *Lab Anim* **38**:55–63. <https://doi.org/10.1258/00236770460734407>.
27. **Šunjić KM, Webb AC, Šunjić I, Palà Creus M, Folse SL.** 2015. Pharmacokinetic and other considerations for drug therapy during targeted temperature management. *Crit Care Med* **43**:2228–2238. <https://doi.org/10.1097/CCM.0000000000001223>.
28. **Taylor DK.** 2007. Study of 2 devices used to maintain normothermia in rats and mice during general anesthesia. *J Am Assoc Lab Anim Sci* **46**:37–41.
29. **Warn PA, Brampton MW, Sharp A, Morrissey G, Steel N, Denning DW, Priest T.** 2003. Infrared body temperature measurement of mice as an early predictor of death in experimental fungal infections. *Lab Anim* **37**:126–131. <https://doi.org/10.1258/00236770360563769>.
30. **Weihe WH.** 1965. Temperature and humidity climatograms for rats and mice. *Lab Anim Care* **15**:18–28.