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Mice Heat Chamber Calibration

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Abstract

Background: Firm conclusions regarding the etiology of heat exposure responses among animals are difficult to draw due to different experimental designs and methodological confounders, such as environmental chamber set-up and heating rates. The purpose of this investigation was to 1) validate the heat test protocol for mice models via cage location and orientation; and to 2) determine the maximum number of cages that can be used without compromising individual heating rates. Methods: A mice temperature transponder (G2 E-Mitter, Mini Meter, Respironics) was centrally positioned inside each mice cage set in the environmental chamber (Thermo Scientific Forma, Model 3961). Two cage orientations (adjacent, left-to-right and parallel, front-to-back) with 3 set-ups (top shelf, bottom shelf and both shelves) using 2 and 4 cages were examined in triplicate and averaged. Transponders equilibrated at 21.5˚C for 5 min, then exposed to 39.5˚C for a minimum of 60 min. Results: A major finding was that adjacent (L-R) top shelf set-up had the smallest temperature difference throughout the heat test (Δ = 0.43˚C vs. Δ = 2.2˚C) and at minute 60 (Δ = 0.2˚C vs. Δ = 1.8˚C). Both orientations for the bottom shelf set-up had a slower rise in temperature (0.04˚C·min^{-1}) than other set-ups (0.3˚C·min^{-1}). Using both shelves, top shelf cages were consistently warmer than bottom shelf cages (1.0˚C - 3.6˚C) for both orientations. Conclusions: We strongly suggest using an adjacent (L-R) top shelf set-up since it enabled uniform chamber heating rates and standardized heat exposure. Bottom shelf is not recommended for use due to poor heating rate performance. Since an increased number of cages may obstruct heat flow patterns, a one shelf set-up with 2 cages should be used.

Keywords

Heat Stress, Core Temperature, Mice Model

1. Background

An endotherm's temperature tolerance range, or thermoneutral zone, is where the basal rate of heat production is

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in equilibrium with the rate of heat loss to the external environment [1] [2]. Outside this thermoneutral zone an organism will exhibit both physiological and behavioral adjustments (e.g., shivering/sweating, huddling together/decreasing food intake) to maintain normothermic internal temperature. When ambient temperature surpasses the upper critical temperature range, heat production is greater than heat loss, which can lead to hyperthermia and possibly death if not treated [1] [2].

Within the current literature, it has been reported that there is interindividual variability in heat exposure responses among humans and animals that is observed via different core temperature (Tc) patterns [3] [4]. Three main types of Tc patterns have been identified: Type I (uncompensable), linear rise and sustained Tc; Type II (compensable), rapid rise then slower increase in Tc; Type III (triphasic), initial increase in Tc, followed by a plateau then a final increase in Tc [5]. Firm conclusions regarding the etiology of these temperature differences are difficult to draw since each investigation varies in their experimental design and have methodological confounders (e.g., chamber heating rates) that influences thermoregulatory profiles generated throughout heat stress [3].

In our laboratory, we utilize a mice heat chamber protocol modified from Michel *et al*. [4] to induce heat stress in mice. Preliminary data [6] has determined that this protocol allows sufficient heat exposure to test sensitivity to heat stress without any risk to the animal. Specifically, the protocol involves 120 min of heat exposure, with the entire trial lasting approximately 180 min (including warm-up and cool-down); during the first 60 min, the environmental chamber is heating and reaches its preset temperature of 39.5˚C ± 0.2˚C [4]. According to the manufacturer of the Thermo Scientific Forma Environmental Chamber, Model 3961 [5], the heat chamber utilizes a directed airflow design that includes a positive pressure feed plenum and a negative pressure return plenum. This airflow mechanism, which is better suited than a top-to-bottom undirected airflow design, directs air across the surface of each solid shelf, with each shelf receiving a consistent flow of conditioned air for temperature uniformity and recovery [7].

Since heat test protocols for animal models have not been standardized, the specific aims of this investigation were to 1) validate the heat stress protocol for murine models by optimizing mice cage location and orientation within the heat chamber; and to 2) determine the maximum number of cages allowed in the chamber without compromising individual heating rates. Furthermore, our main research question was whether or not cage location and/or orientation are important factors in the amount of heat exposure subjected to the animals. Based on the manufacturer's guidelines [7] we hypothesized that parallel (front-to-back) cage orientation would be more efficient than adjacent (left-to-right) cage orientation in providing uniform heat exposure during heat tests. Additionally, we predicted that cage occupancy (*i.e*., shelf location) within the chamber should not affect overall heating rate due to the chamber's directed airflow design.

2. Methods

All procedures were performed at the Laboratory Animal Medicine, Department of Military and Emergency Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, from June to August 2012, and again in May 2013. No animal or human subjects were used for this investigation.

2.1. Experimental Design

An implantable transponder that measures Tc, (G2 E-Mitter, Mini Meter, Respironics), was placed on a Petri dish, which was centrally positioned inside each mice cage. Cages were then placed within the environmental chamber (Thermo Scientific Forma, Model 3961), which was connected to the LabView data acquisition software. It was understood that temperature recordings would not reach maximum temperature, because the transponder was not placed in an animal that has body fat and constant movement. However, the purpose of this investigation was to observe uniform heating rate, not to acquire maximum temperature.

Equipment set-up took approximately 5 min; immediately following set-up, temperature recording began and continued every minute thereafter (**Figure 1**). The first 5 min of temperature recording was to allow the transponder to equilibrate at room temperature (21.5˚C). At minute 5, the heat was turned on (39.5˚C) and remained on for at least 60 min. Since the investigation was concerned with heating rate, recovery or cool down of the chamber was not included or recorded in the protocol.

Two cage orientations, adjacent (L-R) and parallel (F-B) with 3 set-ups, top shelf, bottom shelf and both shelves, were examined (**Figure 2**); each set-up was performed in triplicate and thermal results were averaged across 3 days. Set-up 1 utilized only the top shelf of the environmental chamber, housing 2 cages; set-up 2 utilized only the bottom shelf, housing 2 cages; set-up 3 utilized top and bottom shelves, housing 4 cages.

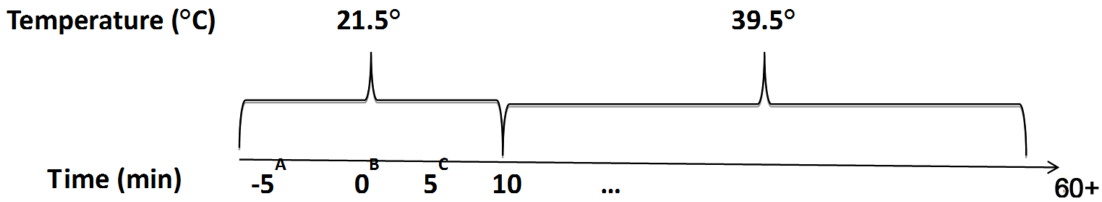


Figure 1. Chamber heating timeline: A: equipment set-up; B: began temperature recording; C: began heating.

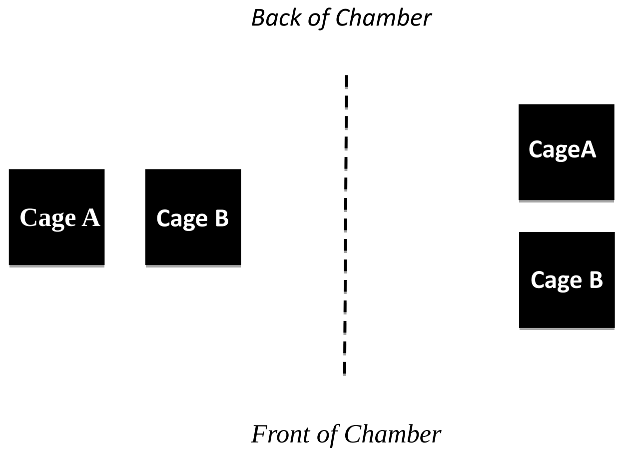


Figure 2. Aerial view of cage orientations: left: adjacent (left-to-right); right: parallel (front-to-back).

2.2. Statistical Analyses

Based on our previous study [6], chamber temperature should reach 39.5˚C ± 0.5˚C within the first 60 min of heating; thus, all data was analyzed up to minute 60. All analyses were performed using IMB SPSS Software (version 20). An analysis of covariance evaluated temperature means across trials controlling for time. A one-way analysis of variance with Tukey's post hoc tests compared temperature means at minute 60. The criterion of significance was set at an alpha level of 0.05.

3. Results

3.1. Adjacent (L-R) vs. Parallel (F-B) Orientation with Top Shelf Set-Up

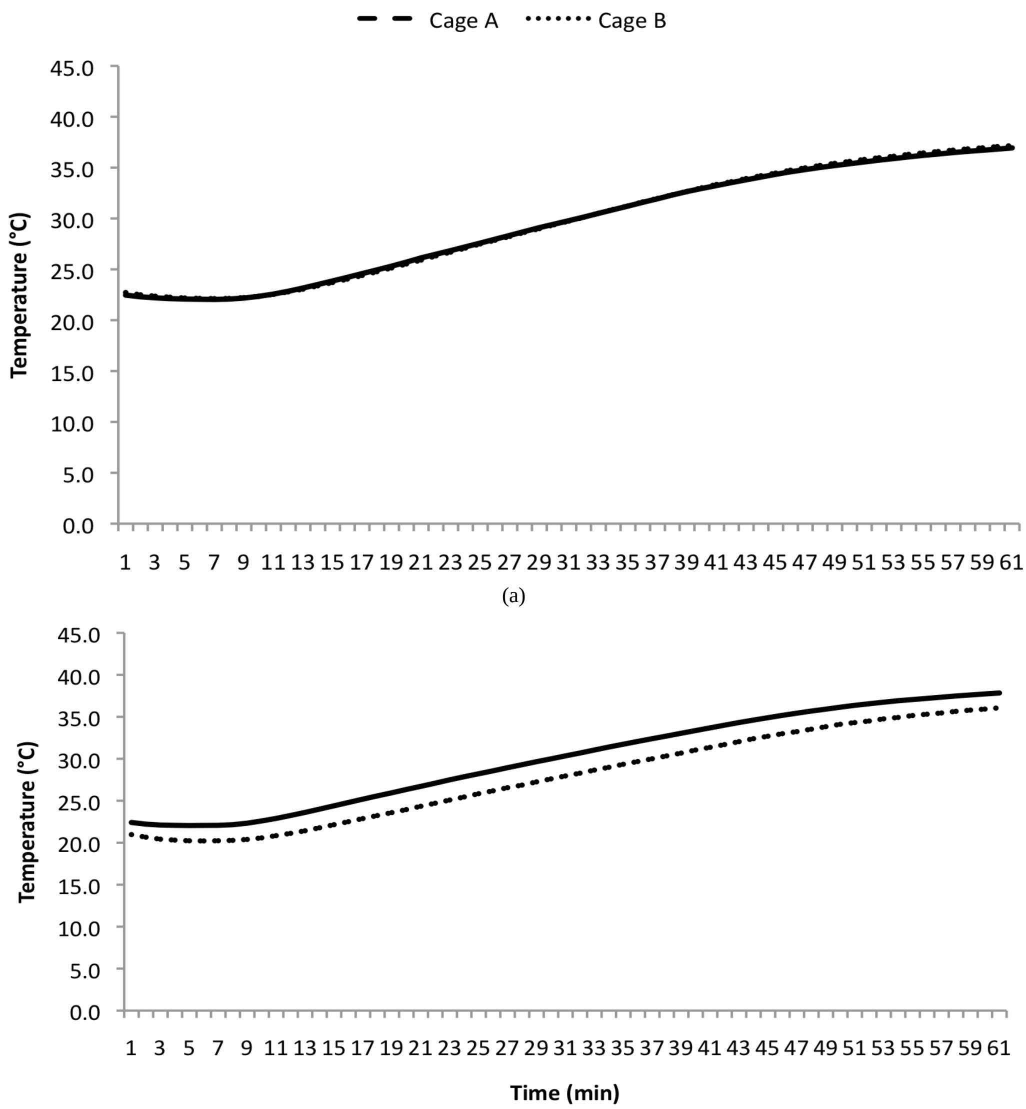
Both adjacent (L-R) and parallel (F-B) orientations with top shelf set-up had an upward linear trend with temperature increasing approximately 0.3˚C every minute. Top shelf adjacent (L-R) cage orientation (**Figure 3(a)**) had the smallest temperature difference across the duration of the heat test ($\Delta = 0.43$˚C, $p > 0.05$) than the parallel (F-B) cage orientation (**Figure 3(b)**; $\Delta = 2.2$˚C, $P = 0.001$). Similarly at minute 60, a smaller temperature difference was observed for the adjacent (L-R) cages ($\Delta = 0.2$˚C, $p > 0.05$) than for the parallel (F-B) cages ($\Delta = 1.8$˚C, $p = 0.001$).

3.2. Adjacent (L-R) vs. Parallel (F-B) Orientation with Bottom Shelf Set-Up

Figure 4 displays the temperature differences for adjacent (L-R) and parallel (F-B) orientations with bottom shelf set-up. Both cage orientations had an upward linear trend with temperatures increasing approximately 0.04˚C every minute. Adjacent (L-R) oriented cages were 2.2˚C ($p = 0.001$) different from one another while parallel (F-B) oriented cages were 1.4˚C ($p = 0.001$) different from each other. Similar temperature differences were observed at minute 60 for both cage orientations.

3.3. Adjacent (L-R) vs. Parallel (F-B) Orientation with Top and Bottom Shelf Set-Up.

Both shelves set-up with adjacent (L-R) and parallel (F-B) orientations are displayed in **Figure 5**. An upward



(b)

Figure 3. Temperature (˚C) recordings of top shelf set-up every minute: (a) adjacent (left-to-right) orientation; (b) parallel (front-to-back) orientation; dashed line: Cage A; dotted line: Cage B.

linear trend between 0.2˚C and 0.3˚C were observed for both cage orientations respectively. Across heating duration, regardless of cage orientation, cages located on the top shelf were warmer than the cages placed on the bottom shelf: 0.3˚C - 1.7˚C, $p = 0.001$ for the adjacent (L-R) orientation; 0.6˚C - 3.6˚C, $p = 0.001$ for the parallel (F-B) orientation. Similar temperature discrepancies were seen at minute 60 for both cage orientations.

4. Discussion

Thermoregulatory responses and adaptations to hot temperatures vary among humans and animals [3] [4]. Three Tc patterns (Type I, uncompensable; Type II, compensable; Type III, triphasic) during heat exposure have been studied and identified [5]. Since investigations utilize different experimental designs each with its own methodological confounders, which can influence thermoregulatory responses and adaptations [3], a standardized heating procedure is warranted. To our knowledge, this is the first investigation to determine and validate uniform heating rate of a mice environmental chamber according to cage orientation (adjacent, left-to-right vs. parallel, front-to-back) and occupancy (*i.e.*, shelf location). Based on this study, we identified three major findings and developed recommendations for future investigations.

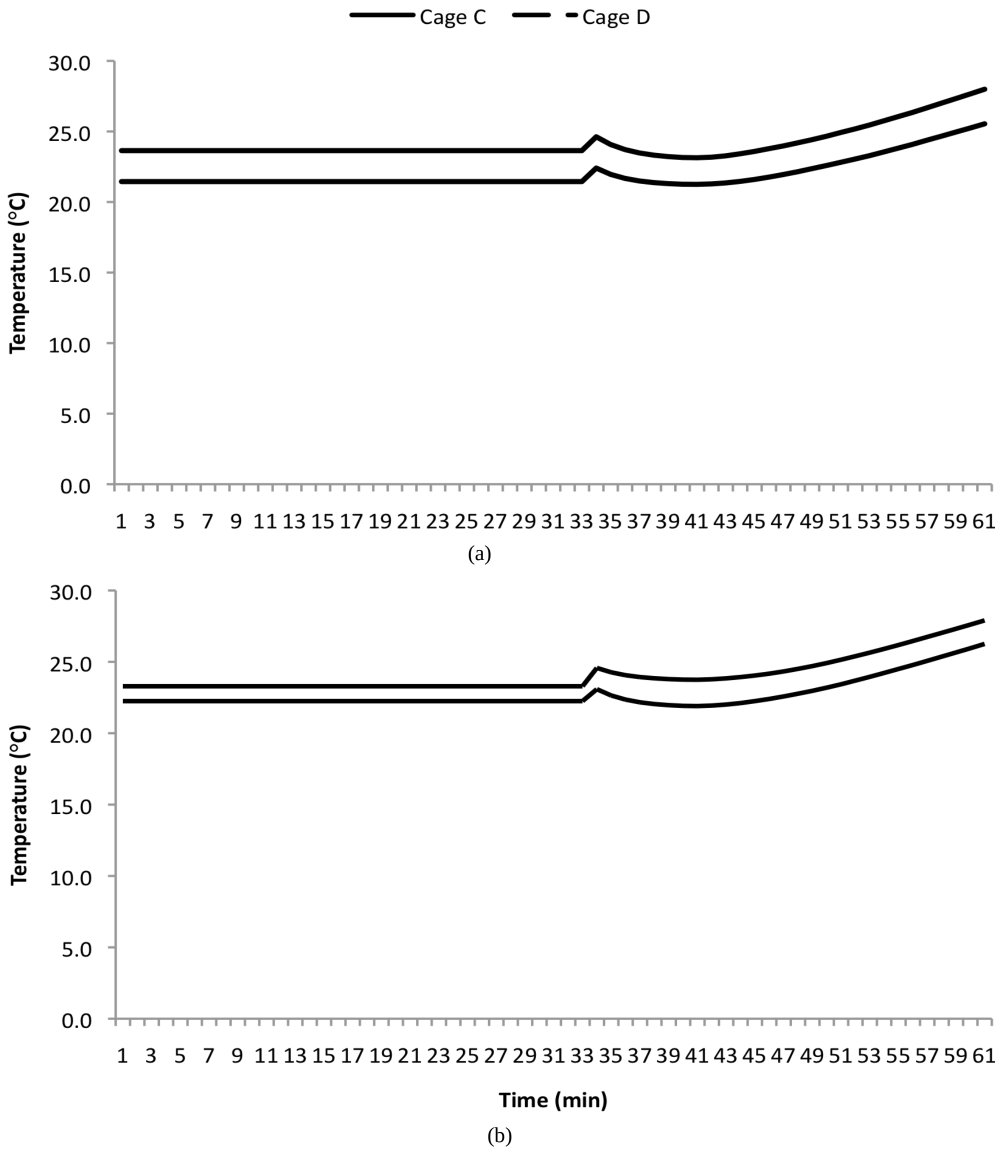
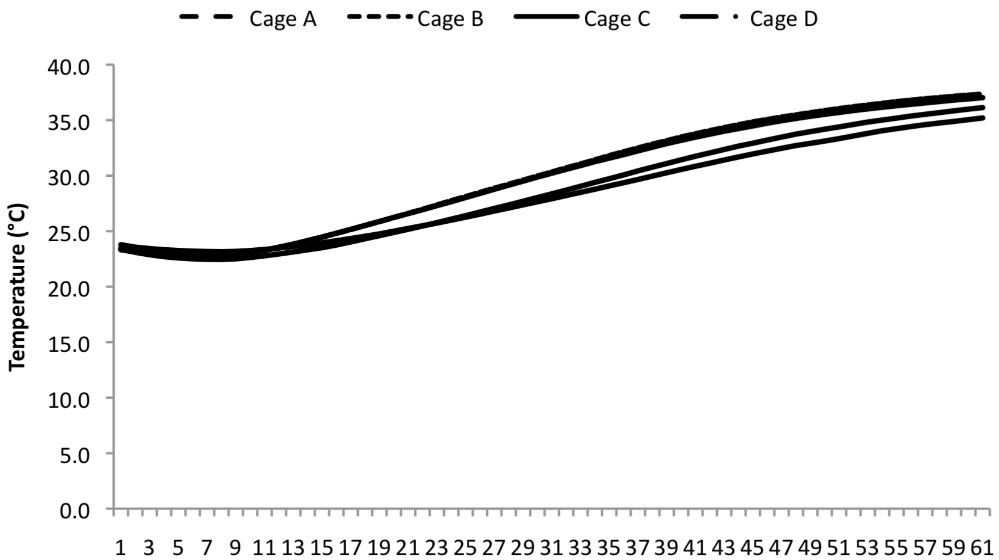


Figure 4. Temperature (˚C) recordings of bottom shelf set-up every minute: (a) adjacent (left-to-right) orientation; (b) parallel (front-to-back) orientation; solid line: Cage C; dash-dotted line: Cage D.

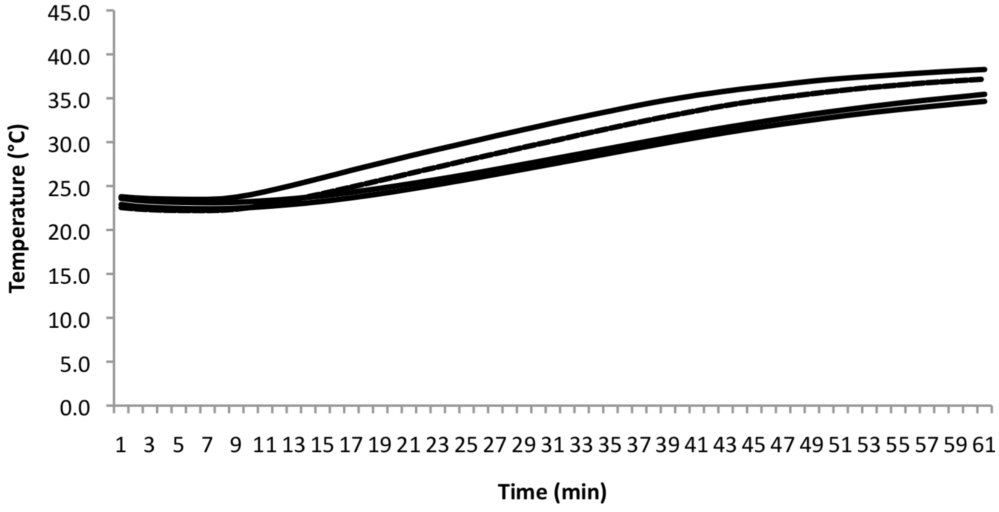
Cages placed on the top shelf in adjacent (L-R) orientation produced the most uniform heating rate ($p > 0.05$) and the smallest temperature differences ($\Delta = 0.43$˚C) compared with other set-ups (bottom shelf, both shelves) and orientations (parallel, front-to-back). This supported the manufacturer's claim of uniformity via the directed horizontal laminar airflow system [7]. Additionally, it supported the experimental protocol of previous studies [4] [6], since we performed the same experimental protocol. Thus, we advise that an adjacent (L-R) cage orientation with top shelf set-up should be utilized to maximize uniform chamber heating rate and subject (mice) heat exposure.

Regardless of orientation, the two-cage set-up (top shelf, bottom shelf) yielded smaller temperature differences in comparison to the four-cage set-up (both shelves). This may be due to an obstruction of heat flow due to the increased number of cages placed in the environmental chamber. Based on this information, a two-cage set-up should take precedence over a four-cage set-up when developing an experimental heating protocol. Additionally, laminar airflow may not be suited for multiple shelves; future studies would be warranted for further justification.

Both adjacent (L-R) and parallel (F-B) orientations with the bottom shelf set-up had slower heating rates



(a)



(b)

Figure 5. Temperature (˚C) recordings of both shelves set-up every minute: (a) adjacent (left-to-right) orientation; (b) parallel (front-to-back) orientation; dashed line: Cage A; dotted line: Cage B; solid line: Cage C; dash-dotted line: Cage D.

(~0.04˚C) compared with the top shelf (~0.3˚C) and both shelves (~0.2˚C - 0.3˚C) set-ups. Ultimately, the bottom shelf alone is not recommended for use in heat tests due to poor heating rate performance perhaps caused by the heat rising paradigm. However, if it must be used, we recommend a parallel (F-B) orientation, since it yielded a smaller temperature difference (1.4˚C, p = 0.001) than the adjacent (L-R) orientation (2.2˚C, p = 0.001).

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