METHODS OF TEMPERATURE & METABOLISM REDUCTION IN RATS AND POSSIBLE INFLUENCE ON HUMAN HEALTH

By

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Abstract

Spaceflight poses unique and significant hazards; the maintenance of human health remains a large part of the National Aeronautic and Space Administration (NASA) strategic goals and work remains to be done if we wish to maintain a long-term presence in space. The effects of ionizing radiation and bone density loss are some of the primary health related problems which need to be addressed. One of the main purposes of this research is to translate aspects of thermoregulation and metabolism reduction in hibernating species to a non-hibernating species in- order to devise alternative methods of preventing DNA damage and loss of bone density in astronauts. A second purpose for this research applies the same approach in emergency medicine, having potential as conjunctive therapy for cardiac arrest victims. Targeted temperature management (TTM; formerly known as therapeutic hypothermia) is the standard of care for these patients and is applied to increase survival rates and reduces neurological deficit.

Stimulating Central Nervous System (CNS) A1 adenosine receptors inhibits shivering and non-shivering thermogenesis, inducing a hibernation-like response in hibernating species. A similar phenomenon occurs when using this technique in non-hibernating species such as rats. The adenosine A1 agonist, N^6-cyclohexyladenosine (CHA) was utilized in all 3 of the experiments to determine how dose, diet, ambient temperature, and finally surface temperature affects the thermoregulatory response in Sprague-dawley rats. In addition to CHA, the partial agonist capadenoson was also tested for thermolytic efficacy (that is, the efficacy to abolish thermogenesis). Surface temperature control using a temperature controlled cage designed and built by myself in combination with IV CHA was found to be most effective in maintaining a target temperature of 32°C without risk of over-cooling. Results from these experiments suggests that the new standard technique in studying TTM using small animals should be similar to what is currently used in clinics; surface temperature modulation.

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Preface

I would like to acknowledge members of the drew lab for support and expertise for assistance in carrying out experiments and obtaining data. I would also like to acknowledge the Alaska space grant program, the Alaska Native Science and Engineering Program and the Alfred P. Sloan foundation for support received during the course of my graduate degree. I also wanted to thank Stormy A. Fields for her patience in my completion of graduate work when at times, a real job would have been nice!

General introduction

Spaceflight poses unique and significant hazards; the maintenance of human health remains a large part of NASA's strategic goals and work remains to be done if we wish to maintain a long-term presence in space. The effects of ionizing radiation and bone density loss are some of the primary health related problems which need to be addressed (Institute of Medicine (US) Committee on Creating a Vision for Space Medicine During Travel Beyond Earth Orbit; Ball JR, 2001). Cardiac arrest is also a significant and growing health problem accounting for up to 20% of all deaths worldwide (Hayashi et al., 2015). Targeted temperature management (TTM; formerly known as therapeutic hypothermia) is the standard of care for out of hospital cardiac arrest patients and reduces mortality and neurological deficits (Malhotra et al., 2013; Vargas et al., 2015). Hypothermia may also decrease detrimental effects of ionizing radiation (Baird et al., 2011) and improves survival rates of hibernating ground squirrels during whole-body radiation exposure (Musacchia and Barr, 1968). Cooling core body temperature, however, is complicated by the cold-defense response such as shivering (Badjatia et al., 2008). One of the main purposes of this research is to translate aspects of thermoregulation and metabolism reduction in hibernating species to a non-hibernating species in- order to devise alternative methods of preventing DNA damage and loss of bone density in astronauts. A second purpose for this research is to develop a method to inhibit shivering during whole body cooling in emergency medicine.

Chapter 1 of this thesis studies N^6-cyclohexyladenosine (CHA) in-order to study the thermolytic and metabolic effects of A1 adenosine receptor agonists on rats. In addition to the full agonist CHA, the partial agonist capadenoson was also studied. These experiments were done at fixed ambient temperatures to determine whether these agonists have potential to lower body temperature for use in emergency medicine and possibly during long term human spaceflight to reduce the effects of ionizing radiation and bone density loss. Results showed that CHA is so effective that it frequently caused over-

cooling of the animals. The project then evolved to a third set of experiments using both IV administration of CHA and using a cage with the capability of real-time temperature adjustment.

Chapter 2 describes how I designed and built the dynamic temperature controlled cage such that the animals would not over-cool when drugs such as CHA are applied to inhibit shivering and other forms of thermogenesi. The reasoning for this was to allow for more consistent and/or accurate biological side effects of this reduced thermogenesis and implement the three R's known throughout the IACUC community: Replacement, Refinement, and reduction animal experiments with the ultimate-goal of minimizing pain and distress. In the case of this new apparatus, now called the "dial-a-temp" cage, it uses all three of these IACUC axioms. The cage operation is based on dynamic temperature control over the cage surface and effectively duplicates what emergency room personnel use to treat cardiac arrest victims. The dial-a-temp replaces old techniques such as spraying animals with water-alcohol mixtures in-order to utilize evaporative cooling (Zhao et al., 2005; Wang et al., 2012; Liu et al., 2013; Bazley et al., 2014). It is a refinement of technique that ultimately reduces the total amount of animals used for cooling experiments by decreasing error through more precise control of core body temperature. As our methods evolved throughout these experiments in the hunt for more accurate temperature control, I anticipate our refined approach will be used in future studies of thermolytics, whether they are adenosine based or not. Appendix B provides design detail and instructions to construct a dial-a-temp.

Chapter 1: Optimization of thermolytic response to A_1 adenosine receptor agonists in rats[1]

1.1 Abstract

Cardiac arrest is a leading cause of death in the United States and currently, therapeutic hypothermia, now called targeted temperature management (TTM), is the only recent treatment modality proven to increase survival rates and reduce morbidity for this condition. Shivering and subsequent metabolic stress, however, limits application and benefit of TTM. Stimulating central nervous system A_1 adenosine receptors inhibits shivering and non-shivering thermogenesis in rats and induces a hibernation-like response in hibernating species. Here, we investigated the pharmacodynamics of two A_1AR agonists in development as anti-shivering agents. To optimize body temperature (T_b) control we evaluated the influence of every-other-day feeding, dose, drug and ambient temperature (T_a) on the T_b lowering effects of N^6-cyclohexyladenosine (CHA) and the partial A_1AR agonist capadenoson in rats. The highest dose of CHA (1.0 mg/kg, IP) caused all ad libitum fed animals tested to reach our target T_b of 32°C, but responses varied and some rats over-cooled to a T_b as low as 21°C at 17.0°C T_a. Dietary restriction normalized the response to CHA. The partial agonist capadenoson (1.0 or 2.0 mg/kg, IP) produced a more consistent response but the highest dose decreased T_b by only 1.6°C.

In order to prevent overcooling with CHA as observed in the previous two experiments, I hypothesized that in-floor cooling and warming could obtain our target temperature faster as well as prevent overcooling. I designed and built a dynamic surface temperature controlled cage floor that utilizes aluminum construction in order to maximize heat transfer and provide an easy to clean surface. After testing, we used this approach to study continuous IV administration in combination with dynamic

surface temperature control. Results show that after CHA administration control of surface temperature maintains desired target T_b better than dose or ambient temperature

1.2 Introduction

Hypothermia is defined as a body temperature colder than 35°C and is a well-known cause of death in cold climates. Despite this, the American Heart Association (AHA, 2015; Callaway et al., 2015a) guidelines for CPR & Emergency Cardiovascular Care strongly recommends induced hypothermia via targeted temperature management (TTM) for treating out-of-hospital cardiac arrest (OHCA), and neonatal resuscitation. Clinical trials for TTM in stroke, the leading cause of adult disability (Mozaffarian et al., 2015) are ongoing (Lyden et al., 2016). While cooling is neuroprotective, clinical application is complicated by side-effects such as shivering.

Paralytics suppress shivering and are used commonly with TTM in comatose patients after cardiac arrest (Bernard et al., 2002). With regard to cooling conscious stroke patients, meperidine (IV) in combination with buspirone (oral) is currently the treatment of choice to suppress shivering. Synergy between these two drugs decreases shivering threshold to a core body temperature (T_b) of 33.5°C with minimal risk of respiratory depression,(Logan et al., 2011) (Mokhtarani et al., 2001; Sessler, 2009) however, a shivering threshold of 33.5°C is not sufficient for optimal control of shivering at colder T_b. The metabolic stress of shivering limits maximum therapeutic benefit of cooling. A recent study(Nielsen et al., 2013) showed no difference in outcome in patients cooled to 33°C vs 36°C and questioned the utility of cooling to 33°C. Importantly, this study reported shivering at 33°C _and_ 36°C but no differences in adverse effects were seen at these temperatures. Other studies confirm shivering at 36°C (Callaway et al., 2015b).

By examining strategies in species which routinely lower T_b, such as hibernators, we sought a safer, alternative method of inducing TTM without harmful side effects such as shivering. In Arctic Ground

Squirrels, stimulation of A_1 adenosine receptors centrally (ICV) or peripherally (IP) using

N^6cyclohexyladenosine (CHA) decreases oxygen consumption ($\dot{V}O_2$) and leads to a subsequent decrease

in T_b in a manner that resembles spontaneous onset of hibernation (Jinka et al., 2011). However, for

unknown reasons, the drug is effective only in the hibernation season. Like the hibernation season in

AGS, dietary restriction (DR) in rats sensitizes animals to the temperature lowering effects of CHA when

compared to their ad libitum (AL) fed counterparts (Jinka et al., 2010). Although CHA effectively lowers

T_b in DR rats, precise control of target temperature has not been achieved in AL rats; and DR is not a

viable option for human emergency medicine. Currently, it is not known how dose and environmental

temperature influences final body temperature in AL rats when given CHA, an A_1 selective full agonist

(van der Wenden et al., 1995) or capadenoson, an A_1 selective partial agonist (Albrecht-Kupper et al.,

2012). The objective of this study was to characterize how dose of CHA, the partial A_1AR agonist

capadenoson, and environmental temperature influence T_b in freely fed rats for the purpose of precise

control of T_b between 32-36°C. We measure the rate of oxygen consumption as an indicator of

thermogenesis, define individual variability in response to capadenoson and to CHA at a dose higher

than tested previously and show that ambient temperature alone is not sufficient to control the depth

of cooling. We report that dynamic control of surface temperature in rats, designed to mimic conductive

cooling used clinically is the most effective means to regulate T_b after CHA.

1.3 Methods

Animals

Experiments were done in accordance with the Guide for the Care and Use of Laboratory animals, 8th

edition (National Research Council, National Academies Press, 2010) and protocols were approved by

University of Alaska Fairbanks Institutional Animal Care and Use Committee. Male Sprague-Dawley rats

(approximately 90 days old) were obtained from Simonson Laboratories (Gilroy, CA) (experiment B) or

from a University of Alaska Fairbanks colony derived from Simonson Laboratories (experiment A and C). All animals were housed in pairs at 21.5-23.0°C on a 12L:12D photoperiod. A summary of experiments and number of animals used can be seen (Table 1).

Experiment A: Dietary restriction & ad libitum, 0.5 mg/kg CHA, IP

Prior research had shown that DR increases sensitivity to the T_b lowering effects of 0.5 mg/kg CHA, but $\dot{V}O_2$ was not measured as an indication of thermogenesis. Here we asked if CHA suppresses $\dot{V}O_2$ prior to the decrease in T_b, consistent with suppression of thermogenesis, and test the influence of 36 days of every-other-day feeding on the thermolytic response to CHA.

Temperature data loggers (iButton; Maxim Integrated, Sunnyvale, CA) were coated with wax and surgically implanted into the abdominal cavity and programmed to record temperature every 10 minutes. After a 10-14 day post-operative recovery period rats were either fed every other day (DR) or AL up to 40 days. Feeding or food removal was done at 10-11AM every day. Body weights were measured every four days. Between 36-40 days after starting the DR protocol animals were moved to a clean cage and housed individually at an ambient temperature of 16.2 ± 0.5°C (mean ± SD) for 24h prior to treatment. Rats were moved to a metabolic chamber for 3 h prior to treatment with CHA (0.5 mg/kg, IP) or Vehicle (1.0ml/kg, IP) and remained in the metabolic chamber for 2h post-injection. $\dot{V}O_2$ was measured by open flow respirometry as detailed below.

Experiment B: Ad lib feeding, 1.0 mg/kg CHA, 1.0 and 2.0 mg/kg capadenoson

We next investigated the effects of 1.0 mg/kg CHA and 1.0 and 2.0 mg/kg of the partial A_1AR agonist capadenoson in AL fed rats. Rats were instrumented with iButton data loggers as described for Experiment A. All animals, housed in pairs, were placed at an ambient temperature of 17.0±0.5°C (mean ± SD) 24h before injections and remained at this ambient temperature until 24h after injection. Each of

the 5 pairs of animals received a different treatment per week based on a balanced cross-over design (Table A1). All treatments were given via IP injections and consisted of CHA (1.0 mg/kg), CHA Vehicle (1.0 mL/kg), capadenoson (1.0 and 2.0 mg/kg), and capadenoson vehicle (1.0 mL/kg). Heart rate was monitored with a digital stethoscope (Littmann© Model 4000 electronic stethoscope; 3M, St. Paul, MN).

Experiment C: Ad lib feeding, IV CHA @ 0.25 mg/kg/h with surface temperature modulation

Finally, we applied dynamic control of surface temperature to optimize control over T_b with CHA administered by continuous IV infusion. A temperature controlled cage was built to modulate T_b in animals treated with CHA. Two male rats were implanted with telemetry transmitters (CTA-F40; Data Sciences International, New Brighton, MN) inside the abdominal cavity and ECG leads were secured to the chest wall. The femoral artery was cannuated using 12cm 3Fr C30PU-RECA1302 polyurethane catheters (Instech, Plymouth Meeting, PA). The femoral vein was also cannulated using C30PU-RJV1420 catheters; both cannula were passed through an inter-scapular incision where they were attached to a 2 channel vascular harness (VAD115AB; Instech). For post-operative recovery animals were housed individually with cotton pads substituted for wood shavings. Sutures were removed 7-10 days post op and catheter maintenance was done by flushing every 5 days using saline followed by filling with a locking solution of heparin/glycerol (500 IU/mL, 50:50) to prevent clotting. On the day of the experiment, animals were placed on the cage surface with the initial surface temperature set to 17°C. CHA was administered by continuous IV infusion (0.25 mg/kg/h). When animals approached a target T_b of 32°C, surface temperature was increased to 32°C to maintain target temperature.

Drugs

CHA (CAS 36396-99-3) is eliminated with a half-life of approximately 2 hours when given subcutaneously (Tuovinen and Tarhanen, 2004) and is a full A_1AR agonist (van der Wenden et al., 1995). CHA (Sigma-

Aldrich; St. Louis, MO) was dissolved in 25% (w/v) hydroxypropyl-β-cyclodextrin (CD) (TCI America, Portland, OR) then diluted to 2.5% in physiological saline. CHA vehicle consisted of 25% (w/v) hydroxypropyl-β-cyclodextrin diluted to 2.5% in physiological saline. Capadenoson (CAS 544417-40-5) is a partial A1AR agonist relative to CCPA (6-chloro-N^6-cyclopentyladenosine) and shows a half-life of approximately 20 hours (Albrecht-Kupper et al., 2012). Capadenoson (>98% purity; Chemexpress, Monmouth Junction, NJ) was dissolved in 100% polyethylene glycol (PEG400; Med Lab Supply, Pompano Beach, FL) then diluted to 60% PEG concentration with sterile water. All substances were USP grade where available. Solutions for injection were sterilized by 0.2μm filtration (Acrodisc syringe filter; Pall corp., Port Washington, NY).

Oxygen consumption ($\dot{V}O_2$)

$\dot{V}O_2$ was measured using open-flow respirometry in conjunction with LabGraph respirometry acquisition and analysis software according to (Toien, 2013) and (Jinka et al., 2011). The accuracy and integrity of the system was calibrated by burning ethanol (100%) following established methodology (Toien, 2013); analyzers were manually calibrated with atmospheric reference air (~0.03% CO_2), zero air (~0% CO_2), and span gas (~0.51% CO_2) before each group of experiments and auto-calibrated subsequently every two hours. $\dot{V}O_2$ data was synchronized with T_b by subtracting a lag time of 4 min calculated as the volume of the chamber and length of the outlet tube.

Statistical Analysis

Variation in T_b, body mass, and $\dot{V}O_2$ was analyzed using repeated measures linear mixed-effect models (Domidenko, 2004) to account for within-rat correlations and to model time trajectories after treatment or feeding regimen. These statistical analyses were conducted using the IBM SPSS Statistics 19. Post-hoc comparisons were performed using t-tests with Bonferonni corrections (Excel 2007). The significance criterion was $\alpha < 0.05$ for all analyses. Data are shown as mean ± SEM unless otherwise indicated.

1.4 Results

Dietary restriction; 0.5 mg/kg IP CHA

We investigated the influence of every other day feeding on whole animal oxygen consumption and on the circadian rhythm in T_b to assess the influence of dietary restriction on thermoregulation. DR decreased T_b compared to animals fed AL (diet x time ($F(1,13.10) = 7.95$, $p = 0.014$) and main effect of diet ($F(1,12.37) = 8.97$, $p = 0.011$)). Post hoc tests show that the T_b in DR animals in comparison to AL were statistically different on days 4-36, except for days 5, 7 and 17 ($p<0.05$) as shown in Figure 1.1A. Next, we asked whether DR affected T_b across the circadian rhythm or only during the light or dark phase of the cycle. Analysis of T_b on the day prior to CHA administration (a feeding day; Figure 1.1B) shows that DR decreases the amplitude during the dark, active period [main effect of time ($F(1,343) = 5.15$, $p = 0.024$) and diet ($F(1,43.51) = 7.74$, $p = 0.008$) with a near-significant interaction between diet and time ($F(1,343) = 3.48$, $p = 0.063$). Assessment of the rhythm in T_b during the lights on (inactive) period was confounded by disturbance associated with feeding and cage cleaning. DR also decreased weight gain relative to AL animals (Figure 1.1C; diet x time ($F(1,13.50)=6.28$, $p=0.026$)).

We next assessed the effects of CHA on T_b or $\dot{V}O_2$ in DR and AL fed rats. Rats in both DR and AL groups maintained T_b at approximately 37.5°C when given vehicle (Figure 1.2A). Both groups responded to 0.5 mg/kg IP CHA but the DR group showed a larger, more consistent response than the AL group (n=4 AL, n=4 DR). Within 120 minutes of injection, T_b in the AL group reached 35.1±1.2°C and T_b in the DR group reached 32.5±0.1°C (diet x time x treatment ($F(3,140.04) = 18.19$, $p < 0.001$) with a significant main effect of time ($F(1,140.04) = 59.86$, $p < 0.001$). Post-hoc t-tests showed that the CHA group was significantly different than vehicle in DR animals ($p<0.05$) at 40-120 min after injection. The T_b in the AL group after CHA was not different from vehicle ($p>0.05$). We observed a bimodal distribution in the 4 AL

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fed rats after giving 0.5mg/kg CHA ; two rats maintained T_b similar to vehicle while the other two showed a decrease in T_b similar to DR rats given CHA [Figure A1].

In order to see if CHA decreased T_b as a result of an inhibition of thermogenesis, we measured $\dot{V}O_2$ as an indirect measure of both shivering and non-shivering thermogenesis in both DR an AL rats. Compared to rats given vehicle, $\dot{V}O_2$ tended to decrease in both AL and DR rats within 10 min after CHA injection (Figure 1.3A). $\dot{V}O_2$ stabilized at minimal levels within 30 to 50 min after CHA administration and tended to be lowest in the DR group. Pair-wise comparisons revealed significant differences between CHA treated and Vehicle treated DR rats at 40-120 minutes ($p<0.05$) and also between AL CHA and vehicle treated rats between 70-120 minutes ($p<0.05$). IP injections with CHA or vehicle tended to produce an immediate increase in $\dot{V}O_2$ except where rats decreased T_b after CHA (Figure 1.3B and Figure A2). In these animals $\dot{V}O_2$ decreased before T_b and is consistent with a decrease in thermogenesis.

Ad lib feeding - 1.0 mg/kg IP CHA, 1.0 & 2.0 mg/kg Capadenoson & Vehicles

We tested a higher dose of CHA (1.0 mg/kg) and two doses (1.0 and 2.0 mg/kg) of the partial agonist capadenson to test the hypothesis that a maximally effective dose or alternative A_1AR agonist would decrease variation in cooling with AL animals. In addition, to assess if the circadian rhythm of T_b influenced drug response we graphed T_b for 3 days prior to drug administration. The circadian rhythm of body temperature was noted visually, but not analyzed further (Figure 1.4). Both doses of capadenoson cooled T_b to a minimum within 2h of injection. Minimum T_b (mean ± SEM) was 37.0 ± 0.2, and 36.6 ± 0.2°C for 1.0 mg/kg, and 2.0 mg/kg respectively (Figure 1.4). Analysis of the minimum core T_b yielded a significant main effect of treatment (capadenoson 1.0 or 2.0 mg/kg, or vehicle) ($F(2,18) = 17.52$, $p < 0.001$). Pair-wise comparisons of minimum T_b revealed a significant difference between 1.0 mg/kg capadenoson and vehicle ($p = 0.008$) and 2.0 mg/kg capadenoson and vehicle ($p < 0.001$) and trended towards significance between 1.0 mg/kg and 2.0 mg/kg injections ($p = 0.081$). Capadenoson lowered

heart rate with a minimum of 77.1% of vehicle baseline for the 1mg/kg dose and 71% for the 2mg/kg

dose within 2h post injection [Figure A3].

While a dose of 0.5 mg/kg of CHA in AL animals resulted in T_b decreases in two out of four animals, the

higher dose of CHA (1.0 mg/kg) produced a notable decline in T_b in all animals (10/10). Nonetheless, the

magnitude and duration of response still varied between animals (Figure 1.4). The lowest *minimum* T_b

recorded was 20.6°C while the highest *minimum* T_b was 32.5°C. We asked whether this variation was

intrinsic to each animal by giving CHA (1.0 mg/kg) to all animals a second time with one to five weeks

separating the two injections. In seven out of ten animals the decline in T_b after the second injection

mirrored closely the response to the first injection (Figure 1.5a); however in three animals it did not.

Statistical analysis on the minimum core T_b within 20.5h after injection showed that CHA produced a

significant decrease in T_b on both injections (treatment (first injection, second injection, or vehicle):

$F(2,18) = 36.57$, $p < 0.001$) with significant differences between CHA first injection and vehicle ($p < 0.001$,

t-test) and CHA second injection and vehicle ($p < 0.001$, t-test)). Moreover, there was no significant

difference between the first and second injection of CHA ($p = 1.000$, paired t-test). Regression analysis

of minimum T_b on the first and second injections yielded an R^2 value of just 0.43, ($p=0.043$) [Figure A4].

Body weight on the day of injection did not predict the magnitude of the cooling response ($p=0.72$, first

injection; $p=0.25$, second injection). Moreover, neither time nor change in body weight between

injections predicted response on the second injection ($p=0.82$) [Table A2]. In addition to lowering T_b,

CHA caused a 74.5% reduction of heart rate in comparison to vehicle on average within 2h of injection

[Figure A3]. Bradycardia resolved between 24-48h after injection [Figure A5].

Ad lib feeding - 0.25 mg/kg/h IV CHA @ 2μl/min

We next asked if surface cooling would normalize T_b and prevent over cooling. We designed and built a

temperature controlled cage to model surface cooling used clinically and adjusted surface temperature

to prevent over cooling. During continuous IV infusion of CHA (0.25 mg/kg/h) in the absence of a bolus loading dose, animal T_b approached 32°C within 3 hours on a surface temperature of 17°C. Increasing surface temperature to 32°C maintained target temperature and prevented overcooling (Figure 1.6). Heart rate declined rapidly at the start of CHA infusion and bradycardia persisted throughout the infusion (Figure 1.6).

1.5 Discussion

Thermolytics include antipyretic drugs such as acetaminophen and certain non-steroidal anti-inflammatory drugs (Section on Clinical et al., 2011). Here, we extend the definition of thermolytic to include drugs that suppress thermogenesis and decrease core T_b. Despite the ability of CHA to suppress thermogenesis precise control of T_b around a predetermined target temperature had yet to be demonstrated prior to this work. Our objectives were to define how dose of CHA and environmental temperature influence T_b in rats treated with CHA. IP Bolus injections using CHA at 0.5 and 1.0 mg/kg failed to produce consistent decreases in T_b, however, use of the higher dose decreased T_b in all animals down to or below our target temperature of 32°C at an ambient temperature of 17°C. From this, we hypothesized that overcooling could be prevented with cage surface temperature modulation and thus facilitate management of target T_b. Here, we report precise control of T_b using CHA coupled with dynamic control of cage surface temperature and show that modulation of dose alone is not sufficient to precisely manage target T_b.

Our results demonstrate robust thermolytic efficacy of CHA in rats and is a refinement of prior attempts with high doses of purine derivatives. AMP was the first purine reported to induce a torpor-like state in rats (Zhang et al., 2006) and both AMP and ATP were later tested in rats to lower T_b for therapeutic benefit (Zhang et al., 2009; Zhang et al., 2013). High doses were necessary in order to promote sufficient cooling, ultimately producing unwanted effects which discouraged further development. AMP induced a

hypothermic response in mice (Swoap et al., 2007) and was later found to act as an A_1AR agonist; (Muzzi et al., 2013) AMP-induced cooling was blocked using an A_1AR antagonist in the CNS (Iliff and Swoap, 2012). Targeting CNS A_1AR using CHA to inhibit thermogenesis shows promise as an effective approach to relieve shivering during therapeutic hypothermia (Jinka et al., 2015).

Other non-purine based thermolytics currently in development include neurotensin receptor agonists (Choi et al., 2012; Wei et al., 2013), transient receptor potential (TRP) agonists and antagonists (Almeida et al., 2012; Feketa et al., 2014; Feketa and Marrelli, 2015), $GABA_A$ agonists (Cerri et al., 2013), and other unique formulations (Katz et al., 2012a; Katz et al., 2012b; Katz et al., 2015). Using a fixed ambient temperature, several studies demonstrate control of target temperature through modulation of dose and dosing regimens alone to maintain T_b or prevent overcooling, (Muzzi et al., 2013; Wei et al., 2013; Feketa et al., 2014) however thermolytic efficacy of other drugs tested to date in rats has not been as great as CHA.

Few preclinical studies combine dynamic temperature control with thermolytics in search for optimal temperature management protocols (Almeida et al., 2012) (Katz et al., 2012b) (Cerri et al., 2013). In the clinic, induction methods vary, but may include packing ice into axillary and groin areas and infusing ice-cold IV saline. Once target temperature is reached T_b is usually maintained with water-blanket surface cooling (Luscombe and Andrzejowski, 2006) (Blanketrol or Arctic Sun, etc) or endovascular cooling. Surface temperature control devices are routinely used in clinical settings and are standard protocol at most hospitals (Callaway et al., 2015a).

Although we found here that surface temperature modulation prevented overcooling, our data does not explain the large individual variation in T_b response to CHA. This variation was unexpected since prior work suggested more consistent responses between animals (Jinka et al., 2010), (Jinka et al., 2015). We did not observe significant variation using the same animals with the partial agonist capadenoson, but

consistency came at the cost of thermolytic efficacy.

Current knowledge suggests A_1AR agonist-induced cooling is due to an inhibition of thermogenesis at a central site of action(Anderson et al., 1994; Tupone et al., 2013). However, peripheral mechanisms such as the inhibition of lipolysis could also impair nonshivering thermogenesis in brown adipose tissue (Asakura, 2004; Viswanadha and Londos, 2006). It is unclear whether these mechanisms are responsible for individual differences in T_b lowering effects of CHA, but our results reflect what might be expected in a diverse clinical population.

Similar variation in response to CHA is seen in ground squirrels where sensitivity to CHA depends on the hibernation season. In Arctic Ground Squirrels (AGS), stimulation of CNS A_1ARs with CHA induces a torpor-like state, but the drug is effective only in the hibernation season (Jinka et al., 2011). Seasonal sensitivity to CHA in AGS precedes a decrease in food intake and is predicted by a gradual decrease in T_b as animals approach the hibernation season (Olson et al., 2013) (Sheriff et al., 2012). In rats, prolonged every-other-day feeding increases sensitivity to the temperature lowering effects of CHA as well as surface expression of A_1AR in hypothalamus (Jinka et al., 2010). While increases in surface expression of A_1AR may contribute to increased sensitivity, prolonged restriction of diet is not a viable approach to normalize response to A_1AR agonists in emergency medicine.

Shivering is one of the most problematic issues in TTM which can impede induction of hypothermia by doubling metabolic rate (Badjatia et al., 2008), which leads to a stress-like response . Despite the importance of metabolism reduction, one of the primary desired effects in administering TTM, limited O_2 consumption data has been reported for other thermolytics in development. Recently however, it was revealed that O_2 consumption was reduced using TRPv3 agonists to induce hypothermia in mice, but these results could not be replicated in rats (Feketa and Marrelli, 2015). Here, evidence supporting inhibition of thermogenesis comes from a decrease in the rate of oxygen consumption ($\dot{V}O_2$) that

precedes a decrease in T_b. A similar hysteresis of $\dot{V}O_2$ and T_b decline is seen during the onset of hibernation and torpor (Jinka et al., 2011).

Generalization of the current results in rats to other non-hibernating species such as swine and humans is likely because rats do not hibernate naturally. By contrast, many strains of laboratory mice spontaneously enter shallow torpor in response to fasting (Geiser, 2004). Results from studies using mice may not translate to species that do not hibernate, as evident in the study of TRPv3 agonists (Feketa and Marrelli, 2015). For this reason mice are less preferred in the investigation of thermolytic efficacy in comparison to rats or swine.

One limitation of this study, and others using small animals to study whole-body cooling is that surface area to weight ratio is far smaller than in larger animals including humans. An important next step in evaluating thermolytic efficacy is to utilize larger animals. In the present study the rate of cooling was faster following IP injection than with continuous IV administration because a loading dose was not given prior to IV infusion. Another limitation not addressed here is the potentially detrimental effects of adenosine receptor-induced bradycardia and hypotension, a side effect of CHA and hypothermia.(Nieri et al., 2001) We have found previously that co-administration of the peripherally acting adenosine receptor antagonist, 8-sulfophenyltheophylline (8-SPT), reverses bradycardia and improves survival and neurologic outcome after cardiac arrest in rats (Jinka et al., 2015) without interfering with the thermolytic effect of the drug. Work is in progress to characterize the effects of 8-SPT on hypotension during CHA assisted cooling.

A recent trial (Nielsen et al., 2013) showed no difference in outcome in patients cooled to 33°C vs. 36°C and questioned the utility of cooling to 33°C. By contrast, an exhaustive number (over 50) of preclinical studies demonstrate that deeper cooling is better (Lyden et al., 2006; Polderman, 2009). Moreover, Nielsen noted shivering at 33°C _and_ 36°C and no differences were found in other adverse effects of 33°C

15

vs. 36°C; other studies confirm shivering at 36°C (Callaway et al., 2015b). Importantly, the benefit to risk of colder T_b may increase as severity of brain injury increases (Yenari and Han, 2012). In response to the Nielsen paper the original International Liaison Committee on Resuscitation (ILCOR) recommendations indicating 32-34°C(Donnino et al., 2015) has been changed to recommend a target Tb between 32°C and 36°C.(Donnino et al., 2015). The current study is the first report to our knowledge of the effects of capadenoson on body temperature. Capadenoson is a partial agonist that produces 75% of full agonist, CCPA (2-chloro-N^6-cyclopentyladenosine), [^{35}S]GTPγS binding in human cortical membranes. In Langendorff heart preparations capadenoson reduces heart rate to a maximal of 10% of the bradycardia produced by the full agonist CCPA. At higher doses CCPA produces complete AV block.(Albrecht-Kupper et al., 2012) The limited bradycardia with capadenoson reported by others is consistent with results reported here. Given the absence of cardiovascular risk the mild hypothermic effect of capadenoson may be useful when a target T_b of 36°C is desired.

In summary, we show pronounced thermolytic efficacy of CHA with unexplained variation that is resolved under dietary restriction, but is not resolved with dose in ad libitum fed animals. Although high thermolytic efficacy produced over-cooling in some animals, dynamic control of surface temperature allowed for fine tuning and maintenance of a prescribed target T_b. This approach to reduce and maintain target T_b in rodents is a refinement over fixed ambient temperatures or evaporative cooling protocols where animals are sprayed with water or alcohol to facilitate heat loss (Klahr et al., 2017), and mimics surface cooling used in the clinic. This new thermolytic class of drugs has potential to facilitate targeted temperature management by inhibiting thermogenesis, providing new avenues for treatment.

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Authorship Contributions

Participated in research design: Drew, Bailey, Laughlin, Moore

Conducted experiments: Drew, Bailey, Laughlin, Bogren, Moore

Contributed new reagents or analytic tools: Bailey, Laughlin

Performed data analysis: Drew, Bailey, Laughlin, Barati

Wrote or contributed to writing of the manuscript: Drew, Bailey, Laughlin

Research Approval

Experiments were done in accordance with the Guide for the Care and Use of Laboratory animals, 8th edition (National Research Council, National Academies Press, 2010) and protocols were approved by University of Alaska Fairbanks Institutional Animal Care and Use Committee

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Endnotes

Table 1. Summary of experiments

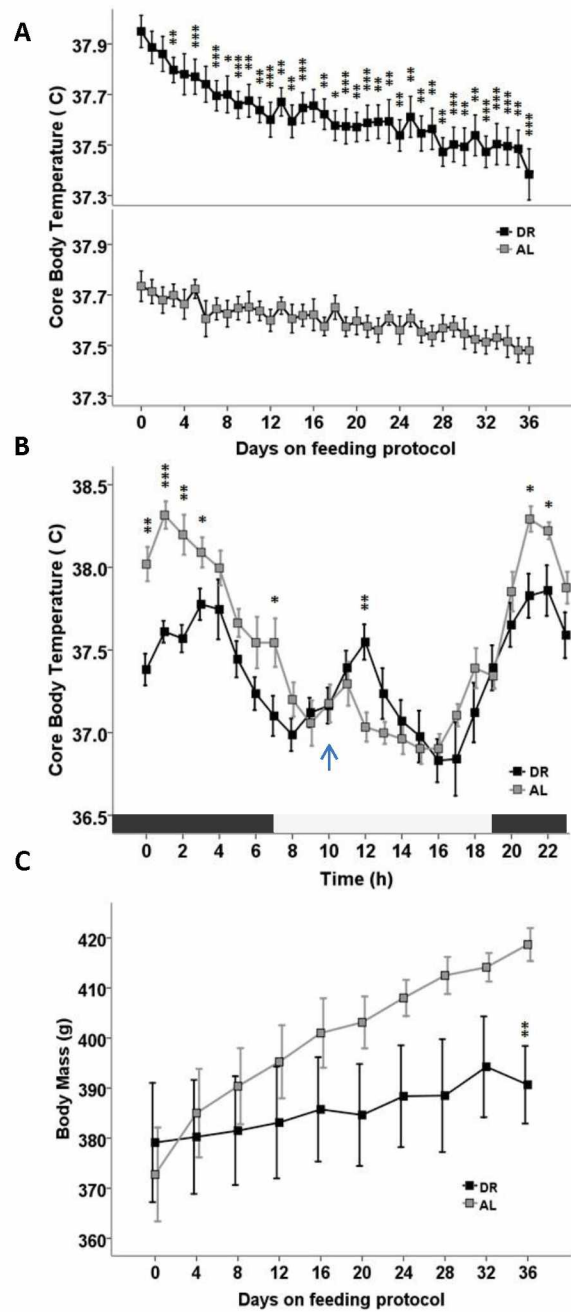| Experiment	Experimental test	Drug	Doses	Route	Ambient temperature	Sample size
1	Diet and Oxygen Consumption	CHA	0.5 mg/kg	IP - bolus	16°C	15
2	↑ Dose of CHA	CHA	1.0 mg/kg	IP - bolus	17°C	10
	Compare Partial Agonist	Capadenoson	1.0 and 2.0 mg/kg	IP - bolus	17°C	10
3	Surface temperature modulation with IV CHA	CHA	0.25 mg/kg/h	IV - continuous	16°C - 32°C	2

Figure 1.1: The effects of every other day feeding (dietary restriction; DR) on body temperature and weight gain over 36 days

Figure 1.1 (continued):

(A) Every other day feeding (dietary restriction; DR) decreases body temperature (T_b) relative to ad libitum (AL) feeding.**(B)** The decrease in body temperature is greatest during the active period. Light:dark cycle is indicated by colored bar below. **(C)** The effects of dietary restriction did not maintain the same rate of weight gain in comparison to rats fed ad libitum. Ta is 20°C, mean ± SEM, n=7 AL; n=8 DR, * p<0.05, ** p<0.01, *** p<0.001 DR vs. AL.
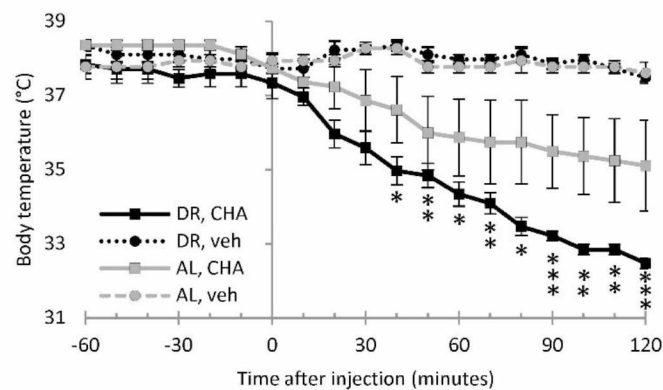

Figure 1.2: The effects of 0.5mg/kg N^6-cyclohexyladenosine (CHA) on body temperature in dietary restricted and ad-libitum fed rats

Treating rats with the A_1AR agonist ^6N cyclohexyladenosine (CHA) at an ambient temperature of 16°C decreases T_b. The decrease in T_b is greater in DR rats than in AL rats. T_a is 16°, mean ± SEM n=4 (DR CHA), n=4 DR VEH, n=4 (AL CHA), n=3 (AL VEH). Error bars not shown are smaller than symbols. * p<0.05, ** p<0.01, *** p<0.001; DR CHA vs. DR vehicle.
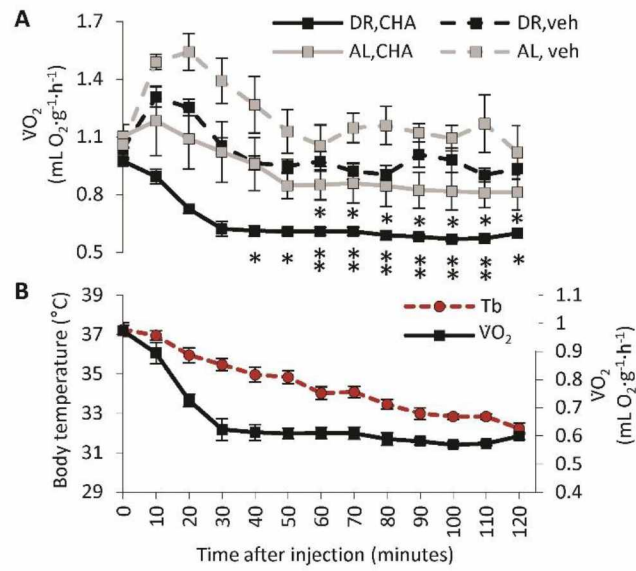
Figure 1.3: The effects of 0.5mg/kg CHA on body temperature vs. oxygen consumption in dietary restricted and ad-libitum fed rats

(A) Dietary restriction significantly lowered oxygen consumption ($\dot{V}O_2$) for both vehicle and CHA treated rats **(B)** Simultaneous measurements of T_b and $\dot{V}O_2$ in the DR group shows that $\dot{V}O_2$ declines prior to T_b. T_a is 16°C, (mean ± SEM, n=4 (DR CHA), n=4 DR VEH, n=4 (AL CHA), n=3 (AL VEH).

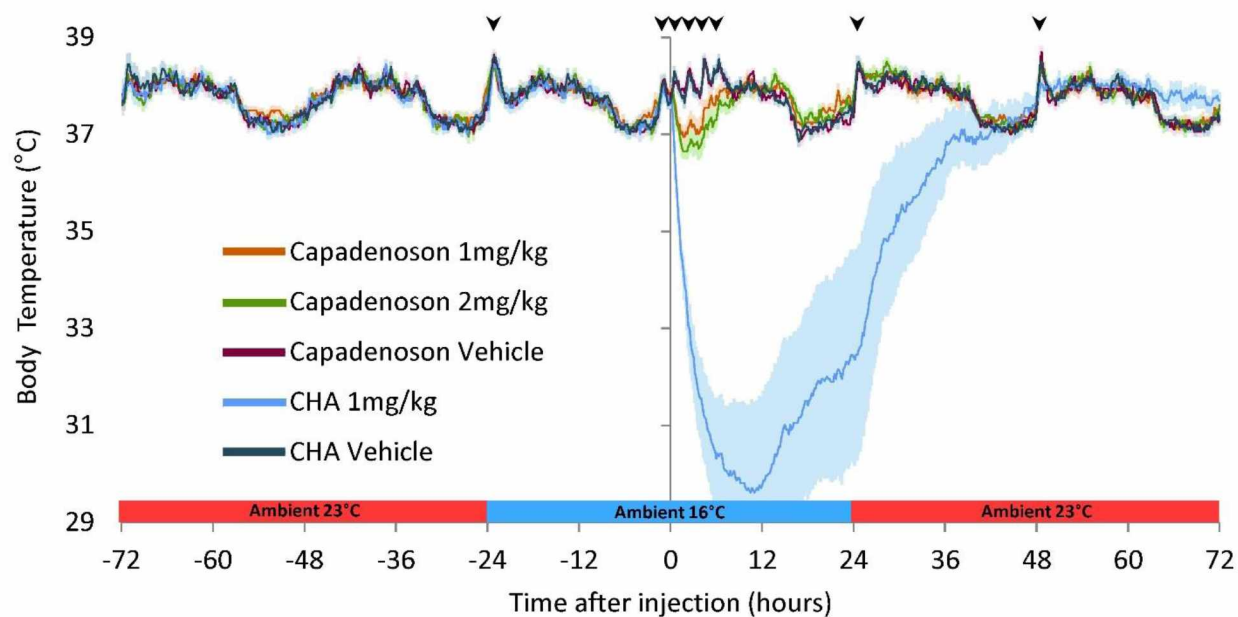**Figure 1.4:** The effects of 1.0mg/kg CHA and capadenoson on body temperature in rats

CHA (1.0 mg/kg) is more effective than either dose of capadenoson (1.0 and 2.0 mg/kg) at reducing body temperature. Variation in both magnitude and duration of response to CHA was not decreased by the higher dose (indicated by SEM; lighter shaded area). Arrowheads indicate time when rats were picked up for heart rate measurements.
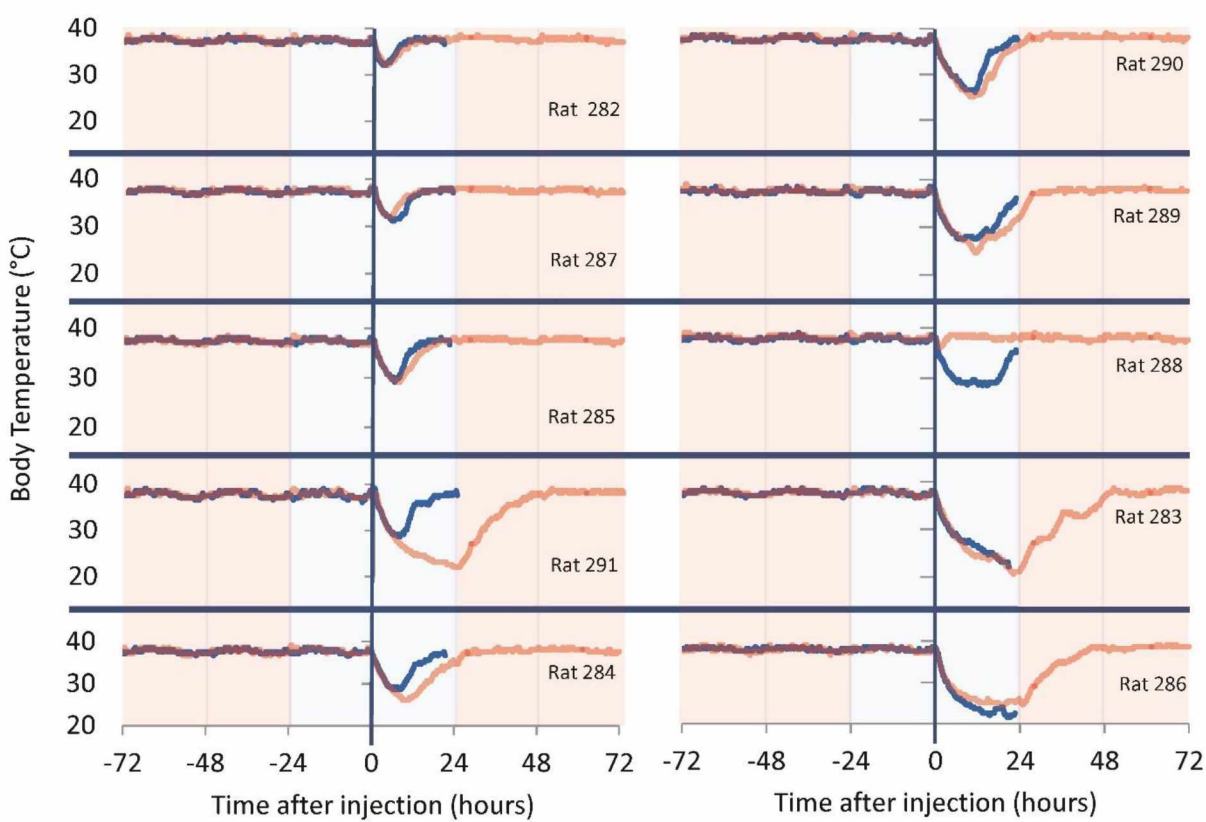
Figure 1.5: Individual animal variation when given two injections of 1.0mg/kg CHA

Response to CHA (1.0 mg/kg) on the first injection (red lines) did not predict the response on the second injection (blue lines) in three out of ten rats. Colored region indicates ambient temperature (red, 23°C; blue, 17°C).
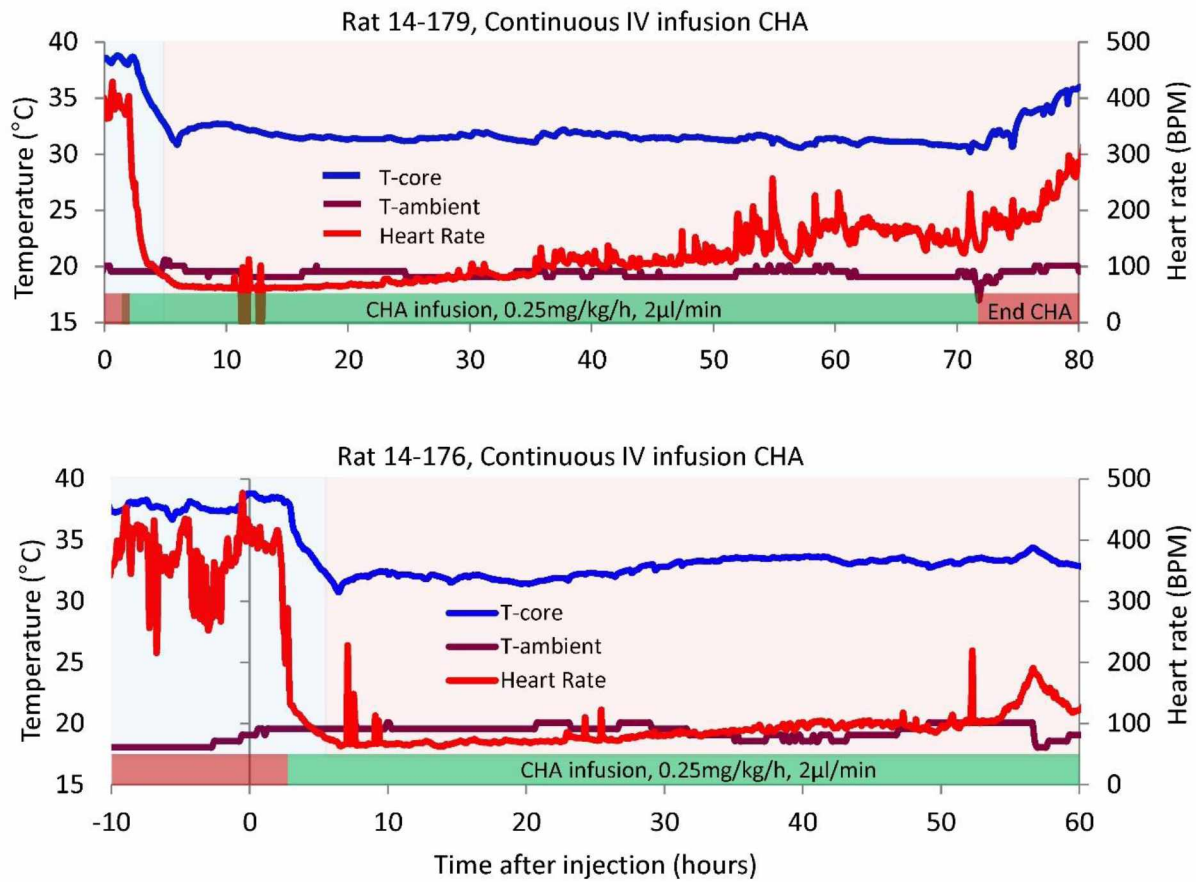
Figure 1.6: Surface temperature controls body temperature using CHA in rats

To prevent animals from over cooling with CHA on board, cage surface temperature was heated or cooled as needed. The cage floor was set to 17°C initially (blue region) and brought up to 32°C (red region) as body temperature reached the target temperature of 32°C to prevent overcooling. At this dose of CHA, heart rate drops to about 20% of baseline and is followed by a decrease in body temperature. This temporal relationship between body temperature and heart rate is similar to what is seen at onset of hibernation and is consistent with CHA-induced inhibition of thermogenesis. Ambient temperature within the cage did not vary significantly.
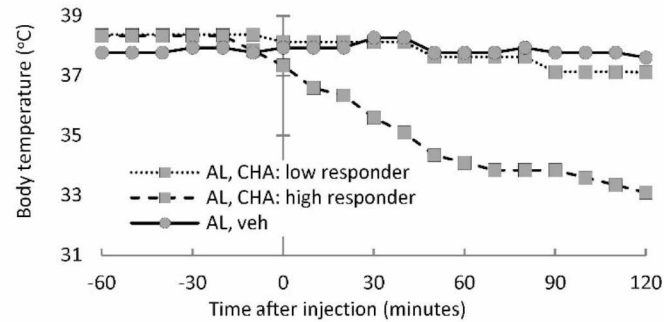
Figure A1: Ad libitum fed rats given 0.5mg/kg CHA diverge into low responders and high responders

In AL animals, CHA (0.5 mg/kg) produced a bimodal response.

Low responding animals resembled vehicle treated animals, and high responding animals resembled

DR fed animals. T_a is 16°C, mean, n=2 AL CHA low responders; n=2 AL CHA high responders;

n=3 AL, Vehicle.
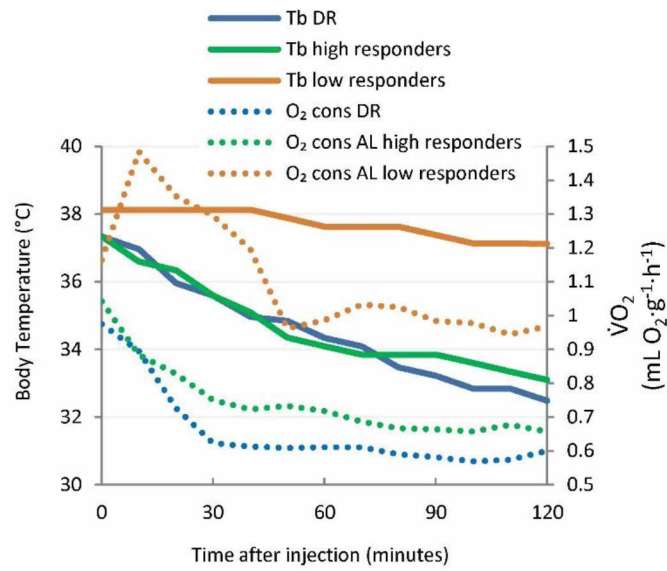
Figure A2: Ad Libitum fed rats responding to 0.5mg/kg CHA show a lower oxygen consumption rate in comparison to the low responding animals.

Oxygen consumption declines sharply after 0.5 mg/kg CHA in DR animals (n=6). After AL feeding, animals that showed a large response to CHA (n=2) decreased $\dot{V}O_2$ at a rate similar to the decline in T_b. By contrast, animals that showed a low response to CHA displayed an abrupt increase and then decrease in $\dot{V}O_2$ with no change in T_b. T_a is 16°C, mean, n=4 (DR) , n=4 (AL).
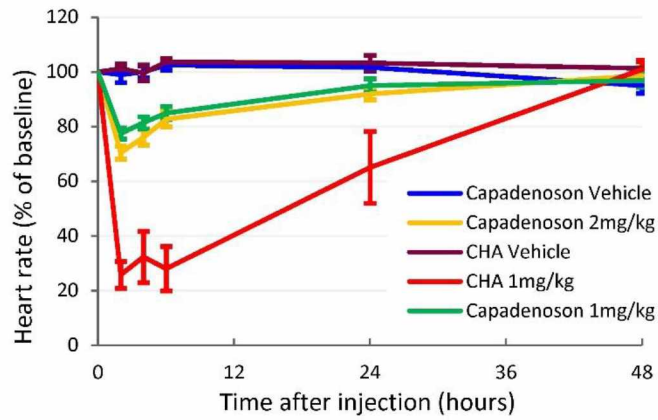
Figure A3: Bradycardia response to CHA and capadenoson injection in rats.

CHA and capadenoson-induced cooling is associated with bradycardia. The magnitude of bradycardia is greatest after CHA. T_a is 17°C, n=10.
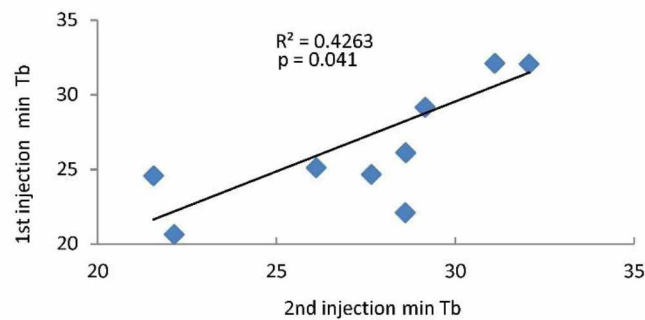
Figure A4: Correlation for animals given two injections of N^6-cyclohexyladenosine.

Minimum T_b after the first injection of CHA predicted weakly minimum T_b after the second injection of CHA with an R^2 of 0.43 (p=0.041), n=10.
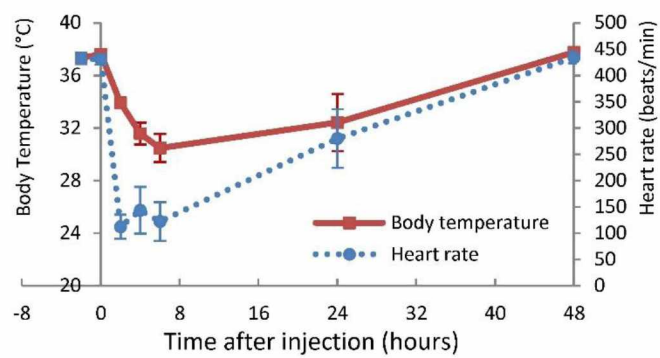
Figure A5: Heart rate follows N[6]-cyclohexyladenosine induced body temperature reduction.

CHA-induced bradycardia tended to precede the decline in T_b. T_a is 16°C, mean, n=10.

Table A1. Balanced block design for experiment 2

Group	Week 1	Week 2	Week 3	Week 4	Week 5
1 (n=2)	CHA Vehicle	Cap Vehicle	CHA 1mg/kg	Cap 1mg/kg	Cap 2mg/kg
2 (n=2)	Cap 2mg/kg	CHA Vehicle	Cap Vehicle	CHA 1mg/kg	Cap 1mg/kg
3 (n=2)	Cap 1mg/kg	Cap 2mg/kg	CHA Vehicle	Cap Vehicle	CHA 1mg/kg
4 (n=2)	CHA 1mg/kg	Cap 1mg/kg	Cap 2mg/kg	CHA Vehicle	Cap Vehicle
5 (n=2)	Cap Vehicle	CHA 1mg/kg	Cap 1mg/kg	Cap 2mg/kg	CHA Vehicle

Table A2. Minimum T$_b$, body weight and time between CHA injections

Rat ID	1st Injection		2nd Injection		Time between
	Body wt (g)	Min Tb (°C)	Body wt (g)	Min Tb (°C)	injection (weeks)
282	402	32.059	408	32.059	3
283	402	20.635	430	22.136	3
284	405	26.113	400	28.612	2
285	404	29.153	412	29.153	2
286	407	24.563	382	21.562	1
287	476	32.103	470	31.104	1
288	380	36.107	432	28.613	5
289	397	24.649	430	27.648	5
290	376	25.107	415	26.107	4
291	400	22.097	453	28.597	4

Chapter 2: A dynamic temperature metabolic cage for studying hypothermia in small animals[2]

2.1 Abstract:

The prior standard in studying therapeutic hypothermia methods in small animals has been done by spraying animals with water or a mixture of water and alcohol in-order to use the high latent heat of vaporization for cooling purposes (Zhao et al., 2005; Wang et al., 2012; Liu et al., 2013; Bazley et al., 2014). In refinement of this technique, we hypothesized that surface temperature control would achieve much better control over body temperature and minimize adverse reactions to the water & alcohol mixtures. This new cage operates in a similar fashion to in-floor heating that is commonly implemented in residential housing. A key difference here is that the floor can be either heated up or cooled down between 4°C and 36°C quickly and is limited to the temperatures for animal comfort when working with Sprague-Dawley rats. The cage could theoretically be cooled to approximately -20°C or warmed past 40°C in the present configuration with limitations coming from the supporting refrigerator cooling power. Here we describe a method of cage construction and provide results that define specifications regarding surface temperature control.

2.2 Methods:

Overview

The primary method of temperature adjustment is done by using the peltier effect to move heat to or from the cage surface and into a heat exchanger located inside the supporting refrigerator. The refrigerator is not needed if desired cage temperature is between approximately 14°C and 37°C; in this case a radiator and fan will reject waste energy from the system. For cooler target temperatures the

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[2] Bailey, I.R., "Methods of temperature & metabolism reduction in rats and possible influence on human health" (M.S. thesis, University of Alaska Fairbanks, 2017), 39-53

radiator, fan, power supply, and water pumps are housed inside the refrigerator to eliminate waste heat

and minimize equipment noise levels. A diagram of the dial-a-temp general configuration for

hypothermia studies in small animals can be seen in figure 2.1.

Cage floor

For construction of the cage floor, a pair of 12"x12"x0.125" 6061 T6 aluminum plates were welded to four sides constructed of 12"x1"x0.125" aluminum flat stock. A strip of 8"x0.75"x0.125" was placed medially to channel water evenly throughout the interior space. Water inlet and outlet fittings were made out of 0.75"x0.125" round tube and welded directly to the side of the cage and then two holes were drilled and de-burred for smooth water passage. All cage construction materials are of the 6061 T6 alloy and was chosen due to a very high Brinell hardness of weldable aluminum alloys. Welding prior to drilling and tapping afforded extra surface area for sealing the threads of a 1/4" NPT male Schrader valve which was used to burp air out of the system before normal operation. During operation, insulation is recommended underneath the cage and will reduce losses due to heat transfer in addition to more accurate temperature sensor input.

Cage top

The cage top was fabricated using five 11"x11"x0.125" thick polycarbonate (plexiglass) sheets with one reserved for the cage lid. A 12"x12"x0.125 sheet was solvent-welded to one of the 11"x11"x0.125" sheets in the center to provide an interference-type fit so that most of the incoming air is limited to the central hole when using respirometry equipment to draw air in. Air and respiratory gasses then exit the cage through four fittings tapped at animal height. These fittings are placed such that any stagnant air respired by animals is captured in addition to a faster cage wash-out. In place of the polycarbonate lid, 1/8" stainless TIG filler wire can be cut and welded together into a wire cage-top to promote open air exchange if respirometry equipment is not used. The polycarbonate lid should NOT be used if respirometry equipment is not continuously pulling in fresh air in as the animal may asphyxiate due to inadequate air flow.

Heat transfer plates

The two identical heat transfer blocks are constructed of 1"x3"x0.125" by approximately 10" long 6061

T6 rectangle tube with 1"x0.125" flat stock welded on both ends as end caps. Each end cap received a 1"

long 0.75"x0.125" round tube for water in and out fittings; after welding the interior was drilled and de-

burred. These two blocks are used in conjunction with four potted Peltier modules and are clamped

together forming the heat transfer block.

Methods of temperature control

Design V1.1 included a home-made H-bridge array of relays controlled digitally by aftermarket

refrigerator controllers, commonly used in kegerator setups. In-order to eliminate failures of mechanical

relays used in the previous design, a stand-alone control unit was obtained (TC-720; TE Technology Inc.,

Traverse City, MI) that uses semi-conductor switching in the form of transistors, increasing reliability.

The control module also uses PWM (pulse width modulation) to drive the Peltier modules in order to

minimize heat cycling of the internal semi-conductors, further increasing reliability.

The TC-720 uses one primary thermistor to use in the PID (Proportional, Integral, Derivative) algorithm

and a secondary thermistor to obtain ambient temperature. This secondary sensor could also be used to

monitor equipment temperature or other experimental parameters.

The only drawback to using this control unit over an H-bridge relay setup is a 20 amp nominal current

limitation. Based on a year of operation, short bursts of up to 25 amps seems to be tolerated by the TC-

720 circuitry and It is suggested that the controller is placed in an area of adequate ventilation for

cooling if frequent over-current loads are expected.

Peltier module matching

The dial-a-temp uses the Peltier effect to move heat forwards or backwards depending on input polarity. Differences in Peltier modules (or pads) exist in terms of temperature and other operation conditions; potted modules can be used for increased reliability at the expense of slightly less efficient heat transfer, something beneficial when working with animals. Differences in module specifications such as voltage and current draw need to be considered when designing a system. Input voltage should always be about half of the total voltage rating the manufacturer specifies for a given Peltier module and usually provides for the best COP (coefficient of performance), where the amount of heat removed per ampere is at maximum. When input voltage approaches the upper limit of the module rating, increased waste heat is generated and may be useful in applications requiring more heating than cooling. We are trying to both cool and heat the cage surface, so a 12V power source was chosen to run both the TC-720 and an array of modules rated to 24V (VT-199-1.4-0.8, potted; TE technology, Traverse City, MI). The maximum COP for these modules is obtained in the 12V range as indicated in literature from TE Technology.

Power converter

There are not many restrictions on the type of 120V AC to 12V DC power transformer as long as adequate current capacity is met. There is variability in current draw depending on the type of Peltier module chosen so a larger power converter is never a bad idea. I chose to go with a 1200W power supply for a PC as they are common, fairly cheap, and have relatively high conversion efficiency. The main limitation to total system power is the main PCB (printed circuit board) utilized in the TC-720 can only operate up to a 20 amp steady state current.

Plumbing

The system uses two segregated water (or coolant) circuits plumbed with 3/4" silicone heater hose and secured using standard worm drive clamps according to figure 1B. Coolant reservoirs in the diagram are

constructed out of 2" schedule 40 ABS Drain waste vent pipe commonly available at hardware stores with end caps. The height of the reservoirs is not critical as long as the liquid level is above the cage surface. End caps for the bottom of these reservoirs are solvent welded, then drilled and tapped for 3/4" NPT (3/4" pipe thread) and sealed using liquid based Teflon sealant. A male-male 3/4" NPT fitting was placed in the reservoir and used to connect to a 3/4" NPT bulkhead fitting that was placed in the refrigerator casing prior as a method of pass-through plumbing. Each water circuit receives its own reservoir in-order to prevent mixing of liquid. Small automotive 12V DC pumps work well in this application due to small size, and low noise; brushless DC water pumps (Varimax PWM coolant pump; TechAFX Inc, Bloomfield, MI) were chosen after utilizing brushed type pumps which failed after about a year of use. Wiring and further specifications and instructions on wiring can be obtained from TechAFX.

In summary, *The cage-side water circuit is plumbed to:*

    A) Cage bottom

    B) One side of the aluminum heat transfer block

    C) Water pump

    D) Water (or coolant) reservoir

The waste energy water circuit is plumbed to:

    A) A small radiator (A new vehicle heater core with dimensions of roughly 10"x10"x1-2" works great)

    B) The other side of the aluminum heat transfer block

    C) Water pump

    D) Water (or coolant) reservoir.

Small in-line Schrader valves can be placed in-line with the water circuits at the top of locations that may accumulate water bubbles. The system must be free of trapped air, something critical if coolant is to be

used for cooler temperatures as foaming will occur; a water-only system will not foam as much but it does reduce system efficiency.

Tuning the system

The TC-720 operates using PWM modulation and uses PID control (Proportional, Integral, Derivative) to regulate the output to the peltier modules. Tuning this type of system requires trial and error time and each system will be unique; if a setup is designed around my design, settings should be similar: Proportional= 0.75; Integral = 10; Derivative = 4. The settings will change depending on temperature differential between the peltier modules and ambient temperature that the radiator is located in. A relatively stable ambient temperature is very important or the PID control will attempt to hone in on the target temperature but will oscillate around it. It's easiest to change these settings after installing TC-720 control software on a PC in addition to serving as a datalogging device for the primary temperature sensor used for cage control and the secondary sensor. Further guidance for using this software can be obtained through TE Technology, Inc.

Further reference materials for the dial-a-temp control unit configuration may be found after figure B1 with photos being taken during construction. The cage floor and heat transfer blocks were welded beforehand and pictures were not taken but construction can be inferred from the above text and configuration pictures below.

Methods for obtaining baseline metabolic rate in rats

All animal procedures were approved by the IACUC at the University of Alaska Fairbanks. Eight naïve Sprague-Dawley rats (UAF colony, derived from rats obtained from Simonson Laboratories, Gilroy, CA) were placed into the dial-a-temp metabolic chamber to obtain baseline metabolic rate in rats vs. surface temperature. The device was programmed to start at 16°C and ramping automatically to 20°C, 24°C,

28°C, 32°C, 34°C and 36°C with a one hour holding (soaking) time at each temperature. Open flow respirometry was used to measure oxygen consumption according to methods established in (Toien, 2013)

2.3 Results:

The dial-a-temp cage automatic temperature ramp and holding programming was tested and then implemented in-order to obtain baseline metabolic rate (figure 2.2 and 2.3). Results using the average minimum O_2 consumption indicate that the basal metabolic rate for rats on a 32-36°C cage surface is approximately 0.9-0.85 mL $O_2 \cdot g^{-1} \cdot h^{-1}$ and rises as cage temperature decreases. This data shows that the dial-a-temp cage is capable of influencing body temperature by surface transfer and can be used to define the surface thermoneutral zone of an animal.

2.4 Discussion:

Some methods of temperature management in humans involves surface cooling achieved through placement of icepacks (Bernard et al., 2002) or water cooled pads with pad temperature adjusted based on target and core body temperature (Arctic Sun™). Here we describe a method for use in rodents that replicates the surface cooling approach used in humans. We found that controlling cage surface temperature provided rapid and effective modulation of core body temperature in animals treated with a drug

2.5 References

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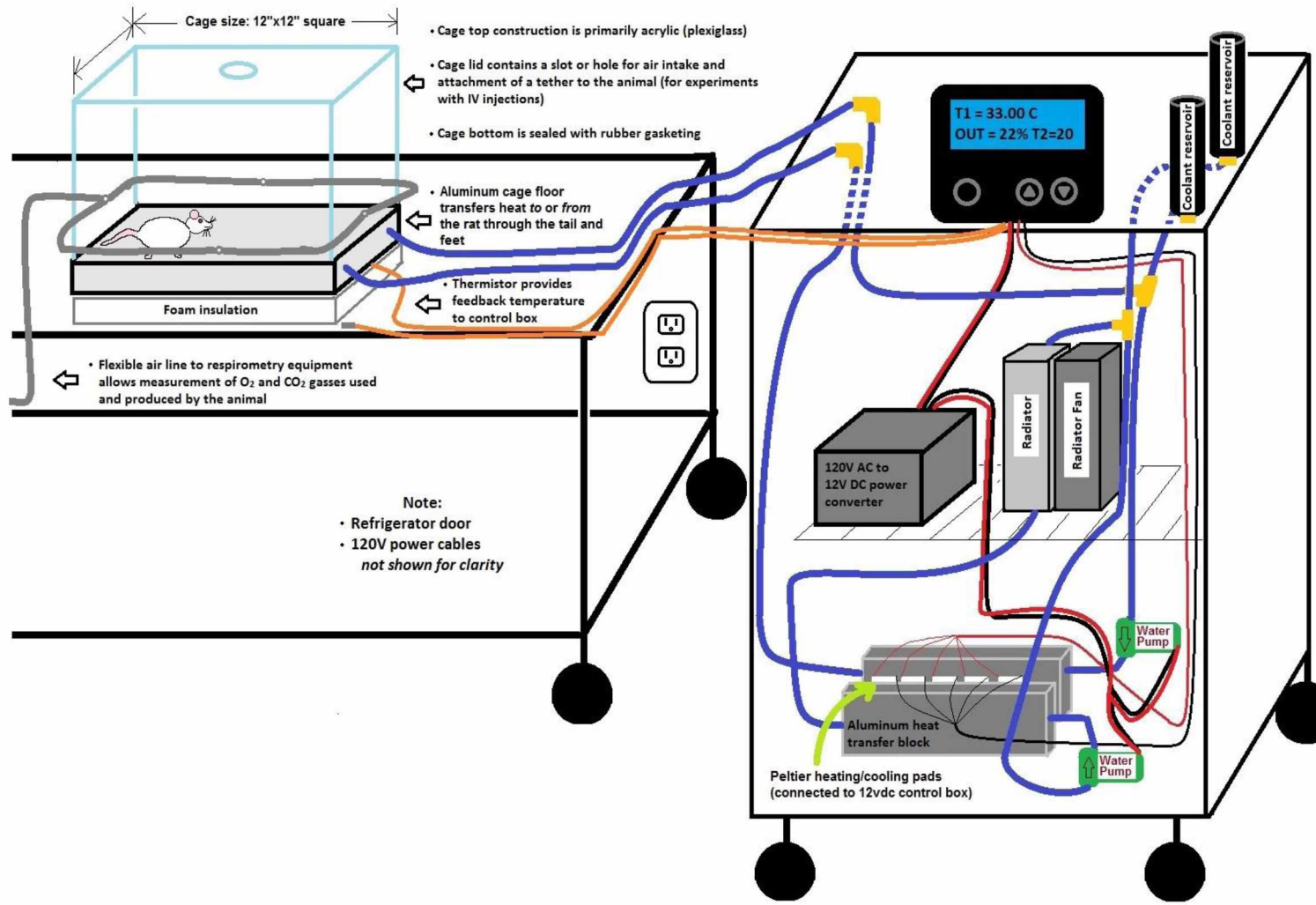
Cage size: 12"x12" square

- Cage top construction is primarily acrylic (plexiglass)
- Cage lid contains a slot or hole for air intake and attachment of a tether to the animal (for experiments with IV injections)
- Cage bottom is sealed with rubber gasketing
- Aluminum cage floor transfers heat *to* or *from* the rat through the tail and feet
- Thermistor provides feedback temperature to control box
- Flexible air line to respirometry equipment allows measurement of O$_2$ and CO$_2$ gasses used and produced by the animal

Foam insulation

Note:
- Refrigerator door
- 120V power cables
  not shown for clarity

T1 = 33.00 C
OUT = 22% T2=20

Coolant reservoir
Coolant reservoir

120V AC to 12V DC power converter

Radiator
Radiator Fan

Water Pump
Water Pump

Aluminum heat transfer block

Peltier heating/cooling pads (connected to 12vdc control box)

Figure 2.1: The Dial-a-temp cage in optimal configuration for studying hypothermia in small animals.

Eqiupment enclosure in a refrigerator allows faster and more significant temperature decreases.
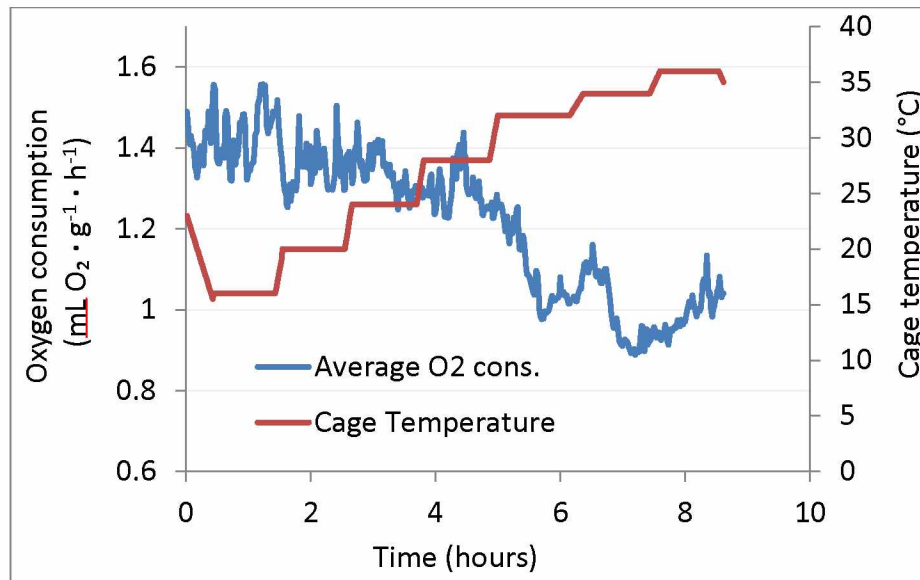
48

Figure 2.2: Naïve Sprague-dawley rats decrease oxygen consumption as surface temperature increases
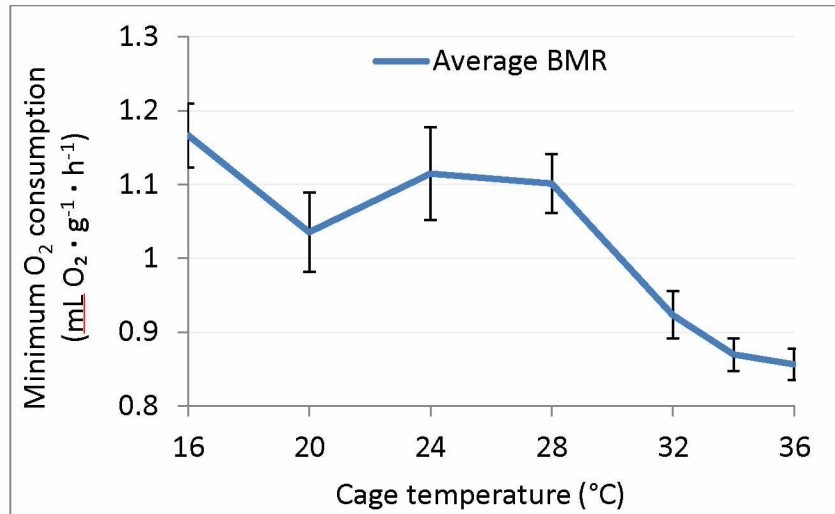
(mean, n=8)

Figure 2.3: The thermoneutral zone was defined by the surface temperature that minimized O_2 consumption. (mean ± SEM, n=8)

General Conclusion:

This thesis asked how to optimize body temperature control with pharmacological agents and ambient temperature control. Prior work using pharmacological agents fail to include ambient or surface temperature control as a parameter in body temperature control. This is despite the fact that clinical application of targeted temperature control often relies on surface temperature to control body temperature (Don et al., 2009). Our work shows that evaluating the performance of thermolytics needs to be done using both dose and ambient or surface temperature. Previous thermolytic agents examined may have more potential than previously thought if research protocols were refined to include a dynamic temperature control device such as the dial-a-temp. Therefore, it is my recommendation that this type of device should be used in the study of future thermolytic agents in order to minimize the use of animals when assessing thermolytic efficacy of pharmacological agents.

The performance of CHA as a thermolytic is promising, but more work needs to be done in-order to establish if the observed bradycardia is problematic. I am confident that thermolytics such as CHA will be delivered in emergency situations in the future, either by itself or in combination with other co-treatments in-order to maximize therapeutic benefit. I am also confident that this approach will be extremely useful in translating aspects of thermoregulation and metabolism to humans in-order to minimize the risks associated with long-term spaceflight. In conclusion, reproducibility is the key when transferring methods from lab to lab in the pursuit of scientific advancement so it is my pleasure in providing the scientific community the dial-a-temp approach.

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