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DEVELOPMENT OF A RAT MODEL OF HYPOTHERMIA

David A. DuBose
David H. Morehouse
Dennis Rufolo
Michael Blaha
Lisa R. Leon

Thermal and Mountain Division

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U.S. Army Research Institute of Environmental Medicine
Natick, MA 01760-5007

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BACKGROUND

As reviewed by Hamlet (4) and Vaughn (18), United States military history has been significantly impacted by cold. It has been recognized as a source of disease among soldiers since the days of the Continental Army during the fight for independence from England. During World War II, more than 7.5 million man-days were lost due to cold injury. Approximately 9,000 cold injuries were experienced in the Korean Conflict. Trenchfoot, frostbite and hypothermia are the major contributors to militarily-related cold injury. However, hypothermia may present a special challenge, since it is geographically and seasonally pervasive, giving it the potential to occur in almost any military setting (2). This compounded by the fact that military operation stress (MOS=exhaustive exercise, caloric restriction and sleep deprivation) may render the soldier uniquely vulnerable to hypothermia illustrates the importance of enhancing the hypothermia knowledge base.

In regard to MOS, it is believed to have contributed to the hypothermia casualties, four of which were lethal that occurred in February of 1995 at the U.S. Army Ranger Training School located in Florida (16). Anecdotal evidence suggests, these Ranger trainees succumbed to relatively moderate hypothermia (core temperature [T_c] decrease of $5\pm 3^\circ\text{C}$), from which they should have been successfully resuscitated, but expired because of the impact of MOS. USARIEM laboratory studies including those conducted with Ranger trainees suggest the threat of MOS-related hypothermia is real (17). However, because of the danger posed to their health, full evaluation of MOS contributions to hypothermia morbidity in human volunteers is unethical. To define how individually and/or concomitantly the various aspects of MOS influence hypothermia and the progression to mortality, an animal model is required. Knowledge gained through animal modeling could impact training regimens and military operations to reduce the influence of MOS on hypothermia in a manner that best supports performance enhancement to ensure mission achievement. Moreover, the availability of well-defined models of various aspects of MOS-related hypothermia may permit prediction of outcomes under a variety of military scenarios. Such a predictive capacity may contribute to casualty reduction associated with special military operations. Thus, an animal model for hypothermia has military relevance. This report describes the methodology used in the development of a rat model of hypothermia. It represents the initial phase in the eventual study of MOS influences on hypothermia morbidity and mortality.

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EXECUTIVE SUMMARY

Hypothermia can significantly impact the outcome of military missions, since it is a seasonal and geographic pervasive physiological phenomenon that reduces not only soldier performance, but may lead to their death. Moreover, MOS such as exhaustive exercise, caloric restriction and sleep deprivation may enhance soldier vulnerability to hypothermia. Understanding the full influence of MOS on hypothermia morbidity and mortality requires an animal model, since ethical considerations in regards to their health preclude the use of human volunteers. A model of hypothermia was developed that employed rats (male; Sprague-Dawley; 250.5 ± 7.3 g) immediately exposed to circulating (0.7 ± 0.3 L/min) cool (10°C) water at a non-full immersion depth of 5 cm. During exposure to cool/wet conditions animals assumed a water avoidance posture of an upright position such that only their hindquarters were exposed to the water. Moderate hypothermia ($32 \pm 3^{\circ}\text{C}$) was induced within a 2 to 4h timeframe in which animal activity and, core (T_c) and brown adipose tissue (BAT) temperature could be monitored. Thermoregulatory temperature profiles for hypothermia induction and recovery were variable among the animals. Markers of hypothermia induction and recovery were identified as: 1) lowest hypothermia T_c ; 2) time to lowest hypothermia T_c ; 3) thermoregulatory maintenance time post lowest hypothermia T_c ; 4) cooling rate; 5) 37°C T_c recovery time from lowest hypothermia T_c ; 6) re-warming rate and 7) length of BAT thermogenic response. This rat model features a hypothermia induction vehicle (immediate cool water exposure) and vehicle exposure pattern (lower extremities) that reflects the militarily relevant scenario of sudden soldier exposure to waist-deep cool water. Its military relevance in conjunction with the identified hypothermia induction and recovery criteria make this model well suited to characterize the influence of MOS on hypothermia morbidity and mortality.

INTRODUCTION

In February of 1995 during student training in Florida at the U.S. Army Ranger Training School, hypothermia casualties resulted, four of which were lethal (16). In this case, trainees were vigorously conducting night exercises in which they suddenly encountered waist-deep water of a temperature ranging from 10 to 15°C. They were under the influence of cool/wet conditions either in or out of the water for a 3 to 5 hr period. It is speculated these trainees succumbed to hypothermia from which they should have recovered, because of the impact of MOS that contributed to thermoregulatory fatigue. Findings (17) that trainees exposed to cold stress (10°C air exposure) within 2h of completion of their nine-week training program have an impaired ability to maintain normal body temperature support this contention. To elucidate which MOS component most contributes to thermoregulatory fatigue, a hypothermia animal model is required. Unfortunately, most hyperthermia models employ anesthesia (3,4-8) or restrain (10, 13-15) that compromise normal thermoregulatory physiological mechanisms or animal behavior. The present report evaluated several cool/wet exposure methods (gradual and immediate) to induce hypothermia in free-ranging rats in a manner that avoided these confounders. The impetus for exploring these various methods was to develop procedures that could account for the ancillary stress associated with an animal's novel experience of being exposed to water. The conditions for the cool/wet exposure associated with Ranger hypothermia morbidity and mortality were used to guide model development. This militarily relevant hypothermia vehicle (cool water) was fortuitous in regards to rats, since they resist T_c reductions for relatively long periods of time when exposed to cool/dry air (4 to 6°C; 5,9), however in cold water (2°C) such reductions occur more rapidly (12). It was hypothesized free-ranging rats exposed to cool water (10°C), at a non-swimming depth (i.e., not full immersion) would experience high anxiety, but would achieve moderate hypothermia ($T_c=32\pm3^\circ\text{C}$) in a 3 to 5h timeframe. Methods to monitor BAT and tail skin temperature were also evaluated. Finally, induction and recovery parameters to characterize a bout of rat hypothermia were defined.

METHODS

Male Sprague-Dawley rats (~60g) were obtained from Harlan Inc. (Indianapolis, IN) and maintained under the NIH Guide for the Care and Use of Laboratory Animals and AAALAC. IACUC approved all study procedures. They were singly housed in polycarbonate cages (50 x 26.8 x 36.4 cm) with wood chip bedding (Pro-Chip, PWI Canada) to permit normal burrowing and behavioral thermoregulation. Rats were given rodent laboratory chow (Harland Teklad, LM-485; Madison WI) and water ad libitum, and maintained at $25\pm2^\circ\text{C}$ in a 12 h light/dark cycle (0700 lights on). During 2 weeks of quarantine, rats were weighed daily to monitor health status. In addition, each day a thermistor (YSI Model 427; Springfield, NJ) was taped to the ventral surface of the tail at the border of its proximal and middle third and allowed to remain there for 2 to 3 mins before removal to accustom the rats to this handling procedure.

Biotelemetry was employed to gather T_c and animal motor activity data, while dataloggers were used to record BAT or subdermal tissue (SDT) temperatures (T_{BAT} or T_{SDT}). The use of such technology reduces the confounding influences of animal handling and restraint that can be associated when employing rectal probes and thermistors. In biotelemetry, transmitting devices emit a frequency proportional to T_c that can be collected at 1-min intervals by a receiver board placed beneath the cage and/or experimental tank. Signals are converted to T_c using pre-determined calibration values. Motor activity is detected by changes in signal strength as the animal moves over the receiver board. This is a general measure of activity, since it can not distinguish the type of locomotor action. Dataloggers are 1.5 cm diameter x 0.5 cm thick cylinders weighing 4.1 ± 0.02 g with a temperature sensing element in the center of the device. These devices do not provide real time viewing of the data, since they require removal from the animal for down-loading of the stored data. Prior to implantation, biotelemetry transmitters (ETA-F20; 4.1 ± 0.01 g; Data Sciences International, St. Paul, MN) and dataloggers (Subcue, Calgary, Canada) were calibrated, the correction factor for each datalogger determined and the dataloggers programmed to initiate data collection for 1.4 days starting at 0700 on the day of experimentation. Following the rat quarantine period, transmitters for T_c and motor activity measurements were implanted by suturing them to the inside abdominal wall just caudal to the sternum, while dataloggers were implanted beneath the dermal skin layer over the subscapular BAT deposit or caudal to this deposit for measurement of T_{BAT} or T_{SDT} , respectively. At the time of surgery, rats were provided intraperitoneally indomethacin (1mg/kg) and ampicillin® (100mg/kg). The following day, rats were given impregnated in an oral treat (BioServ; Frenchtown, NJ) another dose of indomethacin. Oral provision avoided animal handling and analgesic injection stress, which potentially reduced post surgical recovery time. Each day post surgery, rats were weighed and accustomed to the tail thermistor. Seven days post surgery, when pre-surgical body weight (165.4 ± 3.8 g) was exceeded (214.7 ± 3.9) and a robust circadian rhythm for T_c and motor activity established, rats ($N=9$) with tail thermistors were exposed in a counterbalance design to cool/wet or ambient/dry conditions. There was a minimum of 48h of recovery between conditions. The exposure tanks employed were similar to housing cages in dimension (43.5 X 24.7 X 30.6 cm) and like housing cages had water sipper openings (1.9 cm diameter; 8.3 cm from tank bottom) on opposite sides of the tank that were clear of obstruction. The cool/wet tank (Fig. 1) was attached to a refrigerated circulating water bath (Thermo Electron; Newington, NH) by a port from which water could be delivered (0.7 ± 0.3 L/min) into the tank. Diagonal to the water entry port was a siphon tube that was connected to the return vacuum of the water bath. Water depth was regulated by adjusting the distance of end of the siphon tube from the tank bottom. Time 0 (between 0900 and 1130 h) was defined as the 12 h average T_c ($37.27 \pm 0.02^\circ\text{C}$) and activity (1.51 ± 0.16) nadir for rats under the conditions of the USARIEM animal facility. Initiation at nadir values avoided confounding influences of circadian variations in T_c and activity on elicited responses during experimentation. At Time 0, a rat was placed in each of two tanks. One tank remained dry and exposed to ambient air (ambient/dry; $25 \pm 2^\circ\text{C}$) to capture in the absence of water, the robust T_c and motor activity responses associated with rat subjection to a novel environment. After

placement of a rat in the other tank, water ($\sim 37^{\circ}\text{C}$) was allowed to enter until it reached a depth of 4 cm. At which time, water temperature was stabilized at $35 \pm 0.1^{\circ}\text{C}$. The circulating refrigerated water bath reservoir was then directed to obtain an 8.5°C temperature. This cooled the tank water to $\sim 10^{\circ}\text{C}$ (cool/wet) in approximately 1 h to subject the rat to hypothermia. This method was defined as gradual cool water exposure. During rat exposure to the cool/wet or ambient/dry conditions, animal behavior was continuously observed by personnel in the room to ensure their safety. Rats remained in the cool/wet condition until their T_c was reduced to $32 \pm 3^{\circ}\text{C}$. This was defined as a moderate hypothermic exposure, which was selected since it was believed similar to that in Ranger trainee hypothermia cases (16). At moderate hypothermia, rats were removed from both the cool/wet and the ambient/dry tank, and allowed to recover passively in their home cage. The tail skin thermistor was removed when the rat would no longer tolerate its presence, as determined by agitation and attempts to physically remove the thermistor (i.e., chewing/scratching behavior).

Subsequent studies followed modified procedures from those as described above. Rats were studied after 2 rather than 1 week of surgical recovery to support testing of post surgery analgesia procedures not specifically related to the present protocol. The 2 week surgical recovery period ensured adequate body clearance of the different analgesics employed. Such concurrent testing reduces animal use, a major goal of Department of Defense-related research. To facilitate animal comfort and stability during forepaw support of animal posture with water exposure, tanks were modified to include a small shelf beneath the sipper opening (Fig. 1). Tail thermistors were no longer used and personnel no longer remained in the experimental room after the animals were placed in their respective exposure tank. All other modifications were related to the development of an immediate cool/wet exposure method. As such, rather than first exposing animals to 35°C water and then initiating the cooling of the water to 10°C , rats were exposed directly to 10°C water. Water depth was increased from 4 to 5 cm to accommodate the increase in rat body weight associated with the 2 rather than 1 week of surgical recovery. Since water depth was increased, the subdermal tissue datalogger was made less caudal, while the BAT datalogger placement was made more rostral to the BAT deposit. This adjustment in datalogger placement ensured the fur/skin surface above the subdermal tissue datalogger would avoid water immersion that might confound measurements. It also ensured needed separation between the subdermal and BAT dataloggers. In addition to an ambient/dry and cool/wet exposure, rats were exposed to a warm/wet condition in which they were placed directly into 5 cm of 35°C water. Finally, rather than removing animals from the tanks when the rat in the cool/wet condition achieved a $T_c = 32 \pm 3^{\circ}\text{C}$, rats remained in each condition for a set time period of 4h.

To examine the influence of immediate exposure to 35°C water on animal T_c and activity, rats ($N=8$) were exposed (4 h) in a counter balance design to either the ambient/dry or warm/wet condition. Subdermal and BAT datalogger measurements were not made. Animals were permitted a 48h recovery between conditions. Next, rat ($N=24$) thermoregulatory profiles for T_c , T_{BAT} and T_{SDT} were characterized for the

cool/wet, warm/wet or ambient/dry conditions. Counter balance was not employed, since dataloggers could not be re-programmed among conditions without subjecting the animal to an additional bout of anesthesia and surgery.

Data are described as means $\pm$ SEM. T_c is presented as either individual rat 1-min values or group means for cool/wet, warm/wet and/or ambient/dry conditions. Two-way ANOVA with repeated measures followed by Holm-Sidak post hoc testing was employed to determine significant differences in T_c or activity profiles between selected groups. One way ANOVA with repeated measure followed by Tukey post hoc testing distinguished significant differences in temperature over time. One-way ANOVA determined differences in group effects between the gradual or immediate methods of cool water exposure for characteristic features of hypothermia induction and recovery. Significance was set at $p < 0.05$.

RESULTS

In the gradual exposure method, when rats were placed in the dry tanks there was an initial period of exploration. When water began filling the designated wet tank, animal activity decreased, as the rat spent an increasing amount of time at the sipper hole, grasping it with its forepaws. With this forepaw placement, the animal assumed a sitting or crouch-like position, while balancing itself on its toes. As water depth rose and then temperature dropped, this behavior became more pronounced, such that from the time water temperature fell below 20°C to the end of the 10°C cool water exposure, animal activity in cool/wet was significantly less than in the ambient/dry condition (Fig. 2). The immersion of the tail in water discouraged the rat from removing the tail thermistor. However, rats in the dry tank had to be distracted to keep them from removing their tail thermistor.

Figure 3 illustrates water cooling to 10°C and its influence on tail skin temperature. As ~37°C water began filling the tank, it was initially cooled by exposure to the surface of the empty tank, until by recirculation through the heating water bath; tank water achieved a $35 \pm 0.2^\circ\text{C}$ temperature and a depth of 4 cm. When the recirculating water bath reservoir temperature was decreased, tank water temperature began to decrease such that in ~1h it oscillated at $10 \pm 1^\circ\text{C}$. As illustrated in Figure 3, tail skin temperature closely mirrored the changes in tank water temperature. When removed from the cool water after 240 min, rat tail temperature rapidly rebounded. However, tail recordings had to be stopped in ≤ 30 min, since once removed from the cool water after some initial grooming, the rats began to direct their attention to this attached device by gnawing on the thermistor.

As shown in Figure 4a for cool/wet or ambient/dry mean values, Time 0 placement in the novel environment of the tanks induced (~0 thru 30 min) a stress-related hyperthermia in the rats. This was not more pronounced ($p>0.05$) in animals experiencing water exposure. In the ambient/dry condition, the stress-induced hyperthermia gradually dissipated over time. However, in the cool/wet condition, the stress-induced hyperthermia was rapidly driven down by the cooling of the tank water. Exposure to the cool water over time induced significant ($p<0.05$) moderate hypothermia ($31.7\pm0.4^{\circ}\text{C}$) in the rats. It was characterized by variability in the individual thermoregulation T_c profiles for hypothermia induction (Fig. 4b) and recovery to $T_c=37^{\circ}\text{C}$ (Fig. 4c). While hypothermia recovery was characterized by a T_c overshoot relative to the ambient/dry condition, this was only significantly different for a relatively small segment of time (~20 min; Fig. 4a). Table 1 defines the major features of these hypothermia induction and recovery profiles for the gradual cool water exposure method.

In the gradual exposure method from -60 to -30 min, cool/wet T_{BAT} and T_{SDT} significantly differed, however from that point to Time 0 they were similar (Fig 5a). As noted for T_c in the cool/wet condition, T_{BAT} and T_{SDT} reflected a stress-related hyperthermia starting at the Time 0 placement of the rats in the tanks. However, unlike T_c that was rapidly reduced by the cooling of the water, T_{BAT} and T_{SDT} remained above their Time 0 values during much of the cool water exposure. Over 84 to 193 min post Time 0 cool/wet T_c , T_{BAT} and T_{SDT} were significantly ($p<0.05$) different from each other. A positive T_{BAT}/T_{SDT} delta existed from 55 through 255 min (Fig. 5b).

When immediately exposed to warm (35°C) water at a depth of 5 cm, rat behavior was similar to those exposed to a gradual rising water level in which water temperature was adjusted to 35°C . Under both scenarios, rats quickly explored the novel water environment and then found the sipper opening area that allowed them to use their forepaws to support a crouched posture, while standing on their toes. As illustrated in Figure 6a, immediate exposure to the warm/wet compared to ambient/dry condition resulted in a significantly muted stress-induced hyperthermia starting at 1 min. However, by 58 min T_c responses in warm/wet and ambient/dry conditions were no longer significantly different. The muted stress-induced hyperthermia was associated with a significantly reduced level of rat activity from 1 to 12 min (Fig. 6b).

Like those exposed to warm/wet, rats immediately exposed to the cool/wet condition quickly assumed a crouched posture, supporting themselves on their toes by holding with their forepaws the shelf positioned below the sipper opening. Stress-induced hyperthermia in the rats was not noted starting at Time 0 compared to the warm/wet or ambient/dry controls (Fig. 7). Similar T_c values were seen for these controls throughout the exposure period. Following exposure, warm/wet and

ambient/dry rats experienced similar stress-induced hyperthermia upon removal from their tanks and placement in their home cages. As previously noted with gradual exposure (Fig. 4b), rats immediately exposed to 10°C had variable responses to hypothermia induction and recovery (Fig. 7). With the exception of body weight, descriptive hypothermia induction and recovery characteristics were similar for gradual or immediate exposure to cool water (Table 1).

Unlike gradual (Fig. 5a), the 1 hr pre exposure mean for T_{BAT} ($35.57 \pm 0.01^\circ\text{C}$) was significantly ($p < 0.05$) less than T_{SDT} ($36.41 \pm 0.01^\circ\text{C}$) in the immediate cool/wet exposure trials (Fig. 8a). At cool/wet exposure, there was an elevation in T_{BAT} , while T_{SDT} decreased (Fig. 8a), which resulted in similar T_{BAT}/T_{SDT} values throughout the cool wet exposure. Between 4 and 194 mins of cool/wet exposure, T_{BAT} demonstrated a significant ($p < 0.05$) positive thermogenic response relative to its mean Time 0 value (Fig. 8b). This contrasted with T_{SDT} that was significantly ($p < 0.05$) decreased from its mean Time 0 value (Fig. 8c) from 26 thru 146 min. Small rat numbers ($N=8$) in conjunction with increased T_{SDT} variability after 146 min prevented a finding of significant decreases for the entire 240 min of cool/wet exposure. While generally T_{SDT} remained $> T_{BAT}$, these values in both the ambient/dry and the warm/wet condition followed a pattern of first stress-induced temperature elevation and then a slow heat dissipation over time (data not shown).

DISCUSSION

In the development of a hypothermia model that employs water to reduce body temperature, consideration must be given to the psychological stress placed on an animal that has never experienced and perhaps is unlikely to be accustomed to water exposure. Such stress could be a major confounder of thermoregulatory responses, since the novelty of water exposure is not necessarily an issue for humans. It was speculated water exposure would lead to extreme rat agitation as a result of the rat attempting to extricate itself from this novel and threatening environment. It was for this reason that a gradual exposure method was explored, since a gradual water rise and temperature drop might be less threatening. With the gradual exposure method, after an initial exploratory period the rat directed its attention to the sipper opening in the tank, perhaps as a potential escape route. Then as water level gradually rose, the rat used the sipper opening to support itself in a crouched position while standing on its toes. This posture appeared to support the animal's desire to keep as much of its torso out of the water as possible. As such, rat movement was minimal (Fig. 2), since movement away from the sipper hole meant the enhanced potential of slipping from the tank wall into the water. Outwardly, the rat appear relative calm during water exposure, which was reflected by a similar stress-induce hyperthermia over the first 30 min in the gradual cool/wet exposure or ambient/dry condition (Fig. 4). While this suggested water stress did not compound the anxiety of being placed in the novel tank environment, similar T_c may only indicate both conditions were sufficient to obtain the maximum T_c .

inducible by a psychological stress. On the other hand, evaporative cooling on those portions of the rat body that became wet, but which remained above the water may have partially muted stress-induced hyperthermia. Thus, potential confounders clouded the contribution of water on the stress-induced hyperthermia seen in the cool/wet exposures.

Prior to initiation of cooling, dissipation of stress-induced hyperthermia associated with tank and/or water exposure is the ideal. Repeated attempts over 4 days to acclimate rats to the procedures of first placement in a dry tank, waiting for stress-induced hyperthermia to dissipate, then exposure to 35°C water and again waiting for stress-induced hyperthermia to dissipate were without success, since the lengthy time to dissipate body heat did not sufficiently decrease with each subsequent exposure to allow completion of a cooling experiment without interjection of circadian confounders (data not shown). However, when tanks were employed with a shelf below the sipper opening (Fig. 1) to support rat comfort and reduce their slippage from the tank wall into the water when it assumed a typical water avoidance posture, rats placed immediately in 5 cm of 35°C water had significantly reduced T_c (Fig. 6a) and activity (Fig. 6b) compared to ambient/dry rats. Moreover, within ~60 min the stress-induced hyperthermia of ambient/dry rats dissipated such that T_c differences between groups were no longer apparent (Fig. 6a). While this appeared to be a means in which cooling experiments using a gradual exposure design could be initiated when rats were of a similar physiological state, there were still potential confounders embedded with water exposure. For example, it could be assumed that placement in a tank containing water was not less stressful than placement in a dry tank. Thus, the significantly reduced stress-related T_c in warm/wet compared to ambient/dry rats (Fig. 6a) was likely an artifact of the water exposure-related reduction in activity (Fig. 6b) and the evaporative cooling of non-immersed but wetted body areas of warm/wet-exposed rats. While this ultimately led to rats with similar T_c , the pathways to this point was perhaps so different that the milieu of physiological mediators existing in the groups would also be quite different. Thus, full dissipation of the contributions of the ancillary stress of water exposure was perhaps not possible. This was not necessarily fatal to model development, since a design employing immediate exposure that included not only an ambient/dry, but a warm/wet condition in which water presence, not temperature was the major mediator of animal responses provided controls to account for the contributions made by the ancillary stress of tank or water exposure, respectively. In addition, an immediate, rather than a gradual exposure design in which animals maintained an upright posture was militarily relevant, as exemplified by the experience of Ranger trainees who were suddenly exposed to waist-deep cool water that led to hypothermia (10). Finally, motivation for hypothermia model development was the eventual study of MOS influences on thermoregulatory fatigue, which is best supported by an immediate exposure, since this, unlike gradual exposure does not allowing for recovery time from MOS factors like exhaustive exercise before cooling is initiated.

Immediate exposure to cool water eliminated the stress-induced hyperthermia seen in the ambient/dry or warm/wet controls (Fig. 7). Rat behavior and variability in

rat hypothermia induction and recovery thermoregulatory profiles (Fig. 7) were similar to that as noted when a gradual exposure format (Fig. 4b,c) was employed. In addition, no differences in the hypothermia induction and recovery characteristics between gradual or immediate exposure methods were noted (Table 1). The immediate cooling format could be assumed to have an enhanced cooling power, since with gradual cooling ~1 h was required before tank temperature reached 10°C (Fig. 3). However, this did not result in a significantly enhanced cooling rate with immediate exposure, which perhaps reflected the significantly greater body weight of the immediate- compared to the gradual-exposed rats (Table 1). That no differences in rewarming rate existed (Table 1) in rats with significantly different body weights suggested higher metabolic rate to rewarm the larger mass of the heavy rats and/or a surface area to mass ratio in the lighter rats that favored enhanced heat loss through evaporative cooling while animals dried following removal from the cool/wet tank. No difference in lowest T_c or hypothermia induction time even though gradual-exposed rats were significantly lighter (Table 1) was likely another influence of the differences in the gradual relative to immediate exposure procedures. Finally, a beneficial feature of holding cool/wet exposure time constant (4h), as employed in the immediate exposure method was the ability to identify an animal's capacity to maintain thermoregulation after achieving its lowest T_c (Table 1). This could be an important marker in future studies of MOS-related influences on thermoregulation in the cold.

In addition to the considerations of water as a novel and threatening environment, a rat cool/wet hypothermia model should monitor those sites that influence rat T_c when challenged by cold. Small mammals employ BAT to generate heat to sustain body temperature during cold exposure (1). Moreover, blood flow to the tail is restricted during cold challenge to reduce temperature loss from the core (11). Presently, our telemetry system (Data Sciences International, St. Paul, MN) allows only one site of temperature measurement, which in the current study was at the intraperitoneal attachment of the transmitter to the inner abdominal wall. Other systems (Integrated Telemetry Services; Pinckney, MI) could accommodate measurement at multiple sites within the same animal; however after significant investment over many years in the present system conversion to a new system would be prohibitively expensive. As such, the Data Sciences® telemetry system, dataloggers and external thermistors were employed to monitor concurrently T_c , T_{BAT} and tail skin temperature, respectively. As hypothesized, T_{BAT} remained greater than T_{SDT} , when T_c was reduced during hypothermia in the gradual exposure method (Fig. 5a). This demonstrated datalogger capacity to detect BAT heat production during hypothermia, while the positive T_{BAT}/T_{SDT} delta (Fig. 5b) indicated dataloggers could distinguish body temperature differences at or away from BAT deposits. In the immediate exposure method, dataloggers again demonstrated a significant positive thermogenic response by BAT (8b). However, differences at or away from BAT deposits were not distinguished, since T_{BAT} elevation concurrent with T_{SDT} decrease resulted in similar T_{BAT}/T_{SDT} values throughout the cool/wet exposure period (Fig. 8a). This likely resulted because T_{SDT} before and after cool/wet exposure consistently ran above T_{BAT} (Fig. 8a). Moreover, less than optimal BAT and subdermal tissue datalogger placement to accommodate water depth issues in the immediate exposure method may have played a role in this finding. Dataloggers do not provide a discrete point of temperature measurement, since

the temperature sensing element is embedded in the center of the 1.5 cm diameter x 0.5 cm thick device. Thus, appropriate datalogger placement is critical. As such, the use of only a BAT datalogger to ensure placement over the BAT deposit is not compromised by the need to provide adequate separation space from a subdermal tissue datalogger is advisable. In addition to placement issues, datalogger limitations included a fixed window for temperature recording and absence of real time viewing of temperature data. Such limitations interfered with employing a counter balance design and reduce optimization of experiment initiation time relative to starting T_c , T_{BAT} and T_{SDT} . Tail thermistors showed that tail temperature closely followed that of water temperature, which rapidly rose once removed from the water (Fig. 3). Unfortunately, the use of exterior tail thermistors lessened the free-ranging nature of the rats and required human intervention to deter their removal. Though tail skin temperature data is an important element in rat hypothermia modeling, confounders associated with external thermistors made acquisition of such data too problematic to be incorporated into the present model design. Dataloggers could not be used to monitor this site, because of their size and inability to provide a discrete point of temperature measurement.

CONCLUSIONS

A rat model of hypothermia employing cool water was developed. The psychological stress associated with water exposure was identified as a potential confounder. Since it could not be adequately or fully dissipated, a warm/wet control condition was instituted in which water presence not temperature was the salient factor driving animal responses. This should permit water stress contributions to be factored out from those inducing a hypothermia episode in the cool/wet condition. Similarly, the contributions made by stress associated with removal from the home cage to the novel environment of an exposure tank could be captured by the ambient/dry condition. Employing such controls for water stress and placement in a novel environment, immediate rather than gradual cool/water exposure was determined to be not only feasible, but militarily relevant.

Cool water exposure was associated with the rat assuming an upright posture such that only the lower hindquarters of the animal were immersed in water. This was a fortuitous finding in regard to military relevance, since in the Ranger trainee hypothermia cases immersion was only below the waist. Application of a small shelf to assist forepaw support of the animal's water-avoidance posture appeared to improve rat comfort and stability during water exposure. Cool water exposure induced moderate hypothermia ($32 \pm 3^\circ\text{C}$) within 2 to 4 hrs. Hypothermia induction and recovery thermoregulatory profiles showed wide variability. Markers of hypothermia induction and recovery were identified as: 1) lowest hypothermia T_c , 2) time to lowest hypothermia T_c , 3) cooling rate, 4) thermoregulatory maintenance time post lowest hypothermia T_c , 5) 37°C T_c recovery time from lowest hypothermia T_c and 6) rewarming rate.

Unfortunately, external thermistors for tail skin temperature measurements imparted strong confounders to the model. As such, this measurement could not be incorporated into the design. Dataloggers optimally positioned over BAT deposits permitted BAT thermogenic response monitoring during a hypothermic episode. Length of BAT thermogenic response could provide an important marker to characterize hypothermia induction.

In conclusion, rats instrumented with biotelemetry transmitters and dataloggers, and subjected to cool/wet, ambient/dry or warm/wet conditions provided a model for the induction of moderate hypothermia. An experimental design in which animals assumed an upright posture when exposed immediately to the cool/wet environment had similar characteristics to that associated with the hypothermia casualties suffered by the 1995 Ranger trainees. Such a model could contribute to the study of MOS-related thermoregulatory fatigue contributions to hypothermia morbidity and mortality.

RECOMMENDATIONS

It has been demonstrated that rats exposed to cool/wet conditions do develop moderate hypothermia within a workable timeframe. Moreover, the ancillary stress contributions of placement in a novel environment and water exposure can be accounted for within the model design. It is recommended that such a model be used to identify the physiological mediators that drive this cold exposure phenomenon. Moreover, it is recommended this model be employed to study the influence of MOS on hypothermia morbidity and mortality.

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Figure 1. Cool or Warm/Wet Exposure Tank

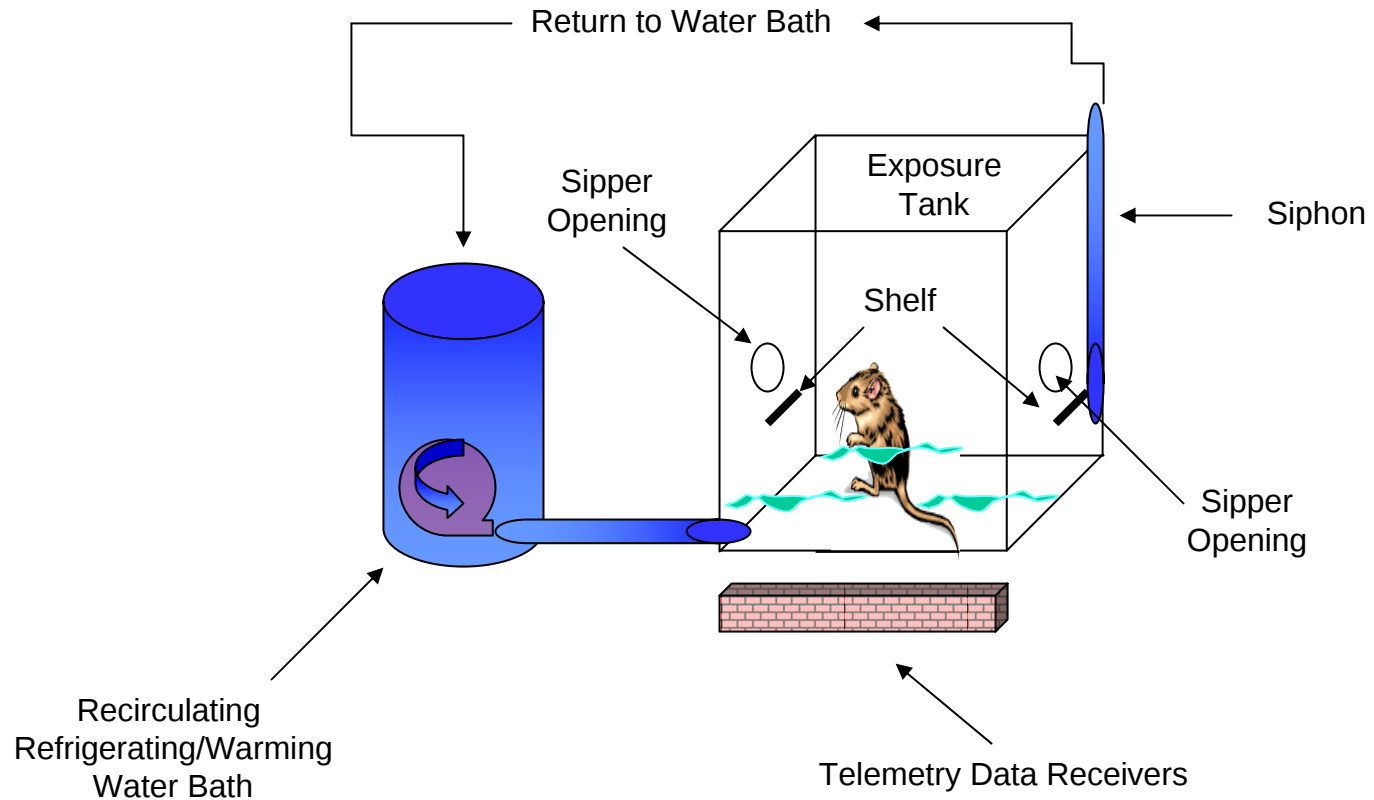


Figure 2. Rat Activity in Gradual Cool/Wet (<20°C) or Ambient/Dry Tank Exposure

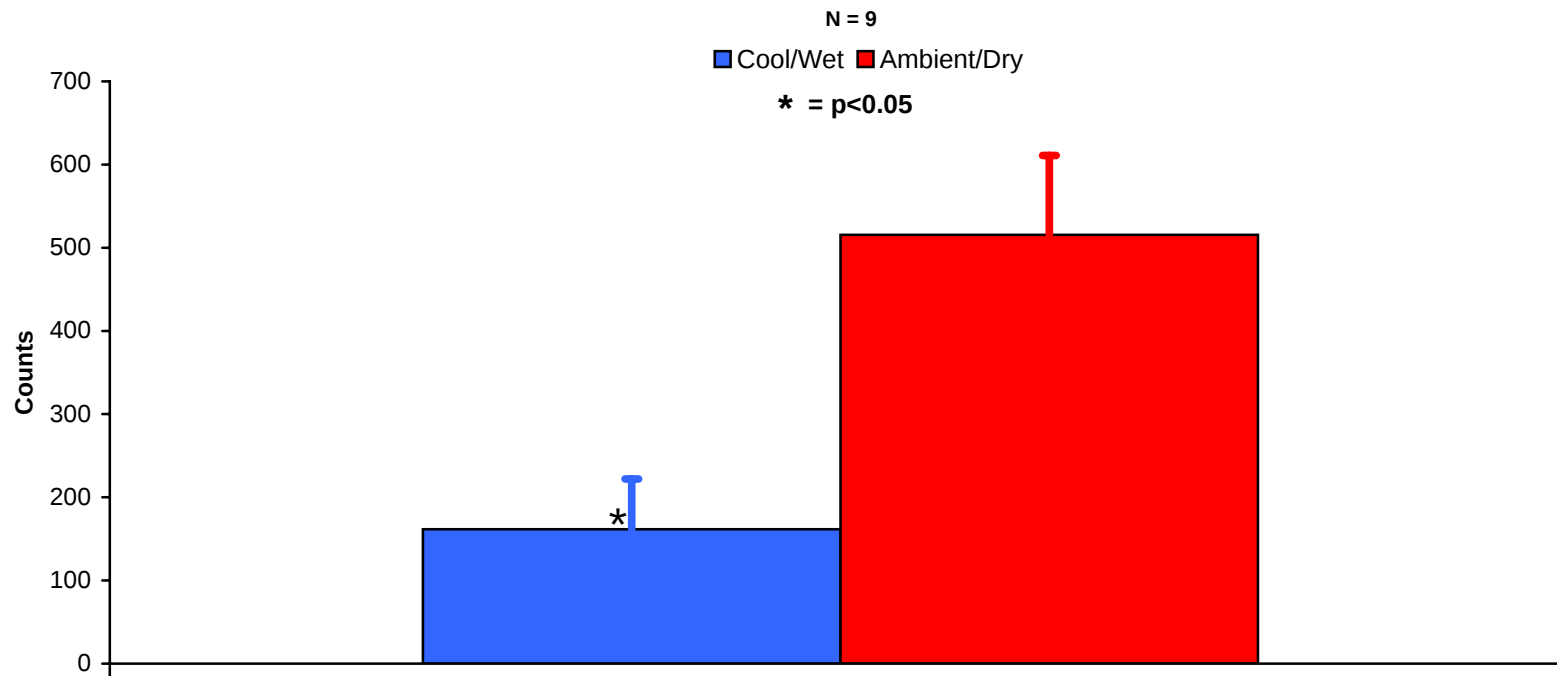


Figure 3. Illustration of the Changes in Rat Tail Skin Temperature Pre and Post Gradual Cool/Wet Exposure

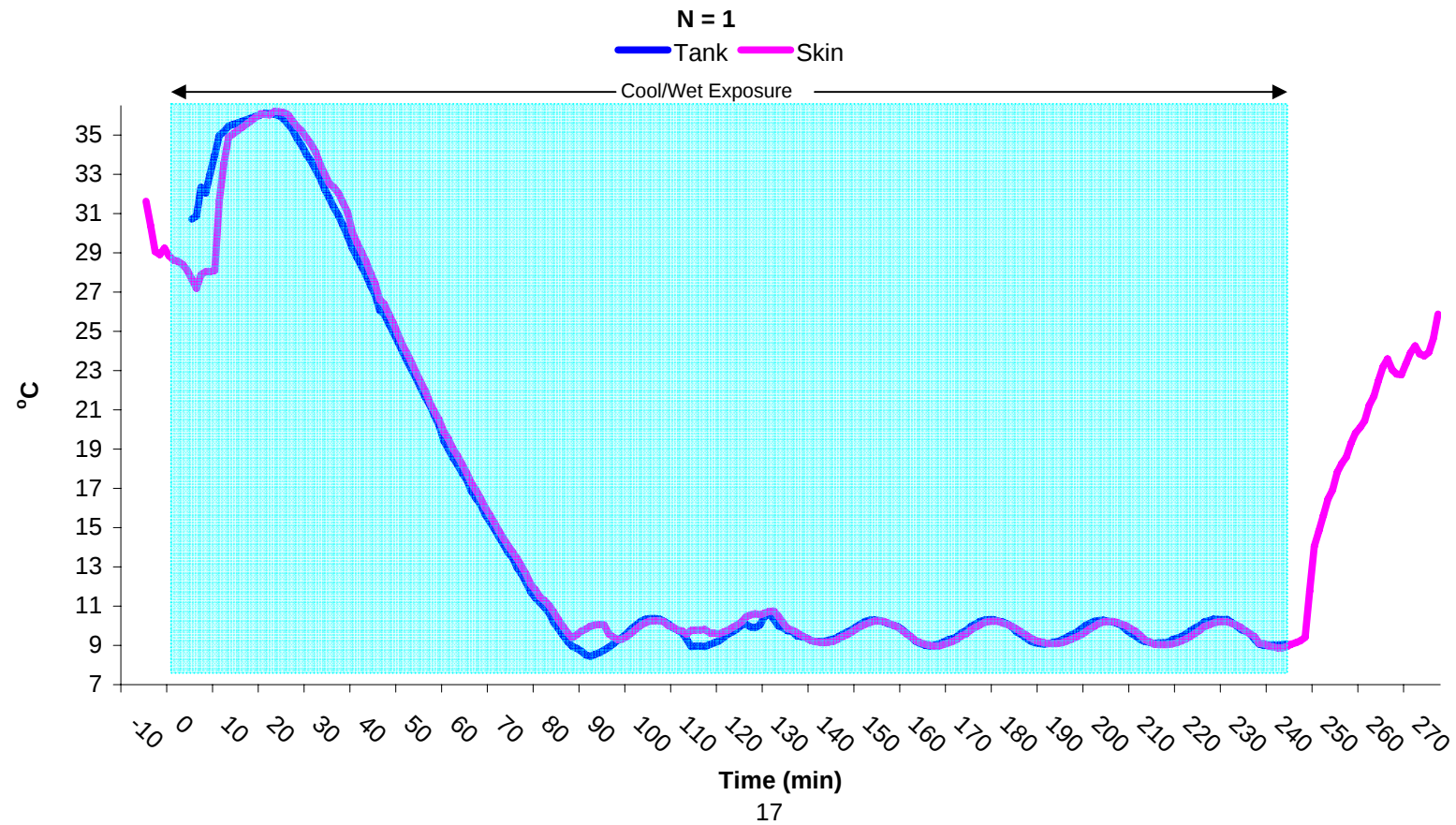


Figure 4a. Comparison of Rat Mean Core Temperatures for Gradual Cool/Wet or Ambient/Dry Exposure.

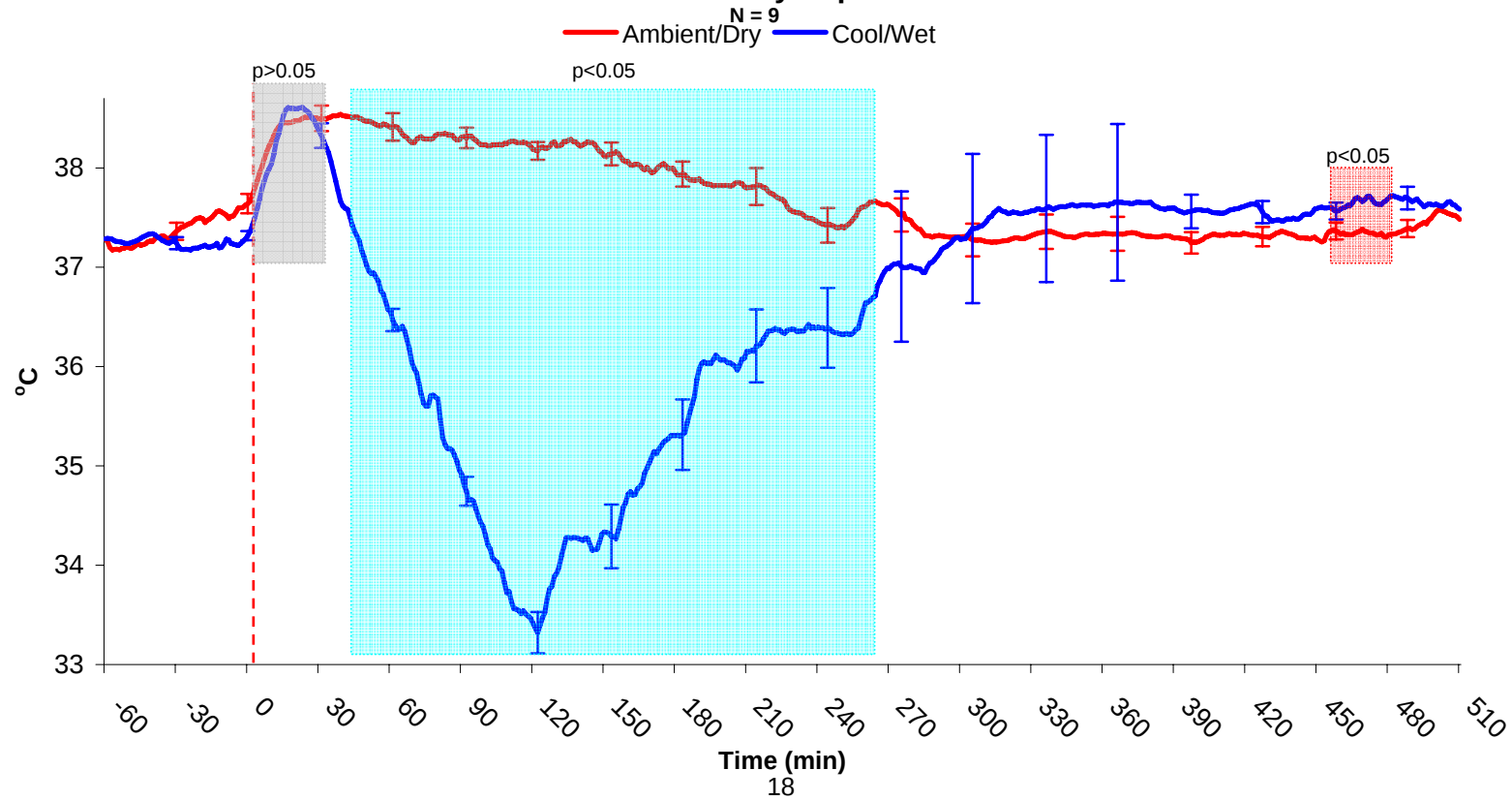


Figure 4b. Individual Rat Core Temperatures Pre and Post Gradual Cool/Wet (CW) Compared to Their Mean Ambient/Dry (AD) Core Temperature

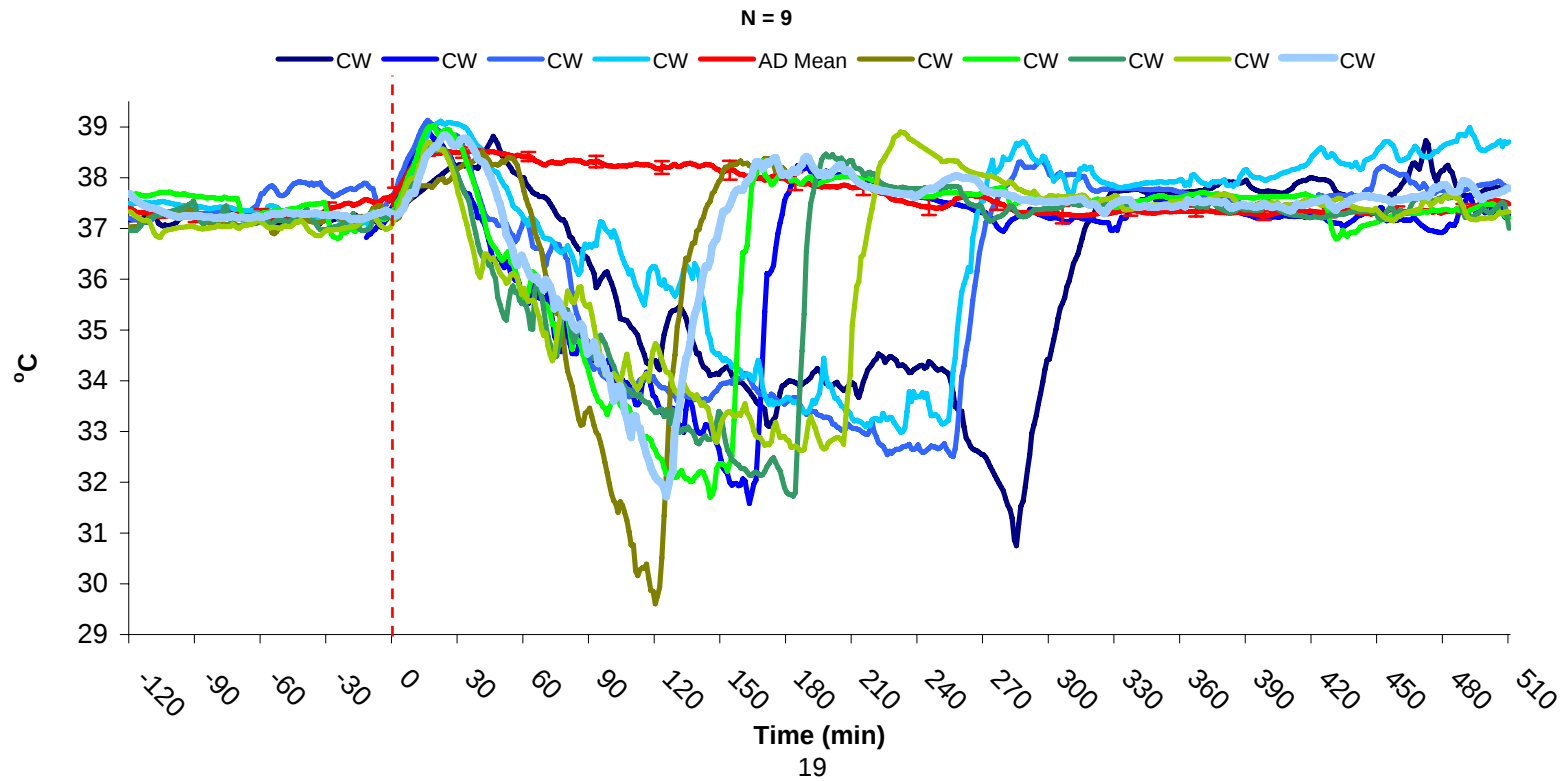


Figure 4c. Individual Rat Core Temperature during Hypothermia Recovery from Gradual Cool/Wet Exposure Back to 37°C

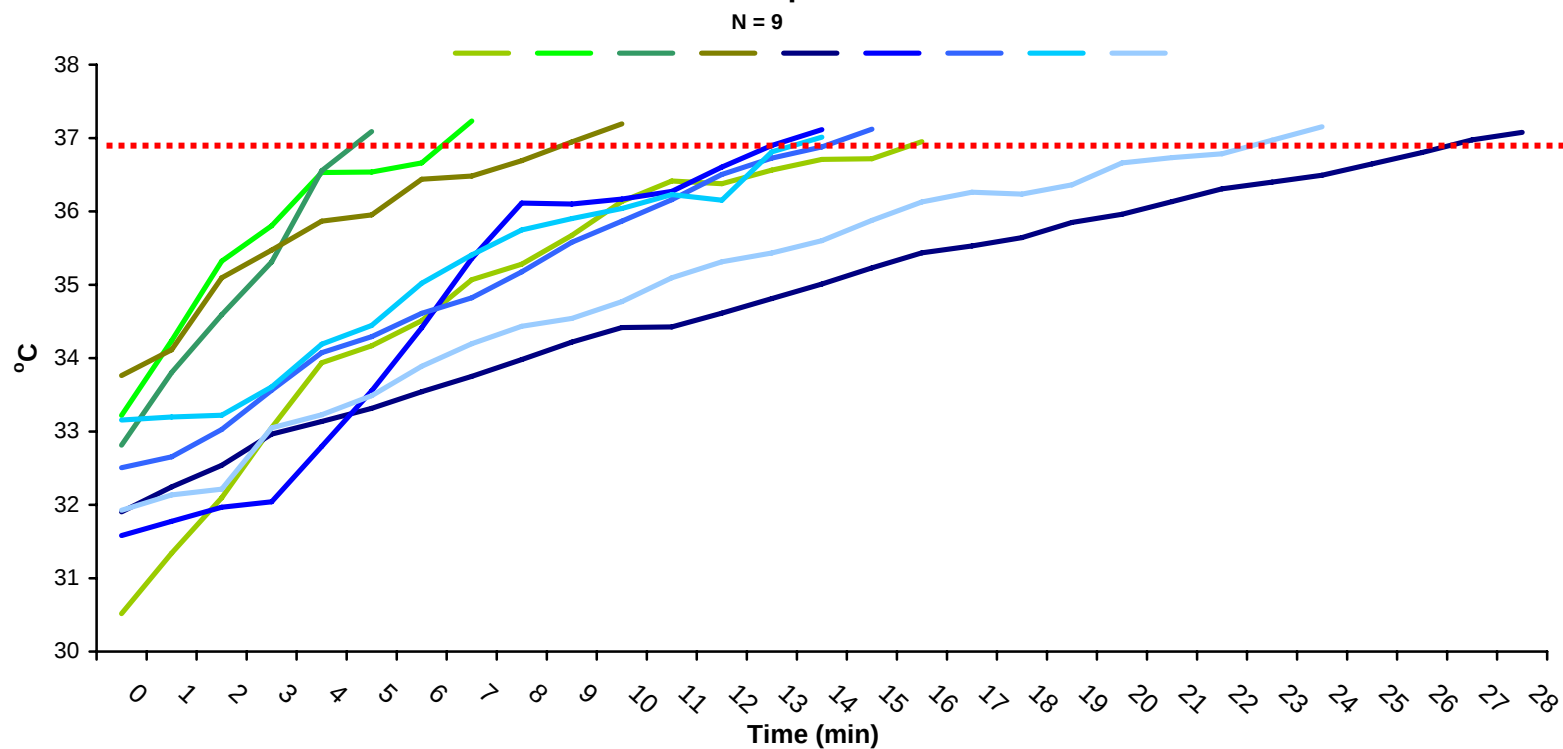


Figure 5a. Comparison among Rat Mean Core (T_c) and, Brown Adipose (BAT) and Subdermal (SDT) Tissue Temperatures for Gradual Cool/Wet Exposure.

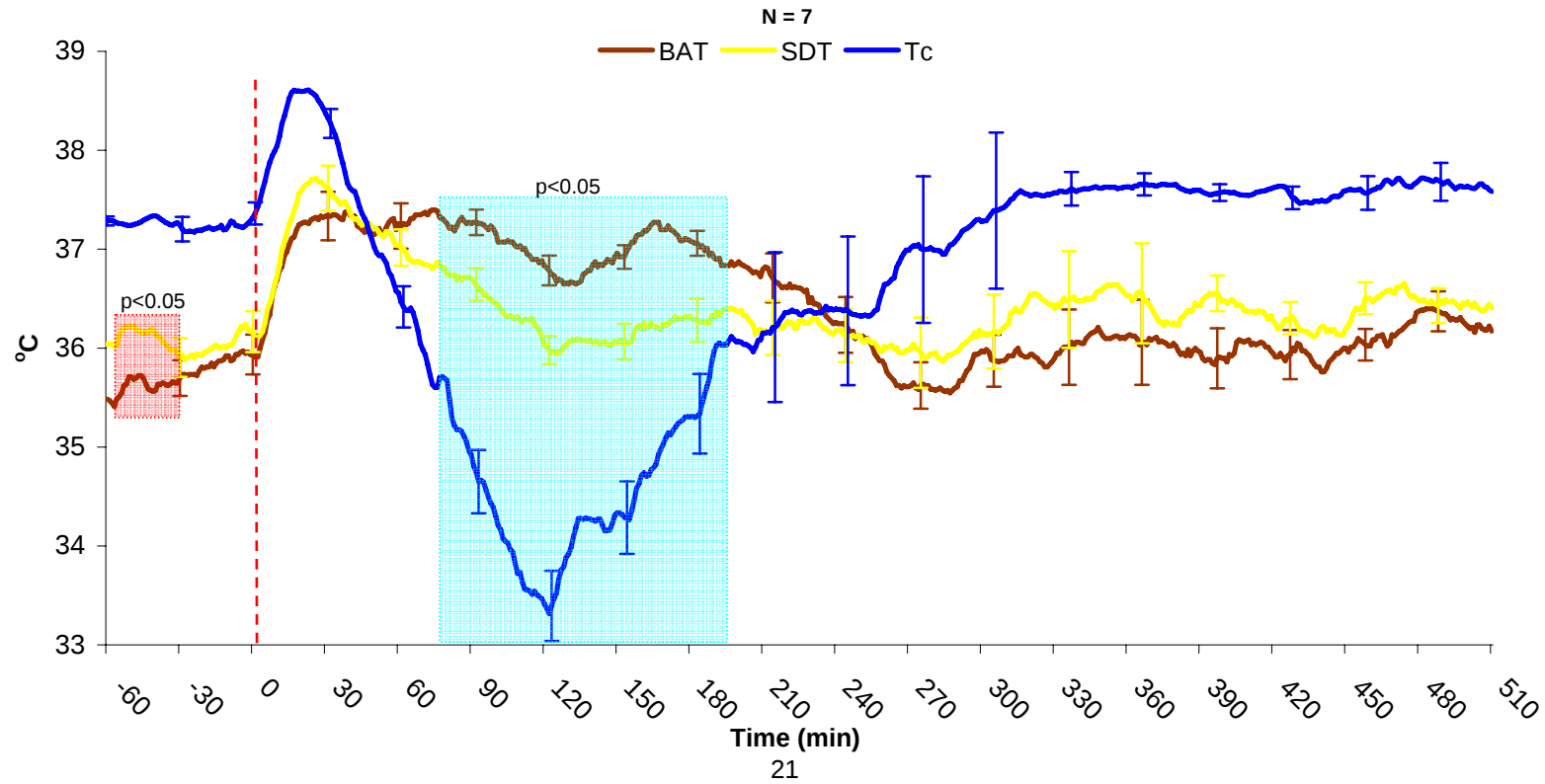


Figure 5b. Rat Brown Adipose/Subdermal Tissue Delta for Gradual Cool/Wet Exposure

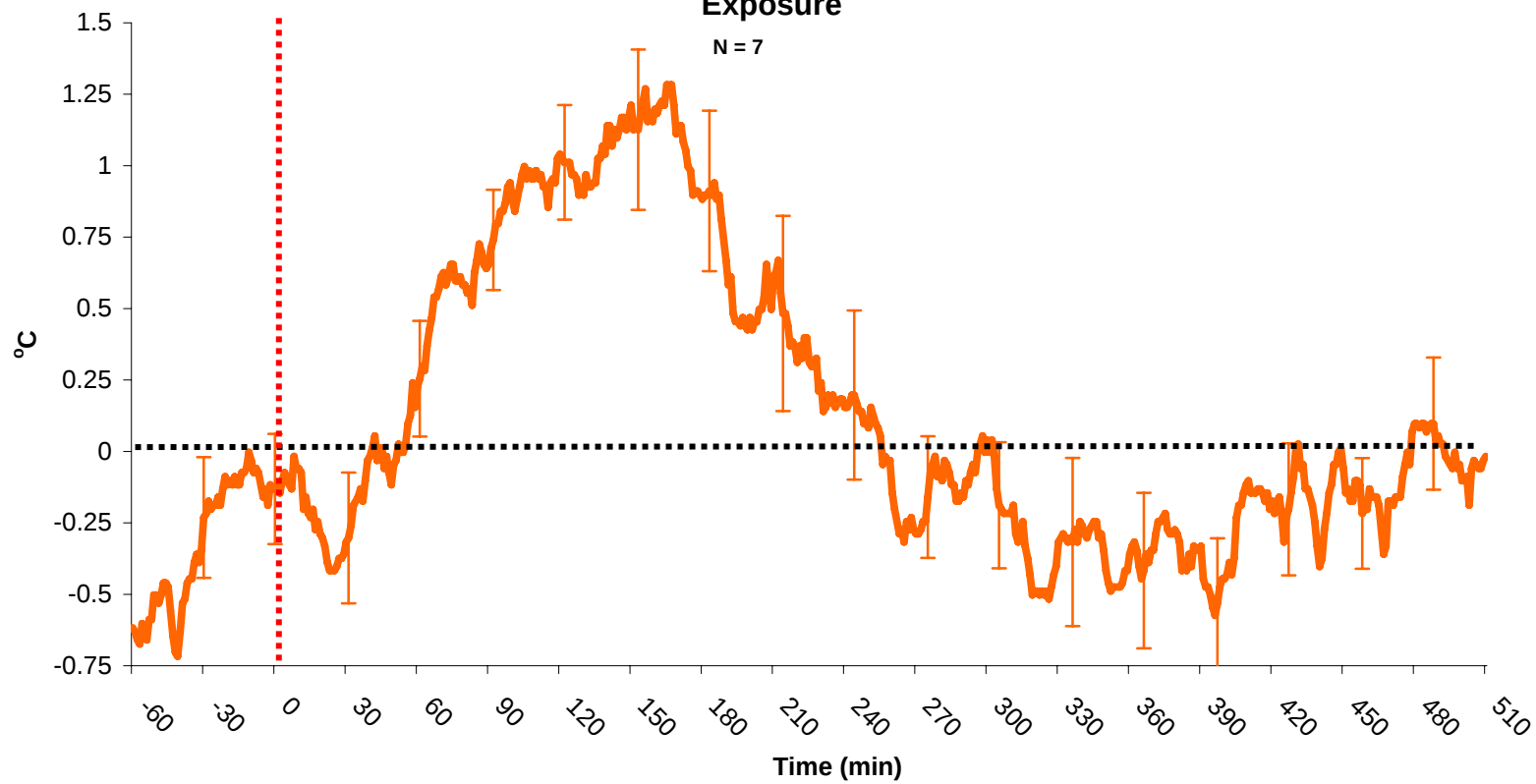


Figure 6a. Rat Core Temperature Comparisons between Immediate Warm/Wet and Ambient/Dry Exposure

N = 8

Warm Wet Ambient Dry

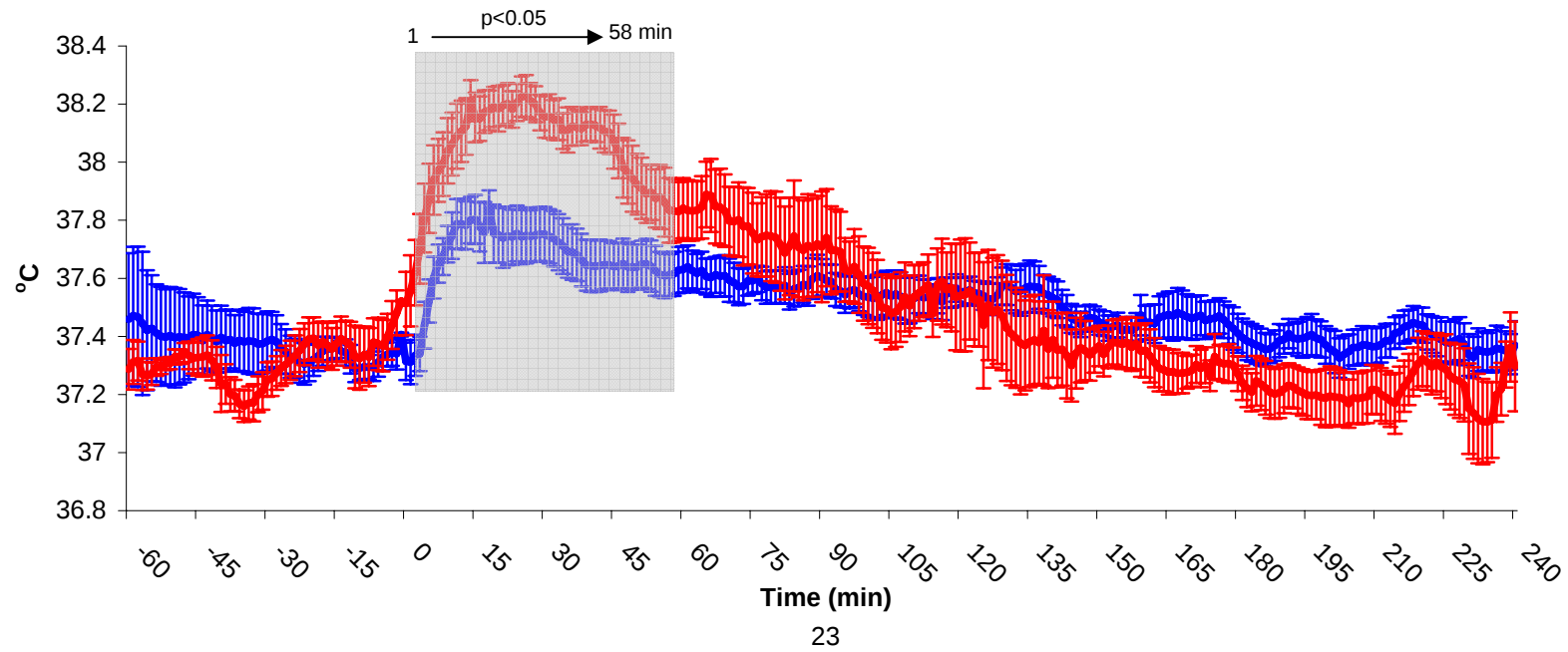


Figure 6b. Rat Activity Comparisons between Warm/Wet and Ambient/ Dry

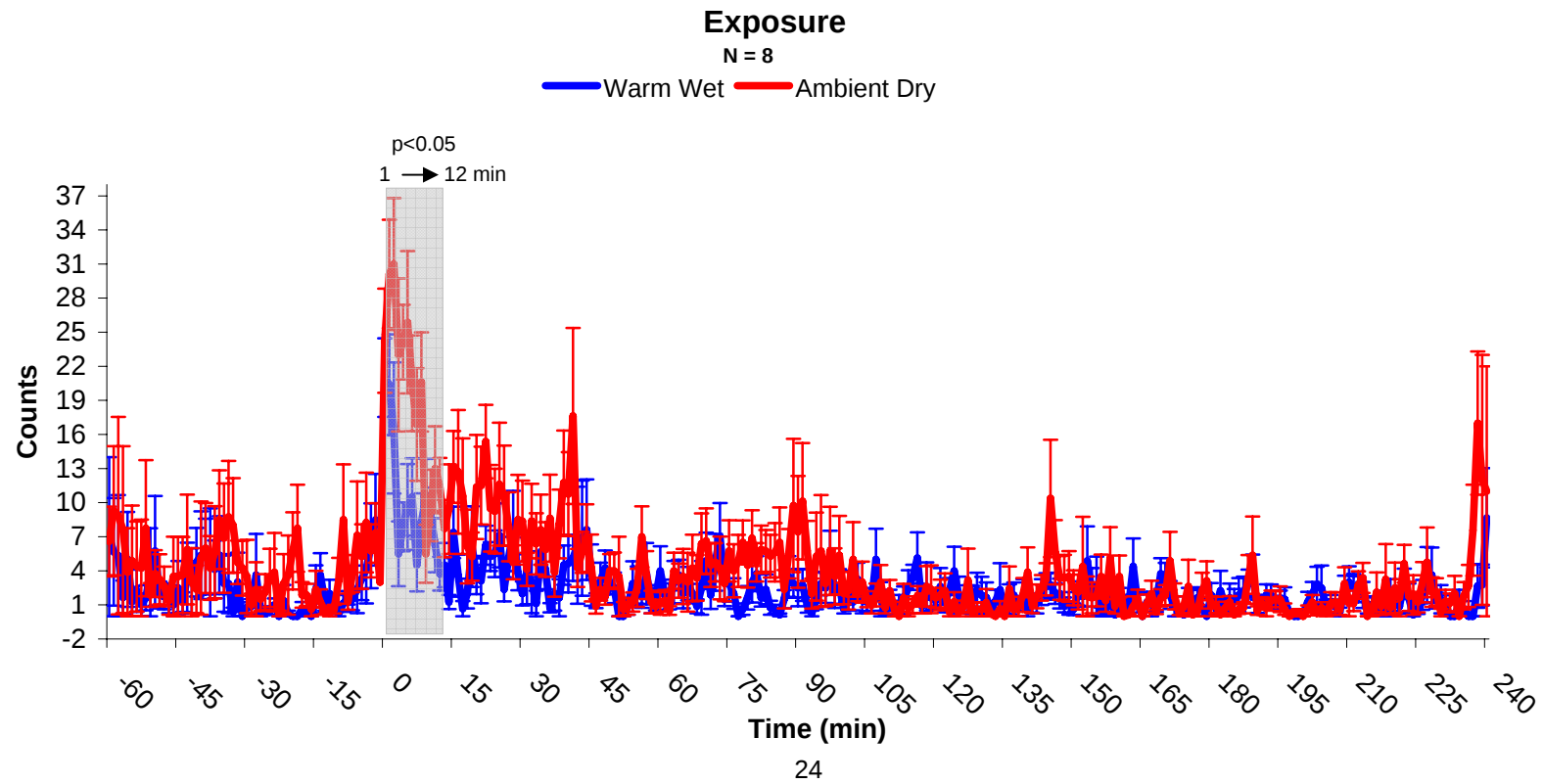


Figure 7. Individual Rat Core Temperatures Pre and Post Immediate Cool/Wet (CW) Exposure Compared to Mean Warm/Wet (WW) or Ambient/Dry (AD) Exposure Core Temperature

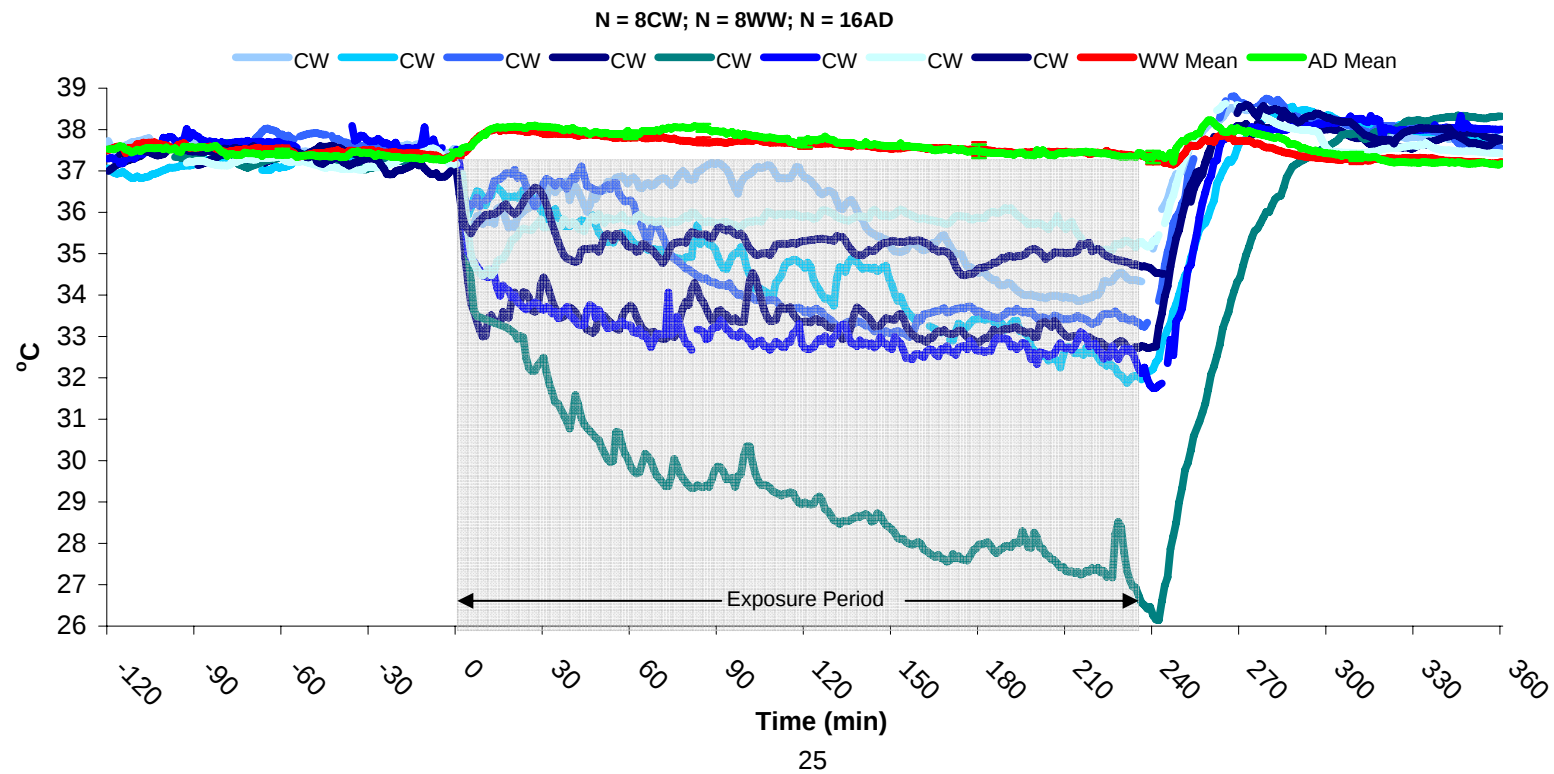


Figure 8a. Comparison Among Rat Core (T_c) and, Brown Adipose (BAT) and Subdermal Tissue (SDT) Temperatures for Immediate Cool/Wet Exposure.

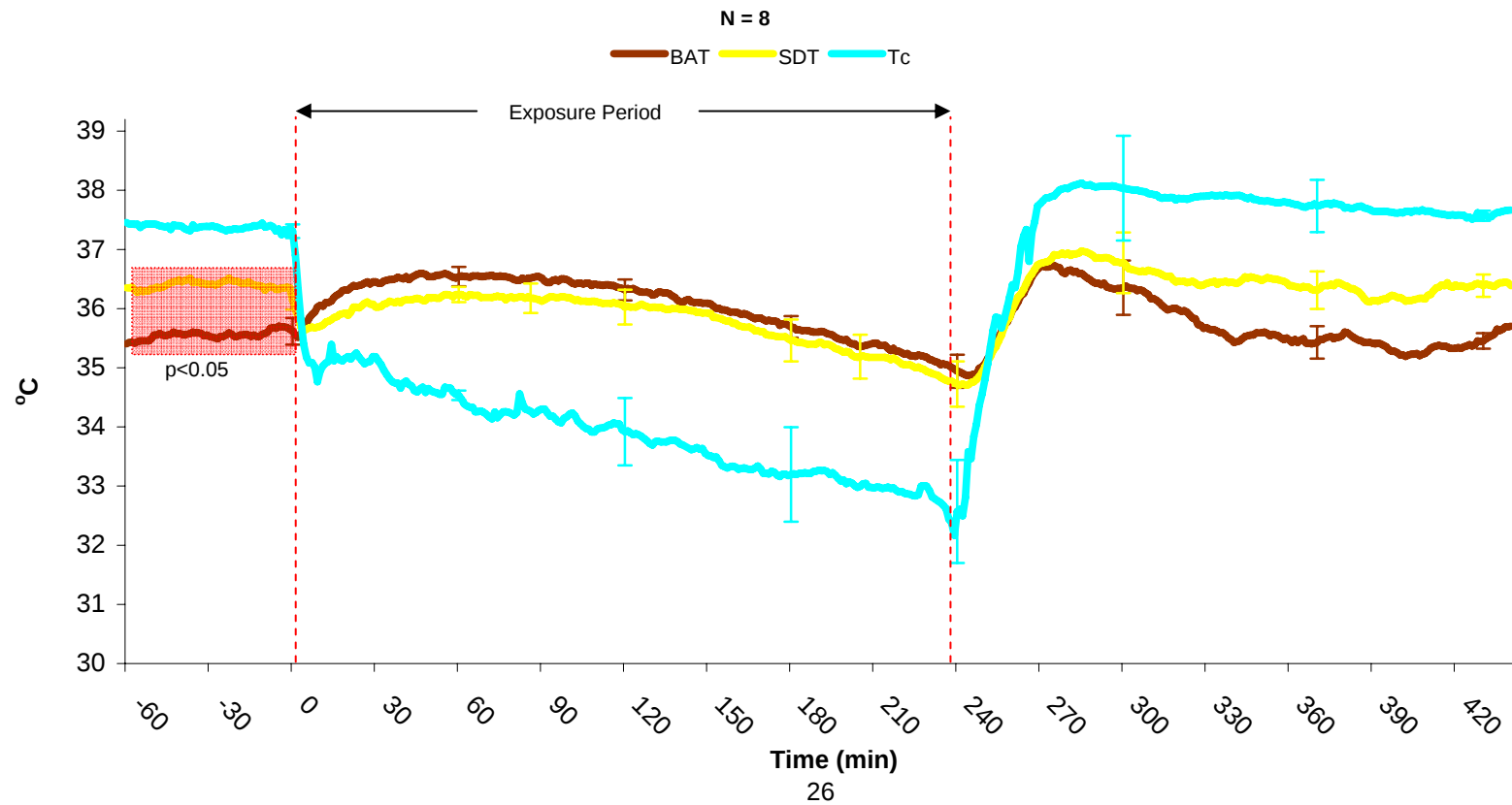


Figure 8b. Rat Brown Adipose Tissue Temperature (T_{BAT}) Pre and Post Immediate Cool/Wet Exposure

N = 8

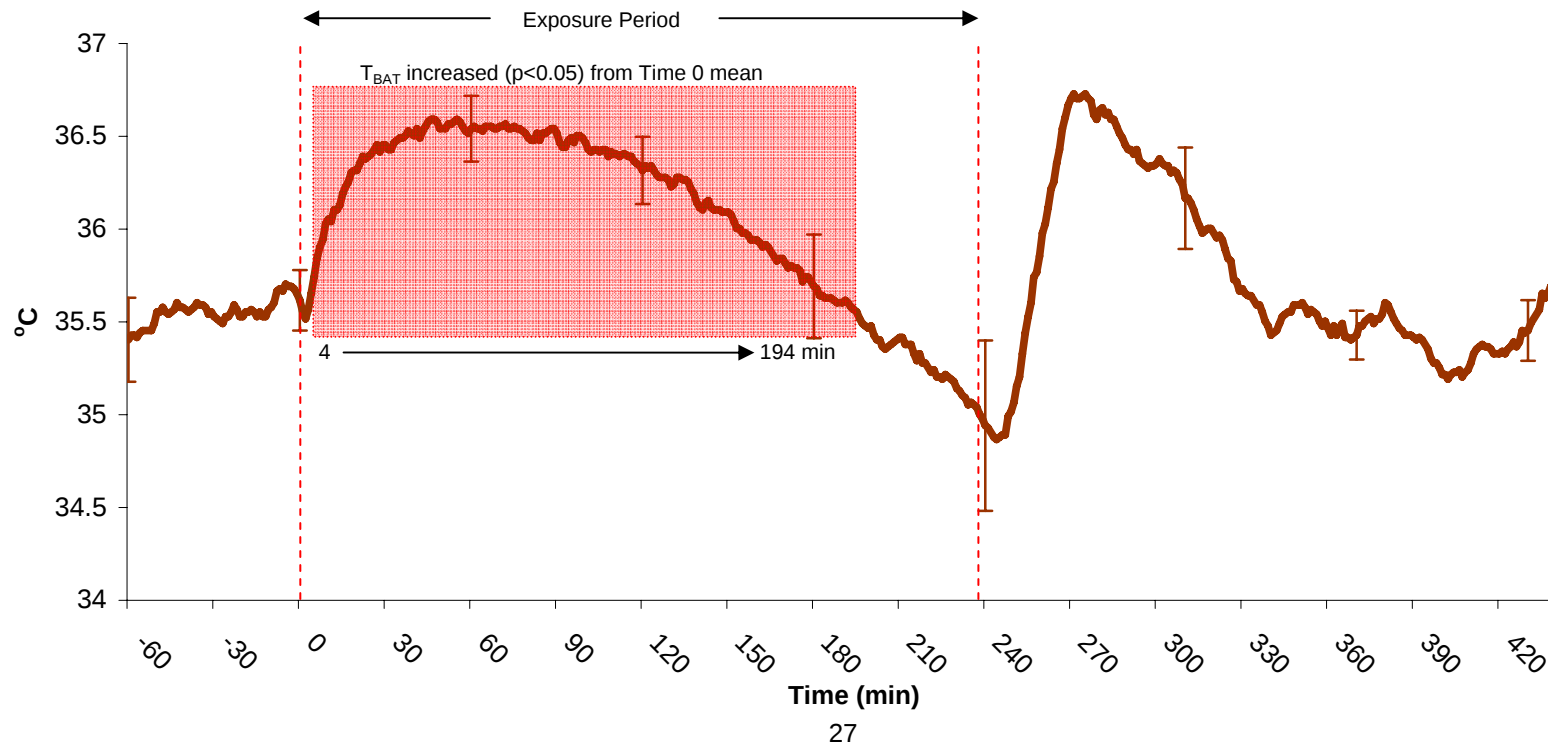


Figure 8c. Rat Subdermal Tissue Temperature (T_{SDT}) Pre and Post Immediate Cool/Wet Exposure

N = 8

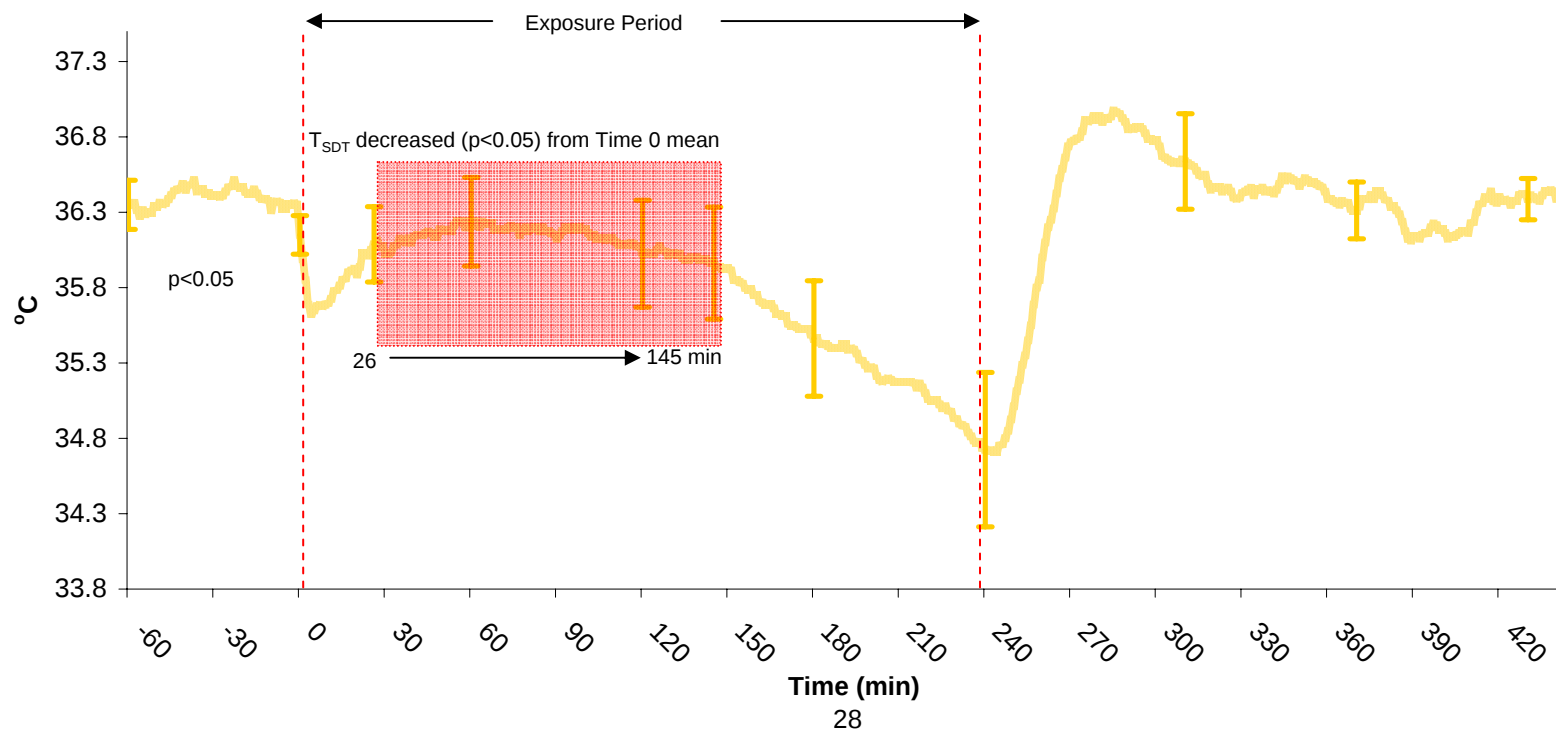


Table 1. Rat Hypothermia Induction and Recovery Characteristics for Gradual or Immediate Exposure to Cool/Wet (CW) Conditions
(Mean $\pm$ SEM)

10°C Exposure Procedure	Body Weight of CW Rats (g)	Lowest CW Hypothermia Core Temperature (T _c ; °C)	Cooling Rate (°C/min) to 34.5°C [#]	CW Thermo-regulatory Maintenance Time (min) after achieving Lowest T _c	Hypothermia Induction Time (min) to Lowest T _c	37°C T _c Recovery Time (min) from T _c =34.5°C [#]	Re-warming Rate (°C/min) from 34.5 to 37°C [#]	T _c (°C) in Ambient (25°C)/ Dry Tank at Lowest T _c in CW Tank	T _c (°C) in Warm (35°C)/ Wet Tank at Lowest T _c in CW Tank
Gradual (N=9)	214.7 $\pm$ 3.9	31.7 $\pm$ 0.4	0.14 $\pm$ 0.07	NT	194.1 $\pm$ 20.0	9.2 $\pm$ 1.3	0.35 $\pm$ 0.07	37.8 $\pm$ 0.2	NT
Immediate (N=8)	250.5 $\pm$ 7.3*	32.3 $\pm$ 1.0	0.27 $\pm$ 0.10	55.3 $\pm$ 27.5	215.7 $\pm$ 13.5	11.1 $\pm$ 1.3	0.23 $\pm$ 0.02	37.5 $\pm$ 0.1	37.5 $\pm$ 0.1

*=p<0.05 for comparison between gradual and immediate exposure; # = a common cooling T_c=34.5°C was selected, since lowest T_c achieved varied among the animals;
NT=Not Tested