Thermoregulation and markers of muscle breakdown in malignant hyperthermia susceptible volunteers during an acute heat tolerance test

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Abstract

Objectives. The study was undertaken to examine the thermal and biochemical responses to a heat tolerance test (HTT) of malignant hyperthermia (MH) susceptible individuals, and compare these with the responses of volunteers who have suffered heat illness (HI) and control volunteers.

Methods. Three groups of male volunteers (n=6 in each group) were recruited to the study:

MHS - civilian volunteers previously diagnosed as MH susceptible;

EHI - military volunteers with a history of exertional HI;

CON - military volunteers with no history of HI or MH.

For the HTT, volunteers walked on a treadmill at 60 % maximal oxygen uptake in an environmental chamber (mean [SD] temperature 35.5 [0.4] °C, relative humidity 43 [1] %). Measurements were made to assess thermoregulation and biochemistry.

Results. There were no differences in deep body temperature, oxygen uptake or serum lactate concentrations between the three groups, although one MHS volunteer and two EHI volunteers failed to thermoregulate. Serum myoglobin concentration and the increase in serum myoglobin was higher in MHS than EHI and CON Post HHT ($P<0.05$).

Conclusion. MH susceptibility does not always predispose an individual to heat intolerance during an acute HTT, but does appear to increase muscle breakdown. The inclusion of serum myoglobin measurements to a HTT may help to distinguish patients that are potentially MHS or have an underlying muscle myopathy, and who otherwise demonstrate thermal tolerance.

Introduction

Exertional heat illness (EHI) describes the condition where an individual is incapacitated during or following exercise as a result of a rise in deep body temperature.[1] In the United States, EHI is the third most common cause of sudden unexpected death in sport.[2] Even in races in the United Kingdom, EHI is a significant occurrence: in the 2009 Great North Run, 55 runners were admitted to the field hospital with deep body temperatures exceeding 41 °C.[3] In the British Army, 361 cases of EHI were reported between 2007-2015 of which 137 were admitted to hospital,[4] and in 2013 the deaths of three soldiers on a military training exercise in the Brecon Beacons were attributed to EHI.[5]

It has been suggested that a skeletal muscle metabolic defect, similar to that responsible for malignant hyperthermia (MH) susceptibility could explain EHI in individuals with no obvious predisposing factors.[6,7] MH presents under general anaesthesia with similar clinical features to EHI. In affected individuals the anaesthetic triggering agents, such as isoflurane and sevoflurane, cause dysregulation of skeletal muscle calcium control leading to a progressive rise in cytoplasmic calcium concentration.[8] The consequences are a rise in skeletal muscle cellular metabolism and contractile activity with increased oxygen consumption, carbon dioxide, hydrogen ion and heat production and rhabdomyolysis. The systemic effects include sympathetic stimulation, respiratory and metabolic acidosis, hyperthermia, hyperkalaemia and myoglobinaemia. The majority of cases of MH susceptibility are associated with variants in the ryanodine receptor 1 (*RYR1*) gene[9] which encodes the skeletal muscle sarcoplasmic reticulum calcium release channel. Genetic screening has limited sensitivity and specificity, so definitive clinical diagnosis of MH susceptibility requires an open muscle biopsy with subsequent exposure of the freshly excised muscle to halothane and caffeine in an *in-vitro* contracture test (*IV*CT).[10]

The Institute of Naval Medicine (INM), UK runs a Heat Illness Clinic (HIC), seeing approximately 140 British Armed Forces personnel a year. These individuals have suffered a

heat illness requiring admission to hospital with either central nervous system disturbance and / or biochemical evidence of organ damage or rhabdomyolysis. Patients referred to the clinic undertake a heat tolerance test (HTT) and those who demonstrate persistent heat intolerance (and in whom there is suspicion of metabolic skeletal muscle defect) are referred for testing for MH susceptibility. Of the 56 referred, 19 have met the laboratory criteria for MH susceptibility. Other studies, similarly report a high incidence of muscle abnormalities amongst individuals that have suffered EHI[11] or rhabdomyolysis.[12] Although several studies have compared the responses of MH susceptible individuals and volunteers to an exercise challenge, the findings are equivocal. On a progressive cycling test, aural but not rectal temperature was higher in MH susceptible volunteers, and it was suggested that the delayed rise in thumb temperature and the greater increase in free fatty acid and cortisol concentrations indicated abnormalities in heat dissipation and sympathetic activity in the MH susceptible volunteers.[13] Whereas, studies using a 15 min cycling test and a two hour treadmill walk found no difference in oxygen uptake, sympathetic activity or muscle metabolism between MH susceptible volunteers and controls.[14,15]

This study was undertaken to determine whether individuals already identified as MH susceptible would demonstrate abnormal thermoregulation on a HTT. It was hypothesised that MH susceptible volunteers would demonstrate a greater rate of rise in deep body temperature, serum lactate concentration and oxygen consumption, and greater changes in concentrations of biochemical markers suggestive of muscle breakdown, in response to a HHT than a control group and a group of volunteers with a history of EHI.

Methods

Three groups of male volunteers with 6 volunteers in each group were recruited to the study; each individual was tested once:

MHS Group: active civilian volunteers with a personal or family history of MH and MH susceptibility confirmed by *IV*CT;

EHI Group: military patients of the INM HIC with previous history of EHI who were scheduled for evaluation by HTT;

CON Group: military volunteers with no personal or family history suggestive of MH and with no history of HI.

Written informed consent of the volunteers was gained in accordance with the Declaration of Helsinki.[16] Absence of MH susceptibility in the EHI and CON groups was assumed rather than confirmed by *IV*CT because of the rarity of the condition and the invasive nature of the test. The volunteers were all European-white other than one volunteer in the EHI group who was non-Caucasian mixed race.

Tests were conducted on a treadmill in an environmental chamber. Fans in front of the treadmill generated a wind speed of 7 $km.hr^{-1}$. Preparation and recovery were conducted in an adjoining room (20-22 °C). Maximum oxygen uptake ($\dot{V}O_2max$) was measured using an incremental running test to volitional exhaustion with the volunteers wearing shorts and T.shirt. After rest for one hour the volunteers undertook the HTT which was conducted in three continuous phases walking on a treadmill with the volunteers wearing combat T.shirt, trousers, jacket, socks and trainers:

Phase 1 (0-30 min): Volunteers carried a 14 kg rucksack, and walked on the treadmill with the speed and gradient set to elicit a work intensity equivalent to 60% $\dot{V}O_2max$.

Phase 2 (30-45 min): At 30 min the rucksack and jacket were removed.

Phase 3 (45-90 min): The T.shirt was removed at 45 min and the volunteers continued to walk on the treadmill until 60 min and were then stopped if a plateau (*i.e.* two consecutive readings the same) or fall in rectal temperature occurred; if rectal temperature was still rising the volunteer continued until a plateau occurred or 90 min had elapsed. If rectal temperature reached 39.5 °C the volunteer was stopped, removed from the chamber and actively cooled. An individual is considered to thermoregulate normally and demonstrate heat tolerance if they

attain a plateau in rectal temperature. Water was not allowed during the test, but drinking was actively encouraged in the recovery periods.

ECG was monitored using a 6 lead ECG on-line telemetry system (VitalJacket, Optima-Life, London, UK).

Rectal temperature (T_{re}) was monitored throughout the HTT using a disposable rectal thermistor (Variohm-Eurosensor Ltd, Towcester, UK) inserted 10 cm beyond the anal sphincter, and measurements recorded on a data logger (Grants, Cambridge, UK).

Intestinal temperature (T_{int}) was measured using a telemetric pill (VitalSense, Mini Mitter Company Inc, Oregan, USA), swallowed two hours before beginning the HTT.

Skin temperature and *heat flow* were measured using sensors (Concept Engineering, CT, USA) taped to the skin (at the right calf, right thigh, right arm, left upper chest, right scapula and mid-forehead). The output was recorded on a data logger (Grants, Cambridge, UK). The heat flow data (mV) were converted to watts and $W.m^{-2}$ using the calibration constants supplied with the sensors and mean skin temperature (M_{sk}) and mean heat flow calculated.

Oxygen consumption and the *respiratory measurements were* made by analysing expired gas using an on-line system (Quark CPET, Cosmed, Rome, Italy).

Whole body sweat loss was calculated from the change in nude body mass measured pre and post the HTT using calibrated scales (Sartorius, Epsom, UK), accuracy 0.001 kg.

Localised sweat rates were measured using sweat capsules attached (using elasticated straps) at the base of the right scapular and thigh (Q-sweat, WR Medical Electronics Co., USA). Data were recorded continuously and 1 min averages calculated.

Skin blood flow was measured by laser Doppler velocimetry (Moors Instruments MBF3D system, Axminster, UK) with probes attached with tape to the middle finger pad on the right hand, right forearm, mid right thigh, and right upper chest and recorded digitally to a computer (PowerLab, ADInstruments Ltd, UK). Data were recorded continuously, 1 min averages calculated and normalised to the percentage of the highest 5 min rolling average.

137 *Blood samples* were taken Pre, Post, 2 Hr Post and 20 Hr Post and analysed for serum lactate,
138 creatine kinase (CK) and myoglobin concentrations. Lactate concentration was determined
139 photometrically (AU680, Beckman Coulter, High Wycombe, UK) CV 2.59%. CK was analysed
140 using the creatine phosphate to adenosine diphosphate method (AU 5800, Beckman Coulter,
141 High Wycombe, UK) CV 3.2% and reference range (males) 25-195 $U.L^{-1}$. Myoglobin
142 concentration was determined using turbiometric analysis (COBAS 6000, Roche, Burges Hill,
143 UK) CV <10% (reference range: 28-84 $\mu g.L^{-1}$).
144 Mean body temperature,[17] rate of metabolic heat production, change in body heat storage,
145 radiative, convective and evaporative heat transfer were calculated.[18]
146
147 Descriptive data were produced and checked for normality. Normally distributed data were
148 analysed using a one-way analysis of variance (ANOVA) or a general linear mixed model
149 ANOVA. *Post hoc* comparisons were made by t-tests with Bonferroni correction. Data not
150 normally distributed were analysed using the Kruskall-Wallis test and *post-hoc* comparisons
151 using the Mann Whitney U with Bonferroni correction.
152
153 **Results**
154 Chamber temperature did not differ between the exposures for the three groups; mean (SD)
155 dry, wet bulb and globe temperatures were 35.5 (0.4), 23.9 (0.2) and 35.2 (0.4) °C producing
156 a mean (SD) WBGT of 27.3 (0.2) °C, relative humidity 43 (1)%. Age, height, body mass and
157 lean body mass were the same for the three groups, mean (SD) values were 27 (5) years, 179
158 (8) cm, 81.9 (12.9) kg and 68.2 (9.9) kg. Percentage body fat differed between the groups,
159 $F(2,15)=6.952$ $p=0.009$; *post hoc* comparisons indicated that the percentage body fat of the
160 MHS group was lower than the EHI group ($P=0.008$), mean (SD) values were 12.3 (3.7) and
161 19.9 (4.4) %. $\dot{V}O_2$max did not differ between the groups, mean values were 4.4 (0.8) $L.min^{-1}$
162 and 54.4 (7.4) $mL.kg^{-1}.min^{-1}$. Two of the MHS volunteers had experienced adverse reactions
163 to anaesthesia and the remaining MHS volunteers underwent *IV*CT screening[15] as they had
164 relatives who had experienced MH complications during anaesthesia. The halothane

threshold for three of the MHS volunteers was 0.5% and for the other three 2%, all six showed a variant in the *RYR1* gene. Two of the MHS volunteers were professional sportsmen and the other four undertook recreational sports.

During the HTT absolute $\dot{V}O_2$ and $\dot{V}O_2$ *as a* % $\dot{V}O_2$max did not differ between groups and there was no interaction between group and time. Rectal temperature for each volunteer is shown in Figure 1. Three volunteers (one from the MHS group and two from the EHI group) were withdrawn as their rectal temperatures reached 39.5°C and were rising. There was no effect of group nor an interaction between group and time on rectal temperature or intestinal temperature. Deep body and skin temperature, heart rate and localised sweat rate data for each phase of the test are shown in Table 1. Statistical analysis indicated that there were no interactions between phase and group for any of these variables. There was an effect of group on thigh temperature x^2=10.491(2) (p=0.005), with MHS lower than CON (p=0.001) median values were 35.4°C and 36.4°C respectively. There was an effect of group on sweat rate at the thigh: x^2=11.273 (2) (p=0.004) with *post hoc* comparisons indicating a higher rate in MHS than CON (p=0.001). Whole body sweat rate (WBSR), absolute and relative to body surface area did not differ between groups; median and (min-max) values for the volunteers were 1.32 (0.75-2.08) $L.hr^{-1}$ and 602 (429-1077) $mL.m^2.hr^{-1}$.

There was no effect of group or an interaction between time and group for total mean heat flow, heat flow at the individual sites, skin blood flow and rate of metabolic heat production or radiative, convective and evaporative heat transfer. There was an effect of group F(2,15)=3.69 (p=0.05) on cumulative heat storage (per kg body mass), with lower values for MHS than EHI (p=0.048). At 30 min mean (SD) cumulative heat storage for MHS was 50.8 (12.8) $W.kg^{-1}$ and for EHI 71.1 (16.6) $W.kg^{-1}$, at 50 min the corresponding values were 60.1 (20.7) $W.kg^{-1}$ and 88.4 (25.2) $W.kg^{-1}$.

Serum myoglobin concentrations for MHS were higher than EHI and CON Post, x^2=6.654 (p=0.010); 2, Hr Post x^2 =5.276 (p=0.022) and 20 Hr Post, x^2 =3.872 (P=0.049). The increase in serum myoglobin was higher in MHS than EHI and CON from Pre to Post (x^2=5.063 [P=0.024]) and from Pre to 2 Hr Post (x^2=5.936 [p=0.015]). There were no differences for serum CK or lactate concentrations, median values are given in Table 2. The serum myoglobin of the MHS volunteers with halothane thresholds of 0.5% were numerically higher than the volunteers with thresholds of 2%, median values Post and 2 Hr Post were 279 and 246 $\mu g.L^{-1}$ compared to 87 and 82 $\mu g.L^{-1}$.

Discussion

Although one volunteer from the MHS group failed to thermoregulate during the HTT, there were no significant differences between the groups in terms of the deep body temperature, oxygen consumption and serum lactate measurements during the HTT. One interpretation of these findings is that, at the least, a large proportion of MH susceptible patients are not at increased risk of EHI and this is consistent with remarkably few reports of heat illness in MH susceptible patients.[19,20] There was, however, evidence to suggest that the MHS group was able to dissipate heat more effectively, as demonstrated by their higher localised sweat rate and lower skin temperature at the thigh. Given that T_{re} and heat production were similar between the groups suggests that this was not sufficient to influence deep body temperature.

Several studies have explored whether the response to exercise differs between volunteers with MHS and controls and in terms of deep body temperature the findings of this study are in agreement with those of Rutberg *et al* (1987)[14] and Green *et al* (1987).[21] Similar to this study, Green *et al* (1987) measured sweat evaporation using a ventilated capsule on the back and also found no significant difference between MHS and control volunteers,[21] although the power to detect a difference in both studies was low because of the high variability of the measurement and low participant numbers. However, despite the small number of volunteers

the sweat rate measured at the thigh in our study was higher in MHS than CON. Interestingly, in an initial study examining the anthropometry of volunteers with MHS, Campbell *et al* (1982) showed that percentage body fat (as in this study) was lower in the MHS group (n=27) and was 16.7% compared with 21.3% in a control group (n=21).[22] The current study used a more physically arduous regimen than the previous work and is the first reported to utilise a HTT with MHS volunteers; although Campbell *et al* (1983) and Green *et al* (1987) measured deep body temperature these only rose to mean values of 37.42 (±0.14) °C and approximately 38.2 °C.[13,21]

The findings are in contrast to the data from RYR1 knock-in mouse models of MH demonstrating consistent heat intolerance.[23-25] To date four murine models have been developed each focusing on a specific variant, the most recent of these involves a variant carried by one of the volunteers in the MHS study group (p.Gly2434Arg),[25] notably this was the volunteer who failed to thermoregulate.

The data do support the hypothesis that the MHS group demonstrate a greater change in biochemical markers suggestive of muscle breakdown in response to a HHT than the CON and EHI groups. Serum myoglobin and muscle enzymes are indirect markers of muscle damage, and in a longitudinal study involving arduous military training, myoglobin was the most sensitive marker of muscle stress.[26] During a MH reaction there is a sustained increase in myoplasmic calcium concentration producing hypermetabolism and contractile activity and it has been suggested that this also occurs with exercise in the heat.[27] Calpain, a nonlysosomal cysteine protease is thought to trigger skeletal muscle protein breakdown and is activated by raised intracellular calcium.[28]

Although there were only six volunteers in the MHS group, those who (on the *IV*CT) responded at 0.5% halothane demonstrated higher serum myoglobin values (at all three sample points after the HTT) than the MHS volunteers who responded to the *IV*CT at 2% halothane. This

suggests that sensitivity to halothane in the *IV*CT may correlate with the degree of muscle breakdown experienced in the HTT.

Our study was limited because of the small number of MHS volunteers recruited, which was due to the low availability of suitable MHS volunteers. A further limitation of the study was the assumption that the control subjects were not susceptible to MH, but confirmation by *IV*CT could not be justified; however, none of these volunteers reported adverse reactions to anaesthesia in themselves or family members. While the HTT is able to discriminate between individuals based on their ability to thermoregulate under standard conditions, it is a surrogate for predisposition to develop EHI. None of the volunteers in the MHS group have a history of EHI, so either they are not susceptible to EHI (including the one MHS individual who failed to thermoregulate during the HTT) or have not been exposed to the same level of exercise or heat as the military patients referred to the HIC and who subsequently fail the HTT.

It is interesting that just one out of 6 MH susceptible individuals, who all had an abnormal IVCT, failed to thermoregulate during the HTT. This contrasts with the observation that 34% of patients referred to the HIC following an episode of EHI and unable to thermoregulate during the HTT have an abnormal *IV*CT.[29] In reconciling these observations it is important to recognise that the *IV*CT is not specific for MH susceptibility and that abnormal findings may be obtained with samples from patients with other muscle disorders.[30] Our working hypothesis is that MH susceptibility and susceptibility to EHI are distinct but overlapping phenotypes. Thus, there are some individuals susceptible to one but not the other, while other individuals are susceptible to both. This is a similar situation to the relationship between MH susceptibility and central core disease.[8]

Conclusions

- Five out of 6 malignant hyperthermia susceptible individuals demonstrated thermotolerance on an acute heat tolerance test.
- Malignant hyperthermia susceptibility appears to increase the magnitude of muscle breakdown on an acute HTT.
- The inclusion of serum myoglobin measurements to a HTT may help to distinguish patients that are potentially MH susceptible.

Acknowledgements

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400 Figure 1. Individual rectal temperatures.
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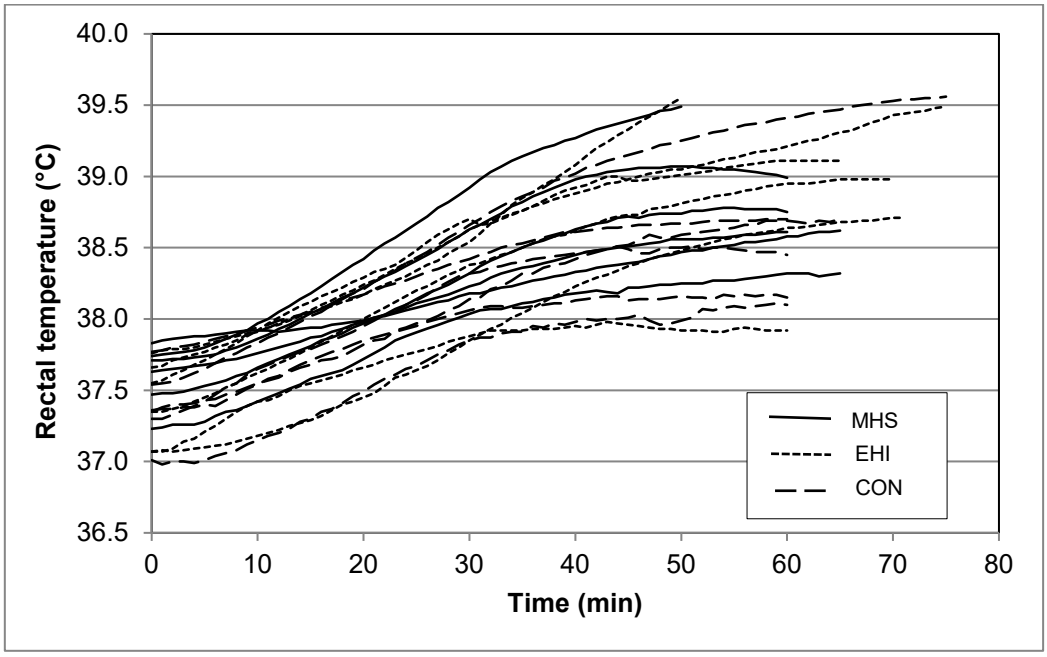

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Table 1. Temperature, heart rate and localised sweat rate (LSR) data at each Phase of the HTT, mean (SD) for variables with normally distributed data, and median (min-max) for variables not normally distributed (denoted by *), # significantly different to CON.

		Phase 1 (0-30 min)	Phase 2	Phase 3
Clothing and equipment worn		Trousers, jacket, T.shirt, rucksack	Trousers, T.shirt	Trousers
Rectal (°C)	All n=18	37.8 (0.3)	38.4 (0.3)	38.6 (0.4)
Rectal rate of rise ($°C.hr^{-1}$)	All n=18	1.7 (0.4)	1.2 (0.7)	0.6 (0.7)
Intestinal (°C)	All n=16	37.8 (0.3)	38.5 (0.3)	38.6 (0.4)
Heart rate ($beats.min^{-1}$)	All n=18	162 (17)	150 (18)	150 (19)
Mean body (°C)	All n=18	37.4 (0.3)	37.9 (0.4)	38.1 (0.4)
M_{sk} (°C)	All n=18	35.9 (0.5)	35.6 (1.1)	35.8 (1.1)
LSR Back ($mL.m^2.hr^{-1}$)	All n=17	0.50 (0.26-0.93)	0.68 (0.31-1.12)	0.64 (0.29-1.03)
LSR Thigh* ($mL.m^2.hr^{-1}$)	#MHS(n=6)	0.21 (0.10-0.26)	0.24 (0.04-0.33)	0.25 (0.02-0.38)
	EHI (n=5)	0.19 (0.09-0.21)	0.17 (0.11-0.30)	0.12 (0.04-0.38)
	CON (n=6)	0.12 (0.07-0.19)	0.06 (0.03-0.22)	0.06 (0.03-0.17)

412 Table 2. Median (range) serum myoglobin, creatine kinase and lactate concentrations.
413

		MHS (n=6)	EHI (n=6)	CON (n=6)
Myoglobin ($\mu g.L^{-1}$)	Pre	60 (27-118)	50 (34-77)	55 (38-75)
	Post	142 (87-378)	79 (65-122)	69 (45-134)
	2 Hr Post	137 (81-280)	73 (52-135)	72 (50-139)
	20 Hr Post	79 (31-101)	52 (41-62)	49 (39-76)
CK ($U.L^{-1}$)	Pre	276 (141-2963)	258 (126-890)	296 (199-412)
	Post	445 (194-2941)	315 (173-825)	314 (223-493)
	2 Hr Post	471 (198-2671)	321 (141-769)	296 (216-478)
	20 Hr Post	609 (176-1633)	336 (144-556)	238 (192-443)
Lactate ($mmol.L^{-1}$)	Pre	1.6 (1.1-1.3)	1.3 (1.0-4.8)	1.6 (1.2-4.5)
	Post	1.4 (0.9-1.7)	1.7 (1.3-3.5)	1.4 (1.0-2.3)
	2 Hr Post	1.4 (1.1-3.2)	1.5 (1.2-2.1)	1.3 (1.1-1.9)
	20 Hr Post	1.5 (1.2-2.3)	1.7 (1.1-2.4)	1.1 (0.8-2.1)

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Table 1b. Temperature, heart rate and localised sweat rate (LSR) data at each Phase of the HTT, mean (SD) for variables with normally distributed data, and median (min-max) for variables not normally distributed (denoted by *), # significantly different to CON.

		Phase 1 (0-30 min)	Phase 2	Phase 3
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Rectal rate of rise ($°C.hr^{-1}$)	All n=18	1.7 (0.4)	1.2 (0.7)	0.6 (0.7)
Intestinal (°C)	All n=16	37.8 (0.3)	38.5 (0.3)	38.6 (0.4)
Heart rate ($beats.min^{-1}$)	MHS	163 (13)	146 (15)	148 (16)
	EHI	170 (15)	160 (16)	161 (20)
	CON	154 (20)	144 (21)	141 (19)
Mean body (°C)	All n=18	37.4 (0.3)	37.9 (0.4)	38.1 (0.4)
M_{sk} (°C)	All n=18	35.9 (0.5)	35.6 (1.1)	35.8 (1.1)
LSR Back ($mL.m^2.hr^{-1}$)	All n=17	0.50 (0.26-0.93)	0.68 (0.31-1.12)	0.64 (0.29-1.03)
LSR Thigh* ($mL.m^2.hr^{-1}$)	#MHS(n=6)	0.21 (0.10-0.26)	0.24 (0.04-0.33)	0.25 (0.02-0.38)
	EHI (n=5)	0.19 (0.09-0.21)	0.17 (0.11-0.30)	0.12 (0.04-0.38)
	CON (n=6)	0.12 (0.07-0.19)	0.06 (0.03-0.22)	0.06 (0.03-0.17)