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Title page

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

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Abstract (words 253)

Objectives. The study was undertaken to compare the thermal and biochemical responses to a heat tolerance test (HTT) of malignant hyperthermia (MH) susceptible individuals, 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 a hot environment. Measurements were made of core and skin temperatures, heat flow, whole body sweat rate and serum lactate, creatine kinase and myoglobin concentrations.

Results. There were no differences in deep body temperature, oxygen uptake or serum lactate and creatine kinase concentrations between the three groups. One MHS volunteer and two EHI volunteers failed to achieve thermal balance with rectal temperature continuing to rise throughout the test and reaching 39.5℃, the rectal temperatures of the other volunteers plateaued at a mean (SD) of 38.7 (0.4)℃ demonstrating thermal tolerance on thi s test. 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, 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 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 ℃.[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 (IVCT).[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, biochemical evidence of organ damage or rhabdomyolysis. The INM HIC was established with a formal protocol in 2001 as a diagnostic tool to identify underlying muscle, metabolic or biochemical disorders and ultimately determine if patients are suitable for normal service duties. The procedure consists of exercise on a treadmill in warm conditions with clothing limitations to initially raise the deep body temperature of the individual. The jacket and rucksack are removed at 30 min and t.shirt after 45 min of exercise, the patient continues to exercise to determine whether thermal balance (i.e. a plateau of deep body temperature) can be achieved. Patients are considered heat intolerant if deep body temperature does not plateau; the test duration is 60-90 min.

Patients 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 patients referred from the HIC, 19 have met the laboratory criteria for MH susceptibility i.e. a positive result on the IVCT. Other studies, similarly report a high incidence of muscle abnormalities amongst individuals that have suffered EHI.[11] However, improving the specificity of the HIC procedure would reduce unnecessary referrals for the invasive IVCT procedure.

In addition, there are concerns that the current procedure is not sufficiently sensitive as there have been instances of patients passing the HIC procedure, returning to duty and sustaining a further exercise related collapse; and subsequently testing positive on the IVCT. Furthermore, a soldier with known MH susceptibility but no history of HI, undertook and passed the HIC assessment, however, additional blood samples (which are not routinely taken) indicated significant metabolic disturbance.

Although several studies have compared the responses of MH susceptible individuals and volunteers to an exercise challenge, the findings are equivocal and none have exposed

individuals to a thermal challenge. On a progressive cycling test, aural temperature was higher in MH susceptible volunteers.[12] 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.[13,14]

This study was undertaken to determine whether individuals already identified as MH susceptible would demonstrate heat intolerance on a HTT. The secondary aim was to determine whether the MH susceptible individuals would have higher concentrations of biochemical markers suggestive of muscle breakdown. It was hypothesised that MH susceptible volunteers would demonstrate a greater rate of rise in deep body temperature, oxygen consumption, serum lactate concentration and greater changes in concentrations of markers suggestive of muscle breakdown, in response to a HTT 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 IVCT;

EHI Group: military patients of the INM HIC with previous history of EHI;

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

The sample size was based on a power calculation using rectal temperature data from the 45^{Th} to 60^{th} min of the HTT from patients (n=11) shown to be heat intolerant and subsequently meeting the laboratory criteria for MH susceptibility and (n=21) heat tolerant patients. A one-sided test with an alpha value of 0.05 and power of 0.9 would require six volunteers in each group.

Written informed consent of the volunteers was gained in accordance with the Declaration of Helsinki,[15] and the protocol was approved by the Ministry of Defence Research and Ethics Committee (Protocol number: 647/MODREC/15). Absence of MH susceptibility in the EHI and CON groups was assumed rather than confirmed by IVCT 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. Testing was conducted between Jan-May 2016, apart from n=3 MHS volunteers who were tested in Sep 2016) and each volunteer was tested at the same time of day. 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 ℃). 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 ℃ the volunteer was stopped, removed from the chamber and ac tively 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.

Hydration status was assessed prior to the $\dot{V}O_{2max}$ test by measuring the specific gravity of urine samples using reagent strips for urinalysis (Multistix 10SG, Siemens, Munich, Germany). 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.[16]

Mean skin temperature (M_{sk}) 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).[17] 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.

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).

Blood samples were taken Pre, Post, 2 Hr Post and 20 Hr Post and analysed for serum lactate, creatine kinase (CK) and myoglobin concentrations. Lactate concentration was determined photometrically (AU680, Beckman Coulter, High Wycombe, UK) CV 2.59%. CK was analysed using the creatine phosphate to adenosine diphosphate method (AU 5800, Beckman Coulter, High Wycombe, UK) CV 3.2% and reference range (males) 25-195 $U.L^{-1}$. Myoglobin concentration was determined using turbiometric analysis (COBAS 6000, Roche, Burges Hill, UK) CV <10% (reference range: 28-84 $\mu g.L^{-1}$).

Mean body temperature (T_{mb}) was calculated (according to the formula: $0.79T_{re} + 0.21T_{msk}$),[18] and change in body heat storage calculated as (change in T_{mb} x mass x specific heat of body tissue [3.48 $kJ.kg^{-1}.℃$])/time). Metabolic heat production, radiative, convective and evaporative heat transfer were calculated using a freely available on-line spreadsheet.[19] Descriptive data were produced and checked for normality. Normally distributed data were analysed using a one-way analysis of variance (ANOVA) or a general linear mixed model ANOVA. Post hoc comparisons were made by t-tests with Bonferroni correction. Data not normally distributed were analysed using the Kruskall-Wallis test and post-hoc comparisons using the Mann Whitney U with Bonferroni correction.

Results

Chamber temperature did not differ between the exposures for the three groups; mean (SD) dry, wet bulb and globe temperatures were 35.5 (0.4), 23.9 (0.2) and 35.2 (0.4) °C producing a mean (SD) WBGT of 27.3 (0.2) ℃ , relative humidity 43 (1)%. The volunteer characteristics and $\dot{V}O_{2max}$ data are shown in Table 1. Percentage body fat differed between the groups, $F(2,15)=6.952$ $p=0.009$; post hoc comparisons indicated that the percentage body fat of the MHS group was lower than the EHI group ($P=0.008$). Two of the MHS volunteers had experienced adverse reactions to anaesthesia and the remaining MHS volunteers underwent IVCT screening as they had 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 regular recreational sports, young active males were sought to match the military volunteers who are habitually active.

The specific gravity of the urine samples from the volunteers were all ≤1.020, suggestive of adequate hydration.[20] During the HTT absolute $\dot{V}O_{2max}$ and $\dot{V}O_2$ as a $\%\dot{V}O_{2max}$ 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℃ and were rising. Deep body and skin temperature and heart rate data are shown in Table 2. Statistical analysis indicated that there were no interactions between phase and group or of group for any of these variables. Whole body sweat rate did not differ between the groups, mean (SD) values were 1.6 (0.4), 1.3 (0.4) and 1.3 (0.5) $L.hr^{-1}$ for the MHS, EHI and CON groups respectively, the corresponding mean (SD) values relative to body surface area were 801 (224), 640 (169) and 618 (182) $L.m^{-2}.hr^{-1}$.

There was no effect of group or an interaction between time and group for total mean heat flow, 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, 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 3. 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.[21,22] 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 IVCT.[23] Furthermore, our findings are not consistent with data from RYR1 knock-in mouse models of MH which demonstrate consistent heat intolerance.[24,25] However, the mouse models have focused on a small number of specific variants, the most recent of these involves the variant (p.Gly2434Arg)[25] which is the same as the variant carried by MHS volunteer in this study who demonstrated heat intolerance.

However, in reconciling these observations it is important to recognise that the IVCT is not specific for MH susceptibility and that abnormal findings may be obtained with samples from patients with other muscle disorders.[26] 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]

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)[13] and Green et al (1987).[14] 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).[27] The greater heat storage in the EHI than MHS group probably reflects the higher body fat and body mass (although this was non-significant) of the EHI group. 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) ℃ and approximately 38.2 ℃. [12,14]

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.[28] 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.[29] Calpain, a nonlysosomal cysteine protease is thought to trigger skeletal muscle protein breakdown and is activated by raised intracellular calcium.[30] Including measurement of serum myoglobin concentration in the HIC protocol may help to identify individuals with an underlying muscle disorder but who demonstrate heat tolerance on the HTT and hence improve the specificity of the procedure. Further work is required to confirm this suggestion.

Although there were only six volunteers in the MHS group, those who (on the IVCT) 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 IVCT at 2% halothane. This suggests that sensitivity to halothane in the IVCT 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 and EHI volunteers were not susceptible to MH, but confirmation by IVCT could not be justified; however, none of these volunteers reported adverse reactions to anaesthesia in themselves or family members. While the HTT can 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.

The aim of the work was to determine whether individuals already identified as MH susceptible would demonstrate heat intolerance on the HIC protocol and hence it was necessary to replicate the HIC protocol and for the volunteers to undertake the $\dot{V}O_{2max}$ and HTT on the same day. The formal protocol was designed in this manner to maximise the number of patients that could be seen in one day and thereby minimise the time waiting for an appointment (during which time service personnel are placed on limited duties) and to complete the testing in one day to reduce the burden on the patients. The study demonstrates that the current HIC protocol will not detect MH susceptibility but including measurement of serum myoglobin concentration may improve sensitivity, however, further work is required to confirm this assertion.

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.

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442 Figure 1. Individual rectal temperatures.

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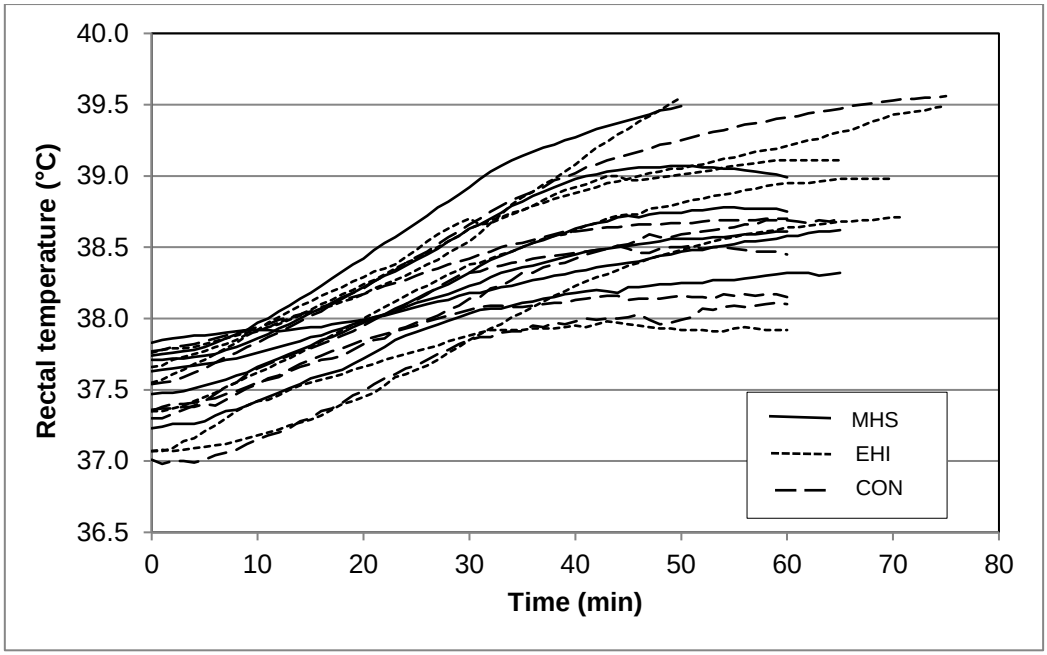

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446 Table 1. Mean (SD) volunteer characteristics and $\dot{V}O_{2max}$ data.

	MHS group (n=6)	EHI group (n=6)	CON group (n=6)
Age (years)	25.2 (3.6)	25.7 (5.9)	29.8 (4.3)
Height (m)	1.80 (0.07)	1.80 (0.09)	1.78 (0.10)
Body mass (kg)	77.2 (9.1)	87.6 (18.2)	79.6 (8.5)
Body surface area (m^2)	1.96 (0.13)	2.07 (0.25)	1.97 (0.16)
Body fat (%)	12.3 (3.7)	19.9 (4.4)	17.3 (2.6)
Lean body mass (kg)	68.2 (8.6)	70.2 (13.6)	66.2 (8.1)
$\dot{V}O_{2max}$ ($L.min^{-1}$)	4.45 (0.67)	4.49 (1.13)	4.36 (0.51)
$\dot{V}O_{2max}$ ($ml.kg.min^{-1}$)	57.7 (9.4)	50.9 (6.6)	54.7 (5.2)

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Table 2. Mean (SD) rectal, intestinal and mean skin temperatures at the end of each phase, rate of rise of rectal temperature and mean (SD) heart rate for each group (n=6 in each group unless stated otherwise).

		Phase 1 (0-30 min)	Phase 2	Phase 3
Clothing and equipment worn		Trousers, jacket, t.shirt, rucksack	Trousers, t.shirt	Trousers
Rectal temperature (℃)	MHS	38.4 (0.3)	38.6 (0.3)	38.8 (0.4)
	EHI	38.3 (0.4)	38.6 (0.4)	38.8 (0.6)
	CON	38.2 (0.3)	38.4 (0.3)	38.6 (0.5)
Rate of rise rectal temperature (℃.hr $^{-1}$)	MHS	1.6 (0.6)	1.2 (0.5)	0.5 (0.6)
	EHI	1.8 (0.3)	1.5 (0.9)	0.9 (0.9)
	CON	1.7 (0.4)	0.9 (0.6)	0.3 (0.4)
Intestinal temperature (℃)	MHS	38.4 (0.4)	38.6 (0.4)	38.8 (0.5)
	EHI (n=5)	38.5 (0.1)	38.7 (0.2)	38.9 (0.2)
	CON (n=5)	38.2 (0.3)	38.4 (0.3)	38.5 (0.5)
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)
M_{sk} (℃)	MHS	36.0 (1.2)	35.0 (1.2)	35.7 (1.3)
	EHI	36.4 (0.8)	35.7 (1.3)	36.3 (0.8)
	CON	36.7 (0.5)	35.9 (0.5)	36.4 (0.6)

455 Table 3. Median (range) serum myoglobin, creatine kinase and lactate concentrations.
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		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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