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RESEARCH PAPER

Thermoregulation during exercise under controlled hot ambient conditions is comparable in individuals with a history of exertional heat stroke, *RYR1*-related malignant hyperthermia, and healthy controls

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ABSTRACT

Exertional heat stroke (EHS) and Malignant Hyperthermia (MH) are potentially life-threatening conditions with overlapping clinical characteristics. In this study, we compared the thermoregulatory response to exercise under increased environmental temperatures in individuals with a history of EHS ($n = 15$) or MH ($n = 14$) to healthy controls ($n = 15$). Groups were age- and sex-matched (31 male, 13 female, 42 ± 10 years). A 60-min exercise test was performed on a cycle ergometer at an ambient temperature of 30.3 ± 0.6°C and a relative humidity of 33.5 ± 4.7%. A stepwise incremental exercise protocol was used to reach a metabolic heat production of 6, 8 and 9 W/kg body mass. Gastrointestinal (T_{gi}) and skin (T_{sk}) temperature were monitored continuously, and partitional calorimetry was used to calculate dry (H_{dry}) and respiratory heat loss (H_{resp}). Whole-body sweat rate (WBSR) was assessed by measuring body mass. Exercise-induced increases in T_{gi} (1.4 ± 0.5°C) and T_{sk} (1.9 ± 0.8°C) were observed, but the magnitude of increase across groups was comparable ($p_{time*group} = 0.80$ and $p = 0.57$, respectively). H_{dry} was significantly lower in EHS participants (54 ± 4 W) compared to controls (65 ± 11 W, $p = 0.023$). No differences were observed in H_{resp} and WBSR. Our results suggest that individuals with MH or a history of EHS do not have an altered thermoregulatory response to exercise in the heat in a controlled setting. Further research is required to determine to what extent the complex accumulation of risk factors contributes to EHS susceptibility.

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Thermoregulation; heat-related illnesses; *RYR1*; heat stress; skeletal muscle; ryanodine receptor

Introduction

Exertional heat stroke (EHS) is the most severe presentation within the spectrum of exertional heat illness and requires early recognition in order to initiate prompt life-saving treatment by rapid cooling, preferably through ice-water immersion, though any available cooling method should be used [1,2]. The current pathophysiological understanding of EHS assumes a systemic inflammatory response due to septicemia, potentially leading to multiple organ failure, coma, or even death [3].

Although numerous external risk factors contributing to EHS have been identified, the literature on a possible genetic susceptibility contributing to EHS remains limited [4]. Animal studies in rats exposed to heat, as well as observational studies of individuals who suffered from EHS, suggest a particular role of the ryanodine receptor-1 (RyR1), the principal skeletal muscle calcium release channel located on the sarcoplasmic reticulum and encoded by *RYR1* [5–8]. Pathogenic variants in *RYR1* may lead to a variety of congenital neuromuscular disorders, as well as malignant hyperthermia (MH), a pharmacogenetic reaction in response to volatile anaesthetics and/or depolarizing muscle relaxants [9,10]. An MH reaction is life-threatening and

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characterized by hypercapnia, tachycardia, hyperthermia (often >40°C), rhabdomyolysis, acute kidney injury, and cardiac arrhythmia. Hence, both EHS and MH involve hypermetabolic states with a high demand for adenosine triphosphate; accelerated oxidative, chemical, and mechanical stress of skeletal muscle cells, and an uncontrolled increase in intracellular calcium [11–13]. Based on the similarities among MH and EHS patients, the term "MH-like syndrome" has been used in previous literature, indicating that heat-related symptoms are relatively common in MH patients. However, the thermoregulatory response to exercise under increased environmental temperatures has rarely been assessed in these patients [14]. The present study aims to investigate the thermoregulatory response to prolonged exercise in hot ambient conditions in patients with a history of EHS or MH, and to compare these responses with healthy age- and sex-matched controls. We hypothesize that EHS and MH individuals would demonstrate a greater rise in exercise-induced core temperature compared to healthy controls. This study will contribute to the understanding of heat stress susceptibility in EHS and MH individuals and help improve patient counseling regarding the risk of EHS recurrence and other heat-related problems in MH individuals.

Methods

The study protocol was approved by the Medical Ethical Committee of the Radboud university medical center, Nijmegen, the Netherlands (#2020–7222), and conformed to the ethical principles outlined in the Declaration of Helsinki. Written informed consent was obtained after study procedures and potential risks had been explained to all participants prior to the investigations.

Participants

A total of 45 participants were recruited, including 15 with a history of EHS, 15 with MH and 15 age- and sex-matched healthy controls. EHS and MH participants were recruited from existing databases from our medical center and were eligible for inclusion if they were aged 18–60 years and performed endurance exercise ≥2 times a week. In addition, EHS participants were recruited from a prior study cohort with self-reported persisting physical complaints at least three months after an EHS event in the past 10 years [15]. A diagnosis of EHS was based on objectively documented exercise-related hyperthermia >40.5°C in the context of neurological symptoms. Participants in the MH group all carried a diagnostic MH *RYR1* variant according to the guidelines of the European Malignant Hyperthermia Group (EMHG) [16,17]. Exclusion criteria were based on contraindications for using an ingestible temperature capsule, including **I)** a body mass <36.5 kg, **II)** an implanted electro-medical device, **III)** a history of inflammatory bowel disease or gastrointestinal surgery, or **IV)** a scheduled MRI scan within five days after the experiment. Furthermore, participants were excluded if they used any medication potentially altering thermoregulatory function, including diuretics, laxatives or antihypertensives (disturbed fluid balance), anticholinergics and certain antiepileptics (reduced sweating), corticosteroids (impaired immune response and cytokine levels), sympathomimetics (reduced vasomotor control), or antipsychotics (reduced sweating and disturbed thermoregulation in the hypothalamus). For the healthy control group, the following additional exclusion criteria were employed: **I)** a (family) history of EHS/MH or a suspected EHS/MH reaction, or *RYR1*-related myopathies, and **II)** a family history of unexplained peri-operative death.

Study design

In this explorative intervention study, participants were invited for one study visit at the department of Medical Biosciences of the Radboud university medical center. Participants were medically screened in order to assess the inclusion criteria. The Short QUestionnaire to ASsess Health enhancing physical activity (SQUASH) was used to estimate the habitual level of physical activity during a normal week over the past month [18]. Venous blood and urine samples were collected at baseline and directly after exercise. Body mass was measured at baseline and directly after exercise, and body mass index (BMI) was calculated after measuring body height. Body surface area (BSA) was calculated by the Dubois and Dubois method and BSA to mass ratio was calculated, as a lower BSA to mass ratio is associated with an increased risk of EHS [19,20]. Participants were instructed to refrain from heavy physical exercise 48 hours prior to the experiment and to

drink 500 ml of water 2 hours pre-exercise [21]. Furthermore, all participants were instructed to wear a short-sleeve shirt and shorts. Next, a 60 min submaximal exercise test on a cycle ergometer was performed in a temperature-controlled room maintained at an ambient temperature (T_{amb}) of 30°C, which is higher than typically encountered during real-world competitive sports settings [22].

Submaximal exercise test

After the participant entered the temperature-controlled room, the cycle ergometer and respiratory gas analyzer mask were fitted to the participant. Indirect calorimetry was performed using a metabolic monitor (Quark CPET, COSMED, Rome, Italy) to estimate metabolic energy expenditure, based on rates of oxygen consumption and carbon dioxide production and accounting for macronutrient energy equivalents. Accordingly, metabolic heat production (H_{prod} in W) was estimated as the difference between metabolic energy expenditure and external work (i.e. workload on the ergometer) [23]. Following a 5 min seated baseline, participants started the 60 min cycling exercise test that was divided into three parts: **I)** 30 min at a H_{prod} of 6 W/kg body mass, **II)** 15 min at a H_{prod} of 8 W/kg, and **III)** 15 min at a H_{prod} of 9 W/kg. H_{prod} was monitored continuously throughout exercise, and workload was adjusted at 1-min intervals to maintain the target H_{prod} allowing a valid comparison between groups in accordance with the matching methods described by Cramer and Jay [24]. Participants were not allowed to drink throughout the exercise protocol. For safety purposes, the study protocol was terminated if core temperature exceeded 40°C, or if the gastrointestinal (T_{gi}) was lost for a certain time (range 2–10 min), depending on the last obtained T_{gi} measurement.

Outcome parameters

Gastrointestinal temperature (T_{gi})

T_{gi} was measured with the myTemp ingestible temperature capsule (MyTemp, Nijmegen, The Netherlands), ingested 3 hours prior to the experiment [25,26]. T_{gi} was measured continuously at 10-s intervals using an external recorder around the waist, and 1-min averages were calculated after data acquisition.

Skin temperature (T_{sk})

T_{sk} was measured using wireless temperature recorders (iButton DS1922L, Dallas Semiconductor Corp, USA) attached to the skin using Tegaderm Film (Tegaderm, Neuss, Germany) in four distinct locations (i.e. neck, right scapula, right shin and left hand) [27,28]. T_{sk} was measured continuously throughout the experiment at 20-s intervals, and 1-min weighted averages were calculated afterward according to international standard operations (ISO-9886) [29].

Heat balance calculations

Partitional calorimetry was used to estimate heat balance parameters, and 1-min averages were calculated and presented in W or W/kg. Metabolic energy expenditure (M) was estimated based on rates of oxygen consumption and carbon dioxide production and accounting for macronutrient energy equivalents. Thus, M was calculated as:

$$M = VO_2 \frac{\left(\left(\frac{RER-0.7}{0.3}\right)e_c\right) + \left(\left(\frac{1.0-RER}{0.3}\right)e_f\right)}{(60 * BSA)}(1000)\left[W/m^2\right]$$

VO_2 is the rate of oxygen consumption in L/min, RER is the ratio of carbon dioxide production to oxygen consumption, e_c represents the caloric equivalent per liter of oxygen for the oxidation of carbohydrates (21.13 kJ) and e_f denotes the caloric equivalent per liter of oxygen for the oxidation of lipids (19.62 kJ) [24]. Subsequently, H_{prod} was calculated as the difference between metabolic energy expenditure (M) and the external work rate (W) that was regulated by the cycle ergometer.

Dry heat exchange (H_{dry}) is the sum of convective (C) and radiant (R) heat exchange through the skin and was calculated as [24]:

$$H_{dry} = C + R\left[W/m^2\right]$$

$$C = h_c(T_{sk} - T_{amb})\left[W/m^2\right]$$

$$R = h_r(T_{sk} - T_{amb})\left[W/m^2\right]$$

The radiative heat transfer (h_r) was calculated as:

$$h_r = 4 * \varepsilon * \sigma * \frac{BSA_r}{BSA} * ((T_{sk} + T_r)/2 + 273.15)^3 \left[\frac{W}{m^2}/K\right]$$

where ε is the skin emissivity (0.95), σ is the Stefan-Boltzmann constant (5.67×10^{-8} W/m^2/K^{-4}), BSA_r/BSA is the effective radiant surface area (0.73) and T_r is the mean radiant temperature, equivalent to T_{amb}.

The convective heat transfer (h_c) was calculated as:

$$h_c = 8.3 * v^{0.6}\left[\frac{W}{m^2}/K\right]$$

where v is the wind velocity, set at 0.2 m/s based on the standard airflow in the climate chamber.

Respiratory heat exchange (H_{resp}) includes evaporative and convective heat loss from the respiratory tract and was calculated as:

$$H_{resp} = 0.0173\left(H_{prod}\right)(5.87 - P_a) + 0.0014\left(H_{prod}\right)(34 - T_{amb})\left[W/m^2\right]$$

where P_a is the ambient vapor pressure (in kPa).

Evaporative heat loss (E_{sk}) was calculated as:

$$E_{sk} = WBSL * \lambda[W]$$

WBSL represents the whole-body sweat loss in g/s and is derived from WBSR (L/h), while λ represents the theoretical latent heat of evaporation, fixed at 2,426 kJ/kg. A sweating efficiency of 100% was assumed.

Heart rate (HR) and exercise intensity

HR was measured continuously at 20-s intervals using a heart rate monitor (Polar RS400, Electro Oy, Kempele, Finland), and minute averages were calculated afterward. Subsequently, exercise intensity was calculated as the fraction of the expected maximal HR, which was estimated using the formula by Tanaka et al. (208–0.7 ×Age) [30].

Fluid balance

Absolute change in body mass during the exercise protocol was used to determine the participants WBSR in L/h, while the relative change in body mass was used to determine whether participants were dehydrated after exercise. Dehydration was defined based on a body mass loss of ≥2%, or a post-exercise urine specific gravity (USG) of ≥1.020 g/ml, measured using a refractometer (ATAGO PAL-10S, Sysmex, Ede, The Netherlands) [31].

Blood sampling and analysis

A venous blood sample was taken at baseline and directly post-exercise. Tubes were centrifuged and stored in aliquots at −80°C for later analysis. Biomarkers were sampled and analyzed using ELISA on a single day to minimize variation, including **I)** serum inflammation markers interleukin-6 (IL-6; Sanquin M9316, Amsterdam, The Netherlands) and Tumor Necrosis Factor-α (TNF-α; R&D DY210, Abingdon, UK) [32], **II)** Intestinal Fatty Acid Binding Protein (i-FABP; Hycult Biotech, HK406, ed 10–16, Wayne, USA), a biomarker used as an indicator of intestinal barrier dysfunction of the bowel [33,34]; **III)** Proenkephalin (PENK; Sphingotec GMbH, Hennigsdorf, Germany) in order to use a novel PENK-based formula to estimate the glomerular filtration rate (GFR), which performed better to calculate GFR compared to most conventional equations [35]. Participants who did not complete the exercise test were not included for biomarker analysis. In case >25% of the test results were below the limit of detection (LOD; PENK = 31 pmol/l, IL-6 = 1.56 pg/ml, TNF-

α = 7,81 pg/ml), data were compared as dichotomous (increased or not increased). In case <25% of the samples were below LOD, one-half the detection limit was assigned as the result [36].

Perceptual outcomes

Thermal comfort and thermal sensation were scored at baseline and every 15 min throughout the exercise test using a 4-point and 7-point scale, respectively [37]. Thermal comfort ranged from 1 (comfortable) to 4 (very uncomfortable) and thermal sensation ranged from −3 (very cold) to 3 (very hot).

Statistical analysis

All analyses were carried out using the Statistical Package for the Social Sciences (SPSS version 25, IBM, Armonk, New York). Dichotomous data were compared using the Pearson χ2 or Fisher's exact test. Dependent continuous variables were assessed at 5-min to perform statistical analyses. Continuous variables were tested for normal distribution using the Shapiro–Wilk test and presented as mean ± SD for normal distribution or median (interquartile range [IQR]) for non-normal distribution, unless indicated otherwise. A one-way ANOVA, or Kruskall–Wallis test for non-normal distributed data, with a post-hoc Bonferroni correction was performed to examine differences in dependent variables between the EHS, MH, and control group, including **I)** baseline characteristics, **II)** thermoregulatory parameters (i.e. Tgi, Tsk, heart rate, heat loss, and fluid balance), **III)** perceptual outcomes, and **IV)** laboratory outcomes. In case of comparing paired samples, the Wilcoxon Signed Rank Test was performed. A linear mixed model was used for analyzing differences in continuous thermophysiological parameters (i.e. T_{gi}, T_{sk}) and perceptual outcomes for a maximum of 14 time points with a 5 min interval, starting 5 min pre-exercise (baseline) up to 60 min. Group was used as a fixed factor (3 levels; EHS, MH and control) and time as a random intercept. The level of significance was set at $p < 0.05$.

Results

A total of 45 participants were recruited, including 15 individuals with a history of EHS, 15 MH individuals, and 15 healthy controls. One participant in the MH group withdrew due to anxiety before starting the test, giving a total sample size of $n = 44$. Of those, 31 were male (70%) and 13 female (30%), aged 42 ± 10 years (Table 1). No group differences were observed in sex ($p = 0.20$), age ($p = 0.79$), weight ($p = 0.38$), height ($p = 0.09$) or BSA ($p = 0.51$). BSA to mass ratio was 0.0252 ± 0.0018 and was comparable across groups ($p = 0.11$). BMI was higher in the MH group (25.9 [23.5–28.5 kg/m^2]) compared to the control group (22.3 [21.2–24.6 kg/m^2], $p = 0.027$). Results of the SQUASH questionnaire did not indicate any difference in baseline physical activity level between the three groups ($p = 0.54$). In the EHS group, the median time frame between the EHS event and the exercise test was 2.7 [2.1–4.5 years]. MH genotypes included p.Val4849Ile ($n = 10$), p.Thr2206Met ($n = 3$), or p.Thr2206Arg ($n = 2$). A comprehensive individual assessment of thermoregulatory outcomes is provided in Supplemental Table S1, and laboratory test results are provided in Supplemental Table S2.

Table 1. Demographics and participant characteristics.

	EHS ($n = 15$)	MH ($n = 14$)	Control ($n = 15$)	Total ($n = 44$)	P-value
Sex					
Male (n(%))	8 (53)	11 (79)	12 (80)	31 (70)	0.20
Female (n(%))	7 (47)	3 (21)	3 (20)	13 (30)	
Age (yr)	41 ± 8	40 ± 12	43 ± 10	41 ± 10	0.79
Height (cm)	178 ± 9	179 ± 8	184 ± 9	180 ± 9	0.09
Weight (kg)	77.9 ± 11.7	83.4 ± 10.6	78.4 ± 12.2	79.8 ± 11.6	0.38
BMI (kg/m^2)	24.3 [22.4–25.9]	25.9 [23.5–28.5]*	22.3 [21.2–24.6]	24.2 [22.2–26.4]	0.027
BSA (m^2)	1.95 ± 0.17	2.02 ± 0.14	2.01 ± 0.19	1.99 ± 0.17	0.51
BSA*mass^{-1} (m^2/kg)	0.0253 ± 0.0018	0.0244 ± 0.0017	0.0258 ± 0.0017	0.0252 ± 0.0018	0.11

Abbreviations: BMI = Body mass index; BSA = body surface area; EHS = Exertional Heat Stroke; MH = Malignant Hyperthermia.
* Significantly different compared to the control group.

Table 2. Exercise characteristics and outcome measures.

	EHS ($n = 15$)	MH ($n = 14$)	Control ($n = 15$)	Total ($n = 44$)	p-value
Test completed (n(%))	12 (80)	11 (79)	15 (100)	38 (86)	0.08
Exercise duration (min)	60 [40 – 60]	60 [25 – 60]	60 [60 – 60]	60 [25 – 60]	0.15
Relative humidity (%)	35.2 ± 4.8	33.6 ± 5.7	31.7 ± 2.8	33.5 ± 4.7	0.17
Ambient temperature (°C)	30.6 ± 0.5*	30.3 ± 0.5	30.0 ± 0.6	30.3 ± 0.6	0.004
Hprod during exercise					
Part 1 (0–30 min, W/kg)	5.9 [5.8–6.0]	5.9 [5.3–6.1]	5.9 [5.7–6.0]	5.9 [5.7–6.0]	0.51
Part 2 (30–45 min, W/kg)	7.7 [7.4–7.9]	7.7 [6.7–7.9]	7.5 [7.5–7.8]	7.7 [7.4–7.9]	0.23
Part 3 (45–60 min, W/kg)	8.8 [8.6–9.1]	8.5 [6.8–9.1]	8.9 [8.5–9.1]	8.9 [8.5–9.1]	0.33
Tgi					
Baseline (°C)	37.3 ± 0.4	37.2 ± 0.4	37.1 ± 0.2	37.2 ± 0.3	0.19
Δ Tgi (°C)	1.4 ± 0.4	1.3 ± 0.6	1.4 ± 0.5	1.4 ± 0.5	0.80
Peak Tgi (°C)	38.7 ± 0.4	38.6 ± 0.4	38.6 ± 0.5	38.6 ± 0.4	0.66
Tsk					
Baseline (°C)	33.9 [33.6–34.3]	34.0 [33.7–34.5]	33.2 [33.0–34.1]	33.9 [33.2–34.4]	0.18
Δ Tsk (°C)	1.7 ± 0.7	1.8 ± 1.0	2.1 ± 0.8	1.9 ± 0.8	0.57
Peak Tsk (°C)	36.1 ± 0.4	36.2 ± 0.6	36.2 ± 0.5	36.2 ± 0.5	0.75
Heart rate					
HR at baseline (bpm)	83 [81–89]	78 [67–84]	76 [71–81]	78 [71–86]	0.06
Δ HR (bpm)	71 ± 20	79 ± 16	80 ± 13	76 ± 17	0.27
Peak HR (bpm)	167 ± 17	161 ± 17	158 ± 18	162 ± 17	0.36
Exercise intensity					
Part 1 (0–30 min, %)	72 ± 8*	70 ± 12	63 ± 8	69 ± 10	0.029
Part 2 (30–45 min, %)	86 ± 8	83 ± 14	79 ± 9	82 ± 10	0.19
Part 3 (45–60 min, %)	90 ± 9	88 ± 10	86 ± 10	88 ± 10	0.63
Mean intensity (%)	82 ± 11	80 ± 14	76 ± 13	76 ± 9	0.07
Dry heat loss					
Part 1 (0–30 min, W)	51 ± 6**	57 ± 8	59 ± 11	56 ± 9	0.029
Part 2 (30–45 min, W)	60 ± 6**	67 ± 10	69 ± 12	65 ± 10	0.025
Part 3 (45–60 min, W)	59 ± 5**	73 ± 10	72 ± 12	65 ± 11	0.006
Total (W)	54 ± 4**	63 ± 9	65 ± 11	61 ± 10	0.023
Evaporative heat loss (W)	606 [539–674]	808 [539–876]	809 [539–876]	741 [539–808]	0.12
Respiratory heat loss					
Part 1 (0–30 min, W)	37 ± 5	39 ± 4	38 ± 5	38 ± 5	0.44
Part 2 (3–45 min, W)	45 ± 5	47 ± 8	49 ± 7	47 ± 7	0.24
Part 3 (45–60 min, W)	49 ± 8	52 ± 10	56 ± 11	53 ± 7	0.26
Total (W)	41 ± 5	45 ± 7	46 ± 7	44 ± 6	0.30
Fluid balance					
Body mass loss (%)	1.3 [0.9–1.4]	1.3 [0.9–1.3]	1.4 [1.3–1.6]	1.4 [1.1–1.4]	0.22
WBSR (L/h)	0.9 [0.8–1]	1.2 [0.7–1.3]	1.2 [0.7–1.2]	1.1 [0.8–1.2]	0.16
≥2% body mass loss (n(%))	0 (0)	0 (0)	0 (0)	0 (0)	
USG baseline (g/mL)	1.0066 ± 0.0054	1.0049 ± 0.0048	1.0056 ± 0.0030	1.0072 ± 0.0042	0.97
USG post-exercise (g/mL)	1.0163 ± 0.0078	1.0134 ± 0.0068	1.0138 ± 0.0054	1.0146 ± 0.0062	0.41
USG ≥1.020 g/mL (n(%))	5 (33)	2 (14)	2 (13)	9 (20)	0.39

Abbreviations: EHS = exertional heat stroke; Hprod = metabolic heat production; MH = Malignant hyperthermia; Tgi = gastrointestinal temperature, Tsk = skin temperature, USG = urine specific gravity, WBSR = whole body sweat rate.
*Significantly different compared to the control group.
**Significantly different compared to the control group and the MH groups.

Exercise characteristics

T_{amb} and relative humidity (RH) were 30.3 ± 0.6°C and 33.5 ± 4.7%, respectively (Table 2). T_{amb} was slightly higher in the EHS group compared to the control group (30.6 ± 0.5°C and 30.0 ± 0.6°C, respectively, $p = 0.004$). Six participants (3 EHS, 3 MH; 14%) were not able to complete the test at the predefined or lower workload, including three EHS individuals due to exhaustion ($n = 2$) or anxiety ($n = 1$), and three MH individuals due to nausea ($n = 1$), self-reported heat intolerance ($n = 1$), or because the signal of the temperature capsule was lost for longer than the predefined interval ($n = 1$). The number of participants

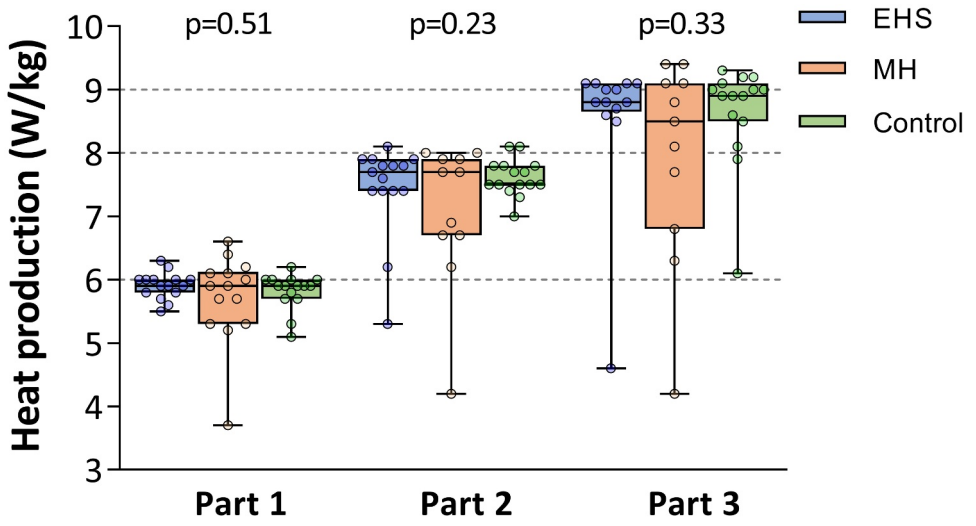

Figure 1. Average Hprod during the three exercise parts for the EHS (blue), MH (orange) and control group (green). The dashed lines represent the target heat production of the exercise parts.

that were not able to complete the test did not differ between the three groups ($p = 0.08$) and all six participants had a T_{gi} ≤38.6°C at exercise cessation and no serious adverse events occurred. Furthermore, four participants (1 EHS, 2 MH, 1 control) completed the exercise test at a lower workload, in order to be able to complete the test. H_{prod} during the exercise test was comparable among the three groups during all three exercise parts (all p-values > 0.05; Figure 1). Subgroup analysis of participants who did not complete the test did not reveal any differences in activity level based on the SQUASH questionnaire ($p = 0.58$) compared to the participants who completed the test, and no differences were observed in T_{gi}, T_{sk}, HR, exercise intensity, H_{dry} or E_{sk} (all $p > 0.05$).

Thermoregulatory response

Tgi and Tsk

Baseline T_{gi} and peak T_{gi} were 37.2 ± 0.3°C and 38.6 ± 0.4°C, respectively, and did not differ between groups ($p = 0.19$ and $p = 0.66$, respectively; Table 2). T_{gi} increased by 1.4 ± 0.5°C during the exercise protocol ($p_{time} < 0.001$), but the magnitude of increase did not differ between groups ($p_{time*group} = 0.80$; Figure 2a). Baseline T_{sk} and peak T_{sk} were 33.9 [33.2–34.4°C] and 36.2 ± 0.5°C, respectively, and did not differ between groups ($p = 0.18$ and $p = 0.75$, respectively). T_{sk} increased with 1.9 ± 0.8°C ($p_{time} < 0.001$), but the magnitude of the increase did not differ between groups ($p_{time*group} = 0.57$; Figure 2b). In the EHS group, no correlation was found between the time frame between the EHS event and the exercise test and peak T_{gi} ($r = 0.05$, $p = 0.84$), nor T_{sk} ($r = -0.24$, $p = 0.34$). The average H_{dry} during exercise was significantly lower in the EHS

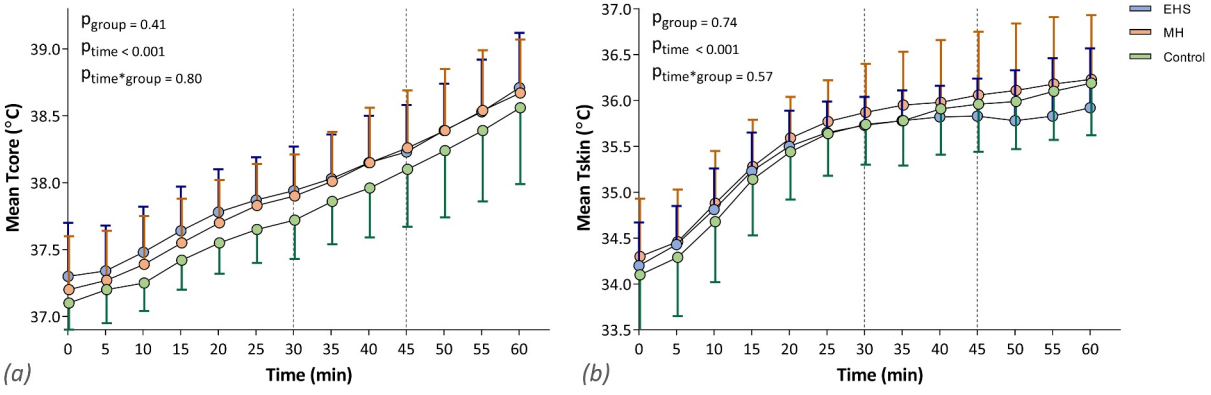

Figure 2. Continuous Tgi (a) and Tsk (b) during the three exercise parts. Data is presented as mean with standard deviation.

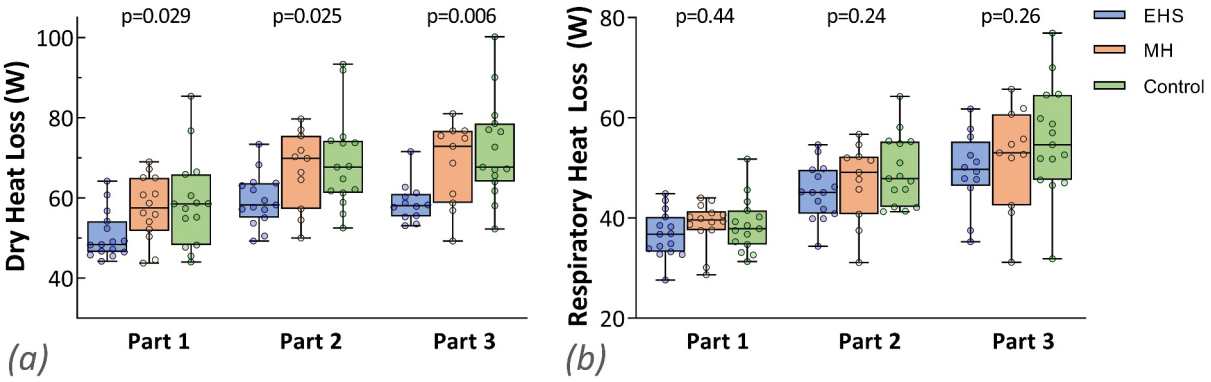

Figure 3. Dry heat loss (a) and respiratory heat loss (b) during the three exercise segments for the EHS (blue), MH (orange) and control group (green).

group (54 ± 4 W) compared to the control group (65 ± 11 W) during all three parts of the exercise test (p = 0.023; Figure 3a). H_{resp} during the exercise protocol was 44 ± 6 W and did not differ across groups (p = 0.30; Figure 3b). WBSR was 1.1 [0.8–1.2 L/h] and did not differ between groups (p = 0.16). Median evaporative heat loss was 606 [539–674 W] in the EHS group, 808 [438–825 W] in the MH group and 809 [539–876 W] in the control group. Evaporative heat loss did not differ between groups (p = 0.12).

Fluid balance

Pre-exercise USG was 1.0072 ± 0.0042 g/mL and increased to 1.0146 ± 0.0062 g/mL post-exercise. Moreover, relative body mass loss was 1.4 [1.1–1.4%] and did not differ between groups (p = 0.22). None of the participants exceeded ≥2% body mass loss indicative of dehydration, but nine participants (20%) could be classified as dehydrated based on USG > 1.020 g/mL (Table 2). Post-exercise USG did not differ between groups (p = 0.41).

Heart rate

Average heart rate at baseline was 78 [71–86 bpm] and did not differ between groups (p = 0.06). Heart rate increased with 76 ± 17 bpm to 159 [148–174 bpm] (p_{time} < 0.001), but the magnitude of the increase was similar between groups ($p_{time*group}$ = 0.27).

Laboratory results

Blood samples were available from 39 participants who completed the test (Table 3). Baseline and post-exercise IL-6 levels were below the LOD more often compared to the EHS group (p = 0.006). Pre- and post-exercise TNF- α did not differ within and between the groups. Throughout all three groups, serum I-FABP at baseline was 442 [220–594 pg/mL] and post-exercise 726 [404–1351 pg/mL], which both did not differ between groups (p = 0.65 and p = 0.08, respectively). The exercise induced increase in serum I-FABP across the three groups was 365 [89–821 pg/mL] (p < 0.001), but the increase was comparable between groups (p = 0.12). Serum PENK at baseline was 44.5 [38.3–58.6 pmol/mL] and post exercise 54.3 [45.9–73.9 pmol/mL], which did not differ between the three groups (p = 0.49 and p = 0.41, respectively). The exercise induced increase in serum PENK across the three groups was 12.1 [1.3–20.2 pmol/mL] (p < 0.001), but the increase was comparable between groups (p = 0.08).

Perceptual outcomes

On the four-point thermal comfort scale, at baseline participants reported to feel comfortable (n = 36, 82%) or slightly uncomfortable (n = 8, 18%; Figure 4). The reported discomfort throughout the exercise test was comparable between the three groups (p = 0.73). After exercise cessation, the majority of participants reported to feel uncomfortable (n = 17, 39%) or very uncomfortable (n = 11, 25%). Thermal comfort increased with 2 [1–2 points] and was comparable between the three groups ($p_{group*time}$ = 0.85). Furthermore, thermal sensation at baseline was most frequently reported as neutral (n = 10, 23%), or slightly warm (n = 31, 71%). The reported thermal sensation throughout the exercise test did not differ between the three groups (p = 0.54). After exercise cessation, participants most frequently reported the thermal sensation as warm

Table 3. Laboratory test results of pre- and post exercise IL-6, TNF-α, I-FABP and PENK serum levels.

	EHS ($n = 15$)	MH ($n = 14$)	Control ($n = 15$)	Total ($n = 44$)	P-value
IL-6 (n(%))	*13 (87)*	*12 (86)*	*14 (93)*	*39 (89)*	
< LOD baseline (n(%))*	*5 (38)***	*8 (67)*	*13 (87)*	*26 (67)*	0.004
< LOD post (n(%))	*7 (54)*	*8 (67)*	*13 (87)*	*28 (72)*	0.029
Baseline (pg/ml)	52.0 [14.3–579.4]	189.2 [45.8–784.55]	10.1	57.1 [20.6–567.3]	0.38
Post-exercise (pg/ml)	321.9 [33.5–643.1]	186.6 [32.6–866.5]	12.9	63.6 [32.1–621.4]	0.45
Δ IL-6 (pg/ml)	19.7 [5.6–37.8]	25.5 [−41.3–82.0]	2.8	9.6 [2.8–39.4]	0.62
TNF-α (n(%))	*13 (87)*	*12 (86)*	*14 (93)*	*39 (89)*	
< LOD baseline (n(%))	*7 (54)***	*8 (67)*	*14 (100)*	*29 (74)*	0.018
< LOD post (n(%))	*10 (67)*	*9 (75)*	*14 (100)*	*33 (85)*	0.25
Baseline (pg/ml)	117.6 [44.3–370.0]	263.7 [100.1–896.2]	–	123.0 [83.6–405.9]	0.29
Post-exercise (pg/ml)	97.4 [42.2–97.4]	398.9 [81.6–398.9]	–	244.5 [71.7–581.0]	0.28
Δ TNF-α (pg/ml)	−2.9 [−10 .7/-3.0]	−14.9 [−17.8/-14.9]	–	−10.7 [−17.8/-2.6]	0.51
I-FABP (n(%))	*13 (87)*	*12 (86)*	*14 (93)*	*39 (89)*	
Baseline (pg/ml)	532 [313–575]	378 [202–593]	396 [163–667]	442 [220–594]	0.65
Post-exercise (pg/ml)	1273 [691–1684]	489 [355–994]	710 [366–1054]	726 [404–1353]	0.08
Δ I-FABP (pg/ml)	680 [325–1233]	209 [−44–537]	338 [1–592]	365 [89–821]	0.12
PENK (n(%))	*13 (87)*	*12 (86)*	*14 (93)*	*39 (89)*	
< LOD (n(%))	–	*2 (17)*	*2 (14)*	*4 (10)*	0.28
< LOD (n(%))	*1 (8)*	–	–	*1 (3)*	0.22
Baseline (pmol/ml)	41.4 [37.2–58.2]	49.8 [40.9–63.7]	43.8 [41.6–62.3]	44.5 [38.3–58.6]	0.49
Post-exercise (pmol/ml)	56.4 [51.5–89.6]	49.5 [39.1–71.1]	55.8 [44.9–69.6]	54.3 [45.9–73.9]	0.41
Δ PENK (pmol/ml)	16.5 [12.5–20.8]	1.0 [−8.8–12.8]	11.1 [2.9–21.3]	12.1 [1.3–20.2]	0.08
GFR (PENK-CR) (n(%))	*13 (87)*	*12 (86)*	*14 (93)*	*39 (89)*	
Baseline (ml/min)	105 [92–112]	95 [82–99]	99 [96–106]	99 [91–108]	0.18
Post-exercise (ml/min)	82 [70–82]	85 [77–90]	89 [83–95]	85 [78–95]	0.39
Δ PENK (ml/min)	−17 [−22/-11.2]	−6 [−18/-1]	−13 [−19/-5]	−13 [−13/-3]	0.24

Abbreviations: EHS = Exertional heat stroke; GFR = Glomerular filtration rate; I-FAPB = intestinal fatty acid binding protein; IL-6 = interleukin-6; LOD = limit of detection; MH = Malignant hyperthermia; PENK = proenkephalin; TNF-α = Tumor necrosis factor-α.
*Lower limit of detection: I-FABP = 47 pg/ml; IL-6 = 1.56 pg/ml; PENK = 31.0 pmol/L; TNF- α = 7,81 pg/ml.
**Statistically significantly different from control group.

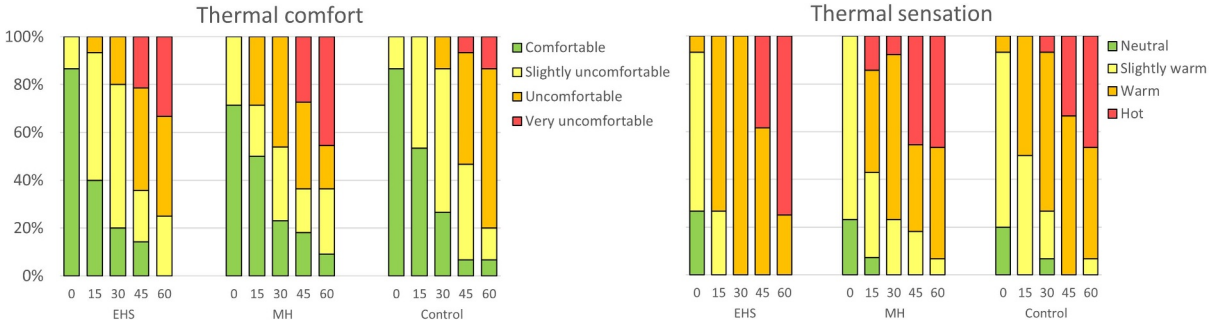

Figure 4. Overview of thermal comfort (A) and thermal sensation (B) at baseline and at 15-min intervals during the exercise test.

($n = 16$, 37%) or hot ($n = 21$, 48%). Thermal sensation increased with 2 [1–2 points] and the increase over time was comparable between the three groups ($p_{group*time} = 0.63$).

Discussion

We investigated the thermoregulatory response to prolonged submaximal exercise in hot environmental conditions in patients with a history of EHS or MH, and healthy controls. No differences were observed between the three groups in terms of exercise-induced changes in T_{gi}, T_{sk}, HR, H_{resp}, or WBSR. These results suggest that EHS and MH patients do not have an altered thermoregulatory response during exercise under the controlled ambient conditions of the current study.

In contrast to our hypothesis, no differences were observed in exercise-induced increase in T_{gi} or T_{sk} between the three groups. H_{dry} was lower in the EHS group compared to the MH and healthy control group. This is likely explained by the slightly higher T_{amb} in the EHS group, leading to a smaller heat transfer gradient between T_{sk} and T_{amb} and thereby a reduced heat loss capacity. However, the modest difference in ambient temperature might not fully explain the difference in H_{dry}.

Our results are in line with a previous study examining the thermoregulatory responses of EHS, MH, and healthy controls ($n = 6$ per group) during an exercise protocol on a treadmill with workload adjusted to the participant's VO_{2max} [14]. Our protocol used a fixed H_{prod} per kg body mass, which is more suitable for comparing thermoregulation across different groups [38], and supports previous findings of a comparable thermoregulation in EHS and MH individuals under such controlled ambient conditions.

Regarding MH, *RYR1* variants have been previously reported in 13% to 46% of EHS individuals; however, the question whether MH individuals are at increased risk for developing EHS remained unanswered in these studies [7,8]. Our results show that the participants with MH do not have signs of impaired thermoregulatory response to exercise in heat in a laboratory setting. However, these results cannot directly be extrapolated to *RYR1* variants in general. The spectrum of phenotypes due to *RYR1* variants is very wide and is likely to be associated with a variable heat sensitivity of the RyR1 protein [39]. Animal studies have demonstrated that knock-in mice with a MH causative variant (Y522S) consistently display heat intolerance [40,41]. Our results suggest that the p.Val4849Ile, p.Thr2206Met, or p.Thr2206Arg genotypes identified in some patients do not result in a greater heat sensitivity of the RyR1 protein. Further research assessing genotype–phenotype correlations will be needed to provide further insights into EHS and/or other heat-related symptoms in MH individuals, also considering that up to 65% of *RYR1* variants detected are still classified as a variant of uncertain significance [42]. In addition to Mendelian genetic defects, several animal studies have suggested epigenetic changes may explain recurrence of EHS [43]. Mice studies demonstrated that after 30 days of recovery after EHS, epigenetic alterations lead to a skeletal muscle phenotype with increased vulnerability to stress [44].

Human studies have examined the thermoregulatory response to exercise in individuals with a history of EHS. Stearns et al. investigated a cohort of athletes at the 11.7 km Falmouth Road Race and reported a recurrence rate of 11% ($n = 37/333$) over a 17-year period, with the highest relative risk (RR) ratio of recurrence in the first two years after the first event (RR = 3.33), declining after 3–6 years (RR = 2.00 to 1.26, respectively) [45]. Furthermore, Sagui et al. investigated military personnel with a history of EHS, demonstrating a recurrence rate of 13% under relatively mild environmental conditions (i.e. T_{amb} 22°C, RH 70%) during a 7 year follow-up [8]. In the present study, we found a comparable thermoregulatory response within the EHS group, irrespective of the timeframe from the event until study. One explanation of a high recurrence rate in military personnel might be that they are potentially exposed to considerably higher levels of heat stress by carrying heavy military equipment and gear and due to the social dynamic of leadership and followership [46–48]. An explanation of the differences between our results and those of previous studies could be that an accumulation of risk factors is required to exceed the threshold for developing EHS (e.g. behavioral and individual factors and health status), which is not present in our study.

Serum IL-6 levels showed an exercise-induced increase in all cohorts, but no differences were observed between the three groups. Previous studies described an exercise induces IL-6 response to physical exertion [49,50]. However, the response does not correlate with body temperature, but does correlate with severity in case of actual EHS, which in our cohort did not occur [51]. Furthermore, exercise-induced serum I-FABP levels increased throughout the three cohorts, but no differences were observed between the three groups. It should be noted that fatty acid-binding proteins are excreted rapidly (e.g. a half-life of 11 min in liver L-FABP) and any differences may thus not have necessarily been captured [52,53]. Our results support previous findings of increased serum I-FABP due to exercise and hyperthermia in the absence of EHS [54]. The question whether the magnitude of exercise-induced serum I-FABP or IL-6 are a predictor of EHS susceptibility could be focus of future investigation.

No differences were observed in thermal comfort nor thermal sensation between the three groups, nor in the six participants who were not able to complete the exercise test due to exhaustion, nausea, lightheadedness, or subjective heat intolerance. A subgroup analysis of the six participants did not reveal any differences in training status nor in any thermoregulatory outcomes at exercise test cessation. Although the role of anxiety in performance remains a matter of debate, anxiety toward exercise has been reported as a

long-term mental health complication in more than half of EHS individuals, which may have contributed to early exercise cessation in these participants [7,50].

A strength of the current study are the well-controlled laboratory conditions that were used to examine the thermoregulatory response to exercise at a fixed metabolic heat production. Environmental conditions were ecologically valid, similar to 18 years of documented conditions during a running race known for a high incidence of EHS [22]. However, some limitations should be considered. First, the workload that was needed to cycle at the predefined H_{prod} was challenging for a proportion of participants, leading to six participants not being able to complete the test and four participants who had to complete the test at a lower workload. Less challenging conditions might have prevented early test cessation in these participants. However, it is unlikely that the missing datapoints had a major effect on the data, which is supported by the comparable H_{prod} as well as the subgroup analysis which did not reveal differences between the participants who did not complete the test and participants who did. Furthermore, evaporative heat loss through the skin surface was estimated based on the whole-body sweat loss during exercise. However, this estimate does not account for interindividual differences in sweating efficiency. Moreover, body mass loss during exercise was measured to the nearest 100 grams, and post-exercise body mass was measured after a 15-min resting recovery period, during which additional sweat loss may have occurred. Therefore, the E_{sk} should be interpreted with caution. Finally, genetic testing was only performed in three EHS participants as a part of the clinical assessment, and no *RYR1* variants were found. Since genetic testing was not routinely included in 12 EHS individuals the current study, we cannot answer the question if genetic variants are present in the majority of individuals within the EHS group. Nonetheless, this did not interfere with answering the research question whether EHS/MH individuals have an altered thermoregulatory response compared to healthy controls. The question whether specific *RYR1* genotypes lead to an increased heat susceptibility would be an interesting focus for future research, preferably by performing genetic testing in a large cohort of EHS individuals. Furthermore, it would be particularly interesting to investigate to what extent the complex accumulation of other risk factors contributes to developing EHS, given the absence of signs of impaired thermoregulation in the current conditions.

Conclusion

We demonstrated that in a cohort of individuals with a history of EHS or MH, no differences are observed in thermoregulatory response to exercise in hot environmental conditions compared to healthy controls under controlled conditions. These results suggest that individuals with a history of EHS or MH do not have an increased risk of developing EHS while performing strenuous exercise in hot ambient conditions. Given the absence of signs of impaired thermoregulation in our experimental setup, future research should assess to what extent the complex accumulation of other risk factors contributes to developing EHS.

Abbreviations

BMI	Body mass index
BSA	Body surface area
EHS	Exertional heat stroke
EMHG	European Malignant Hyperthermia Group
GFR	Glomerular filtration rate
H_{dry}	Dry heat loss
H_{resp}	Respiratory heat loss
HR	Heart rate
I-FABP	Intestinal Fatty Acid Binding Protein
IL-6	Interleukin-6
MH	Malignant hyperthermia
RH	Relative humidity
RYR1	Ryanodine receptor-1 gene
RyR1	Ryanodine receptor-1 protein
SQUASH	Short QUestionnaire to ASsess Health enhancing physical activity

T_{amb}	Ambient temperature
T_{gi}	Gastrointestinal temperature
T_{sk}	Skin temperature
TNF-α	Tumor necrosis factor-α
USG	Urine specific gravity
WBSR	Whole-body sweat rate

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The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. Moreover, the results of the present study do not constitute endorsement by the American College of Sports Medicine, Indianapolis, Indiana, USA.

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Disclosure statement

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Data availability statement

The anonymized dataset used for this study is available from the corresponding author upon reasonable request.

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