Title Neurobiomarker and body temperature responses to recreational marathon running

Abstract

Objectives To assess how biomarkers indicating central nervous system insult (neurobiomarkers) vary in peripheral blood with exertional-heat stress from prolonged endurance exercise.

Design Observational study of changes in neuron specific enolase (NSE), S100 calcium-binding protein B (S100β), Glial Fibrillary Acid Protein (GFAP) and Ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCHL1) at Brighton Marathon 2022.

Methods In 38 marathoners with in-race core temperature (Tc) monitoring, exposure (High, Intermediate or Low) was classified by cumulative hyperthermia - calculated as area under curve of Time x Tc>38 °C - and also by running duration (finishing time). Blood was sampled for neurobiomarkers, cortisol and fluid-regulatory stress surrogates, including copeptin and creatinine (at rested baseline; within 30 minutes of finishing; and at 24 h).

Results Finishing in 236 ± 40 min, runners showed stable GFAP and UCH-L1 across the marathon and next-day. Significant ($P<0.05$) increases from baseline were shown post-marathon and at 24 h for S100β (8.52 [3.65, 22.95] vs 39.0 [26.48, 52.33] vs 80.3 [49.1, 99.7] $ng.L^{-1}$) and post-marathon only for NSE (3.73 [3.30, 4.32] vs 4.85 [4.45, 5.80] $ug.L^{-1}$, $P<0.0001$). While differential response to hyperthermia was observed for cortisol, copeptin and creatinine, neurobiomarker responses did not vary. Post-marathon, only NSE differed by exercise duration (High vs Low, 5.81 ± 1.77 vs. 4.69 ± 0.73 $ug.L^{-1}$, adjusted $P=0.0358$).

Conclusions Successful marathon performance did not associate with evidence for substantial neuronal insult. To account for variation in neurobiomarkers with prolonged endurance exercise, factors additional to hyperthermia, such as exercise duration and intensity, should be further investigated.

Keywords brain injuries, traumatic; copeptin; cortisol; heat illness; GFAP protein, human; heat stroke; Ubiquitin carboxyl-Terminal Hydrolase L-1, human.

Introduction

Exertional hyperthermia indicates a state of increased physical strain characterised by augmented sympathoadrenal and neurohumeral responses.[1] As the severity of heating increases with prolonged or strenuous exercise bouts, performance may be impaired and the risk of incapacity rises. Exertional heat stroke (EHS), a life-threatening disorder of extreme hyperthermia and organ injury, lies at the extreme end of the pathology spectrum reported with collapse under physical stress.[2]

Brain injury is a hallmark of fatal illness and may cause long term debility in survivors.[3] Lesser forms of exertional heat illness and other maladies affecting central nervous system (CNS) function also contribute to the syndrome of Exercise Associated Collapse (EAC), but markers of CNS insult do not feature in standard clinical biochemistry panels and knowledge of neuronal stress in such circumstances is limited.[2]

For non-exertional causes of CNS failure, such as Traumatic Brain Injury (TBI) and Sepsis Associated Encephalopathy (SAE), the application of peptide 'neurobiomarkers' is increasingly reported to extend diagnostic and prognostic capabilities.[4-6] This suggests a potential role for their assay in the assessment of brain insult associated with, or sustained under, exertional hyperthermia, so long as they prove robust to potential confounding from the physical stress of exercise itself.

Titres of the glial-derived peptide S100 calcium-binding protein B (S100β) are known to rise in the peripheral circulation in association with both oxidative[7,8] and thermal[8,9]stress and a relationship has been shown with core body temperature (Tc) response.[10] We have previously reported circulating elevations in S100β and the neuronal enzyme neuron specific enolase (NSE) in the setting of cool weather marathon running, for NSE to a degree significantly greater in collapsed cases diagnosed with heat illness than in healthy controls.[11] Other

evidence has also been cited in support of a role for each peptide in prognosticating for neurological outcomes following heat stroke.[12]

Yet the use of these two markers in isolation presents challenges to differentiating changes associated with integrity of the blood-brain barrier (BBB) itself and exercise in general from true neuronal insult.[10,13] Two further neurobiomarkers, glial fibrillary acid protein (GFAP) and ubiquitin C-terminal hydrolase-L1, are approved for near-care application to help exclude more severe forms of TBI following head injury. In SAE, neurological insult is thought to result from sepsis-driven impairments to BBB function and inflammatory changes in the brain matter[14] and UCH-L1 performs better than GFAP in discriminating SAE from sepsis uncomplicated by encephalopathy.[6] However injuries incurred from sports accounted for only 2.5% of 1959 cases used to validate assay of these markers in TBI[4] and greater clarity on how they may vary with exercise is required.

Therefore the primary aim of this study was to investigate neurobiomarker responses to prolonged endurance exercise in a group of recreational marathon runners. We hypothesised that neurobiomarkers would increase with cumulative hyperthermia, as quantified with continuous monitoring of core body temperature (Tc) in-race. We further explored relationships with finishing times and also had opportunity to characterise responses in hyperthermic cases incapacitated during the same event and treated at an on-course Medical Treatment Facility (MTF). As we have previously observed substantially elevated plasma copeptin - the glycopeptide surrogate for arginine vasopressin (AVP) secretion – with higher levels of thermal strain and in heat illness cases,[11,15] we explored its response to help corroborate and potentially explain our findings.

Methods

The study was conducted at the Brighton Marathon held on 10th April 2022 in Brighton, United Kingdom (UK). Ethical approval was obtained from the UK Ministry of Defence Research and Ethics Committee (approval number 1030/MODREC/19) and complied with the standards set in the Declaration of Helsinki. The study was funded internally by the UK Surgeon General and practical support was given by Human Telemetrics (London, UK). The authors retained the right to collect, analyse and interpret data independently and to approve or disapprove publication of the finished manuscript.

Data were collected from two groups of runners – people who volunteered for in-race monitoring of Tc and blood and anthropometric assessments before and after completing the full marathon, 'successful finishers' (SF) - and a separate group of individuals, not enrolled as prospective SF, who collapsed during the marathon and required care at one of two MTFs temporarily sited on-course for clinically diagnosed exertional heat illness (termed Exertion Associated Collapse, EAC, for purposes of this manuscript). There was no overlap between these two groups.

The study was publicised on social media and through marathon organiser emails prior to the event and 44 participants were prospectively recruited at the pre-race exhibition. Demographic information including age, prior number of marathons completed and details of previous performance was collected for SF. Inclusion criteria for SF were age 18-55 years with an expected finishing time of 4 h or less. Exclusion criteria were new symptoms of an acute infectious illness (coryzal symptoms, cough, gastrointestinal disturbance, rash); congenital renal disease, or acquired renal dysfunction requiring medication; brain injury sufficient to cause loss of consciousness in the preceding four weeks; or any other significant chronic health condition (cardiac problems, asthma, diabetes) un-mitigated by appropriate medical advice on participation in the event..

Anthropometric and physiological measurements and blood tests were taken between 10.00 and 19.00 on the day prior to the event. Unshod standing height and minimally clothed body mass were recorded for each control participant using a stadiometer and scales. Each participant received two e-Celsius ingestible capsules for the measurement of core temperature (BodyCap, Caen, France). Participants were instructed to swallow the two pills, respectively, 12 and 3 h before the event. Core temperature for both pills (precision 0.1°C; sampled every 30 s) was continuously recorded using e-Celsius ingestible capsules (BodyCap, Caen, France).

Venepuncture was performed at the antecubital fossa after 10 min seated rest. Blood samples (13 ml per draw) were taken at the following time-points: (1) pre-race baseline, at race registration the day before the marathon; (2) post-race, as close to the time of successful completion of event as feasible (<30 minutes); (3) the following day, as close to 24 hours post-run as feasible. Blood was centrifuged at 1500G for 15 minutes, with serum having stood for 30 min minimum. Serum and plasma were then aliquoted and frozen to -20 °C on site, prior to subsequent shipping and assay.

Criteria for recruitment of EAC cases were age 18 to 55 years old with a clinical diagnosis of heat illness. Runners who collapsed during the marathon were assessed for study enrolment following evacuation to the nearest MTF, clinical re-assessment and immediate essential medical treatment. The cases in question were triaged, treated and confirmed by clinicians experienced in the management of EAC and the heat illness subgroup of interest. Body temperature was measured rectally (Intellivue integrated thermistor, Philips Healthcare, Amsterdam, Netherlands) The presence or absence of exclusion criteria, as described above, was documented for later review, but did not prevent recruitment. Heat illness was diagnosed where excess body heat was deemed the primary cause of incapacity. This was defined as Tc around the point of incapacity shown to be – or, based on cooling trajectory, suspected to have been - ≥38.5°C, in association with CNS impairment, such as abnormal

motor control, loss of responsiveness, amnesia for the episode. These features were required to have occurred spontaneously during or soon after marathon run, and to have been followed by failure to make a prompt recovery with prostration and initial medical care. Runners diagnosed with exertional or post-exertion syncope were excluded from recruitment or further participation in the study, as were those re-categorised with an aetiology other than heat-related, based upon response to initial treatment and subsequent investigations.

Level of consciousness upon presentation to medical staff was defined according to the widely used Alert-Confused-Voice-Pain-Unresponsive (AVPU) scale, which assigns the best casualty response to stimulation in a graded fashion. For cases who initially lacked mental capacity to consent for themselves, we proceeded with presumed consent until they were deemed able to give it retrospectively. As clinical considerations allowed, blood samples (13 ml per draw) were taken at time-points (1) T0, as close to the time of collapse as feasible (within 30 minutes of presentation to the treatment facility) and (2) T1, one hour further on from T0. Per the MTF standard operating procedures, active cooling was applied to EAC with Tc>38 °C, using ice for Tc>40 °C and spraying and fanning below this. Samples were processed as for successful finishers. No EAC cases were available for next-day sampling despite ethical approval being in place to do so.

Samples from SF and EAC were moved from -20 °C storage on-course, to -80 °C storage locally. Dry ice was used for shipping by courier to definitive storage and analysis at Affinity Biomarker Labs (London, UK). Upon thawing, serum was analysed for serum creatinine (sCr), creatinine kinase (CK) and cortisol on a commercial platform (Siemens Advia 1800, Siemens Healthcare Diagnostics Ltd, Camberley, UK). Serum NSE (R & D Systems Europe, Abingdon, UK), serum S100β (EMD Merck-Millipore, St. Louis, USA), plasma GFAP (Oxford Biosystems, Abingdon, UK) and plasma UCH-L1 (R & D Systems Europe, Abingdon, UK) were measured by commercially available immunoassays with intra- and inter-assay variability of <8.0% and upper limits of detection of 20ug.L^{-1}, 2000 ng.L^{-1}, 25 ug.L^{-1} and

2500ng.L^{-1} respectively. Plasma copeptin was measured by Time-Resolved Amplified Cryptate Emission technology (Thermo Fisher-Brahms, Hennigsdorf, Germany), with inter-assay coefficient of variation of 2.5–3.7% and a lower limit of detection of 0.9 pmol L^{-1}. Plasma osmolality (pOsm) was measured by depression of freezing point.

Minute-sampled values of Tc were used to construct Tc x time plots for each individual marathon performance by successful finishers. Where Pill 1 and Pill 2 overlapped, the higher Tc value was taken. This was to allow for artefactual depression from fluid ingestion, anticipated to operate to a greater extent with more recent pill ingestion. Where such artefact was clearly evident and limited to <10 min duration, the curve was smoothed using interpolated values; if artefact was prohibitive to reasonable interpolation, the participant was removed entirely from the analysis. Following the approach of Cheuvront et al,[16] cumulative hyperthermia was defined as the area of the curve above the pre-selected thermal threshold of Tc >38 °C, the latter representing the upper limit of response expected for most humans engaged in moderate-intensity exercise in thermoneutral conditions.[17] The resulting Area of the Tc-time curve was categorised as High, Medium or Low exposures, based upon the distribution of absolute values observed. Additionally, relationships to the total duration of exercise undertaken (marathon finishing time) were explored, again according to High, Medium or Low exposure (in minutes).

All data were assessed for normality and expressed as mean ± SD or median [IQR]. Linear relationships between changes in biochemical variables with marathon running versus hyperthermia exposure were assessed for significance by Pearson's or Spearman's rank tests, for parametric and non-parametric variables respectively. Pairwise comparisons were by t-test (parametric data) or Mann-Whitney test (non-parametric data) for pre to post sampling. One way ANOVA (or Kruskal-Wallis non-parametric equivalent) was applied, with post hoc corrections for multiple comparisons, to compare tertiles of runner characteristics and marathon responses by exposure category (hyperthermia or marathon finishing time).

Two-way ANOVA was performed on parametric data, (or the natural logarithm thereof if non-parametric), again with post hoc corrections for multiple comparisons, to assess variation in biochemical parameters across the marathon. Significance was set to alpha=0.05. A formal power calculation was not attempted prospectively, due to the study protocol's dependence upon a convenience sample of willing volunteers and uncertain availability of heat illness cases. However, our previous study[11] had indicated sufficient power with thirty successful finishers and eight exertional heat illness cases to demonstrate significant elevation in S100β and NSE across the marathon and differential response for finishers vs collapsed hyperthermic cases.

Results

On the day of the marathon, ambient temperature measured at the local meteorological station increased from 0 °C during the event muster (race start time 09:45 am) to peak at 12 °C with runners still on the course. Of 44 SF who took part in Tc monitoring, deleterious artefact necessitated removal of six from further analysis. The remainder all achieved Tc> 38 °C, a majority (n=27) >39 °C and three peaked >40 °C. All 38 retained participants completed blood pre sampling (within 24 hours of the marathon start) and post sampling (within 30 minutes of completion). A further 10 - two female, eight male – accepted the invitation to return for blood sampling again the day following the marathon.

Baseline and intra-/post-race characteristics for SF – including finishing time - are presented, by tertile of body temperature response, in Table 1. Representing the area under the curve of [Time x Tc >38 °C], respective values for High, Intermediate and Low categories of hyperthermia were 246 ± 61, 144 ± 16 and 60 ± 32 min.°C. Supplementary Table 1 presents equivalent data displayed by tertile of exercise duration (High 293 ± 33 min, Medium 243 ± 8 and Low 199 ± 21 min).

Baseline and post-run biochemical results for the 38 SF are presented in Figure 1 (by hyperthermia tertile), Figure 2 (by finishing time) and Supplementary Table 2 (pooled results, including next-day measures). Supplementary Table 3 presents linear associations for the changes in biochemical variables with marathon running versus hyperthermia exposure.

Of ten marathon runners presenting with collapse, seven were confirmed clinically as heat illness cases and formed the study EAC group. For these seven casualties, two manifested more extreme hyperthermia in keeping with fulminant, ongoing EHS. Qualifying characteristics and individual biochemical results are displayed in Table 2. One casualty had been actively cooled prior to reception at the treatment facility; in the others, initial sampling was conducted

at the very outset of treatment i.e. before active cooling or intravenous fluid therapy were instituted. Exclusion criteria were not fulfilled in any of the seven.

Discussion

This is the first field investigation to quantify thermal stress in relation to neurobiomarkers and, to the best of our knowledge, the first to assess GFAP and UCH-L1 with either marathon running or EAC. We also believe that this study is the first to assess core temperature directly in relation to markers associated with organ injury from marathon running, including copeptin, with previous work having relied upon heart-rated derived estimates.[18] Novel findings include stable levels of GFAP and UCH-L1 in SF, without variation by Tc response across the marathon or by finishing time; and high levels of S100β, UCH-L1 and copeptin levels in the small group of EAC cases characterised close to the point of incapacitation, this elevation being particularly marked in two experiencing EHS at point of sampling.

S100β and GFAP are expressed primarily by the glial (astrocyte) population of cells, whose roles include preservation of the integrity of blood-brain barrier (BBB).[5] Resting blood concentrations of S100β escalate promptly with increasing BBB permeability, from lower values upon the infusion of hypertonic solutions, to higher with frank CNS damage (~180 $ng.L^{-1}$).[19] Fluid deprivation combined with exertional-heat stress has been used to evidence a preserved influence of increasing extracellular tonicity on BBB opening with exercise.[9] A systematic review of peripheral S100β levels following exercise concluded that, in health, elevations are largely attributable to increased BBB permeability, with the severity of compromise dependent upon the intensity of the preceding exertion.[13]

In the present work, S100β levels in SF appear to have been broadly stimulated by exercise – to levels previously reported to associate with mild TBI[20] - rather than following Tc response *per se*. Stratifying by finishing time revealed no difference in S100β response. We do not anticipate that the tertiles of exercise duration concealed major differences in overall relative exercise intensity, as the degree of hyperthermia - known to align with $\%VO_2max$ upon submaximal exercise[21] – did not differ across the groups, nor did cortisol and copeptin as indicators of global stress. Rather, the significant variation in previous personal best times for

the discipline suggested that slower runners were simply less well trained. Failure of S100β to follow the rise in osmolality across declining tertiles of finishing time may indicate that factors other than blood tonicity influenced BBB permeability and its release into the peripheral circulation. Secondary release of CNS-derived S100β from peripheral (extracranial) sites of storage may contribute to changes observed with exertional heat-stress and could conceivably confound the association of S100β with neuroglial function and injury.[13] However sampling within one to two hours seems to preserve the specificity of a rise in of S100β for a preceding CNS challenge, such as sports-related head injury.[22] Post-marathon measures on the day of the event were made comfortably within this window.

In contrast to the early rise observed with S100β, GFAP shows a relatively delayed (~20 h) peripheral peak following CNS insult,[23] suggesting that its passage into the blood proceeds by alternative routes to the BBB[5] and that it may not reliably delineate isolated disruption. In TBI studies, elevated GFAP has been reported to associate with mass lesions in anatomically discrete regions of the brain, as with focal bleeding. Discriminant peripheral titres may rely upon particular patterns of underlying structural damage, such as axonal injury.[4,24] This may help to explain the lack of change in GFAP with marathon running, such that even next-day levels failed to vary from baseline.

Unlike the astroglial markers S100β and GFAP, both NSE and UCH-L1 derive from neurons, though their *de novo* appearance in blood is assumed contingent upon co-existence of disruption to the BBB with neuronal insult. NSE does not rise with peak exercise testing and 60 min exercise bouts, conducted at a variety of exercise intensities in laboratory conditions, have also failed to show significant changes.[8] Nevertheless NSE elevations have been reported with running activities in field settings over marathon and ultramarathon distances.[11,25] NSE is also known to rise with activation of peripheral neuroendocrine tissues,[26] thus it is possible that high levels of physical stress in the present and previous field investigations account for differences in response against shorter or less competitive

laboratory exposures. This hypothesis is supported by the findings that SF completed the event slower than they predicted, indicating additional challenges, and that those who spent longer running exhibited a higher NSE. Haemolysis is known to artefactually elevate NSE and may also have arisen, either intravascularly or with sample collection.[27]

Conversely, UCH-L1 did not rise across the marathon and, instead, remained highly particular to the individual being sampled, with 92.0% of variance attributable to participant factor in SF. Mean post-run concentrations, while encompassing outlier values at the top of the assay cut-off, appeared substantially lower than those observed in the EAC cohort and in those affected by EHS in particular, as did S100β, blood osmolality, creatinine and copeptin concentrations. Conclusions based on these findings must necessarily be cautious due to lack of comparative baseline data for EAC and the inability to correct for changes in plasma volume engendered by the study setting and design; a direct statistical comparison was not attempted owing to the heterogeneous nature of EAC observed and differences in the timing of initial sampling due to on-course delays in casualties presenting for treatment at the MTFs. Nevertheless, it is possible to speculate that variation in S100β and UCH-L1 may have reflected greater BBB disruption and CNS insult in EAC.

It was not possible to undertake collateral investigations to support increased BBB permeability, such as neuroimaging, on the marathon course due to time and logistic constraints. This could be considered to provide extra information in future investigations. Furthermore the precise nature of elevation in neurobiomarkers in this context remains to be elucidated. For example, a further peptide released with neuronal stress, brain-derived neurotrophic factor (BDNF), plays various roles in neuroprotection, with levels in the peripheral circulation found to be relatively increased by physical exertion combined with heat stress.[28,29] Moreover, absolute values of BDNF better reflect end-exercise core body temperature than the duration of exercise/amount of work performed.[29] Future work could consider whether peptides examined in the present work may be elevated under conditions of heating, exercise

or supervening illness as simple bystander molecules, or whether they contribute to processes supportive to homeostasis and recovery.

The relative inconvenience and potential negative connotations of further participation in the study may have hampered our attempts to secure next-day sampling in EAC runners. This would otherwise have offered an important comparison with neurobiomarker response in SF at 24 hours post-marathon/exertional collapse. The availability of methods to assay both UCH-L1 and S100β in saliva[5,] may aid the construction of larger or more targeted studies to address heat illness diagnosis and prognostication among EAC cases presenting to medical care, especially if sampling can be achieved remote from the study hub.

Considering other limitations, female runners were relatively under-represented for our findings to be fully generalisable . We also observed only modest fluid-regulatory stress in most successful finishers, with <3% loss of body mass across all categories of hyperthermia, and a commensurate, modest rise in plasma osmolality and copeptin. More severe conditions of environmental stress could exacerbate fluid loss and potentially increase osmotic effects at the BBB and on neurobiomarker response. Variation in individual strain could also account for different responses, as may have been the case in the EAC sample we report. These issues should be examined in follow up investigations, with characterisation of disruption at other natural barriers (e.g. the gastrintenstinal tract[30]) incorporated to link to other potential mechanisms underlying heat illness and EHS.

Conclusions

Stability of GFAP and UCH-L1 across the marathon, coupled with failure of S100β and NSE to show a graded response to cumulative hyperthermia, contradicted our hypothesis that neurobiomarker values in peripheral blood would vary with increasing thermal stress. Incredibly stable UCH-L1, including at 24 h, argues against the evolution of critical neuronal strain in successful marathon performance. Lack of change in GFAP was also reassuring for use of these assays in the evaluation of TBI casualties where there is a preceding history of endurance exercise and hyperthermia. Further work is required to understand and isolate factors related to BBB function, and the use of S100beta in surrogate for this, with prolonged exercise under heat stress.

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Practical implications

- The neurobiomarkers GFAP and UCH-L1 were stable from pre-marathon baseline to completion and the following day, suggesting that substantial neurological insult does not routinely manifest in recreational marathon participants.
- Thus should Traumatic Brain Injury occur in the context of preceding endurance exercise, it is unlikely that the use of the markers in the first 24 hours will negatively impact clinical assessment.
- While cumulative exercise-heat stress does not appear to affect immediate neurobiomarker response in health, relative elevations in collapsed runners merits further investigation.

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Figure Legends

Figure 1. Biochemical responses (mean, SD) with marathon running according to hyperthermia category. Main effect of time (pre to post), ***P<0.0001. Interaction (time x hyperthermia response), §§P <0.01. Post-hoc difference between Post measures, adjusted P [a]<0.01 (High vs Low), [b]<0.05 (Intermediate vs Low).

Figure 2. Biochemical responses (mean, SD) with marathon running according to finishing time (exercise duration High, Intermediate or Low). Main effect of time (pre to post), ***P<0.0001. Interaction (time x exercise duration), §§P <0.005. Post-hoc difference between Post measures, adjusted P[a]<0.05 (High vs Low).

Tables

Table 1. Baseline and race characteristics of 38 control runners who completed the marathon while monitored for core body temperature (Tc) response. [a]Area Under Curve for time x Tc>38 °C. Adjusted P <0.05 *High vs Low, †Intermediate vs Low, §High vs Intermediate.

	Hyperthermia category (min. °C)[a]			P
	High (n=13)	Intermediate (n=13)	Low (n=12)	
Age (years)	34 ± 8	36 ± 11	44 ± 12	0.0541
Female (%)	30.1	15.4	8.3	0.3310
Baseline BMI (kg/m^2)	23.1 ± 3.6	23.9 ± 2.3	23.3 ± 2.3	0.7802
Previous personal best (min/marathon)	216 ± 43 *n=9*	210 ± 25 *n=10*	217 ± 39 *n=11*	0.9140
Maximum Tc (°C)	39.7 ± 0.4*§	39.2 ± 0.3	38.8 ± 0.3	<0.0001
Mean Tc (°C)	38.7 ± 0.4*	38.6 ± 0.3†	38.0 ± 0.5	0.0002
Mean Tc, second half (°C)	38.9 ± 0.6*	38.6 ± 0.5†	38.0 ± 0.5	0.0007
Finishing time (min)	255 ± 47	241 ± 37	243 ± 52	0.7081
Δ Time (predicted – actual finishing)	-17 ± 20 (n=10)	-14 ± 27 (n=13)	-22 ± 29 (n=8)	0.8333

Δ Race placing (Halfway – final)	-321 [-756, 295]	57 [-321, 345]	179 [-995, 535]	0.4539
Δ Body mass (%)	-2.4 ± 2.0	-2.4 ± 2.4	-1.1 ± 1.6	0.1907

Table 2. Clinical and biochemical results for seven EAC cases sampled within 30 minutes of presentation to medical treatment facility (T0 sampling point) and/or 60 minutes later (T1 sampling point). Following collapse these runners received first aid ± cooling on-course +/- cooling while being evacuated forwards to the nearest Medical Treatment Facility (MTF). Loc. 1. MTF stationed at 14 mile-point on course (course design resulted in runners up to 21 miles being received and treated here). Location II. MTF stationed 100 m behind finishing line (26.3 miles, receiving casualties from 21 miles onwards).
A-CVPU, Alert-Confused-Voice-Pain-Unresponsive scale; EHI – Exertional Heat Illness; EHS – Exertional Heat Stroke; GCS – Glasgow Coma Scale; Tc -first measured core temperature upon admission to medical facility; U/K – unknown.
*declined further blood sampling **pre-MTF Tc anticipated >38.5 °C based upon delay in presentation (diagnosed with heat illness clinically).

Distance complete at collapse (km)	On-course history *(time to MTF, min)*	Time-point	ACVPU at MTF	Tc at MTF	GFAP	UCH-L1	S100β	NSE	pOsm	sCr	CK	Cortisol	Copeptin	Discharge diagnosis
				°C	$\mu g.L^{-1}$	$ng.L^{-1}$	$ng.L^{-1}$	$\mu g.L^{-1}$	mosm. Kg-1	$\mu mol.L^{-1}$	$IU.L^{-1}$	$nmol.L^{-1}$	$pmol.L^{-1}$	
<33.8	Collapse *(30)*	T0 T1*	A -	38.6 37.7	<0.05 -	462.5 -	66.4 -	3.29 -	296 -	117 -	222 -	1275.9 -	88.3 -	EHI
33.8	Incapacitated, tympanic temperature 39.6 °C *(95)*	T0 T1	C A	38.1 37.0	<0.05 <0.05	546.5 408.9	121.7 101.6	14.75 3.82	312 306	176 127	680 781	1179.9 1213.8	431.4 114.6	EHS
35.4	Collapse, GCS 3 *(U/K)*	T0 T1	P P	40.2 35.4	<0.05 <0.05	1468.3 1230.1	622.7 371.3	6.85 5.82	317 329	186 173	2924 5245	1371.3 1304.4	3236.0 2117.1	EHS
>28.2	Confusion, amnesia; actively cooled (U/K)	T0 T1	C -	38.4 -	<0.05 -	509.6 -	151.2 -	4.07 -	312 -	78 -	532 -	1419.4 -	89.1 -	EHI
35.4	Weakness, stumbling, amnesia (75)	T0** T1	A -	38.0 -	<0.05 -	>2500 -	176.0 -	7.18 -	303 -	191 -	913 -	1387.8 -	237.1 -	EHI
>33.8	Collapse, GCS 3	T0 T1	V A	41.8 38.3	<0.05 <0.05	>2500 >2500	201.7 147.5	5.08 6.56	309 301	155 132	430 714	1031.1 1169.7	1062.0 217.5	EHS

	(U/K)													
42.2	Confused *(U/K)*	T0 T1	A -	38.5 -	<0.05 -	243.5 -	115.9 -	5.73 -	307 -	89 -	734 -	1424.7 -	275.3 -	EHI

Supplementary material

Supplementary Table 1. Baseline and race characteristics of 38 control runners who completed the marathon while monitored for core body temperature (Tc) response, stratified by exercise duration (High 293 ± 33 min, Medium 243 ± 8 and Low 199 ± 21 min). Adjusted P <0.05 *High vs Low, †Intermediate vs Low.

	Exercise duration (min)			P
	High (n=13)	Intermediate (n=13)	Low (n=12)	
Age (years)	38 ± 13	36 ± 11	40 ± 10	0.6577
Female (%)	30.8	15.4	8.3	0.3310
Baseline BMI (kg/m^2)	24.2 ± 3.1	23.5 ± 2.6	22.4 ± 2.4	0.2857
Previous personal best (min/marathon)	232 ± 29* *n=9*	237 ± 28† *n=9*	190 ± 24 *n=9*	0.0019
Maximum Tc (°C)	39.1 ± 0.5	39.4 ± 0.5	39.3 ± 0.5	0.7236
Area under the curve of [Time x Tc >38 °C] (min. °C)	157 ± 101	160 ± 76	141 ± 85	0.8486
Δ Body mass (%)	1.7 ± 2.2	2.1 ± 2.4	2.2 ± 1.5	0.8174

Supplementary Table 2. Changes in biochemical variables from pre- to post-marathon in 38 control runners. *p-value for next day vs baseline, adjusted for multiple comparisons (pre-post-next day).

	Pre	Post	p	*Next day (n=10)*	*Adj p**
GFAP, $ug.L^{-1}$	0.05 [0.05, 0.05]	0.05 [0.05, 0.05]	0.5000	*0.05 [0.05, 0.05]*	*>0.9999*
UCH-L1, $ng.L^{-1}$	98.1 [39.1, 578.1]	93.9 [39.1, 512.0]	0.7667	*109.1 [39.1, 651.5]*	*0.5391*
NSE, $ug.L^{-1}$	3.73 [3.30, 4.32]	4.85 [4.45, 5.80]	<0.0001	*3.96 [3.73, 4.58]*	*0.3526*
S100β, $ng.L^{-1}$	8.52 [3.65, 22.95]	39.0 [26.48, 52.33]	<0.0001	*80.3 [49.1, 99.7]*	*0.0010*
pOsm, $mosm.kg^{-1}$	292 ± 4	296 ± 6	<0.0001	*291 ± 5*	*>0.9999*
sCr, $umol.L^{-1}$	78 ± 11	117 ± 24	<0.0001	*77 ± 13*	*>0.9999*
CK, $IU.L^{-1}$	132 [109, 204]	361 [229, 566]	<0.0001	*1105 [704, 2050]*	*<0.0001*
Cortisol, $nmol.L^{-1}$	393.2 ± 31.0	1113.0 ± 271.0	<0.0001	*296.1 ± 103.4*	*>0.9999*
Copeptin, $pmol.L^{-1}$	4.19 [3.06, 7.53]	30.72 [14.47, 75.32]	<0.0001	3.96 [2.78, 6.67]	P>0.05

Supplementary Table 3. Spearman relationships of hyperthermia burden versus change in biochemical analytes (pre to post) in 38 marathon control runners

	GFAP	UCH-L1	NSE	S100β	pOsm	sCr	CK	Cortisol	Copeptin
AUCTc38	r=-0.09, P=0.57	r=0.27, P=0.10	r=0.14, P=0.41	R=0.23, P=0.17	r=0.04, P=0.79	r=0.33, P<0.005	r=0.21, P=0.21	R=0.34, P<0.05	r=0.55, P<0.01