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Title of the article: Changes in gastrointestinal cell integrity after marathon running and exercise-associated collapse

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ABSTRACT:

Purpose

Endurance exercise and hyperthermia are associated with compromised intestinal permeability and endotoxaemia. The presence of intestinal fatty acid binding protein (I-FABP) in the systemic circulation suggests intestinal wall damage, but this marker has not previously been used to investigate intestinal integrity after marathon running.

Methods

Twenty-four runners were recruited as controls prior to completing a standard marathon and had sequential I-FABP measurements before and on completion of the marathon, then at four and 24 hours later. Eight runners incapacitated with exercise-associated collapse (EAC) with hyperthermia had I-FABP measured at the time of collapse and one hour later.

Results

I-FABP was increased immediately on completing the marathon (T0; 2593 ± 1373 $ng \cdot l^{-1}$) compared with baseline (1129 ± 493 $ng \cdot l^{-1}$; $p < 0.01$) in the controls, but there was no significant difference between baseline and the levels at four hours (1419 ± 1124; $p = 0.7$), or at 24 hours (1086 ± 302; $p = 0.5$). At T0, EAC cases had a significantly higher I-FABP concentration (15389 ± 8547 $ng.l^{-1}$) compared with controls at T0 ($p < 0.01$), and remained higher at one hour after collapse (13951 ± 10476 $ng.l^{-1}$) than the pre-race control baseline ($p < 0.05$).

Conclusion

I-FABP is a recently described biomarker whose presence in the circulation is associated with intestinal wall damage. I-FABP levels increase after marathon running and increase further if the endurance exercise is associated with EAC and hyperthermia. After EAC, I-FABP remain high in the circulation for an extended period, suggesting ongoing intestinal wall stress.

KEY WORDS:

Athletes, gastrointestinal tract, fatty acid binding protein, heat stress.

DECLARATIONS

Funding

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Competing interests

No authors declare any competing interests.

Ethics approval

Ethical approval was obtained from the London - South East Research Ethics Committee.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Authors' contributions

All authors contributed to the design of the work, and the acquisition and analysis of the data. All authors were involved with the writing of the paper, and all have seen and approved the final version. OG provided further statistical analysis of the data.

ABBREVIATIONS

DBP Diastolic blood pressure

EAC Exercise-associated collapse

EHI Exertional heat illness

EHS Exertional heat stroke

GI Gastro-intestinal

HR Heart rate

I-FABP Intestinal fatty acid-binding protein

MAP Mean arterial pressure

SBP Systolic blood pressure

SpO_2 Peripheral oxygen saturation

T_{TYM} Tympanic temperature

WORD COUNT: 3469

INTRODUCTION

Long-distance running is associated with gastro-intestinal (GI) disruption and disturbance, which may affect one third of runners (Halvorsen 1990). After commencing endurance exercise, the GI barrier integrity may become compromised, increasing permeability between the GI tract and the systemic circulation (Smetanka 1999). Exercise-induced increases in gut permeability (March 2017) may precede a pro-inflammatory response and endotoxaemia (Camus 1997).

Exercise-associated collapse (EAC) has been estimated to affect 1.5 in every 1000 endurance runners (Lüning 2019), and is often due to venous pooling in the lower limbs at the cessation of exercise. More significant causes include cardiac conditions, heat illness and metabolic derangements (Jaworski 2020). The definition of hyperthermia varies but has been defined as a core temperature greater than 38.0°C (Niven 2016). Heat illness, and subsequent inflammatory and endotoxaemic responses, whether due to exertional activity or due to passive heat gain, carries a significant risk of morbidity and mortality. Heat stroke, the most extreme form of heat strain on the EAC spectrum, is associated with a risk of multi-organ failure (Walter 2016) and a high mortality rate. Exertional heat stroke (EHS), defined as a core temperature greater than 40°C accompanied by central nervous system dysfunction (Armstrong 2007), is among the leading causes of sudden death in athletes, with the incidence of sports-related EHS deaths continuing to increase (Nichols 2014). As with endurance exercise, heat strain also damages gut wall integrity (Koch 2019) and allows translocation of intestinal bacteria or endotoxins into the systemic circulation (Lim 2017). Systemic complications from heat strain are likely to be due to a combination of direct thermal injury and intestinal bacterial endotoxin translocation. Direct thermal injury may account for around 63% of the variance in changes in intestinal permeability (Pires 2017). Reducing intestinal

bacterial endotoxin translocation with antibiotics against intestinal bacteria in animal models of heat illness has shown some promise in improving cardiovascular dysfunction and mortality (Walter 2020). The combination of heat and exercise causes higher levels of endotoxin than exercise of equivalent intensity with lower temperature gain (Snipe 2018), suggesting that intestinal permeability in exertional heat illness (EHI) is multi-factorial and may not be entirely contingent on core temperature change alone (Laitano 2019). Increases in intestinal permeability may additionally be related to reduction in splanchnic blood flow, which may decrease by up to 60% in conditions of heat stress (Crandall 2015).

Intestinal fatty acid-binding protein (I-FABP) is a 15 kDa cytosolic protein involved in the cellular uptake and metabolism of fatty acids. It is present in the mature enterocytes of the small intestinal villi and is released once cell membrane integrity is compromised, subsequently appearing in the circulation following enterocyte injury (Memet 2019). It is gaining validity as a sensitive biomarker of intestinal injury (Funaoka 2010) and may have a role in the detection of intestinal ischaemia (Kanda 2011), including during exercise (van Wijck 2011) and mirrors the bacterial translocation through a permeable intestinal wall in pancreatitis (Coelho 2016). I-FABP serum levels increase after exercise and are associated with changes in permeability (March 2017), suggesting that intestinal cell damage is partly responsible for the increase in permeability. It is thought to have a short half-life in the circulation of a few minutes (van Wijck 2011, van de Poll 2007). The effect of endurance running and exertional heat strain on I-FABP levels, and whether this recently described biomarker adds further understanding to GI wall damage after exercise and thermal stress is unknown. We therefore sought to establish the I-FABP response to running a marathon (Brighton Marathon) and compare and contrast the response between healthy finishers and those where the initial clinical diagnosis was EHI. It was hypothesised that I-FABP would

increase following a marathon, and that greater increases would be observed in those incapacitated during or at the end of the event.

MATERIALS AND METHODS

Participants

Following favourable ethical approval from the London - South East Research Ethics Committee, participants were recruited from entrants into the 2019 Brighton Marathon (14 April 2019, event start 9:45 am). The Brighton Marathon (Sussex, UK) is an annual event which has run since 2010. Around 10,000 runners take part each year. A medical facility with sports medicine and critical care doctors is provided at the finishing line to provide immediate treatment to runners. All participants completed prospective or retrospective informed consent for inclusion in the study.

Experimental design

Baseline anthropometric and physiological data and venous blood samples were collected from control participants, between 10.00 and 19.00 on the day prior to the event (BASE). Upon completion of the event, repeat measurements were made and a further blood sample was drawn (T0; as soon as possible after crossing the finishing line), and at four (T4) and 24 hours (T24) after finishing.

Runners clinically diagnosed with incapacitation from EHI during or immediately after the event, and managed clinically as such, provided equivalent physiological data to that of the controls was collected as soon as possible after EAC (T0), and 60 minutes later (T1). For incapacitated runners, a core (rectal) temperature measurement was taken. A clinical diagnosis of EHI for the purposes of this study was defined as loss of consciousness or altered mental status occurring spontaneously during or soon after marathon run, in combination

74 with core temperature elevation (≥38.5°C at the point of collapse) and failure to make a
75 prompt recovery, requiring medical care.
76
77 **Materials and Methods**
78 Anthropometric and physiological measurements
79 Unshod standing stature and minimally clothed body mass were recorded for each control
80 participant using a stadiometer and scales. Participants were then seated for around 10 min
81 prior to the measurements of resting heart rate (HR), systolic (SBP) and diastolic blood
82 pressure (DBP) and subsequent calculation of mean arterial pressure (MAP), and peripheral
83 oxygen saturation (SpO_2), all obtained with an integrated patient monitoring device (GE
84 Carescape V100, UK). Tympanic temperature (T_{TYM}; Braun Thermoscan 3020, Kronberg,
85 Germany) was also assessed at this time.
86
87 Blood samples
88 Venepuncture was performed at the antecubital fossa. For the analysis of I-FABP, whole blood
89 (5 ml) was collected into EDTA vacutainers and inverted. Whole blood was immediately
90 centrifuged, and the plasma extracted and distributed between two aliquots. Samples were
91 immediately frozen in liquid nitrogen and stored at –86°C until analysis. The analysis was
92 performed by a commercial analytical laboratory (Affinity Biomarker Labs, London, UK);
93 standards and samples were pipetted into a microplate well pre-coated with a monoclonal
94 antibody specific for, and which bound to, human I-FABP. After washing away any unbound
95 substances, an enzyme-linked polyclonal antibody specific for human I-FABP was added to
96 the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate
97 solution was added to the wells and colour developed in proportion to the amount of bound

I-FABP. The intensity of the colour was measured. The lower limit of detection was 3.63 $ng.l^{-1}$; levels exceeding 20,000 $ng \cdot l^{-1}$, despite further dilution and re-analysis (n = 6) were assumed to be 20,000 $ng \cdot l^{-1}$ for statistical analysis, as described below. An intra-assay coefficient of variation of between 2.9 and 4.1% was calculated.

Statistical analysis

No formal power calculation was undertaken, as sampling was subject to the availability of runners (collapsed cases in particular) and reagents (I-FABP assay numbers limited due to global logistical challenges associated with the pandemic). Nevertheless, in previous human exercise studies conducted at moderate-severe relative intensity (60-80% VO_2peak) over 20-120 minutes (March 2017, Snipe 2018, Van Vijck 2011), 18-20 healthy volunteers provided adequate power to demonstrate significant elevation in I-FABP compared with rested baseline (91-99% increase; March 2017, Van Vijck 2011). Comparison of 10 relatively hyperthermic participants exercised in warm conditions with 10 participants terminating exercise with core temperature approximately 0.5 °C lower also showed a significant difference, approximately 45%, in I-FABP (Snipe 2018).

Paired samples t-tests were used to assess changes in physiological data before and immediately after completing the marathon in controls. Data were assessed for normality prior to analysis, and non-parametric tests used where data did not conform to a normal distribution. A non-parametric Wilcoxon rank sign test was used to compare changes in I-FABP between baseline and T0, T4 and T24 in controls, and between physiological data at T0 and T1 in cases. A non-parametric Mann-Whitney U test was used to compare I-FABP levels between controls and cases of heat illness. A Spearman's rank correlation test was used to

assess for correlation between time to complete marathon and I-FABP levels, and between BMI and I-FABP levels. Statistical significance was set at $p < 0.05$.

RESULTS

Participants

The ambient temperature at the start was 8°C, relative humidity 66%, pressure 1025 mbar, and wind speed 14.5 $km \cdot h^{-1}$. The ambient temperature at the mean finish time of controls remained at 8°C, with humidity reducing to 64% and pressure to 1024 mbar. The peak city temperature on the day was 10°C. Solar radiation data are not available. Twenty-four runners volunteered for inclusion into this phase of the research study to serve as apparently healthy controls, and all subsequently completed the marathon and provided a blood sample at T0. A further eight participants were recruited from runners incapacitated with suspected EAC during (n = 4; two at 14 miles, one at 21 miles, one at 25 miles) or immediately after the event (n = 4).

Participant characteristics for the control group are described in table 1 with specific sample sizes stated for each variable. Six participants returned four hours after completing the marathon for an additional assessment (T4), and four participants volunteered for a further assessment 24 hours after finishing (T24).

Of the cases, five (62.5%) were male and one (12.5%) was female, with the gender not recorded in two (25.0%) and not retrievable following the event due to data protection. The time to finish or collapse in the collapse group was 234 ± 63 min. Three of this cohort were still in the medical tent receiving medical attention, and had a further blood sample taken 60 min after the initial blood sample (T1), with the remainder abstaining from further participation due to recovery and fitness for discharge from the medical facilities.

Physiological responses in control participants and cases

A significant change in body mass ($p < 0.001$), HR ($p < 0.001$), SBP ($p < 0.001$), DBP ($p < 0.001$), MAP ($p < 0.001$) and SpO_2 ($p = 0.031$) was observed between baseline and T0 in the controls; see table 2.

The heart rate was significantly higher (121 ± 24 vs 87 ± 15 bpm, $p < 0.05$) but no difference in blood pressure or oxygen saturation between the incapacitated cases and controls respectively at T0. The core (rectal) temperatures of cases at T0 was 39.7 ± 1.2°C, and tympanic temperature of the controls at T0 was 36.2 ± 0.8°C.

There were no significant differences in temperature ($p = 0.125$), systolic ($p = 0.685$), diastolic ($p = 0.125$) or mean blood pressures ($p = 0.312$), or heart rate ($p = 0.125$) between the collapsed cases at T0, and T1.

I-FABP levels

The I-FABP levels at baseline and after completing the marathon in the controls and EAC cases are shown in table 3.

Healthy controls

A significant increase ($p < 0.01$) was observed in I-FABP concentration between baseline (1129 ± 493 $ng \cdot l^{-1}$) and immediately on completing the marathon (T0) (2593 ± 1373 $ng \cdot l^{-1}$) in the controls (figure 1 and table 3). There was no significant difference between the baseline I-FABP levels and the levels at four hours ($p = 0.7$), or at 24 hours ($p = 0.5$) (see table 3 and figure 1). There was no correlation in the time taken to complete the marathon and I-FABP rise ($p = 0.13$), or between BMI and I-FABP rise ($p = 0.8$).

Of the six controls who provided samples at four hours, the mean ± SD I-FABP levels were 2096 ± 698 $ng \cdot l^{-1}$ at T0, not significantly different to the complete T0 cohort ($p = 0.99$).

Cases with exercise-associated collapse

Post-hoc review of the eight collapsed cases indicated insufficient data to corroborate the clinical diagnosis of EHI. These cases were therefore assigned EAC for purposes of data interpretation.

All EAC cases were admitted to one of the two onsite medical centres for immediate medical management of the collapse. No cases required advanced airway, ventilatory or cardiovascular support. All patients underwent active cooling primarily by dowsing with cool water, in order to achieve rapid reduction in core temperature, according to current guidelines (Walter, 2018). Patients were discharged if the temperature and other physiological parameters and point-of-care biochemistry results were not significant deranged and were fully alert and orientated. After one hour, the core temperature had reduced to 37.92 ± 0.82°C. Seven patients were discharged from the medical centre, and one patient was transferred to the local hospital.

Of the EAC cases, six of the T0 I-FABP were above the limit of the assay (20,000 $ng \cdot l^{-1}$), despite serial dilution and re-analysis. Rank statistical analyses were therefore performed, as described above, to compare the control with the heat illness cases without absolute values of I-FABP. For descriptive statistics in table 3, values greater than 20,000 $ng \cdot l^{-1}$ were assumed to be equal to 20,000.

At the time of collapse (T0), cases demonstrated a significantly higher I-FABP concentration in comparison to controls at the time of completion of the marathon (T0) (15389 ± 8547 $ng.l^{-1}$ vs 2593 ± 1373 $ng.l^{-1}$ respectively; $p < 0.01$), as shown in figure 2 and table 3.

At one hour after collapse (T1), the average I-FABP concentration in the collapses remained significantly higher than the pre-race baseline in the controls (13951 ± 10476 $ng.l^{-1}$ vs 1129 ±

493 ng.l^{-1} respectively; $p < 0.05$). There were too few data to determine if a correlation exists between I-FABP levels and the rise in temperature in the EAC cases.

DISCUSSION

This study is the first to show the existence of a raised I-FABP level immediately after completing a marathon in controls and a much greater rise at the time of collapse in those with EAC and hyperthermia.

I-FABP levels after marathon running

These data support the hypothesis that endurance exercise is associated with intestinal wall damage, consistent with previous studies showing an increase in gastro-intestinal symptoms and permeability of the GI wall, after endurance exercise (Costa 2017). I-FABP levels are not significantly different to baseline four hours later suggesting that the compromised wall integrity is reversed following cessation of the exertion. The tympanic temperature of control runners before and immediately after completing the marathon was not different suggesting that a moderate degree of intestinal damage may arise after prolonged endurance exercise without sustained core temperature rise, although it is acknowledged that runners may have displayed increased temperature during the run which subsequently returned to baseline by the finish of the race. Damage to intestinal integrity with endurance exercise is at least partly caused by a reduction in splanchnic blood flow. A reduction in splanchnic blood flow as assessed by gastric tonometry correlates with the degree of intestinal damage (van Wijck 2011), and improvement in splanchnic blood flow, by administration of L-Citrulline, reduces intestinal damage (van Wijck 2014).

Levels of I-FABP after exercise-associated collapse

Levels of I-FABP at the time of EAC were significantly elevated compared with controls sampled within the same time window (<30 min of marathon completion or exertional

collapse). The primary cause of collapse was not determined, but the average core temperature in the collapse group was above 39.5°C, with two meeting the criteria for EHS. Whether the collapse is associated with increased intestinal permeability, leading to greater endotoxin translocation and heat retention, or the raised temperature per se contributes to greater intestinal damage and translocation in cases of collapse is not clear. Thermal stress, in addition to and independent from normothermic endurance exercise, affects intestinal integrity (Snipe 2018), as a result of direct thermal damage and microvascular damage, vascular stasis and extravasation (Vlad 2010). Mesenteric blood flow is reduced by around 20% at 38°C compared with 37°C (Badoer 2010), suggesting that at even the milder degrees of hyperthermia experienced in the EAC group, intestinal barrier function may be compromised, and is consistent with dysfunction of other organs occurring at 1-2°C higher than baseline (Walter 2016). The intestinal wall is sensitive to damage from exercise and from hyperthermia (Lim 2018) and these data present more evidence that the combination of multiple stressors are likely to cause cumulative damage. High temperatures appear to be tolerated better by athletes after previous repeated exposure to hyperthermia, suggesting that adaptations following repeated heat stress (i.e. acclimation/acclimatisation/acquisition of the endurance athlete phenotype) mitigates against EHI (Stevens 2020, Racinais 2019) and changes in intestinal permeability. This may be a result of increased heat shock proteins (in particular HSP72), which are transcribed following elevations in core temperature during interventions such as heat acclimation (Gibson 2015, Gibson 2016), ultimately leading to increased basal protein production (Nava 2020), or due to increases in plasma volume (Crandall 2015) and left ventricular end-diastolic volume, contributing towards reduced gut ischaemia during heat stress (Périard 2016). The impact of training, finishing time and prior heat exposure on levels of I-FABP rise was not assessed in this study, therefore further work

examining the impact of training, and prior heat exposure on thermal strain and gastro-intestinal permeability changes following prolonged marathon running is required.

Levels of I-FABP remained substantially raised after one hour in cases of EAC, compared with pre-race baseline in controls. The exact half-life of I-FABP is not known, but a closely related isoform (L-FABP) present in the intestine has a half-life of only 11 mins (van de Poll 2007), which suggests that there may have been ongoing intestinal cell damage and leak despite cessation of exercise and a return to a normothermic core temperature (37.9°C), a reduction of around 2.0°C within one hour. This is consistent with in vitro findings, suggesting a deterioration in cellular resistance on exposure to high temperatures, which subsequently improves over a few hours on return to around 37°C (Dokladny 2006). The substance I-FABP appears to be excreted unchanged in urine (Thuijls 2011) but the authors recognise the possibility that hepatic metabolism may occur, with the possibility of raised or prolonged serum levels with hepatic dysfunction.

Two of the cases displayed T0 I-FABP levels not significantly different to the T0 levels in the controls, and the variation in I-FABP overall was large. Both cases met the criteria for EAC for this study, and in both, the core temperature at collapse was greater than the control group, but corresponding I-FABP rise was not seen. The cause of this, and the large variability, is not clear. There is likely to be variation in the temperature at which an individual's cellular integrity becomes compromised, so that the cases in whom IFABP levels were lower may have been more resistant to heat stress. There is known to be a larger inter-person variation in tolerance adaptation to hyperthermic conditions (Tyler 2016) The duration of hyperthermia as well as the peak is known to affect organ damage (Vicario 1986) so the runners with a higher IFABP may have been hyperthermic for longer. Alternatively, IFABP is a relatively new

biomarker whose characteristics are not fully known, but may not fully correlate with intestinal permeability (March 2017).

Mechanisms of systemic damage

The mechanisms of systemic damage after exercise and with EHI are incompletely understood but are considered by some to be a combination of direct thermal damage, and translocation of endotoxins through an intestinal wall rendered permeable by exercise or heat stress (the so-called Dual Pathway Model (Lim 2018)). This study adds further evidence of intestinal wall damage after exercise and heat illness, and in addition proposes a novel use of this biomarker in investigating intestinal wall damage in exercise and heat illness.

Limitations of study

The authors acknowledge a number of practical limitations with the data and interpretation. There are no baseline data for the runners who subsequently collapsed as it was not possible to identify this group prospectively. It is assumed that the pre-race I-FABP levels between the cases and controls were similar. The authors also acknowledge the significant logistical issues of data collection from a large number of control and collapsed runners, presenting in a short period of time, to different sites across the marathon in which clinical care was also being administered. There was only a small number of collapsed cases for whom data were available after one hour, limiting the robustness of the interpretation between T0 and T1. Dehydration, especially in the EHI group, may have altered the plasma volume and subsequently the plasma I-FABP concentrations. The authors consider that the plasma volume changes would be insignificant compared with the large differences in I-FABP observed between the collapsed and control runners. Tympanic thermometers may not accurately reflect core temperatures,

294 estimated to be on average 1°C lower than a rectal temperature during outdoor exercise in
295 the heat, less than oral axillary or temporal methods (Casa 2007), and making comparisons
296 with core temperatures unreliable
297 As discussed above, the impact of training status and heat acclimation was not assessed in
298 this study, which may have affected GI integrity and stability. The rate of cooling and impact
299 on I-FABP levels was also not assessed in either the control or collapse cohort. That the
300 permeability of an in vitro GI cell line changes with heating and subsequent cooling (Dokladny
301 2006), and the association between rapid cooling to below 38.9°C within 60 min in classical
302 heat stroke and improvement in mortality exists (Vicario 1986) suggests that systemic I-FABP
303 levels are likely to be associated with the temperature-time profile in endurance running.

CONCLUSIONS

Intestinal FABP is a recently described biomarker whose presence in the circulation is associated with intestinal wall damage. This study has shown that I-FABP levels increase after marathon running and increase further if the endurance exercise is associated with the development of EAC and hyperthermia. After EAC, levels remain high in the circulation for an extended period, suggesting ongoing intestinal wall damage. This may raise the possibility of targeted therapies to prevent or mitigate incapacitation and endotoxaemia associated with EAC and hyperthermia.

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The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation.

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TABLES

Table 1. Participant characteristics of controls. Data are mean ± SD [min, max].

Variable	**Controls**
Sex	M = 10 (41.6%) F = 10 (41.6%) Not recorded = 4 (16.7%)
Age (years)	39.3 ± 9.6 [20, 63] (n = 23)
Height (cm)	175 ± 12 [151, 196] (n = 23)
Mass (kg)	73.5 ± 10.5 [48.0, 96.6] (n = 23)
Body surface area (m^2)	1.88 ± 0.19 (n = 23)
Body mass index ($kg.m^{-2}$)	24.0 ± 2.6 [23.2, 33.2] (n = 23)
Time to finish (min)	249 ± 46 [166, 336] (n = 21)

427 **Table 2.** Physiological responses at BASE and T0 for controls, and upon incapacitation (T0) in
428 cases. Data presented are mean ± SD [min, max].

429 Note: * - difference between control at baseline and at T0 ($p < 0.05$); ** - difference
430 between incapacitated cases at T0 and control at T0 ($p < 0.05$); ND – no data; T0 – time of
431 completion or incapacitation; T1 – one hour after time of incapacitation (T0)

	Controls		**Cases**	
	Baseline	**T0**	**T0**	**T1**
Body temperature (°C)	36.2 ± 0.7 [34.4, 37.7] (n = 23)	36.2 ± 0.8 [34.5, 38.0] (n = 21)	39.7 ± 1.2 [38.5, 41.5] (n = 8)	37.9 ± 0.8 [37.2, 39.5] (n = 5)
Heart rate ($b.min^{-1}$)	60 ± 14 [39, 92] (n = 22)	87 ± 15 [60, 111]* (n = 21)	121 ± 24 [93, 155]** (n = 5)	95.2 ± 9.9 [82, 106] (n = 5)
Systolic blood pressure (mmHg)	137 ± 17 (n = 23)	117 ± 16* (n = 21)	117 ± 14 (n = 5)	119 ± 21 (n = 5)
Diastolic blood pressure (mmHg)	81 ± 9 (n = 23)	69 ± 9* (n = 21)	58 ± 10 (n = 5)	79 ±17 (n = 5)
Mean arterial pressure (mmHg)	100 ± 10 (n = 23)	78 ± 26* (n = 21)	78 ± 10 (n = 5)	92 ± 17 (n = 5)

Peripheral oxygen saturation (%)	98 ± 1 (n = 21)	97 ± 1 (n = 19)	ND	ND

434 **Table 3.** I-FABP concentrations over time for both controls, and incapacitated cases (mean ±

435 SD [min, max] (ng.l^{-1})).

436 Note: * - difference in I-FABP concentrations between incapacitated cases at T0 and control

437 at T0 ($p < 0.05$); ** - difference in I-FABP concentrations compared with baseline in controls

438 ($p < 0.05$); ND - no data; †I-FABP levels greater than 20,000 ng.l^{-1} are marked

	Controls	**Cases**
Baseline	1129 ± 493 [443.9, 2443] (n = 23)	ND
T0	2593 ± 1373 [905.7, 6174.2] (n = 24)**	15389 ± 8547 [810.8, 20000†] (n = 8)*/**
T1	ND	13951 ± 10476 [1854, 20000†] (n = 3)**
T4	1419 ± 1124 [512.0, 3607] (n = 6)	ND
T24	1086 ± 302 [872.1, 1518.3] (n = 4)	ND

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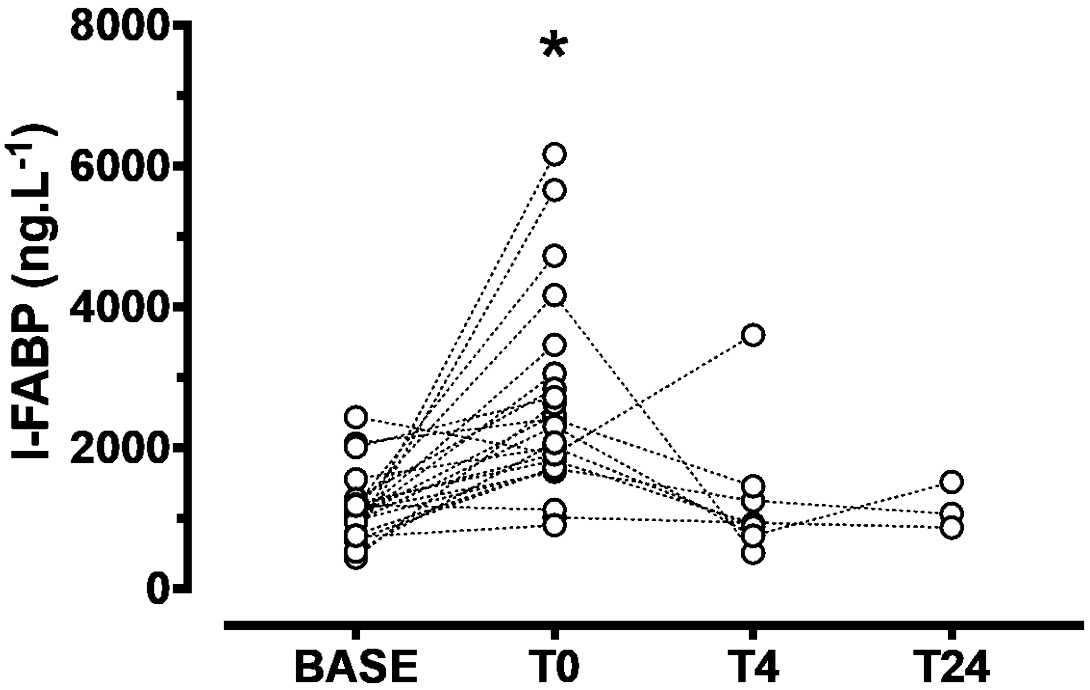
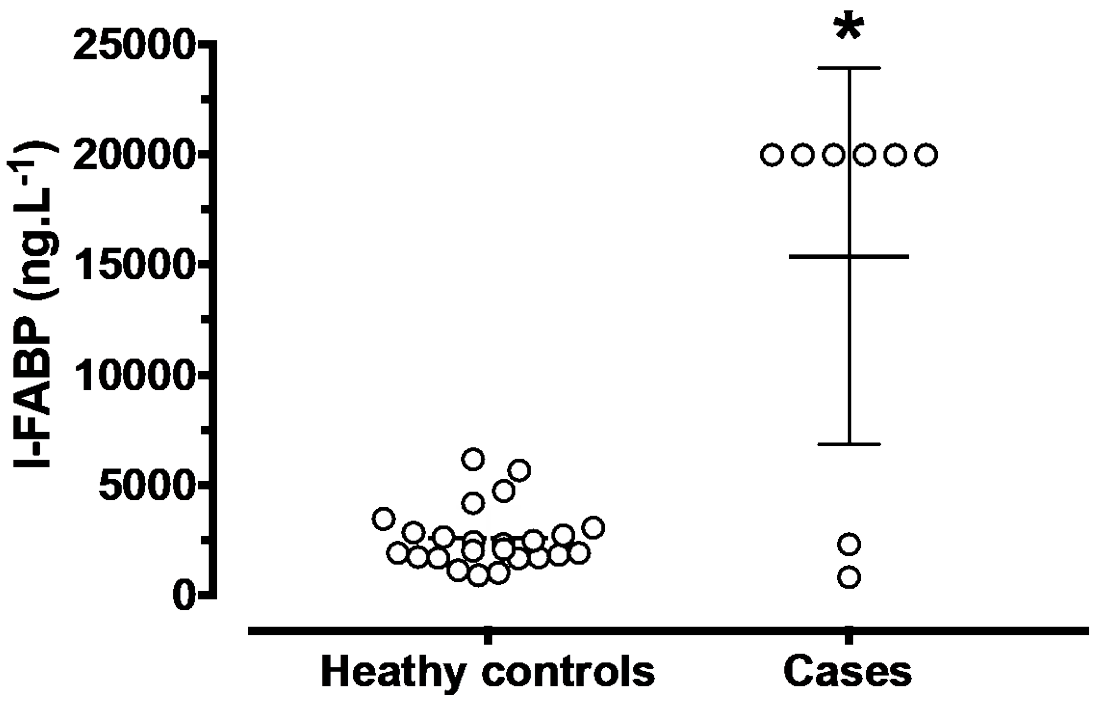
FIGURE LEGENDS

Figure 1. Individual I-FABP concentrations at BASE, T0, T4 and T24 for controls. Note: * - difference from BASE ($p < 0.05$).



444 Figure 2. Individual, mean ± SD I-FABP concentrations at T0 in controls and cases. Note: * -

445 significant difference ($p < 0.05$).



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APPENDICES

Nil