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Title	Physiological adaptations in ultra#endurance athletes during a 5# day multisport adventure race: an assessment of serological and inflammatory cytokine profiles
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Publication date	2017-05-04
Original Citation	Curran T.#I., Cronin O., Coffey F. T., Keohane D. M., McCarthy Y., Dahly D. L., Molloy M. G. and Falvey E. C. (2018) 'Physiological adaptations in ultra#endurance athletes during a 5#day multisport Adventure Race: An assessment of serological and inflammatory cytokine profiles', Translational Sports Medicine, 1(3), pp.120-131. https://doi.org/10.1002/tsm2.25
Type of publication	Article (peer-reviewed)
Link to publisher's version	https://onlinelibrary.wiley.com/doi/abs/10.1002/tsm2.25 - 10.1002/tsm2.25
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Download date	2026-09-06 18:50:18
Item downloaded from	https://hdl.handle.net/10468/6241

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Article type : Original Article

Title: Physiological adaptations in ultra-endurance athletes during a five-day multi-sport Adventure Race: An assessment of serological and inflammatory cytokine profiles.

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/tsm2.25

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Funding

No external funding was received for this study.

Keywords

Sports Medicine, Adventure Racing, Endurance Exercise

Abstract:

Background:

Multi-day endurance sports expose athletes to multiple physical stressors. Little is known about the athletes' physiological responses to these stressors. A detailed understanding of the serological changes that occur during competition may improve the treatment of athletes suffering from illness or injury.

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Objective:

This prospective, observational study aims to characterize serological changes in AR athletes across multi-day competition.

Methods:

Athletes underwent venipuncture at the start, mid-point, and end of a 5-day, multi-discipline event. A variety of serological and inflammatory factors were measured and then analyzed to describe their changes over the course of the race.

Results:

27 AR athletes (29.6% female, 70.4% male) met inclusion criteria out of 33 recruited initially. The mean age was 37.7 (IQR 32.5, 41) The median race-time for athletes was 133 hours (IQR 123, 142). Serum creatinine, sodium and potassium tended to remain stable as the race progressed. Conversely, serological measures, including haemoglobin, interleukin-6 and C-reactive protein levels, tended to change substantially during the race.

Conclusions:

Participants demonstrated the ability to maintain homeostasis, despite significant physiological threat. Renal function, electrolyte balance and hormonal profiles were stable. However, a pro-inflammatory response and decrease in red cell availability were evident by the mid-point of the race.

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1. Introduction.

Multi-day adventure racing is a relatively young sport, undertaken by mixed-sex teams of 4 athletes continuously racing more than 72-hours. Participants race supported only by their teammates, and must use a variety of methods, such as mountain biking, kayaking, and trail running, to navigate the race. Multi-day adventure racing shares many physiological and technical demands with traditional endurance events such as triathlons, Ironman®, and ultra-marathons. However, adventure racing poses additional challenges to the athletes, including the longer duration, the absence of a support crew to optimize nutrition and hydration, significant sleep deprivation, and the cognitive demands of navigating a demanding course under these circumstances. Furthermore, environmental conditions, particularly large swings in ambient temperature, may place additional demands on the athletes.

While the short-term intensity of exercise during multi-day adventure racing tends to be lower than that of events such as the triathlon, the prolonged duration of physical and mental effort presents risks to both professional and amateur competitors alike. The total energy expenditure for a fit male during an expedition-length race is approximately 10,000 to 13,000 kilocalories per day (1), making it difficult for athletes to consume enough water and nutrients to meet the physiological demands of the race (2). Previous evidence suggests that exercise intensity in multi-day adventure racing is more than 60% of heart rate reserve in the first 12 hours of a race, before progressively falling to approximately 40% of heart rate reserve at 24 hours, which is usually sustained until race completion (3, 4).

These stresses can have knock-on effects on the risk of illness. At the 2005 adventure racing World Championship, the incidence of illness within the race was reported to be one per 1,000 race-hours (5). This is a seemingly low rate of health related events but it is tempered by the prolonged race-hour exposure of 16,774 hours during the event. Furthermore, it is estimated that up to 12% of adventure racing athletes sustained a new illness within 1 week of race completion (5). This rate is significantly higher than the 5% illness rate reported by athletes during the 2016 Summer Olympics (6). The majority of illnesses relate to gastro-intestinal and respiratory disturbance (7), though episodes of exertional rhabdomyolysis with myoglobinuria have also been reported (8).

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Several studies have reported that endurance exercise induces an acute phase inflammatory response similar to that seen in sepsis and trauma (9). Cytokines mediate this inflammatory response to exercise and their release during exercise is known to be affected by exercise intensity and duration (10). The endothelial and muscular disruption encourages the migration of leukocytes to the area of injury. This local reaction induces a systemic inflammatory reaction known as the acute phase response. This response includes the production of a large number of hepatocyte-derived acute phase proteins, such as C-reactive protein (CRP) (11). Pederson offers a comprehensive review of the topic of the exercise-induced inflammatory cytokine response in his summary article *Exercise and Cytokines* (Pederson). After a marathon race Tumour Necrosis Factor alpha (TNF- α) and Interleukin 1β (IL-1β) levels increase twofold and Interleukin 6 (IL-6) levels increase up to 100-fold. This is followed by a marked increase in the concentration of Interleukin 1 receptor alpha (IL-1ra). This release of pro-inflammatory mediators is balanced by the release of cytokine inhibitors (IL-1ra and TNF receptors) and the anti-inflammatory cytokine Interleukin 10 (IL-10). In addition, the concentrations of the chemokines Interleukin 8 (IL-8), macrophage inflammatory protein -1α (MIP-1α) and MIP-1β are elevated after a marathon. These findings suggest that cytokine inhibitors and anti-inflammatory cytokines tightly restrict the magnitude and duration of the inflammatory response to exercise as previously thought. The presence of multiple cytokines (TNF-α, IL-1β, IL-6, IL-2 receptors and interferon-γ) in the urine following exercise provides further evidence of that a broad spectrum of cytokines are expressed in response to exercise (9)

To date, there has been little research on the physiological adaptations of adventure racing athletes during multi-day races. The increasing popularity and competitiveness of the sport underlines the importance for attending medical teams and sports medicine practitioners to understand the physiological stressors that competing athletes may experience so that they can plan and treat accordingly (1). The aim of this study is to thus describe the physiological profiles of athletes during an expedition-length adventure race. The authors hypothesis that a significant pro-inflammatory response will be noted in adventurer racers as is seen in other prolonged endurance exercise above but we postulate that these athletes' serological profiles will remain overall stable given the low rate of

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illness reported in this athlete group. This study will help us to better understand the physiological demands of these events, allow us to identify areas of potential harm to this athlete population, and to ultimately enhance medical provision at adventure racing events. To do this, we profiled the haematological, biochemical, hormonal, and immunological alterations occurring in adventure racing athletes competing in the ITERA Adventure Racing World Series event held in the West of Ireland in August 2016. Here, we describe the serological changes throughout the grueling 5-day race in the largest cohort of adventure racing athletes measured to date.

2. Materials and Methods

2.1 Study Design and Setting

This observational study was prospective in design and included participants in the 2016 ITERA Expedition Race, one of ten races that take place each year in the Adventure Race World Series. This continuous, 5-day expedition-length adventure race took place on the west coast of Ireland from the 17th to 21st August 2016. The event involved 33 mixed-sex teams from nine different countries. The race covered 579 km of nonstop racing, over five days, across five Irish counties. This included 293 km mountain biking, 156 km on foot (running and trekking over 3 separate mountain ranges), 130 km on kayaks in open seas and lakes, a total climb of 15000 m which took in the summits of the highest and second highest mountains in Ireland, and additional challenges such as coasteering, cave navigation and swimming.

2.2 Study Population

All registered participants of the ITERA race were invited to take part in the study via email and social media. To be included in the study, the participants needed to be a registered competitor for the ITERA Expedition Race 2016 that completed more than one full day of racing. The exclusion criteria were under 18 years of age, suffering with an acute or chronic illness at the start of the race, on long-

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term medication, and pregnancy. Eligible participants were provided with written information about the purposes and details of the study in advance of enrollment. All athletes were required to provide written informed consent prior to enrollment. Enrolled participants could withdraw at any time. The Clinical Research Ethics Committee of the Cork Teaching Hospitals (CREC) approved the study prior to commencement.

2.3 Data Collection

2.3.1 Participant Characteristics

A preliminary session was held at race registration to measure participant weight and height, from which body mass index (BMI) was calculated. Height was measured using a free-standing stadiometer. Weight was measured using calibrated electronic scales placed on a hard level surface. Participants completed a pre-race survey that included age, sex, nationality, team country of origin, presence or absence of team sponsorship, previous adventure race experience (including only races of greater than 24 hours duration), background medical history, current medications, and illness or injury resulting from previous expedition racing. Athletes were asked to describe their typical weekly training volume during the competitive season. This was recorded in hours of activity per day. Final race position and total race time were recorded from the race website upon publication of the official ITERA Adventure Race results.

2.3.2 Blood sampling

Blood sampling took place at race registration, at a midrace transition zone on day 2 -3 of racing (kayaking to mountain bike stage), and at the finish line. The final blood sampling and a second weight measurement were taken on race completion within 30 minutes of crossing the finish line. Trained personnel performed venous blood sampling from the antecubital vein. One 3ml K_3EDTA, one 9ml K_3EDTA, one 4ml Z serum Separation Gel Clotting Activator, and one 2ml sodium

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fluoride/K_3EDTA vacutainer tubes (Vacuette®, Greiner Bio-One GmbH, Kremsmünster Austria) were taken at each sampling time point. Athletes were monitored for 5 minutes after each sampling episode to observe for any ill-effects.

The 2-ml sodium fluoride/K_3EDTA vacutainer tubes were immediately frozen on crushed ice. The 9ml K_3EDTA samples stored at 0-4°C during the sampling period, prior to transfer to the laboratory for plasma cytokine measurement. All other samples were maintained at room temperature. All samples for cytokine analysis were transferred to the laboratory within 6 hours of blood draw. On arrival, these samples were centrifuged at 2,000g for 20 minutes and plasma was aliquoted into plain 2ml vials stored at -80°C.

2.3.3 Outcome analysis

All samples were processed in the relevant departments (biochemistry and haematology) in the clinical sciences laboratory of Cork University Hospital, Cork City, Ireland. This large, university teaching hospital laboratory conducts a significant quantity of clinical blood testing using standardized methodology and regular quality control. Extensive haematological, biochemical, hormonal, and lipid profiles were performed in additional to inflammatory marker testing including C-reactive protein. The concentrations of subjects' circulating inflammatory cytokines including interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10) and tumour necrosis factor alpha (TNF-α) in plasma supernatant were determined using Meso Scale Discovery multi-spot analyte micro-plates (Rockville, Maryland, USA). This system is an electrochemiluminesence-based solid-phase multiplex assay. Specifically, the ultra-sensitive human pro-inflammatory I, V-Plex immunoassay panel, was used. Stored samples were allowed to thaw fully at room temperature before dilution. Samples were diluted x2 according to the manufacturer's recommendations.

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2.4 Statistical Methods

2.4.1 Descriptive Statistics

Categorical variables were described by their counts and percentages in each category. Continuous variables were described by their means and standard deviations, by their medians and inter-quartile ranges, and by their full ranges, where appropriate.

2.4.2 Estimation of Trends

Trajectories for each outcome over the three time points were estimated in an exploratory manner using linear mixed effects models with a random intercept for athlete to account for the clustered nature of the data. First, for each outcome, two models were estimated: one that included linear time in the model (coded as 0/1/2) and one that did not. Models were estimated with maximum likelihood estimation, and the likelihood ratio test (LRT) of the nested models was used to evaluate the importance of the inclusion of time in the model. Where the LRT chi squared p value was > 0.05, it was concluded that there was no appreciable time trend in that outcome, and these were labeled as *no trend*.

For the remaining outcomes, a second set of models were estimated: one that included linear time in the model (as before), and another that also included time squared, to capture any quadratic trends. Again, the LRT of the nested models was used to evaluate the importance of the quadratic term the model. Where the LRT chi squared p value was > 0.05, it was concluded that the trend could be adequately described as *linear*; otherwise the trend was described as *quadratic*. All models were further adjusted for sex and age. The trends for each outcome were also plotted for each participant to verify conclusions from the mixed effects models. Prior to model estimation, the distributions of each outcome at each time point were visually inspected to determine if a log-transformation would be helpful. All analyses were conducted using the R Project for Statistical Computing (Version 3.2.2). Linear mixed effects models were estimated using the lme4 package (12)

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3. Results

3.1 Sample population characteristics

A total of 33 athletes initially enrolled in the study (28 expressed interest by email in advance of the race; 5 volunteered on the day of race registration). Six athletes withdrew or did not meet the inclusion/exclusion criteria. The final sample population comprised 27 athletes from 11 teams (20% of the total race population). This was made up of 19 males and 8 females, which matched the 3:1 ratio of males to females in most adventure racing teams. Sixty-three percent of study participants received sponsored external aid either through direct finance or equipment. The study group was predominantly made up of experienced adventure racers, with 77.8% of participants having previously raced in at least one similar race. The average time spent training was 10.7 hours per week (range 3 –20 hours). Anthropometric and demographic characteristics of the sample population are outlined in Table 1.

The median time spent racing was 133 hours (5.5 days). 7.4% of the sample finished the entire race (long course) achieving all mandatory checkpoints. 37% of enrolled athletes were converted to the short course. 11.1% (n=3) of our sample retired before completion of the race. 24 athletes (88.8%) were measured at all 3 time points. Those who retired did so after the second time point, and were not sampled at race completion. The highest placed team in the study population finished 6th and the lowest was 32nd.

3.2 Effects of time on outcome variables

Tests of a linear time trends across the outcomes are given in Figure 1. For most outcomes, the mixed effects model which included a linear effect of time fit the data better than model, based on

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Among the outcomes where the p-value for the test of a linear time trend was < 0.05, we then tested whether the inclusion of a quadratic time effect would further improve model fit. These tests for quadratic time trends across all outcomes are given in Figure 2. For most outcomes, the inclusion of a quadratic time effect improved model fit, based on the p-value of the LRT chi-squared test of the nested models.

3.3 Effect of sustained physical exertion on the haematological profile of adventure racers

Haemoglobin concentration decreased linearly as the race progressed (Fig. 3). The pattern is consistent with destruction or loss of red blood cells through hemolysis caused by race trauma (concurrent increase in serum bilirubin levels). The decrease in haemoglobin was mirrored with a proportional decline in both haematocrit and red blood cell (RBC) values. Mean cell volume (MCV) remained unchanged suggesting the fall in haemoglobin was not the result of blood loss.

Total white blood cell (WBC) count, predominantly composed of neutrophils and lymphocytes, increased from baseline during the race and remained elevated in keeping with an acute phase inflammatory response. Both lymphocyte and neutrophil numbers increased from baseline to midrace but lymphocyte response decreased below baseline by the finish line whereas neutrophils remained slightly elevated (mean concentrations outlined in supplementary Table 1).

3.4 Effect of sustained physical exertion on the renal function and electrolyte balance of adventure racers

Overall, athletes' electrolytes and renal function remained tightly regulated throughout the race (Table 2). Serum urea trended upwards as athletes progressed through the race indicating the presence of dehydration in the competitors. However, serum creatinine levels remained relatively unperturbed, suggesting an ability to preserve renal function in the face of prolonged physical effort. All electrolytes including sodium, potassium, phosphate and magnesium showed no apparent change in the sample.

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3.5 The effect of sustained physical exertion on the liver function of adventure racers

Liver function tests can be divided into the direct assessment of liver enzymes (Alanine aminotransferase (ALT); total bilirubin (tBili)) and the assessment of synthetic liver function (albumin and hydroxylation of cholecalciferol to active vitamin D). In response to prolonged strenuous exertion, ALT and tBili levels demonstrated a marked curved elevation with progression of race time possibly reflecting hepatic insult (Figure 4). Serum albumin, an indicator of hepatic synthetic function followed a declined linearly. Vitamin D levels were stable throughout the race (mean values presented in supplementary Table 2).

3.6 Effect of sustained physical exertion on hormonal levels of adventure racers.

Analysis of serum cortisol levels did not appreciable change over the course of the race (Table 3). Thyroid stimulating hormone (TSH) linearly increased, while free thyroxine (fT4) levels decreased, during the race. However, overall values for thyroid function tests remained within normal limits for both genders.

3.7 The effect of prolonged exertion on serological markers of muscle damage and circulating inflammatory cytokines in adventure racers.

Figure 5 demonstrates a distinct rise in serum creatine kinase levels (CK) mid-race with a subsequent downward trend towards normal at the end of the race. At baseline CK levels were within normal limits in all athletes, peaking mid-race in 78% of the sample with a subsequent downward trend towards normalization at race end. Conversely, venous lactate levels remained within normal limits throughout the race (Table 4).

A marked change in inflammatory cytokine levels occurred during the multi-day adventure race (Table 4 and Figure 6). Significant elevations were noted in circulating IL-6, IL-8 and to a lesser extent TNF-α occurred as the race progressed and was associated with a concurrent rise in C-reactive

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protein and total white cell count. Conversely, the anti-inflammatory cytokine IL-10 remained stable throughout all measured time points. Interpretation of these results suggests that the evident elevation in IL-6 and IL-8 in this cohort of adventure racers results in a pro-inflammatory effect.

4. Discussion

4.1 Overview of the findings

Here, we describe the physiological alterations reflected in haematology, biochemical, hormonal and inflammatory profiles of adventure racers during a continuous 5-day adventure race event. Our cohort of athletes was representative of usual adventure racing participants, comprising a mixture of experienced and inexperienced athletes, both professional and amateur, with a gender ratio consistent with other races. As has been described previously, the anthropometric characteristics of our study population were comparable to those seen in triathletes (1). This race was an Adventure Race World Series championship event, a highly competitive race, featuring some of the world's best adventure racing athletes. Approximately three quarters of the study participants reported competing previously in similar races. The winning time of 90 hours was indicative of a race which had a slightly slower pace than the average for the 6 other races in the series (81 hours) reflecting a challenging course; and unseasonable, inclement weather (wind and rain) which challenged the athletes throughout the week.

The results highlight the demand placed on athletes' homeostatic mechanisms during such events, but also serve to reassure event medical teams of a robust ability amongst racers to preserve organ function despite prolonged physical and psychological stressors. First, our results indicate a significant decline in total red cell number and haemoglobin concentration as the raced progressed. In several respects, this is unsurprising considering increased iron loss through sweat production in combination with increased haemolysis secondary to recurrent trauma and foot strike during exercise. This phenomenon has been described previously in runners (13). Despite the reduction in haematological parameters, clinically significant quantities of blood loss or reductions in haemoglobin were not witnessed. Reassuringly, athletes demonstrated preserved renal function and tight electrolyte

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control throughout the event. While the presence of warm weather may have led to different outcomes, it is reassuring that athletes maintained stable levels of renal function under severe physiological stress. During extended endurance exercise splanchnic blood flow to intra-abdominal organs is reduced, diverting blood to actively respiring tissues i.e. lungs, cardiac and skeletal muscle (14). Despite this, hepatic function remained relatively stable across the race. Hepatic production of albumin, and hydroxylation of cholecalciferol by the liver, indicated by serum vitamin D levels, remained relatively stable throughout the 5-day event. While a mild rise in serum alanine aminotransferase (ALT) and total bilirubin levels were evident as the race progressed (supplementary table 2), mean levels did not increase greater than twice the upper limit of normal. These findings can be explained by increased ALT leak from damaged skeletal muscle tissue (15), and increased haemolysis of red cells causing hyperbilirubinaemia respectively (13). Furthermore, hormonal assessment indicated maintained thyroid stimulation and thyroxine production throughout the race.

4.2 Changes in inflammatory profiles in athletes during multi-day adventure racing

In our study, the statistical models suggested that IL-6 levels were the most sensitive biomarker to time spent exercising. We cannot be certain that the sustained IL-6 elevation seen in our cohort is definitively a pro-inflammatory effect as it is known to exert pleiotropic effects bringing about positive and negative physiological adaptions depending on the tissue it is produced in. However the concurrent increase in other pro-inflammatory markers and absence of increase in Il-10 suggests that it may be operating an inflammatory as well as a metabolic effect during exercise of this type (16-18). In the acute period immediately following exercise, circulating interleukin-6 levels surge following release from contracting skeletal muscle tissues. Increases in IL-6 levels post-exercise offer distinct functions to its more traditional pro-inflammatory effect when released from immune cells (19). The quantity of IL-6 released following exercise is subject to activity intensity and duration (20).

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In this study, cytokine and inflammatory marker dynamics depict a clear and acute inflammatory response to exercise that was maintained until race-end. For all athletes, a marked increase in circulating levels of IL-6, IL-8, creatine kinase, TNF-α, C-reactive protein and total white cell count was evident as the race progressed. Furthermore, levels of IL-10, a cytokine with predominantly anti-inflammatory actions, remained stable throughout the race. This suggests that IL-6 release in our study is predominantly pro-inflammatory in nature. These findings suggest that extended physical activity, such as that which occurs during adventure racing, leads to a measurable and unopposed pro-inflammatory response. Previously, evidence has demonstrated that increases in circulating levels of TNF-α are evident *only* after extreme forms of exercise (21, 22). In the current analysis, levels of TNF-α rose appreciably in a curved manner highlighting the magnitude of physical exertion and the degree of inflammatory stress elicited by this form of exercise.

The potential effects of the described inflammatory response to athletes' health are unclear. However, awareness of this phenomenon is important for medical staff who may be treating adventure racing athletes who are injured or unwell. Previous analysis has demonstrated the negative psychological effects resulting from lipopolysaccharide-induced IL-6 release, leading to depressed mood, malaise and fatigue (23, 24). Work by Anglem *et al*. has demonstrated the occurrence of altered mood states in adventure racing athletes persisting for up to 2-weeks following expedition-length races (7). Whether the sustained IL-6 and pro-inflammatory responses demonstrated during multi-day competitions are directly responsible for mood disruption in adventure race athletes is plausible but is currently unproven. Furthermore, the contribution of sleep deprivation to these effects may be disparate to those physiological effects caused by prolonged aerobic activity. The examination of cytokine dynamics in the period following multi-day adventure races may provide useful information in this regard in the future.

In addition, extreme and unaccustomed physical effort, as seen in multi-day adventure racing, predisposes athletes to elevated risk of infectious disease. Spikes in immune cell activity (e.g. natural killer cells) resulting from intense, prolonged physical activity is frequently followed with a transient window of elevated infection incidence upon cessation of activity (25, 26). This phenomenon has

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previously been illustrated in adventure racing (7) and is supported by the immunological profiles of the present study.

4.3 Strengths and limitations of the study

The major strength of the study was the comprehensive measurement of serological changes of adventure racers using high-quality procedures in a real-life field setting. Further, the presence of pre-race testing allowed for accurate assessment of changes in measured variables as the race progressed which adds strength to the study. The study is limited by its observational and exploratory nature; and by the relatively small but heterogeneous sample, which limits the generalizability of the findings.

5. Perspective

Adventure racing is a unique sport with a multitude of substantial environmental, physiological, psychological and cognitive stresses that result in each athlete performing at the limits of their capabilities. Multi-day, expedition-length races expose both experienced and non-experienced participants to the health risks of sustained endurance activity in the setting of extreme sleep deprivation. This study indicates that participating athletes can preserve internal organ homeostasis despite extreme physical stress over a 5-day period. Pro-inflammatory cytokine dynamics during this race indicate an acute and sustained inflammatory response to continuous physical exertion. Potential negative effects of such responses are unknown, but may affect athlete's mood, innate immune function, and infection risk. Further study of adventure racing athletes in the days to weeks post-race would provide a greater understanding of the potential health risks and benefits of multi-day competition.

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Notes:

Compliance with ethical standards

Before study commencement, the Clinical Research Ethics Committee of the Cork Teaching Hospitals (CREC) granted ethical approval. All participants provided written informed consent before enrolment.

Funding

No external funding was received this study.

Conflicts of interest

The authors declare no conflicts of interest pertaining to this work or related publication.

Acknowledgements

The authors would like to thank and acknowledge the organizing team of the ITERA adventure race, namely race organizer James Thurlow, event doctor Charlotte Hattersley and Avril Copeland in permitting this study to be performed. The authors extend their gratitude to Mark Butler, Mary Ring, Professor John O'Mullane, Professor Mary Cahill and all the staff at the biochemistry and haematology laboratories at Cork University Hospital for their assistance. Finally, to the race participants who voluntarily enrolled in this study and allowed us to test them in such trying conditions, thank you for your patience and for allowing us to find out more about your fascinating sport.

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Figures/Tables

Table 1. Athlete characteristics

Variable	Observations (n)	Mean ± SD Or N (%)	Median [IQR]	(Min, Max)
Sex	27			
Female		8 (29.6%)		
Male		19 (70.4%)		
Age (years)	27	37.8 ± 8.1	36 [32.5, 41]	(24, 62)
Weight (kg)	27	73.9 ± 9.5	74.5 [68.3, 79.8]	(55.5, 93)
Height (m)	27	1.7 ± 0.1	1.8 [1.7, 1.8]	(1.6, 1.9)
BMI (kg/m^2)	27	24.1 ± 2	24.5 [22.7, 25.2]	(20.6, 28)
Sponsored	27			
No		10 (37%)		
Yes		17 (63%)		
Course type	27			
Full		2 (7.4%)		
Non-competitive		12 (44.4%)		
Retired		3 (11.1%)		
Short		10 (37%)		
Finishing place	27	18.5 ± 8.3	19 [11, 25]	(6, 32)
Finish time (hours)	27	125 ± 27.8	133 [123, 142]	(57, 166)
Previous experience	27			
No		6 (22.2%)		
Yes		21 (77.8%)		
Training time (hours per week)	27	10.7 ± 4.1	11 [8, 13.5]	(3, 20)

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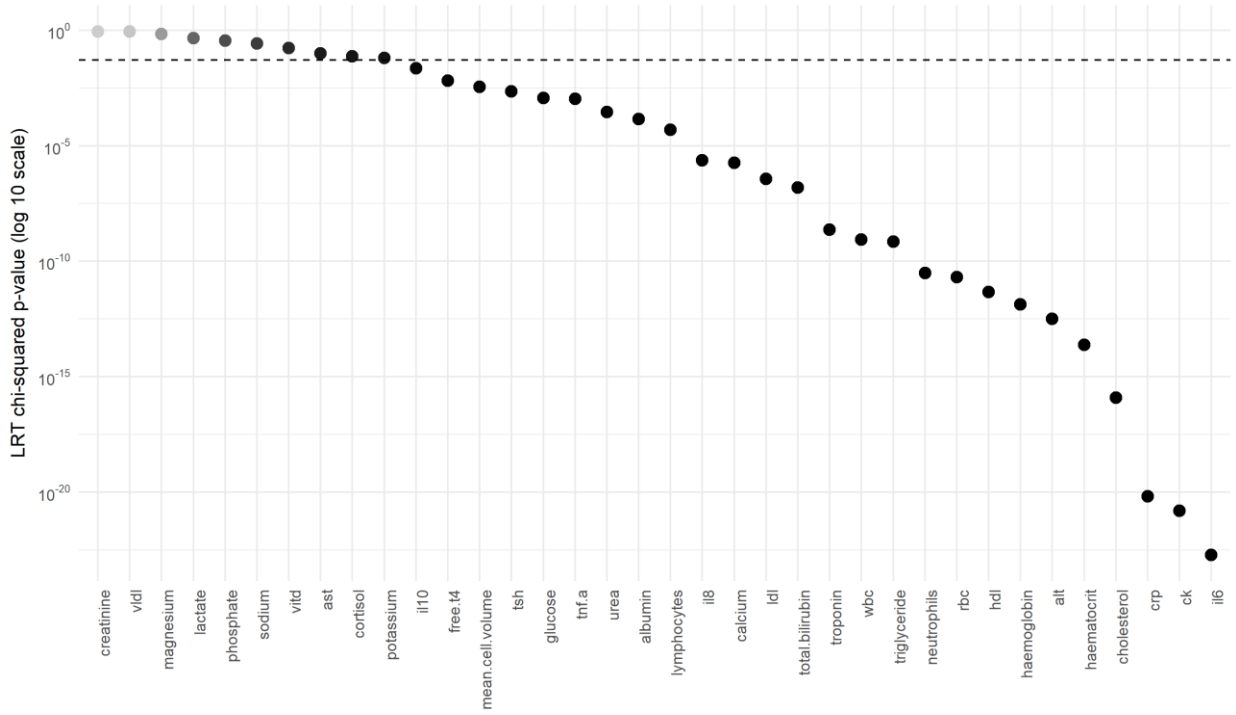
Fig. 1 – Tests of linear time trends across all outcomes



Caption:

The plot displays the LRT chi-squared p-value for each outcome, comparing two, nested linear mixed effects models: a model with no effect of time, and a model with a linear effect of time. The null hypothesis is that the inclusion of the time effect does not improve model fit. Low p-values suggest discordance between the observed data and the null hypothesis. P-values are plotted on the log 10 scale to help distinguish values very close to zero. A p-value of 0.05 is highlighted by the dashed-line.

alt *alanine aminotransferase,* ***ast*** *aspartate aminotransferase,* ***ck*** *creatine kinase,* ***crp*** *c-reactive protein,* ***free.t4*** *free thyroxine,* ***hdl*** *high density lipoprotein,* ***il6*** *interleukin-6,* ***il8*** *interleukin 8,* ***il10*** *interleukin 10,* ***ldl*** *low density lipoprotein,* ***rbc*** *red blood cell count,* ***tnd.a*** *tumour necrosing factor alpha,* ***tsh*** *thyroid stimulating hormone,* ***vitd*** *vitamin d,* ***vldl*** *very low density lipoprotein ,* ***wbc*** *white blood cell count*

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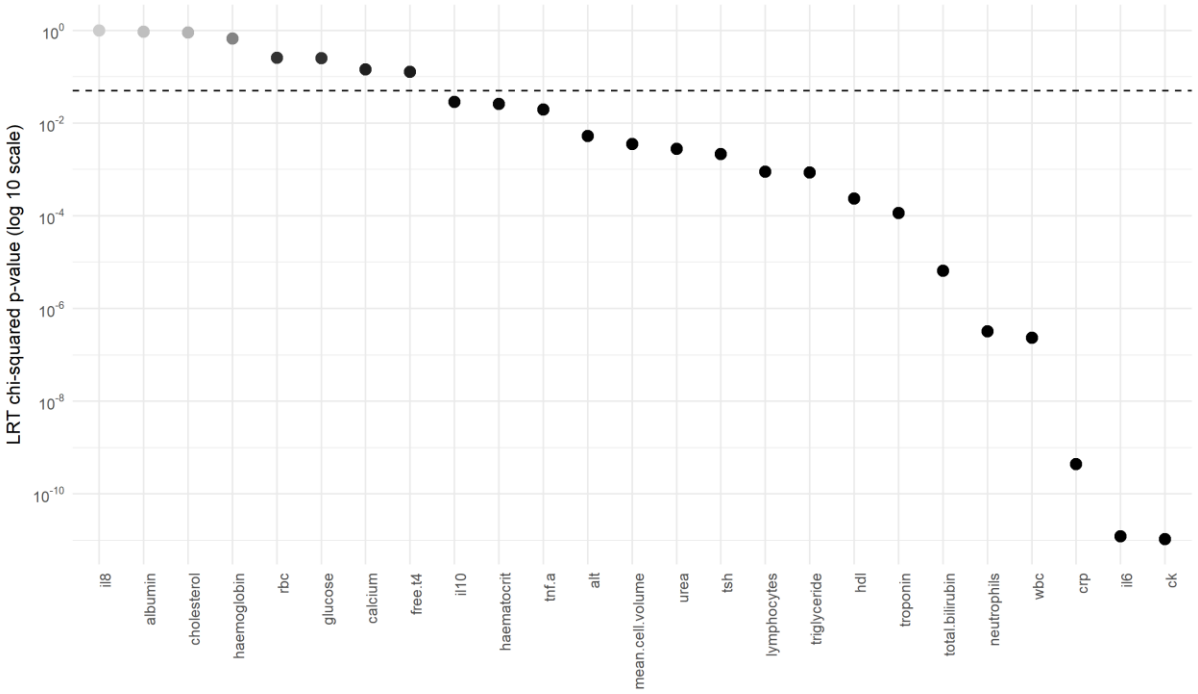
Fig. 2 - Tests of quadratic time trends across all outcomes



Caption:

The plot displays the LRT chi-squared p-value for each outcome, comparing two, nested linear mixed effects models: a model with a linear effect of time, and a model that adds a quadratic effect of time (i.e. time squared). The null hypothesis is that the inclusion of the quadratic effect does not improve model fit. Low p-values suggest discordance between the observed data and the null hypothesis. P-values are plotted on the log 10 scale to help distinguish values very close to zero. A p-value of 0.05 is highlighted by the dashed-line.

***alt** alanine aminotransferase, **ck** creatine kinase, **crp** c-reactive protein, **free.t4** free thyroxine, **hdl** high density lipoprotein, **il6** interleukin-6, **il8** interleukin 8, **il10** interleukin 10, **ldl** low density lipoprotein, **rbc** red blood cell count, **tnd.a** tumour necrosing factor alpha, **tsh** thyroid stimulating hormone, **wbc** white blood cell count*

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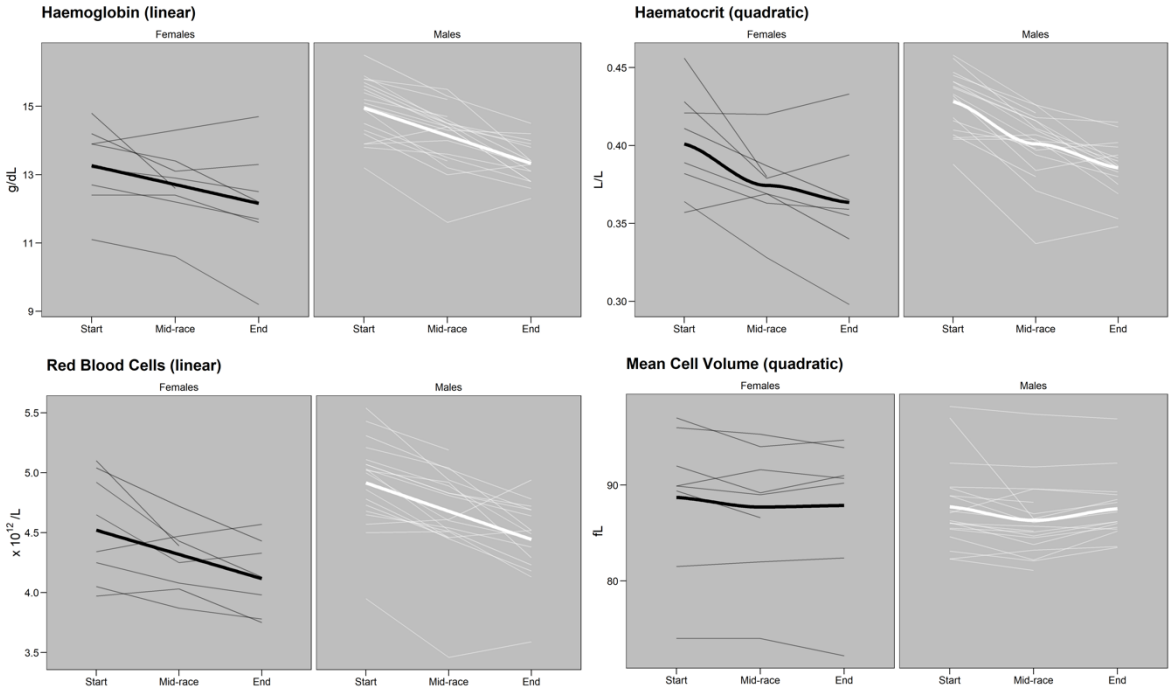
Fig. 3 – Trajectories for haematological indices



Caption: The plots display individual trajectories, as well a summary of these trajectories (the heavier line), by sex. For outcomes labelled as no trend or linear, the summary trajectory is estimated by ordinary least squares. For outcomes labelled as quadratic, it is estimated with a local regression smoother. Natural log transformed outcomes are noted as such on the y axis label.

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Table 2. Summary of mean renal and electrolyte panel results

		Start		Mid-race		End		Normal Range
Item	**Sex**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**Units**	
Urea	F	8	5.3 ± 0.6	8	6 ± 1.8	7	5.8 ± 1.9	mmol/L	2.8-8.4
	M	19	5.9 ± 1	19	7.6 ± 1.6	16	7.1 ± 2.2		
Creatinine	F	8	63 ± 4.3	8	62.2 ± 4.2	7	69.9 ± 5.9	umol/L	64-104
	M	19	76.5 ± 8.8	19	77.4 ± 9.9	16	73.2 ± 8.3		
Phosphate	F	8	1.2 ± 0.2	8	1.3 ± 0.2	7	1.2 ± 0.1	mmol/L	0.8-1.5
	M	19	1 ± 0.2	19	1.1 ± 0.2	16	1.1 ± 0.2		
Magnesium	F	8	0.9 ± 0	8	0.9 ± 0.1	7	0.9 ± 0.1	mmol/L	0.7-1
	M	19	0.9 ± 0.1	19	0.9 ± 0.1	16	0.9 ± 0.1		
Calcium	F	8	2.4 ± 0.1	8	2.3 ± 0.1	7	2.3 ± 0.1	mmol/L	2.1-2.65
	M	19	2.4 ± 0.1	19	2.4 ± 0.1	16	2.3 ± 0.1		
Sodium	F	8	137.5 ± 1.3	8	137.6 ± 1.7	7	137.7 ± 1.7	mmol/L	132-144
	M	19	139.3 ± 1.5	19	139 ± 1.6	16	138.2 ± 1.5		
Potassium	F	8	4.1 ± 0.3	8	3.9 ± 0.5	7	4 ± 0.2	mmol/L	3.5-5.1
	M	19	4.2 ± 0.3	19	4 ± 0.4	16	4.1 ± 0.4		

SD standard deviation.

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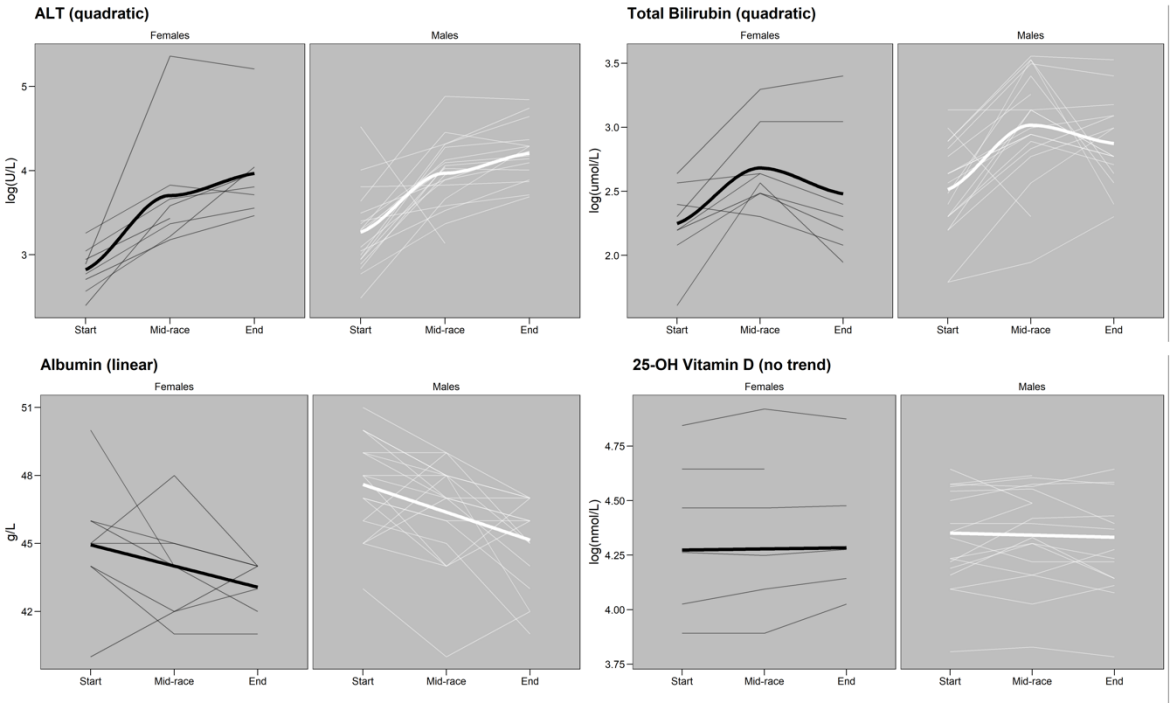
Fig. 4 – Trajectories for liver function tests



Caption: The plots display individual trajectories, as well a summary of these trajectories (the heavier line), by sex. For outcomes labelled as no trend or linear, the summary trajectory is estimated by ordinary least squares. For outcomes labelled as quadratic, it is estimated with a local regression smoother. Natural log transformed outcomes are noted as such on the y axis label.

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Table 3. Summary of mean hormone levels.

		Start		Mid-race		End		Normal Range
Item	**Sex**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**Units**	
Cortisol	F	8	328 ± 147	8	271 ± 92.8	7	245 ± 90.9	nmol/L	
	M	19	339.5 ± 98.8	19	336.5 ± 108	16	287 ± 82.2		
Free T4	F	8	13.8 ± 2.4	8	15.1 ± 2	7	15.2 ± 1.9	pmol/L	9-19.1
	M	19	14.6 ± 1.2	19	15 ± 1.7	16	14.8 ± 1.5		
TSH	F	8	1.7 ± 1.2	8	2.2 ± 0.8	7	1.2 ± 0.6	mIU/L	0.35-4.94
	M	19	1.6 ± 0.9	19	1.8 ± 0.9	16	1.4 ± 0.9		

T4 thyroxine, TSH thyroid stimulating hormone.

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Fig. 5 - Temporal pattern of creatine kinase levels throughout the 5-day adventure race.

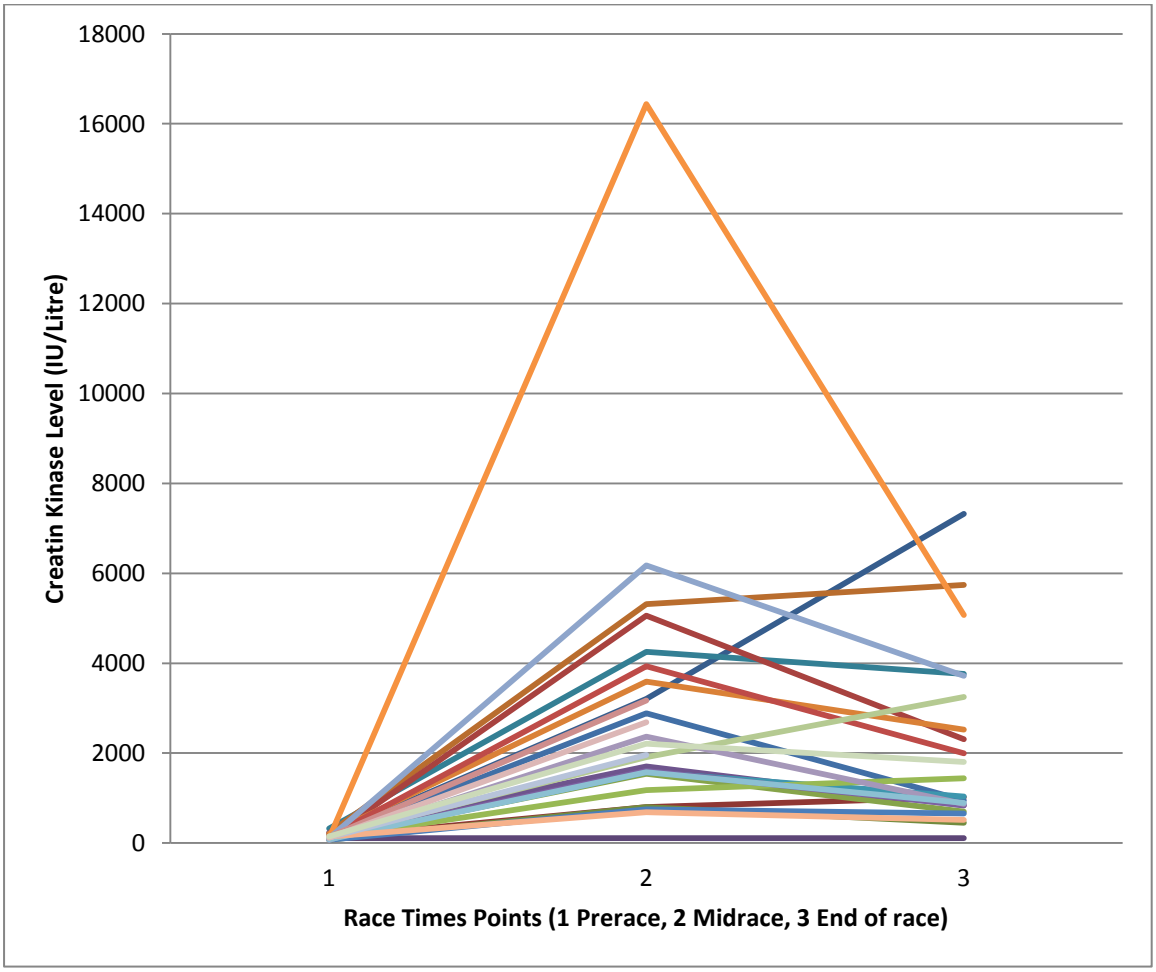

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Table 4. Variation in mean inflammatory and serological muscle damage biomarkers.

			Start		Mid-race		End		Normal Range
Item	**Sex**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**Units**	
CK	F	8	96.7 ± 28	8	3272 ± 5354	7	1347 ± 1654	U/L	40-180
	M	19	392 ± 1066	19	2883 ± 1567	16	2781 ± 2166		
Lactate	F	8	1.4 ± 0.4	8	1 ± 0.2	7	1.3 ± 0.5	mmol/L	0.5-2.2
	M	19	1.2 ± 0.4	19	1.3 ± 0.6	16	1.3 ± 0.5		
TNF-α	F	8	1.8 ± 0.3	8	2.4 ± 0.6	7	2.1 ± 0.5	pg/ml	
	M	19	2.5 ± 0.7	19	2.8 ± 0.55	16	2.9 ± 0.7		
IL-10	F	8	0.3 ± 0.1	8	0.5 ± 0. 2	7	0.4 ± 0.2	pg/ml	
	M	19	0.6 ± 1	19	0.6 ± 0.3	16	0.6 ± 0.2		
IL-8	F	8	3.8 ± 3	8	6.0 ± 4.5	7	7.9 ± 5.8	pg/ml	
	M	19	1.9 ± 5.7	19	6.9 ± 5.2	16	9.6 ± 4.4		
IL-6	F	8	0.56 ± 0.2	8	3.48 ± 2.6	7	2.3 ± 1.2	pg/ml	
	M	19	0.4 ± 0.5	19	4.8 ± 5	16	4.4 ± 2.7		
CRP	F	8	0.63 ± 0.2	8	11.4 ± 6.1	7	6.9 ± 2.6	mg/L	0-10
	M	19	0 ± 1.3	19	3 ± 1	16	2.9 ± 0.8		

CK creatine kinase, TNF tumour necrosis factor, IL interleukin, CRP C-reactive protein.

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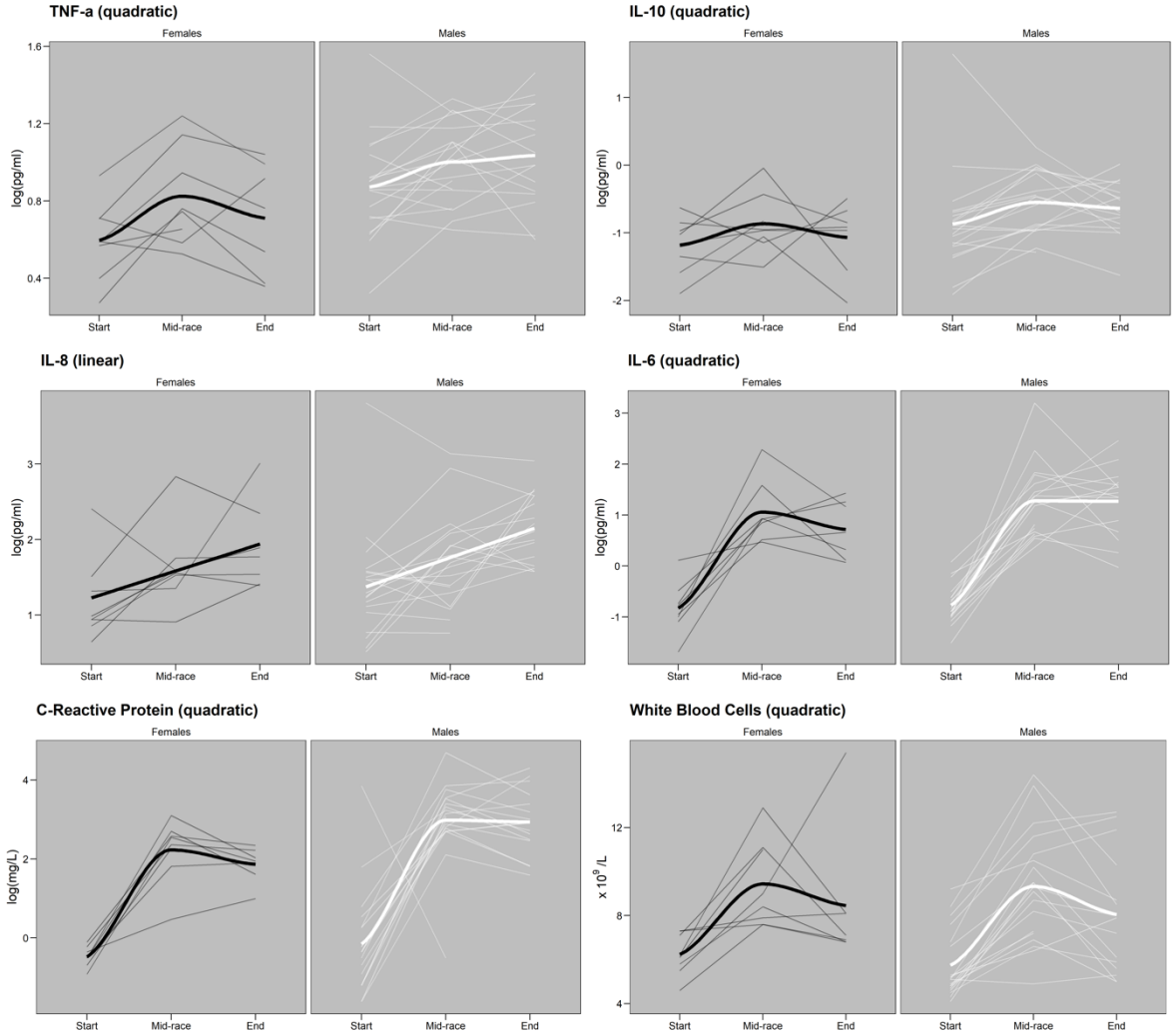
Fig. 6 – Trajectories for cytokines, C-reactive protein and total white cell count.



Caption: The plots display individual trajectories, as well a summary of these trajectories (the heavier line), by sex. For outcomes labelled as no trend or linear, the summary trajectory is estimated by ordinary least squares. For outcomes labelled as quadratic, it is estimated with a local regression smoother. Natural log transformed outcomes are noted as such on the y axis label.

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Appendices

Supplementary Table 1. Summary of haematological results.

		Start		Mid-race		End		Range
Item	**Sex**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**Units**	
Haemoglobin	F	8	13.3 ± 1.2	8	12.7 ± 1.1	7	12.2 ± 1.7	g/dL	11.7-15.9
	M	19	15 ± 0.9	19	14.1 ± 0.9	16	13.3 ± 0.6		13 - 17
Haematocrit	F	8	0.4 ± 0	8	0.4 ± 0	7	0.4 ± 0		0.35-0.46
	M	19	0.4 ± 0	19	0.4 ± 0	16	0.4 ± 0		0.38 – 0.49
RBC	F	8	4.5 ± 0.4	8	4.3 ± 0.3	7	4.1 ± 0.3	$x10^{12}$/L	3.9 – 5.3
	M	19	4.9 ± 0.4	19	4.7 ± 0.4	16	4.5 ± 0.3		4.2 – 5.6
MCV	F	8	88.7 ± 7.6	8	87.7 ± 6.9	7	87.9 ± 8	fL	80 - 96
	M	19	87.7 ± 4.3	19	86.3 ± 3.8	16	87.5 ± 3.4		80 - 96
WBC	F	8	6.2 ± 1	8	9.4 ± 2	7	8.5 ± 3.1	$x10^{12}$/L	4.4 – 11.3
	M	19	5.7 ± 1.5	19	9.3 ± 2.5	16	8 ± 2.6		4.4 – 11.3
Lymphocytes	F	8	1.9 ± 0.6	8	2.3 ± 0.7	7	1.7 ± 0.8	$x10^{9}$/L	0.9 - 3.2
	M	19	1.8 ± 0.4	19	1.8 ± 0.5	16	1.5 ± 0.5		0.9 – 3.2
Neutrophils	F	8	3.6 ± 0.8	8	6.1 ± 2.3	7	5.9 ± 3.3	$x10^{9}$/L	1.4 - 6.6
	M	19	3.3 1.1	19	6.5 ± 2.2	16	5.7 ± 2.2		1.4 – 6.6

SD standard deviation.

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Supplementary Table 2. Summary of liver function tests.

		Start		Mid-race		End		Range
Item	**Sex**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**Units**	
ALT	F	8	17.1 ± 4.7	8	55.4 ± 64.1	7	63.7 ± 53	U/L	0-45
	M	19	28.9 ± 18.3	19	57.4 ± 24	16	71 ± 24		
tBili	F	8	9.78 ± 2.9	8	15.4 ± 5.7	7	13.7 ± 8.6	umol/L	2-20
	M	19	12.8 ± 4.7	19	22.05 ± 8.3	16	18.7 ± 6.4		
Albumin	F	8	45 ± 2.8	8	43.9 ± 2.2	7	43.1 ± 1.2	g/L	35-52
	M	19	47.5 ± 2.1	19	46.5 ± 2.3	16	45.1 ± 2		
Vit. D	F	8	71 ± 29	6	84 ± 32.2	7	73.4 ± 28.6	nmol/L	>50
	M	19	78.3 ± 19.1	17	79.5 ± 16.8	16	77.3 ± 22.2		

ALT alanine aminotransferase, tBili total bilirubin, Vit. Vitamin, SD standard deviation.

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