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ORIGINAL ARTICLE

Influence of long-distance trail running on blood hemostasis at the World Mountain Trail Running Championship 2023—a pilot study

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Abstract

Background: Severe exercise performed over longer duration can involve multiple prothrombotic alterations in blood coagulation markers. Standard coagulation tests are not robust in identifying hyper- or hypocoagulability due to indirect determination of changes in hemostasis and fibrinolysis. Conversely, viscoelastic tests might do so.

Objectives: The aim of this pilot study was to assess the exercise-induced changes in coagulatory and fibrinolytic processes by applying viscoelastic tests in a mountain ultramarathon. Seven elite athletes participating in the World Mountain and Trailrunning Championship 2023 were examined. The 86.9 km track involved 6500 m of climbing and 6920 m of descent.

Methods: Venous blood samples were taken the day prior to and within 3 hours following the competition. Plasma coagulation tests and whole blood viscoelastic coagulation tests (ClotPro) were used to assess changes in hemostasis.

Results: Plasma coagulation testing revealed prolonged prothrombin time, with correlatively decreased factor (F)V, FVII, and FX activities. As a consequence of increased intrinsic coagulation factor activities (FVIII, FIX, FXI, and FXII) and von Willebrand factor, activated partial thromboplastin time was shortened. In addition, plasminogen decreased, whereas α2-antiplasmin and D-dimer showed significant elevations. Maximal lysis, examined by viscoelastometric tests, was observed to be slightly diminished postrace, whereas fibrin polymerization increased.

Conclusion: The trail-long race generated substantial alterations in coagulation that were linked to a higher inflammatory state, with characteristic increases in FVIII, von Willebrand factor, and fibrinogen levels. Viscoelastic coagulation monitoring used in

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our study was able to reflect the summation of pro- and anticoagulants, as well as pro- and antifibrinolytic changes, in hemostasis.

KEYWORDS
blood coagulation, core temperature, fibrinolysis, inflammation, ultramarathon, viscoelastic tests

Essentials

- Intense, long-duration exercise can cause multiple changes in hemostasis.
- After an 86.9 km race, elite mountain trail runners displayed several changes in coagulation.
- Postrace, coagulation factors increased, whereas fibrinolysis markers decreased.
- Coagulation changes are associated with an exercise-induced activation of inflammation.

1 | INTRODUCTION

Regular physical exercise is known to prevent thromboembolic diseases and reduce all-cause mortality, and is therefore considered an important part of a healthy lifestyle [1,2]. However, data on the effects of acute and chronic exercise on the coagulation system are conflicting [3,4]. High-intensity, long-duration, and extreme exertion are reported to induce a rebalanced hemostatic state by causing hypercoagulability and concomitantly enhanced fibrinolysis [5–8]. In contrast to the persistence of the activation of coagulation (until 24 hours after exertion), some studies have found that either coagulation activation was more pronounced than fibrinolysis, or fibrinolysis returned to baseline much earlier, indicating that the rebalanced hemostatic state may be lost during recovery [9–11]. Trail running races also impact blood viscosity with a reduction after an ultramarathon run [12].

In addition, extreme environmental conditions (heat, cold, and high-altitude hypoxia), as well as exercise-driven immune activation, can play a central role in these hemostatic changes [7,11,13–17]. Several prothrombotic changes in blood coagulation markers are observed after severe exercise, independent of environmental factors, including prolonged prothrombin time (PT), D-dimer, von Willebrand factor (VWF), and coagulation factor (F)VIII. As a consequence, a significant shortening of activated partial thromboplastin time (aPTT) is reported (for review, see Skouras et al. [4] and Kicken et al. [9]). However, those markers are only indirect indicators of ongoing hemostatic and fibrinolytic processes.

Moreover, the hemostatic changes related to acute strenuous exercise seem to be similar to those associated with inflammation and infection. Elevated D-dimer, FVIII, and VWF are related to inflammation, infection, and endotheliopathy due to the release of inflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor-α, and the activation of the microthrombotic pathway due to the exocytosis of hemostatic factors [18]. Any disturbances in the fibrinolytic system affect D-dimer. At first glance, it may seem surprising that high D-dimer is associated with hypofibrinolysis rather than hyperfibrinolysis. However, in the end, an elevated D-dimer merely indicates coagulation activation. This was also observed in critically ill COVID-19 intensive care unit patients who experienced hypofibrinolysis or a fibrinolysis shutdown [19]. Routine plasma coagulation tests cannot reliably differentiate whether hyper- or hypocoagulability is present [20]. Viscoelastic methods in whole blood might be more suitable to show the net effect of an altered pro- and anticoagulant situation in the blood, as is the case in a severe inflammatory situation [21]. Therefore, viscoelastic tests could determine whether increased D-dimer is a result of hypo- or hyperfibrinolysis (recombinant tissue plasminogen activator [r-tPA] in an extrinsic pathway-based assay [TPAtest] [Haemonetics Corporation]) and whether increased FVIII levels result in a clinically relevant shortening of the clotting time (ellagic acid-activated assay [INtest]) (Haemonetics Corporation). To our knowledge, this has never been explored until now, and it is also of particular clinical interest, given the fact that long-distance trail running events are showing increasing popularity among older participants. Among them, it is difficult to predict the consequences of an unknown risk of cardiovascular effects, which can be acutely increased by such physical activity, alongside alterations in hemostasis and inflammation. Moreover, a certain percentage of runners may suffer from unknown coagulation pathologies (eg, activated protein C resistance, prothrombin G20210A polymorphism, or deficiency/dysfunction of antithrombin, protein C, or protein S), and it is speculated that the combination of prolonged extreme physical activity and systemic inflammation can promote hypercoagulation, which is associated *per se* with thromboembolic complications [19]. In addition, hypercoagulation can be triggered by inflammation and hyperthermia, which have been shown to occur in the clinical setting [11], but such an association has never been studied in athletes, although signs of systemic inflammation and increases in core body temperature (T_{core}) are well documented [22,23]. Therefore, study results on blood hemostasis can add important knowledge about the consequences of long-distance trail runners' health. The aim of this pilot study was to investigate the hemostatic system alteration pre- and postcompetition with focus on fibrinolysis under extreme environmental conditions.

2 | METHODS

2.1 | Experimental design

This single-center, cross-sectional study was performed in tier 4 elite/international athletes [24] competing in an 86.9 km mountain trail run (ie, "trail long" competition) as part of the World Mountain and Trail Running Championship 2023, held on June 8 in Innsbruck, Austria. The race course had an overall ascent of 6500 m and a descent of 6920 m, and was composed of hiking trails (72%), gravel paths (12%), and tarmac roads (16%). The highest altitude reached during the trail was 2398 m, whereas the lowest point of the course was at an altitude of 574 m. The race started at 6:30 AM at an altitude of 994 m (Neustift/Stubaital, Austria), with an outdoor temperature recorded at 10 °C. During race day, outdoor temperatures ranged from 13 °C to 30 °C (average, ∼24 °C) at 574 m and from 5 °C to 15 °C (average, ∼10 °C) at an altitude of 2287 m. The study was approved by the Ethical Committee of the Medical University of Innsbruck, Austria (vote no. 1083/2023). Participants provided written informed consent after obtaining verbal and written information about the objectives, procedures, and potential risks of the study. The research was conducted in accordance with the ethical principles outlined in the 1975 Declaration of Helsinki.

2.2 | Participants

Participants were recruited through an email communication to all trail running athletes registered for the "trail long" competition and by introducing the planned study directly to national team coaches. To be eligible to participate in the study, athletes had to be 18 years of age or older. In addition, athletes were not allowed to participate or were excluded from the study if 1 of the following exclusion criteria was met: any chronic illness treated with medications that could interfere with the test results (eg, any autoimmune disease), acute illness or injury before or during the study phase, active anticoagulant therapy, or the presence of an acquired or hereditary coagulation disorder.

Three out of 10 athletes who volunteered to take part in the study had to interrupt the race at early stages. Therefore, data are presented for 7 athletes (median [IQR], age: 33 [29–38] years; body height: 179 [171-181] cm; and weight: 69.5 [66-77] kg). In detail, 6 athletes (5 males and 1 female) completed the race, and 1 female athlete stopped the race after 12 hours (53 km).

2.3 | Blood sampling

One day prior to the competition, the first venous blood sample was taken from the antecubital vein using a minimal tourniquet. A second blood sampling was performed within 3 hours after the completion of the race. To maintain temperature stability during transport, all samples were placed in thermally insulated boxes and transported at ambient temperature to the University Hospital Innsbruck. Samples were carefully handled during transport to avoid physical disturbances and were immediately analyzed upon arrival at the laboratory.

2.4 | T_{core}

Each participant was provided with an ingestible, calibrated telemetric capsule for the measurement of T_{core} (e-Celsius Performance, BodyCap Medical). T_{core} was continuously recorded from the athlete competing until postrace (30-second sampling). Data were logged onto the capsule and downloaded wirelessly immediately after the race. Participants were asked to ingest the capsule immediately after waking up in the morning. This technique has been described in greater detail elsewhere [23].

2.5 | Laboratory analyses

2.5.1 | Plasmatic coagulation tests

Citrated plasma samples were analyzed on a BCS XP and Atellica COAG 360 system (both Siemens Healthcare Diagnostics GmbH) using the following reagents (all Siemens Healthcare Diagnostics GmbH): Pathromtin SL for aPTT, Thromborel S for PT, Multifibren U for fibrinogen (modified Clauss method), BC Thrombin for thrombin time, Berichrom Antithrombin III for antithrombin III via thrombin (FIIa), Coagulation FVIII/FIX/FXI/FXII Deficient Plasma for functional intrinsic coagulation factor activities (one-step assay using Pathromtin SL aPTT reagent), Coagulation FII/FV/FVII/FX Deficient Plasma for functional extrinsic coagulation factor activities (one-step assay using Thromborel S PT reagent), and Berichrom FXIII (assay using bovine thrombin in the presence of a fibrin polymerization inhibitor to detect FXIIIa-dependent ammonia release and nicotinamide adenine dinucleotide reduction) for the activity of coagulation factor. Plasminogen was also measured kinetically using a streptokinase assay (Berichrom Plasminogen, Siemens Healthcare Diagnostics GmbH), as was α2-antiplasmin (Berichrom α2-Antiplasmin, Siemens Healthcare Diagnostics GmbH). Binding activity (VWF) to the platelet-specific glycoprotein Ib receptor (Innovance VWF Ac, Siemens Healthcare Diagnostics GmbH), VWF antigen concentration, and D-dimer (Innovance D-Dimer, Siemens Healthcare Diagnostics GmbH) were measured using turbidimetric measurements of agglutination assays. Cellular blood components, ie, white and red blood cells and platelets, were detected using a combination of impedance and optical methods (Sysmex XN, Sysmex Austria GmbH). Standard chemical parameters were performed on the Roche Cobas 8000 system using Roche reagents (Roche Diagnostics). All tests were performed according to the original instructions.

2.5.2 | Whole blood viscoelastometric coagulation tests

ClotPro was a commercially available, Conformité Européenne-marked viscoelastic *in vitro* coagulation analyzer (Haemonetics Corporation) used primarily in central Europe as a point-of-care test. It uses pipettes prefilled with starting and modifying agents and 340 µL of citrated whole blood to initiate the measurement. A stationary pin is inserted into a clockwise and counterclockwise moving cup, from which the reduction in movement is captured and recorded as amplitude, resulting in thromboelastometry curves similar to those of other viscoelastic testing methods, such as rotational thromboelastometry (ROTEM). Viscoelastic tests were performed within 4 hours after blood sampling. The standard tests used in the study were tissue factor (TF)-activated assay (EXtest, Haemonetics Corporation), INtest, functional fibrinogen assay, and EXtest-based assay supplemented with aprotinin to block fibrinolysis (APtest) (Haemonetics Corporation). Fibrinolysis was tested using TPAtest, which measures fibrinolysis by adding 650 ng mL^{-1} r-tPA to TF-activated whole blood. The lysis time is the time it takes for r-tPA to dissolve 50% of the clot (defined as maximum clot firmness) once maximum clot firmness is reached. Further parameters reported include clot formation time and maximum clot lysis.

The routine laboratory parameters shown in Table 1 were measured by standard analytical methods.

2.6 | Statistical analysis

Differences in blood parameters and T_{core} are reported using medians and IQRs (type 7 quantile method), and results were compared using dependent sample Wilcoxon matched-pairs signed-rank tests. Statistical significance was accepted at $P \leq .05$, with exact effect sizes (r) and P value calculations, except for variables with ties, where an approximate P value was calculated. The rank-based r was calculated by dividing the test statistic Z by the square root of the number of observations (N), ie, $r = Z/\sqrt{N}$ (according to Cohen's conventions, $r = .1$ indicates a small effect, $r = .3$ a moderate effect, and $r = .5$ a large effect). Values of C-reactive protein (CRP) and IL-6, which were below the detection limit, were treated as left-censored and imputed with the lowest detectable value for all subsequent analyses. All calculations were performed using R (version 4.2.2, R Foundation for Statistical Computing) in RStudio (version 2024.12.1+563, Posit Software PBC), applying the packages tidyr (Wickham H, Vaughan D, Girlich M), effectsize (Ben-Shachar M, Lüdecke D, Makowski D), and rstatix (Kassambara A). Plots were generated using ggplot2 (Wickham H) and ggpubr (Kassambara A). The figure was assembled using Inkscape (version 1.4.2, http://www.inkscape.org).

3 | RESULTS

T_{core} at start of the race was 37.2 °C (37.2-37.3 °C) and was significantly increased after race termination (to 38.5 °C [38.3-38.9 °C], $P = .016$; individual maximum T_{core} was 40.4 °C).

As markers of inflammation, both IL-6 and CRP significantly increased after the race. We also observed a 10-fold increase in total creatine kinase (CK) and a 2.5-fold increase in creatine kinase isoform MB from pre- to postrace. Respective data on myoglobin showed a 43-fold elevation (Table 1).

All 3 parameters of the red blood cell count—number of red blood cells, hemoglobin, and hematocrit—were not significantly changed after termination of the race compared with baseline values. However, white blood cell count increased significantly postrace (Table 1).

Though a discrete increase in platelets was detected postrace, the share or count of immature platelets and platelet volume did not change significantly (Table 2).

3.1 | Plasmatic coagulation tests

After the race, the PT (%) decreased significantly (ie, prolongation of PT in seconds; Table 3). PT is dependent on fibrinogen and FII, FV, FX, and FVII. With the exception of unchanged fibrinogen and FII, the levels of FV, FVII, and FX were significantly reduced postrace (Figure and Table 3). Thrombin time, which reflects fibrinogen polymerization, remained unaffected, as did FXIII activity levels. The aPTT was significantly shortened postrace, which was associated with a significant increase in all intrinsic coagulation factors (FIX, FXI, FXII, and especially FVIII; Table 3 and Figure). Moreover, we observed highly elevated levels of VWF antigen and VWF glycoprotein Ib activity after the endurance competition. Plasminogen was decreased, and α2-antiplasmin, as well as D-dimer, were increased after the race. Antithrombin levels remained unchanged (Table 3).

TABLE 1 Selected parameters of inflammation and muscle injury before and after the race.

Parameter	Prerace	Postrace	*P* value	*r*
CRP (mg/dL)	**0.06 (0.06-0.06)**	**0.50 (0.30-0.54)**	**.016**	**.91**
IL-6 (ng/L)	**3.5 (3.5-3.5)**	**52.9 (34.1-68.1)**	**.016**	**.91**
CK (U/L)	**180 (125-243)**	**1842 (1505-3884)**	**.016**	**.91**
CK-MB (U/L)	**23 (21-30)**	**58 (50-107)**	**.016**	**.91**
Myoglobin (µg/L)	**35 (28-46)**	**1518 (1449-3115)**	**.016**	**.91**

Data are medians and IQRs. A P value $\leq .05$ was considered statistically significant (bold values). Effect sizes are reported as rank-based effect sizes (r).
CRP, C-reactive protein; CK, total creatine kinase; CK-MB, creatine kinase isoform MB; IL, interleukin.

TABLE 2 Full red and white blood cell count, immature platelets, and mean platelet volume.

Parameter	Prerace	Postrace	*P* value	*r*
RBC (Mio./μL)	4.9 (4.7-5.0)	4.9 (4.6-4.9)	.219	.46
Hemoglobin (g/L)	148 (145-152)	147 (135-149)	.089	.64
Hematocrit (%)	43 (41-45)	42 (39-43)	.078	.67
WBC (Mio./μL)	**6.5 (6.4-6.9)**	**14.8 (12.7-17.4)**	**.016**	**.91**
Granulocytes (10^3/μL)	**3.7 (3.5-4.2)**	**12.9 (9.8-14.9)**	**.016**	**.91**
Lymphocytes (%)	**33.3 (27.9-38.1)**	**7.4 (7.0-9.3)**	**.016**	**.91**
Monocytes (%)	7.4 (7.1-8.9)	7.2 (7.6-8.3)	.375	.34
Eosinophils (%)	**1.5 (1.3-1.8)**	**0 ± 0**[a]	**.018**	**.90**
Basophils (%)	**0.9 (0.7-1.1)**	**0.3 (0.3-0.5)**	**.018**	**.90**
Platelet counts (G/L)	313 (281-332)	324 (297-347)	.078	.67
Immature platelets				
Absolute (G/L)	8 (8-17)	8 (7-12)	.078	.67
Relative (%)	3 (2-6)	3 (2-4)	.078	.67
Mean platelet volume (fL)	10.0 (9.8-10.8)	9.9 (9.8-10.6)	.495	.26

Data are medians and IQRs. A *P* value ≤ .05 was considered statistically significant (bold values). Effect sizes are reported as rank-based effect sizes (*r*).
RBC, red blood cell count; WBC, white blood cell count; Mio, million.
[a]Decrease to zero.

3.2 | Whole blood viscoelastometric coagulation tests

After the race, the maximal lysis in the EXtest and APtest decreased significantly. The other parameters did not change postrace apart from a nonsignificant increase in fibrin polymerization, as determined by the functional fibrinogen assay (Table 4).

4 | DISCUSSION

In the present study, we showed that extreme, long-lasting physical stress in elite trail runners induced an inflammatory reaction with multiple changes in blood coagulation and characteristic increases in FVIII, VWF, and fibrinogen levels.

Physical exercise itself acts as a stressor to the human body and can induce an acute systemic inflammatory response, which is dependent on the type, intensity, and duration of physical exercise, as well as on environmental conditions. For example, a higher degree of inflammation has been reported after an ultramarathon compared with a marathon competition [25]. Pronounced increases in white blood cell numbers are almost always reported, mainly due to neutrophils and higher numbers of macrophages, accompanied by an increase in the hepatic acute-phase protein CRP. White blood cells release TF, enhance thrombin formation through the formation of neutrophil extracellular traps (NETs), activate platelets via P-selectin,

TABLE 3 Changes in parameters of the plasmatic coagulation after the trail run.

Parameter	Prerace	Postrace	*P* value	*r*
PT (%)	**95 (90-100)**	**79 (78-83)**	**.016**	**.91**
aPTT (s)	**28 (26-30)**	**26 (25-27)**	**.041**	**.77**
Thrombin time (s)	21 (20-23)	21 (20-22)	.414	.31
Antithrombin (%)	102 (98-104)	105 (99-108)	.249	.44
Plasminogen (%)	**96 (94-99)**	**86 (83-90)**	**.018**	**.90**
α2-antiplasmin (%)	**91 (89-97)**	**104 (103-106)**	**.016**	**.91**
D-dimer (μg/L)	**288 (216-317)**	**476 (380-592)**	**.031**	**.81**

Data are medians and IQRs. Parameter normal ranges: PT, 70% to 130%; aPTT, 26 to 37 seconds; thrombin time, 15 to 23 seconds; antithrombin, 79% to 112%; plasminogen, 75% to 150%; α2-antiplasmin, 80% to 120%; D-dimer, 0 to 500 μg/L. A *P* value ≤ .05 was considered statistically significant (bold values). Effect sizes are reported as rank-based effect sizes (*r*).
aPTT, activated partial thromboplastin time; PT, prothrombin time.

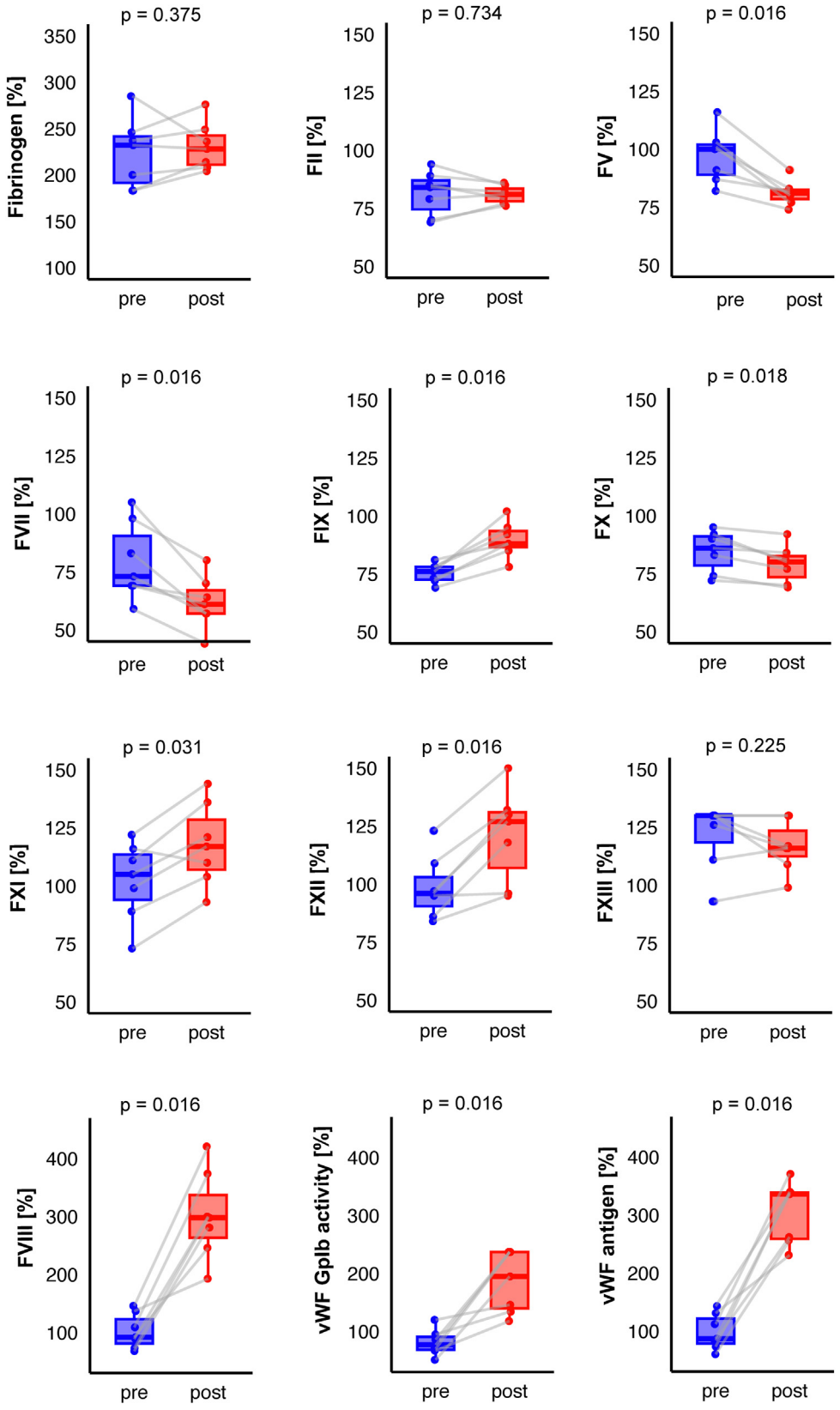

FIGURE Changes in single coagulation factors from pre- (blue) to post- (red) race. Normal ranges for parameters are as follows: factor (F)I (fibrinogen; 210-400 mg/dL), FII, FV, FVII, FIX, FX, and FXI (70%-120%), FVIII and FXII (70%-150%), FXIII (70%-140%), von Willebrand factor (VWF) glycoprotein 1b activity (VWF GpIb; 48%-173%), and VWF antigen (58%-174%). Boxplots display median and IQR; whiskers show minimal and maximal values; individual paired values are shown as connected dots; *P* values are derived from Wilcoxon matched-pairs signed-rank tests.

TABLE 4 Changes in viscoelastic parameters from pre- to postrace.

Parameter	Prerace	Postrace	*P* value	*r*
EXtest CFT (s)	65 (56-70)	52 (51-65)	.375	.34
INtest CFT (s)	75 (71-91)	70 (69-83)	.066	.70
APtest CFT (s)	60 (57-80)	67 (65-74)	.865	.06
EXtest MCF (mm)	**57 (55-58)**	**60 (60-61)**	**.017**	**.90**
INtest MCF (mm)	**55 (51-57)**	**58 (57-59)**	**.027**	**.83**
FIBtest MCF (mm)	**14.0 (12-16)**	**15.0 (14-17)**	**.038**	**.78**
TPAtest lysis time (s)	185 (169-191)	157 (149-176)	.063	.70
INtest maximum lysis (%)	**10 (9-11)**	**5 (4-8)**	**.027**	**.83**
EXtest maximum lysis (%)	**11 (9-12)**	**6 (4-8)**	**.016**	**.91**
APtest maximum lysis (%)	**10 (9-11)**	**6 (5-7)**	**.017**	**.90**

Data are medians and IQRs. A *P* value $\leq$ .05 was considered statistically significant (bold values). Effect sizes are reported as rank-based effect sizes (*r*). AP/EX/FIB/IN/TPAtest, standard Clotpro assays; CFT, clot formation time; MCF, maximum clot firmness.

inhibit fibrinolysis by upregulating α2-antiplasmin and suppressing PAI-1, and downregulate anticoagulant pathways, including protein C, through inflammation-mediated downregulation of thrombomodulin on endothelial surfaces, which occurs within 24 hours and reduces the activation of protein C. Furthermore, IL-6, which was increased significantly postrace, reduces expression of thrombomodulin on endothelial cells, thereby also impairing protein C activation and promoting a procoagulant state related to inflammation [26]. Prolonged aerobic exercise induces changes in a variety of anti/and inflammatory markers. In a systematic review and meta-analysis [22], long-distance running was shown to promote an increase in IL-6, IL-1ra, IL-1β, IL-8, IL-10, and tumor necrosis factor-α, and a decrease in IL-2 concentrations.

In the present study, we found coagulation changes that mirror an inflammatory status. Increases in FVIII and VWF activity are typically observed in critically ill patients suffering from severe infection [18]. However, unlike bacterial sepsis, eg, we did not detect a decrease in antithrombin or consumption of FXII, which is activated and consumed by the presence of pathogen-associated molecular pattern molecules, damage-associated molecular patterns, and NETs [27].

The significant changes in PT and aPTT can be explained by the decreased FVII, FV, and FX levels or the increased FVIII, FIX, FXI, and FXII levels. These fluctuations were not detectable in the functional viscoelastic measurements and might, therefore, not be clinically relevant on their own. Instead, only an increase in fibrin polymerization and slightly diminished lysis were noted, potentially linked to an inflammatory response following extreme physical exercise [22].

The effects on fibrinolysis remain unclear. To our knowledge, this is the first study to employ standardized viscoelastic functional tests (ClotPro) after physical exercise. These viscoelastic tests are already well-established in critical care settings, particularly among COVID-19 patients [28]. They offer a comprehensive functional assessment of the fibrinolytic potential in whole blood, whereas measurements of plasma levels alone fail to capture the cumulative effects of pro- and antifibrinolytic enzymes. Hypofibrinolysis plays a crucial role in various critical conditions, including infection, sepsis, trauma, and disseminated intravascular coagulation (DIC). These conditions often lead to fibrinolysis shutdown, predisposing patients to thrombosis and microvascular complications. The ClotPro tissue plasminogen activator (tPA) test is particularly sensitive in detecting hypofibrinolysis as it measures fibrinolytic activity directly and in a standardized manner under controlled conditions. The ClotPro tPA test uses tPA to actively induce fibrinolysis. Under normal physiological conditions, this should lead to a controlled breakdown of the fibrin clot formed. In hypofibrinolysis or fibrinolysis shutdown, the clot remains intact because plasminogen activation is insufficient or because fibrinolytic inhibitors (eg, PAI-1 and α2-antiplasmin) are overly active. The ClotPro tPA test is highly sensitive in detecting hypofibrinolysis as it directly assesses fibrinolysis under controlled conditions. To our knowledge, this is the first study where this test has been used in athletes under extreme physical stress.

The plasma parameters showed decreased plasminogen and increased antiplasmin levels, and thus could indicate reduced or consumed fibrinolysis. This finding correlates with a nonspecific inflammatory reaction, resulting in reduced fibrinolysis, also shown in the INtest and APtest (Table 4). Nevertheless, it is particularly noteworthy that lysis appears to be impaired after the race. In critically ill patients, hypofibrinolysis or a shutdown of fibrinolysis is related to the severity of inflammation and sepsis and is also consistently associated with poorer prognosis, increased complications, and organ failure in such patients [29].

4.1 | Possible influence of environmental factors: downhill running, hypoxia, and heat

The participants in the trail showed extremely high plasma CK, creatine kinase isoform MB, and myoglobin levels, which suggest muscle

damage. However, these markers are indirect indicators and are a common finding in different types of sports. Extraordinary values for CK as a muscular enzyme have been published for ultramarathon runners (for review, see Knechtle and Nikolaidis [30] and Tiller and Millet [31]). For example, Babcock et al. [32] reported a >100-fold increase in serum CK after a 100-mile endurance run. These observations are in line not only with long-lasting endurance exercise itself, but also with the high contribution of eccentric muscle load due to downhill running [5,6]. Despite the fact that eccentric muscle exercise is metabolically less demanding, it induces more pronounced muscle fiber damage, a stronger inflammatory response, and a higher rate of oxidative stress than concentric exercise [33]. Moreover, it seems that eccentric muscle exercise not only influences the hemostatic balance but may result in a more pronounced fibrinolysis impairment than concentric workload [34]. Therefore, the long distances of downhill running could have been a cofactor for the observed coagulation changes after the trail run.

Over the 86.9 km trail run, the athletes had to pass mountain peaks up to an altitude of 2398 m, which reflects moderate hypoxia. In the literature, there are, in general, conflicting results on the possible impact of hypoxia on blood hemostasis. However, respective data on moderate hypoxia alone and exercise at moderate hypoxia are scarce [14]. Even passive exposure to 4500 m for 8 hours results in only small changes within the reference limits of all coagulation parameters [35]. Compared with passive transfer, in women who actively ascended to 3883 m, increased thrombin generation, as well as increased levels of FVIII and VWF, were reported. Platelet activation was reduced and delayed [36]. Maximal ergometry at 2650 m induced a more pronounced platelet activation than exercise at low altitude [16]. Under acute normobaric hypoxia (FIO_2 = 15.3%), exercise did not amplify changes in coagulation observed in normoxia [15]. Since the evidence published so far suggest there are either no hemostatic changes or only minor alterations after exercise at moderate altitude, and since the maximum altitude was < 3000 m and the duration of the sojourn at moderate altitudes was short, there is no clear evidence that the intermittent slightly hypoxic conditions during this ultramarathon had an effect on the hemostatic system.

During the whole race, we recorded T_{core}. Although there were intraindividual differences in the maximal T_{core}, all athletes showed significant increases during and after the run, with peak values >40 °C. It is well known that exertional heat stroke, the third leading cause of mortality in athletes and most severe manifestation of exercise-induced heat illnesses, can lead to multiorgan injury with pronounced coagulopathy, including DIC [37]. In the clinical setting, hyperthermia leads to activation of coagulation and can even, in extreme cases such as heat stroke, lead to consumption coagulopathy, which is clinically manifested by the simultaneous occurrence of intravascular thrombotic obstruction and an increased bleeding tendency [11]. In detail, hyperthermia directly modulates platelet function. Heat alters platelet function through excessive inflammation and production of cytokines and heat shock proteins. Aberrant hyperthermia induces interactions between leukocytes and endothelial cells, which are also involved in platelet dysregulation (Iba T). In a mouse model, the role of NETs in this context was identified. NETs originating from heat stroke promoted the development of DIC. Degradation of NETs reduced the risk of developing DIC and improved the survival rate of mice [38].

Dehydration may support these processes. In general, dehydration also contributes to inflammation, endotheliopathy, and disbalanced coagulation (Iba T). After the trail run, there were no changes in the red blood cell count; thus, dehydration and hemoconcentration do not seem to be involved in the hemostatic changes. After a 5 km run with increased T_{core} (up to 40 °C), there was accelerated thrombin generation and an attenuated plasmin response [13]. A 164 km road cycling event in a hot environment resulted in platelet activation and increased concentrations of coagulation and fibrinolytic markers [7]. Since there are no studies comparing extreme endurance exercises under different ambient temperatures, we cannot provide clear information on whether or not the increase in T_{core} influences coagulation, in addition to the exercise-induced impacts of long-distance running.

4.2 | Limitations

Although the total number of participants in this trail run was about 300 athletes, the number of runners that we could finally include in this study was unexpectedly low. Since the race was classified as a World Championship Trail Run, we assume that the motivation for participation was limited. In addition, we had only 2 time points for blood collection (prerace and within 3 hours after finishing), which makes interpretation of the data over a longer period difficult. This can be explained by the fact that most of the top athletes left the city within a few hours after the competition ended. On the other hand, in contrast to other studies, our study participants were highly trained athletes. Since the total time of exercise, plus the time until blood collection, was up to 15 hours, the whole study observation period legitimizes our interpretation of the results. Unfortunately, due to the low number of participants, possible sex differences (ie, males vs females) could not be evaluated.

5 | CONCLUSION

In summary, the changes in the hemostatic system after the long trail race indicated a pronounced inflammatory state, similar to those observed in critically ill patients with systemic immune activation. These inflammation-related coagulation changes are typically characterized by elevated FVIII and VWF levels in the acute phase, followed by increased fibrinogen levels, which act as an acute-phase protein, alongside slightly impaired fibrinolysis. Our data needs to be confirmed and extended with other ultradistance races with a higher number of participating athletes.

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AUTHOR CONTRIBUTIONS

W.S., D.F., P.E.A., F.G., and S.B. contributed to the conception and design of the study. T.D., A.K., B.S., and B.T. were involved in the acquisition of data. W.S., D.F., A.K.T., G.W., and C.I. analyzed and/or interpreted data. W.S., D.F., and A.K.T. drafted the article. T.D., A.K., B.S., P.E.A., F.G., S.B., G.W., C.I., and B.T. revised the article critically for important intellectual content. All authors gave final approval of the version to be submitted.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

DATA AVAILABILITY

The authors did not engage in open practices for this study. Due to confidentiality agreements, the data and materials for this study are not publicly available.

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