

1 **The impact of exercise-induced core body temperature elevations** 2 **on coagulation responses**

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1 ABSTRACT

2 **Objectives:** Exercise induces changes in haemostatic parameters and core body temperature
3 (CBT). We aimed to assess whether exercise-induced elevations in CBT induce pro-
4 thrombotic changes in a dose-dependent manner.

5 **Design:** Observational study.

6 **Methods:** CBT and haemostatic responses were measured in 62 participants of a 15-km road
7 race at baseline and immediately after finishing. As haemostasis assays are routinely
8 performed at 37°C, we corrected the assay temperature for the individual's actual CBT at
9 baseline and finish in a subgroup of n=25.

10 **Results:** All subjects (44±11 years, 69% male) completed the race at a speed of 12.1±1.8
11 km/h. CBT increased significantly from 37.6±0.4°C to 39.4±0.8°C (p<0.001). Post-exercise,
12 haemostatic activity was increased, as expressed by accelerated thrombin generation and an
13 attenuated plasmin response. Synchronizing assay temperature to the subjects' actual CBT
14 resulted in additional differences and stronger acceleration of thrombin generation
15 parameters.

16 **Conclusions:** This study demonstrates that exercise induces a prothrombotic state, which
17 might be partially dependent on the magnitude of the exercise-induced CBT rise.
18 Synchronizing the assay temperature to approximate the subject's CBT is essential to obtain
19 more accurate insight in the haemostatic balance during thermoregulatory challenging
20 situations. Finally, this study shows that short-lasting exposure to a CBT of 41.2°C does not
21 result in clinical symptoms of severe coagulation. We therefore hypothesize that prolonged
22 exposure to a high CBT or an individual-specific CBT threshold needs to be exceeded before
23 derailment of the haemostatic balance occurs.

24

25 **Key-Words:** Athletes, Haemostasis, Heatstroke, Hyperthermia, Thermoregulation.

1 Introduction

2 Strenuous exercise induces a hypercoagulable state, hallmarked by an increased factor VIII
3 concentration and a subsequently increased (in vitro) thrombin generation¹⁻³. The increased
4 thrombin enables fibrin formation, which stabilizes the platelet plug⁴. Simultaneous to these
5 prothrombotic changes, increased levels of tissue plasminogen activator and reduced levels
6 of plasminogen activator inhibitor hallmark increased fibrinolysis^{1 3 4}. In this manner, the
7 prothrombotic effects of exercise are offset by increased fibrinolysis, which prevents the
8 formation of excess blood clots. Whilst the magnitude of these haemostatic changes are
9 generally well balanced during general aerobic exercise, they are associated with an
10 increased risk of cardiovascular complications such as acute coronary syndrome in individuals
11 with previous cardiovascular disease⁵.

12

13 In addition to prothrombotic changes, exercise also leads to an increase in core body
14 temperature (CBT)^{6 7}. Whilst this exercise-induced CBT increase is a normal result of
15 metabolic heat production⁸, excessive increases in CBT to values above 40°C can potentially
16 result in heatstroke^{6 9 10}. Heatstroke is characterized by neurological symptoms (delirium,
17 coma) and a derailed haemostatic response that results in a disease state similar to diffuse
18 intravascular coagulation⁹⁻¹¹. Potential consequences of heatstroke are multi-organ failure, in
19 part due to thrombotic complications, and may result in death⁹. Hence, strong CBT rises during
20 exercise bear the risk of developing potential serious thrombotic complications. Conversely,
21 hypothermia (a CBT <35°C) has been shown to negatively impact on haemostatic activity,
22 resulting in an increased bleeding diathesis^{12 13}. These observations suggest that CBT directly
23 impacts on the activity of the haemostatic system, and the procoagulant responses during
24 exercise could thus theoretically be partly caused by an increased CBT. However, whether an
25 increased CBT during exercise actually induces prothrombotic changes in a dose-dependent
26 manner is currently unknown.

27

1 Therefore, the first aim of this study was to investigate whether the exercise-induced elevation
2 in CBT induces prothrombotic changes in a dose-dependent manner. To that end, we
3 measured thermal and haemostatic responses in 62 participants of a 15-km road race at
4 baseline and immediately after finishing.

5

6 Interestingly, haemostatic assays are routinely performed at a temperature of 37°C, whilst the
7 *in vivo* CBT in the present study population was expected to be substantially higher ($\geq 39.0^\circ\text{C}$)
8 at the finish line⁷. Routinely obtained haemostasis data might thus provide inaccurate
9 information due to potentially changing properties of the clotting factors at elevated
10 temperatures¹⁴⁻¹⁶. Therefore, as a second aim, we corrected the temperature at which the
11 assay is performed to approximate the subjects' actual CBT at baseline and after finishing in
12 a subgroup of 25 individuals and investigated whether this leads to different results compared
13 to the routine assessment at 37°C. We hypothesized that an increased CBT at the finish line
14 would enhance prothrombotic responses, and that adjusting the temperature at which the
15 haemostasis assay is performed to approximate the subjects' actual CBT would show a more
16 pronounced haemostatic activation.

17

18 **Methods**

19 Sixty-two individuals (43 males, 19 females; age 44 ± 11 years; height 178 ± 8 cm; body weight
20 72.8 ± 10.5 kg; body mass index 22.9 ± 2.3 kg/m²) who participated in a 15-km road race (Seven
21 Hills Run, Nijmegen, the Netherlands) were recruited for the present study. Recruitment took
22 place identically as previously described by the present authors⁷. Before being included in the
23 present study, all subjects provided a written informed consent and all subjects were screened
24 for the presence of any exclusion criteria for using the temperature pill: 1. A history of
25 obstructive or inflammatory bowel disease or prior abdominal surgery, 2. The presence of any
26 implanted electric (medical) device, 3. A scheduled MRI scan within 1 week after the event, or
27 4. Pregnancy. None of the subjects had a history of cardiovascular or thrombotic disease,

1 none of the subjects used any anticoagulant medication. The average training status of our
2 subjects was 3.6 ± 2.1 hours of running exercise per week for the past year. Study procedures
3 were approved by the Radboud university medical center Ethics Committee and accorded to
4 the principles of the declaration of Helsinki.

5

6 Baseline measurements were performed 2 hours before the start of the race in a laboratory
7 set up 50 meters from the start/finish line. CBT was measured at baseline, 1 minute before
8 the start, and within 15-seconds after finishing. Venous blood samples were obtained via
9 venipuncture from an antecubital vein at baseline and within 5 minutes after finishing. No
10 measurements were performed during exercise, and subjects completed the race at a self-
11 selected pace. Body weight was measured at baseline and immediately after finishing (Seca
12 888, Hamburg, Germany), and relative changes in body weight were calculated to determine
13 the hydration status of participants. Dehydration was defined as a body weight loss $\geq 2\%$ ¹⁷.

14 Subjects ingested an individually calibrated telemetric temperature pill at least five hours (8
15 a.m.) before the race (start 1 p.m.) to prevent interaction of the CBT measurements with fluid
16 ingestion during testing¹⁸. CBT was measured using a portable telemetry system (CorTemp™
17 system, HQ Inc., Palmetto, USA), which has been demonstrated to safely and reliably
18 measure CBT^{19 20}. The average of three consecutive measurements for each time point was
19 used for further analyses.

20 Based on finish CBT, subjects were classified as low- (CBT <39) moderate- (39 \geq finish CBT
21 <40) or high-responders (CBT \geq 40). The exercise-induced increase in CBT (Δ CBT) was
22 calculated by subtracting baseline CBT from finish CBT. Again, three subgroups were created
23 to classify low- (Δ CBT < 1.5) moderate- (1.5 \geq Δ CBT <2.5) and high-responders (Δ CBT \geq 2.5).

24

25 Venous blood samples were collected from an anticubital vein in CTAD (sodium citrate
26 theophylline, adenosine dipyridamol) buffered collection tubes in our on-site laboratory.
27 Samples were centrifuged at 4200 rpm for 15 minutes, after which the plasma was
28 immediately transferred into new uncoated Eppendorf tubes which were subsequently snap

1 frozen in liquid nitrogen and stored at -80°C until further analysis. Thrombin and plasmin
2 generation were simultaneously measured using the 'Nijmegen Haemostasis Assay'²¹. This
3 assay allows simultaneous measurement of *in vitro* thrombin and plasmin generation in the
4 same blood specimen in a single well (Supplemental Figure 1). Lag time thrombin generation
5 describes the lag time between activation of the coagulation cascade and the time at which
6 the initiation of thrombin generation is measured. Time to thrombin peak refers to the time
7 between activation of the coagulation cascade and reaching the peak thrombin generation
8 value. Thrombin peak height refers to the maximal thrombin generation rate. The area under
9 the curve reflects the total thrombin potential ($\text{AUC}_{\text{thrombin}}$). Plasmin peak time refers to the time
10 between activation of the coagulation cascade and the time at which the plasmin peak is
11 reached. Plasmin peak height refers to the maximal plasmin generation rate. Fibrin lysis time
12 refers to the time between the start of plasmin generation and the moment at which plasmin
13 peak height is attained. In all instances, each plasma sample was analysed in duplicate and
14 the average of each duplicate was used for further analysis. The inter-assay variation of
15 thrombin generation parameters varies from 5.9% ($\text{AUC}_{\text{thrombin}}$) to 25% (lag time thrombin
16 generation). The inter-assay variation of plasmin generation parameters varies from 10%
17 (plasmin peak height) to 14% (plasmin potential).

18 For aim 2, we re-analysed the plasma samples at an assay temperature nearest to the
19 subject's CBT at baseline and finish in a subgroup of $n=25$. For example, if a subject had a
20 baseline CBT of 37.4°C , the assay was performed at 37°C , whereas in case of a baseline
21 CBT of $\geq 37.5^{\circ}\text{C}$ and $\leq 38.4^{\circ}\text{C}$ the assay was performed at 38°C . Likewise, a finish CBT of
22 39.4°C was analysed at an assay temperature of 39°C and finish CBT $\geq 39.5^{\circ}\text{C}$ at 40°C . Within
23 this subgroup we also measured additional haemostatic parameters, in order to quantify the
24 *in vivo* haemostatic activity. Plasma prothrombin fragment 1+2 was measured to assess the
25 *in vivo* thrombin generation using an enzyme-linked immunosorbent assay (ELISA; Enzygnost
26 F1+2, Behring Diagnostics GmbH, Frankfurt, Germany), while plasma D-dimer levels were
27 measured to quantify the *in vivo* fibrinolysis activity using an ELISA (Zymutest D-dimer,
28 Hyphen BioMed, Neuville-sur-Oise, France). Furthermore, we determined the isolated effect

1 of the assay temperature on haemostasis parameters by performing the analyses at 37°C,
2 38°C, 39°C and 40°C in the subgroup of n=25.

3

4 Statistical analyses were performed using the Statistical Package for the Social Sciences (IBM
5 SPSS Statistics for Windows, Version 20.0, IBM Corp., Armonk, NY, USA). Data were
6 reported as mean \pm standard deviation unless otherwise indicated. All haemostasis data were
7 visually inspected for normality distribution. In case of a non-Gaussian distribution, log-
8 transformation was performed and data were re-inspected. If a normal distribution could not
9 be attained, non-parametric analyses were performed. The impact of exercise on changes in
10 haemostatic parameters was assessed using a paired Student's T-Test. To test the hypothesis
11 that a higher finish CBT would aggravate the exercise-induced haemostatic responses two-
12 way repeated measurements analysis of variance (Gaussian distributed data) and Kruskal-
13 Wallis or Friedman test (non-Gaussian distributed data) were used. The relationship between
14 finish CBT and post-exercise haemostasis parameters was calculated using a Pearson
15 (Gaussian distributed data) or Spearman's rank correlations (non-Gaussian distributed data).

16

17 **Results**

18 All subjects completed the race in cool environmental conditions (Wet bulb globe temperature
19 12.5°C, Dry-Bulb temperature 10.5°C, relative humidity 87%, wind speed 3.4 – 5.4 m/s) at an
20 average running speed of 12.1 \pm 1.8 km/h. The average body weight loss during the race was
21 -1.4 \pm 0.6% of total body weight, and 18% of all subjects classified as dehydrated. Post-
22 exercise, lag time in thrombin generation (baseline 4.5 \pm 1.1 *versus* finish 3.8 \pm 0.7 min; p<0.001)
23 and time to thrombin peak (9.0 \pm 1.4 vs. 8.0 \pm 0.9 min; p<0.001) were significantly shortened,
24 whilst thrombin peak height (207 \pm 29 vs. 208 \pm 25 nmol/L; p=0.70) and AUC_{thrombin} (1293 \pm 172
25 vs. 1292 \pm 172 nmol/L/min; p=0.94) did not differ between baseline and post-exercise. Plasmin
26 peak time (36.6 \pm 9.1 vs. 35.3 \pm 8.2 min; p=0.20) and fibrin lysis time (30.6 \pm 8.6 vs. 30.4 \pm 8.3 min;
27 p=0.81) did not change after exercise, whereas a significant reduction in plasmin peak height

1 (19.2±12.2 vs. 10.0±9.0 nmol/L; Wilcoxon Signed Ranks Test $p<0.001$) was observed after
2 finishing.

3

4 Baseline CBT was 37.6±0.4°C and increased significantly (1.8±0.9°C, $p<0.001$) to 39.4±0.8°C
5 at the finish line (range: 38.0–41.2°C). We identified 24 low-responders, 20 moderate-
6 responders and 18 high-responders for finish CBT. Coagulation and fibrinolysis responses did
7 not differ across low-, moderate- and high-responders for all but one parameter (Table 1). A
8 group effect ($p<0.02$) was found for AUC_{thrombin} , with significantly higher values in moderate-
9 and high-CBT responders compared to low-CBT responders. Similarly, the magnitude of
10 ΔCBT did not interact with changes in haemostatic parameters (Supplemental Table 1). Lastly,
11 neither finish-CBT nor ΔCBT correlated significantly with post-exercise haemostasis
12 parameters, body weight loss or running speed (Supplemental Table 2).

13

14 Synchronisation of the haemostatic assay temperature to actual baseline and finish CBT
15 resulted in a significant decrease of lag time thrombin generation, time to thrombin peak,
16 thrombin peak height and AUC_{thrombin} between baseline and finish (Figure 1A-D respectively).
17 Plasmin peak time synchronized to actual CBT and plasmin peak height decreased
18 significantly between baseline and finish (Figure 1E + F), whilst fibrin lysis time did not change
19 significantly (Figure 1G). Interestingly, a high finish CBT resulted in a prolonged fibrin lysis
20 time, which was in contrast with subjects that reported a moderate and low finish CBT and
21 demonstrated a shorter fibrin lysis time (Table 2). In addition, fragment 1+2 increased from
22 223±121 pmol/L to 482±623 pmol/L ($p<0.001$), while d-dimer increased from 247±128 ng/L to
23 1223±2821 ng/L ($p<0.001$). No correlation was found between the change in Fragment 1+2
24 and finish CBT ($r=-0.01$; $p=0.96$) or the change in d-dimer and finish CBT ($r=0.04$; $p=0.87$). A
25 similar effect of assay temperature on haemostasis parameters was observed in the subgroup
26 analyses of $n=25$ (Supplemental Table 3).

27

1 **Discussion**

2 The purpose of this study was to investigate whether the exercise-induced CBT-rise causes
3 prothrombotic changes in a dose-dependent manner, and whether correcting the temperature
4 at which the haemostasis assay is performed to approximate the subjects' actual CBT alters
5 the haemostasis outcomes. Our study demonstrates that thrombin generation peaked
6 significantly earlier after the race compared to baseline and that plasmin peak height was
7 significantly lower after finishing. However, changes in haemostatic parameters were not
8 related to finish CBT or Δ CBT. The novelty of our findings lies in the adjustment of the assay
9 temperatures to approximate the subjects' CBT. Correcting the assay temperature resulted in
10 more pronounced changes of time to thrombin peak and AUC_{thrombin} , and resulted in the
11 additional identification of a significantly shortened lag time thrombin generation, decreased
12 thrombin peak height and a significant impact of finish CBT on fibrin lysis time. These results
13 suggest that increases in CBT may partially contribute to the exercise-induced haemostatic
14 activation, but that (short lasting) exposure to CBTs up to 41.2°C does not result in clinical
15 symptoms of increased coagulation. Hence, prolonged exposure or a higher CBT is needed
16 to induce excessive coagulation that is typically observed in athletes diagnosed with
17 heatstroke. Most importantly, our results demonstrate that synchronization of the temperature
18 at which haemostasis assays are performed significantly influences the results. In situations
19 where CBT is outside of normal (36.0 – 37.5°C) range, it is essential to adjust the assay
20 temperature to obtain the most accurate results.

21

22 Participants of our study demonstrated a large ($1.8 \pm 0.9^\circ\text{C}$) and significant increase in CBT
23 after running a 15 km road race, with an average finish CBT of $39.4 \pm 0.8^\circ\text{C}$. These
24 thermoregulatory responses are in agreement with a previous study⁷. Our subjects were well
25 trained (3.6 ± 2.1 hrs/week) and were thus well accustomed to the elevated CBT caused by the
26 exercise itself. We also observed a significant activation of the haemostatic balance, as
27 expressed by a faster thrombin generation and an attenuated plasmin response. These

1 findings confirm the prothrombotic effects of exercise that were reported previously^{4 5 22}. Since
2 prolonged endurance training has previously been shown to attenuate the prothrombotic
3 effects of exercise²³, our findings might have been more pronounced in an untrained control
4 group. Independent of training status, the combination of a large inter-individual variation in
5 CBT with a significant haemostatic activation allows us to explore the potential relationship
6 between these exercise-induced phenomena.

7

8 Haemostatic responses were largely comparable between low, moderate and high responders
9 of finish CBT. Although the AUC_{thrombin} was significantly higher in moderate- and high-
10 responders compared to low-responders, the other exercise-induced haemostatic changes
11 did not differ across finish-CBT groups. In contrast to our hypothesis, these data suggest that
12 the exercise-induced pro-thrombotic changes do not depend on finish CBT. The observation
13 of a massive haemostatic activation in patients with heatstroke suggests that excessive
14 thrombotic responses may only occur above a certain CBT threshold or after prolonged
15 exposure to a high CBT^{24 25}. Hence, the peak finish CBT that was reported in our study
16 (41.2°C) was apparently either too low to induce substantial activation of the coagulation
17 cascade, or too short lasting to induce substantial changes. Data from several animal studies
18 reinforced this hypothesis and showed that prolonged (2.5 - 3 hours) passive heating with a
19 $\text{CBT} > 42^{\circ}\text{C}$ was needed to induce excessive activation of the haemostatic response^{11 26 27}.
20 Indeed, the finish CBTs that were observed in our subjects did not result in a derailed
21 haemostatic response nor in clinical symptoms. This was supported by the statistically
22 significant, though clinically irrelevant, increase in plasma prothrombin fragment 1+2 and d-
23 dimer levels in absence of a correlation of these parameters with finish CBT. Therefore,
24 physical exercise typically performed by the general population does not necessarily result in
25 a derailment of blood haemostasis.

26

27 Importantly, the absence of an interaction between CBT and haemostasis may relate to the
28 fact that analyses were performed at 37°C instead of the actual CBT at the time of blood

1 collection. When synchronizing the assay temperature to the individual's baseline and finish
2 CBT, we observed additional and stronger baseline-to-finish changes in several haemostatic
3 parameters compared to the findings from the assay at 37°C (Figure 1). Differences were not
4 limited to finish results, but also showed altered baseline values when synchronizing for
5 baseline CBT. Athletes with a finish CBT $\geq 40.0^{\circ}\text{C}$ demonstrated a significantly larger fibrin
6 lysis time compared to peers with a lower finish CBT who demonstrated a reduced fibrin lysis
7 time. These findings were confirmed by the analysis in which we varied the assay temperature
8 (Supplemental Table 3) and suggest a direct impact of CBT on exercise-induced haemostatic
9 responses. Interestingly, we have observed this effect after analysis of frozen plasma samples
10 which had already been exposed once *in vivo* to an elevated temperature during the exercise
11 bout. The fact that we were able to identify additional significant changes or stronger baseline-
12 to-finish changes compared to when the assays were performed routinely at 37°C suggests
13 that at least part of the observed procoagulant effects are temperature-dependent. Evidence
14 from previous studies in hypothermic patients support our observations. These studies
15 showed that lowering the haemostatic assay temperature to mimic the real-life CBT resulted
16 in a prolonged initiation of blood clotting, whilst the absolute concentration of clotting factors
17 remained unchanged^{15 28 29}. Altogether, these data suggest that altering the CBT causes the
18 enzymatic coagulation reaction to become slower at lower temperatures *versus* faster at
19 higher temperatures²⁹. Our findings therefore demonstrate that it is essential to adjust the
20 assay temperature to approximate the subject's CBT in order to obtain the most accurate
21 results. This may not only apply to exercise-induced CBT rises, but also to other conditions
22 where CBT is outside of the normal physiological range.

23

24 We found that running a 15 km road race resulted in a pro-coagulant state, which is partially
25 dependent on the magnitude of the exercise-induced rise in CBT. None of our subjects
26 experienced clinical coagulation-related problems, which indicates that changes in
27 haemostatic parameters that are accompanied by a CBT up to 41.2°C are of minor clinical
28 relevance. Hence, our data suggest that heatstroke-induced disseminated intravascular

1 coagulation, which is a serious threat for endurance athletes³⁰, occurs when CBT passes a
2 certain threshold value. Once an athlete exceeds this threshold and is diagnosed with
3 heatstroke, our data underline the importance of rapid and aggressive cooling, which may
4 reduce the CBT related stimulation of the coagulation cascade and could result in a better
5 outcome. Notably, given the diverse range of peak core body temperatures that individuals
6 develop during exercise⁷, this CBT threshold might be individually determined.

7

8 A potential limitation of the present study is that blood samples were collected within 5 minutes
9 after finishing, while CBT was assessed directly at the finish line. Due to the time difference,
10 subjects may have had a slightly lower CBT at the time of blood collection due to passive
11 cooling. Nevertheless, it is unlikely that these minor CBT differences would impacted our
12 haemostasis results, apart from the fact that it would even underestimate our findings. Another
13 potential limitation of the present study is that we did not correct for exercise-induced changes
14 in plasma volume. However, 82% of our study population was well hydrated, making it unlikely
15 that shifts in plasma volume had a large impact on study outcomes. Importantly, all
16 measurements for the temperature adjusted assays were performed on the same plasma
17 samples and the only manipulated factor was assay temperature. Therefore, the adjusted
18 assay temperature data were not affected by plasma volume changes. Lastly, whilst the
19 applied haemostasis assay has a low inter-assay variability for analyses performed on a single
20 say, the variability is slightly larger when comparing analyses performed on separate days (for
21 example Table 1 *versus* Supplemental Table 3). All results reported within each table were
22 therefore acquired on a single day and all reported results can thus be safely interpreted within
23 each table. However, even though the assay variability when performed on separate days was
24 limited, caution should be applied when comparing several tables with one another.

25

26

1 **Conclusion**

2 In conclusion, this study demonstrates that exercise induces a procoagulant state, and our
3 results suggest that this might partially be dependent on the magnitude of the exercise-
4 induced CBT rise. Moreover, athletes with a finish CBT as high as 41.2°C do not necessarily
5 demonstrate haemostatic activation leading to clinical symptoms. We therefore hypothesize
6 that prolonged exposure to, or a specific CBT threshold needs to be exceeded before
7 derailment of the haemostatic balance occurs. Most importantly, our results show that
8 adjusting the assay temperature to approximate the subject's CBT is highly recommended to
9 obtain more accurate insight in the haemostatic balance when CBT lies outside the
10 physiological range.

11

12 **Practical implications**

- 13 • 15-km running exercise resulted in a significant increase of core body temperature to
14 39.4±0.8°C, and significant procoagulant haemostasis activity by increasing thrombin
15 generation and attenuating plasmin responses.
- 16 • Whilst haemostatic responses were comparable between individuals with a low or
17 high finish core body temperature, adjusting the assay temperature to approximate
18 the individual's actual core body temperature revealed a significant interaction
19 between core body temperature and fibrinolytic activity.
- 20 • These findings suggest that the prothrombotic state induced by exercise might be
21 partially dependent on the exercise-induced core body temperature rise. Adjusting
22 the assay temperature to approximate the individual's actual core body temperature
23 is essential to obtain the most accurate results when body temperature is outside the
24 normal (36.0-37.5°C) range.

25

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17

Figure Legends

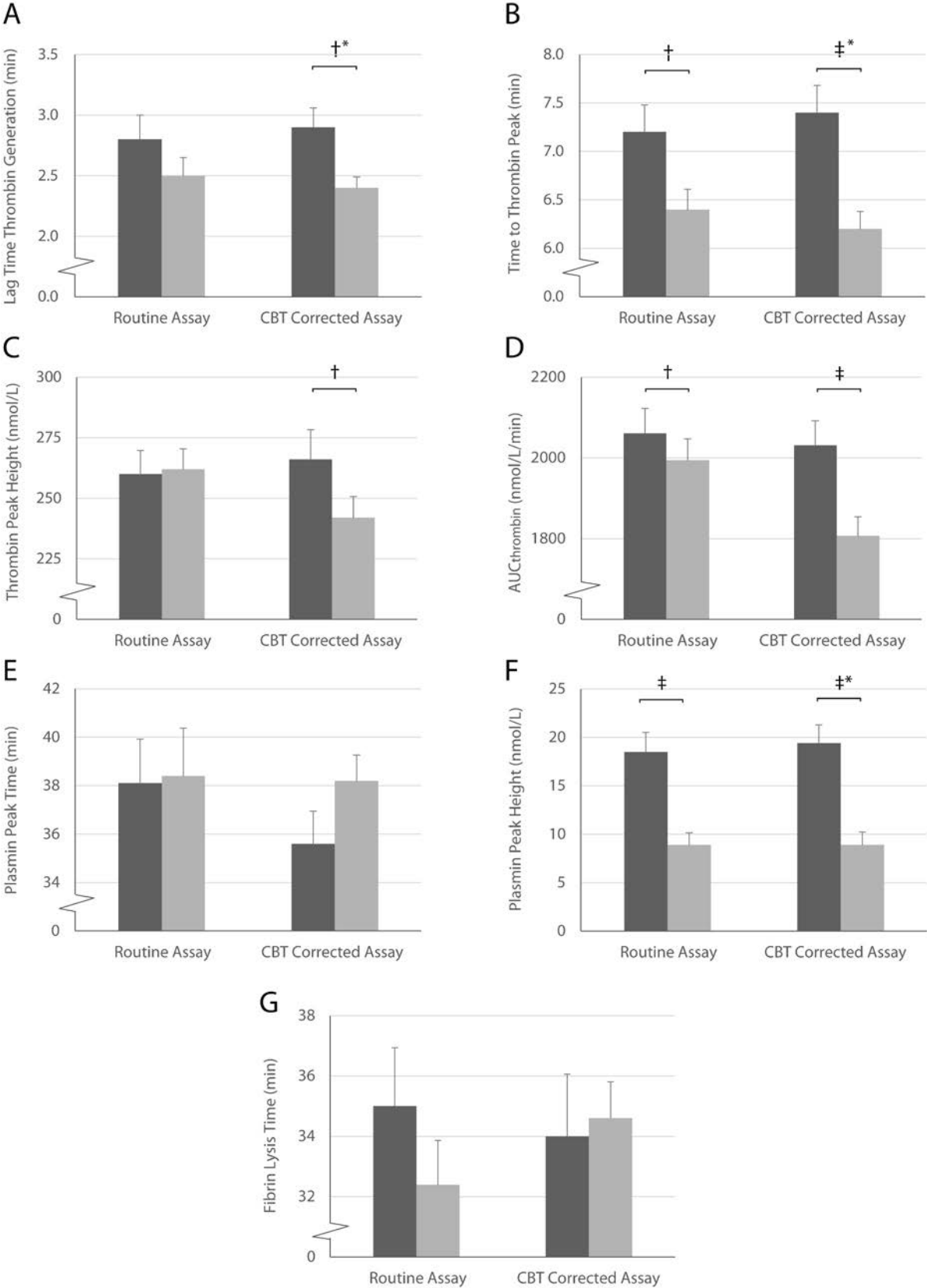
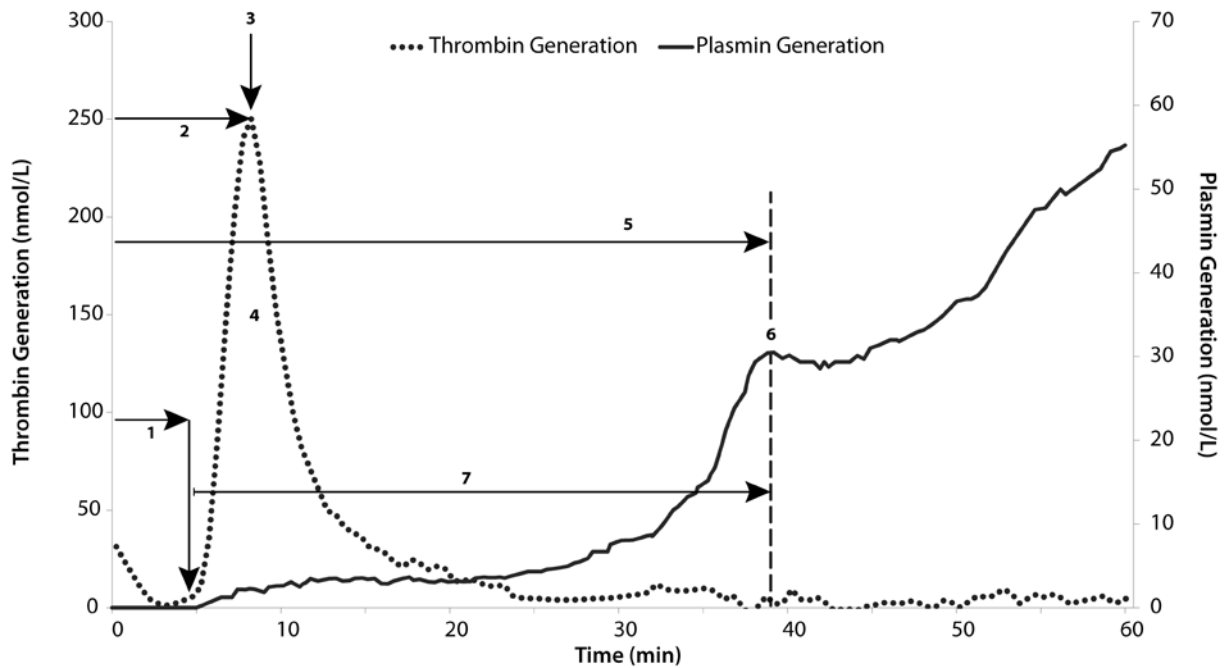


Figure 1: Results of the routinely performed assays (left hand side of graph) *versus* the assay results corrected for the actual core body temperature (right hand side of graph) at baseline and post-finish (black and grey bars respectively). All corrected results for the baseline samples were corrected for CBT at baseline, and all corrected results for the finish samples were corrected for CBT at the finish line. (A) Lag Time Thrombin Generation; (B) Time to Thrombin Peak; (C) Thrombin Peak Height; (D) AUC_{thrombin}; (E) Plasmin Peak Time; (F) Plasmin Peak Height; (G) Fibrin Lysis Time. Legend: # = p<0.05; † = p<0.01; ‡ = p<0.001; * = Statistics are based on non-parametric analysis. Data are presented as mean ± standard error of the mean. CBT = Core Body Temperature



Supplemental Figure 1: Schematic overview of the Nijmegen Haemostasis Assay. The dotted line represents real-time measurement of thrombin generation, whilst the continuous line represents the simultaneous real-time measurement of plasmin generation in the same blood sample. For the purposes of the present study, four parameters were derived from thrombin generation: (1) Lag Time Thrombin Generation (min), (2) Time to Thrombin Peak (min), (3) Thrombin Peak Height (nmol/L) and (4) AUC_{thrombin} (nmol/L/min). From plasmin generation, three parameters were derived: (5) Plasmin Peak Time (min), (6) Plasmin Peak Height (nmol/L) and (7) Fibrin lysis time (min). Adapted from Van Geffen *et al.* (2011).²¹

