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The impact of exercise-induced core body temperature elevations on coagulation responses.

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ABSTRACT

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

Design: Observational study.

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

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

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

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

Introduction

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

In addition to prothrombotic changes, exercise also leads to an increase in core body temperature (CBT)[6 7]. Whilst this exercise-induced CBT increase is a normal result of metabolic heat production[8], excessive increases in CBT to values above 40°C can potentially result in heatstroke[6 9 10]. Heatstroke is characterized by neurological symptoms (delirium, coma) and a derailed haemostatic response that results in a disease state similar to diffuse intravascular coagulation[9-11]. Potential consequences of heatstroke are multi-organ failure, in part due to thrombotic complications, and may result in death[9]. Hence, strong CBT rises during exercise bear the risk of developing potential serious thrombotic complications. Conversely, hypothermia (a CBT <35°C) has been shown to negatively impact on haemostatic activity, resulting in an increased bleeding diathesis[12 13]. These observations suggest that CBT directly impacts on the activity of the haemostatic system, and the procoagulant responses during exercise could thus theoretically be partly caused by an increased CBT. However, whether an increased CBT during exercise actually induces prothrombotic changes in a dose-dependent manner is currently unknown.

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

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

Methods

Sixty-two individuals (43 males, 19 females; age 44±11 years; height 178±8 cm; body weight 72.8±10.5 kg; body mass index 22.9±2.3 kg/m^2) who participated in a 15-km road race (Seven Hills Run, Nijmegen, the Netherlands) were recruited for the present study. Recruitment took place identically as previously described by the present authors[7]. Before being included in the present study, all subjects provided a written informed consent and all subjects were screened for the presence of any exclusion criteria for using the temperature pill: 1. A history of obstructive or inflammatory bowel disease or prior abdominal surgery, 2. The presence of any implanted electric (medical) device, 3. A scheduled MRI scan within 1 week after the event, or 4. Pregnancy. None of the subjects had a history of cardiovascular or thrombotic disease,

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

Baseline measurements were performed 2 hours before the start of the race in a laboratory set up 50 meters from the start/finish line. CBT was measured at baseline, 1 minute before the start, and within 15-seconds after finishing. Venous blood samples were obtained via venipuncture from an antecubital vein at baseline and within 5 minutes after finishing. No measurements were performed during exercise, and subjects completed the race at a self-selected pace. Body weight was measured at baseline and immediately after finishing (Seca 888, Hamburg, Germany), and relative changes in body weight were calculated to determine the hydration status of participants. Dehydration was defined as a body weight loss ≥2%[17].
Subjects ingested an individually calibrated telemetric temperature pill at least five hours (8 a.m.) before the race (start 1 p.m.) to prevent interaction of the CBT measurements with fluid ingestion during testing[18]. CBT was measured using a portable telemetry system (CorTemp™ system, HQ Inc., Palmetto, USA), which has been demonstrated to safely and reliably measure CBT[19 20]. The average of three consecutive measurements for each time point was used for further analyses.
Based on finish CBT, subjects were classified as low- (CBT <39) moderate- (39≥ finish CBT <40) or high-responders (CBT ≥40). The exercise-induced increase in CBT (ΔCBT) was calculated by subtracting baseline CBT from finish CBT. Again, three subgroups were created to classify low- (ΔCBT < 1.5) moderate- (1.5≥ ΔCBT <2.5) and high-responders (ΔCBT ≥2.5).

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

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

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

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

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

Results

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

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

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

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

Discussion

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

Participants of our study demonstrated a large (1.8±0.9°C) and significant increase in CBT after running a 15 km road race, with an average finish CBT of 39.4±0.8°C. These thermoregulatory responses are in agreement with a previous study[7]. Our subjects were well trained (3.6±2.1 hrs/week) and were thus well accustomed to the elevated CBT caused by the exercise itself. We also observed a significant activation of the haemostatic balance, as expressed by a faster thrombin generation and an attenuated plasmin response. These

findings confirm the prothrombotic effects of exercise that were reported previously[4 5 22]. Since prolonged endurance training has previously been shown to attenuate the prothrombotic effects of exercise[23], our findings might have been more pronounced in an untrained control group. Independent of training status, the combination of a large inter-individual variation in CBT with a significant haemostatic activation allows us to explore the potential relationship between these exercise-induced phenomena.

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

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

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

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

coagulation, which is a serious threat for endurance athletes[30], occurs when CBT passes a certain threshold value. Once an athlete exceeds this threshold and is diagnosed with heatstroke, our data underline the importance of rapid and aggressive cooling, which may reduce the CBT related stimulation of the coagulation cascade and could result in a better outcome. Notably, given the diverse range of peak core body temperatures that individuals develop during exercise[7], this CBT threshold might be individually determined.

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

Conclusion

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

Practical implications

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

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Figure Legends

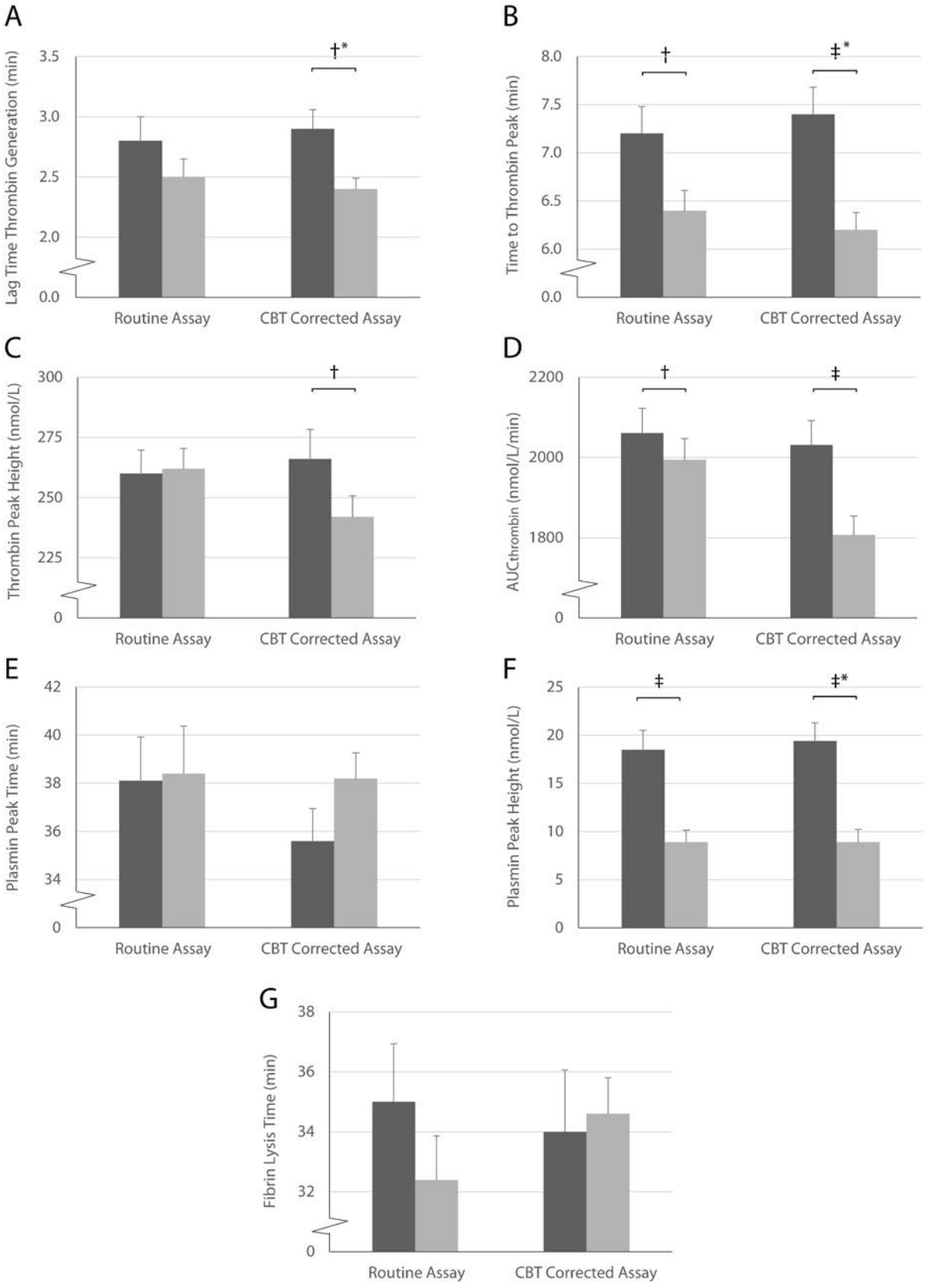
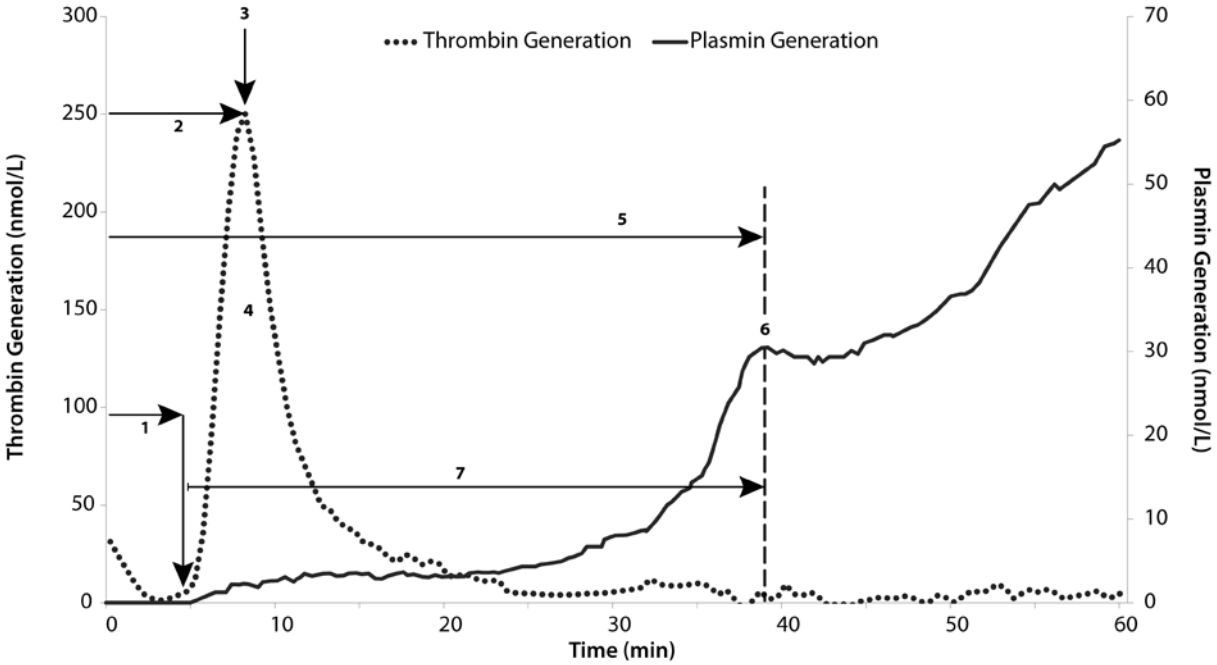


Figure 1: Results of the routinely performed assays (left hand side of graph) *versus* the assay results corrected for the actual core body temperature (right hand side of graph) at baseline and post-finish (black and grey bars respectively). All corrected results for the baseline samples were corrected 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]