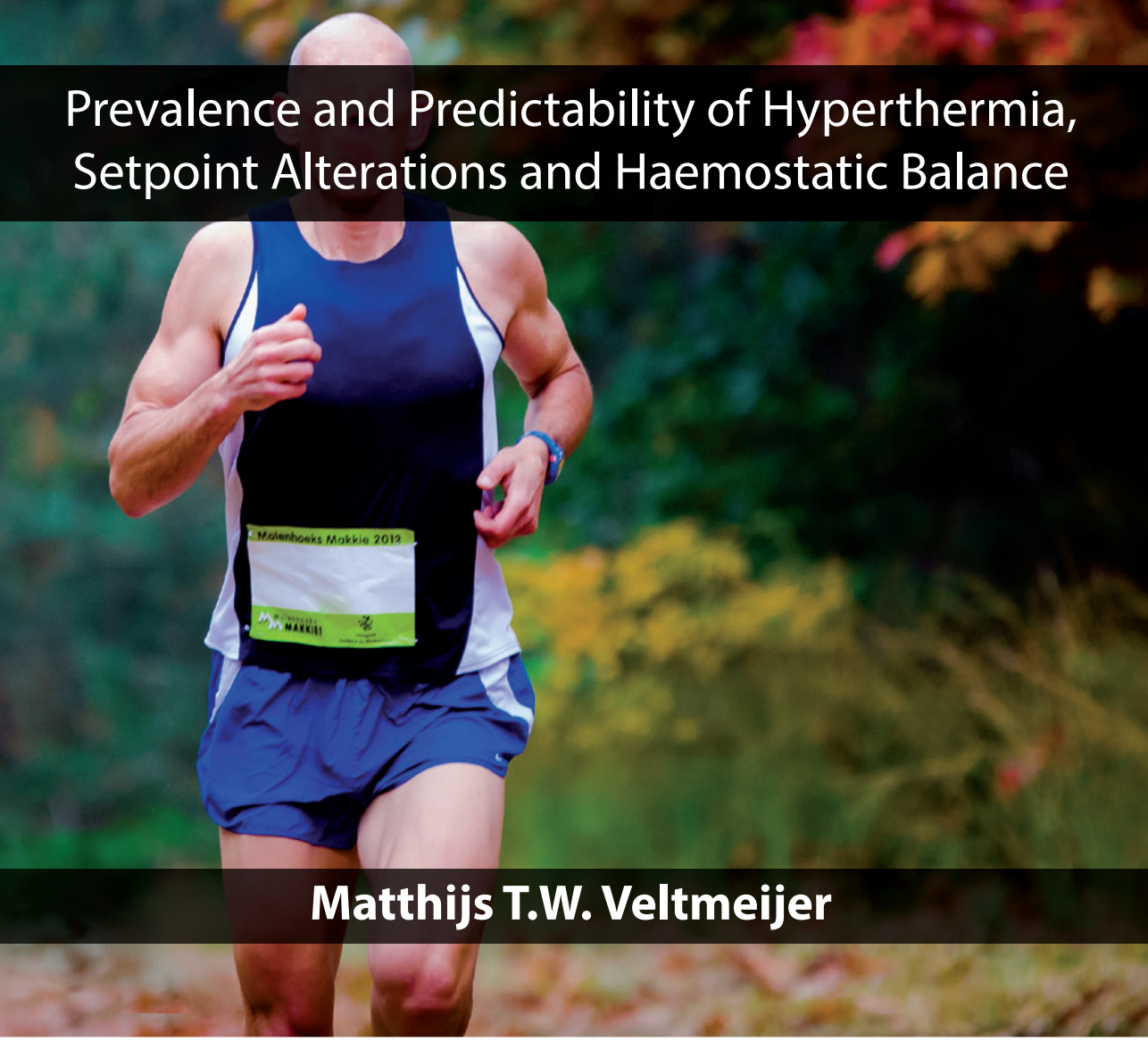
The Thermoregulatory Burden of Running:

Prevalence and Predictability of Hyperthermia, Setpoint Alterations and Haemostatic Balance



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Promotor	Prof. dr. M.T.E. Hopman
Copromotor	Dr. T.M.H. Eijsvogels
Manuscriptcommissie	Prof. dr. M. Olde Rikkert Prof. dr. M.J.R. Edwards Prof. dr. W.D. Van Marken Lichtenbelt (Universiteit Maastricht)

Contents

Chapter 1

General Introduction and Outline of Thesis

General Concepts of Human Thermoregulation

Being the world's most sophisticated piece of machinery, the human body is able to sustain itself nearly fully automatically with very little need for conscious effort such as gathering food or taking shelter from extreme weather conditions. It self-regulates respiration, circulation, removal of (toxic) waste products and maintains the optimal core body temperature for all these processes. The temperature at which all cell processes are performed optimally lies between 36.0 – 37.5°C.[1-4] Within this temperature range proteins fold into their optimal molecular shape at which enzymatic reactions are best catalysed.[5-7] Since core body temperature plays a key-role in all bodily processes, it is strictly maintained within this optimal range.[2-4 7]

In terms of temperature, the body can be divided into two main compartments: 1) the core, constituting the cerebral, thoracic and abdominal area, and 2) the shell, constituting the region outside of the core including the extremities and the skin (Figure 1-1).[4 8] The actual core body temperature is measured by the body at different locations: the core body temperature is measured centrally in the pre-optic area of the hypothalamus and possibly also in the spinal cord, and shell temperature peripherally through skin thermosensors.[4 8-10] The central thermosensors are the primary sensory afferents for core body temperature, and are most sensitive to normal and elevated temperatures. The peripheral temperature sensors are most sensitive to lower temperatures and give rise to early heat-conserving responses for example in case of cold weather conditions, even before any drop in core body temperature is observed.[2 8 11] The afferent signals of both the core and the shell are integrated in the body's thermoregulatory centre, located in the hypothalamus.[4 10] This thermostat continuously compares the measured core body temperature with the hypothalamic setpoint, typically lying between 36.0 – 37.5°C in healthy subjects.[2-4]

If the body temperature deviates too much from the hypothalamic setpoint, central and peripheral effector mechanisms are activated to either conserve and produce heat or to dissipate excess body heat to the outside world.[2 4] In essence, a cold environment will first be sensed by the body's peripheral thermosensors located in the skin, and induce vasoconstriction of peripheral arterioles which restricts the flow of warm blood to the shell well before any drop in core temperature is noticeable. Other mechanisms to prevent the core temperature from dropping are based on increased heat production due to muscle activity (increased physical activity pattern, shivering), and heat generation in brown adipose tissue.[4 12-14] Conversely, even the slightest increase in body temperature will be sensed in the preoptic area of the hypothalamus and will induce powerful heat dissipating responses.[4 10] One of the primary heat dissipating mechanisms of the body constitutes

peripheral vasodilatation, increasing the flow of heated blood from the core to the shell, allowing increased heat loss to the outside world.[2 4 8 15 16] However, the most important heat-releasing method is the evaporation of sweat from the skin, which extracts 58 kcal of heat per 100mL of evaporated sweat. Lastly, a semi-autonomic mechanism to either conserve or even gain heat, or maximize the gradient between the shell temperature to the outside world, are behavioural adjustments.[18-20] Examples are the instinct to either get out of the cold or to look for shade, to reduce the physical activity level, or to add or reduce extra layers of clothing.

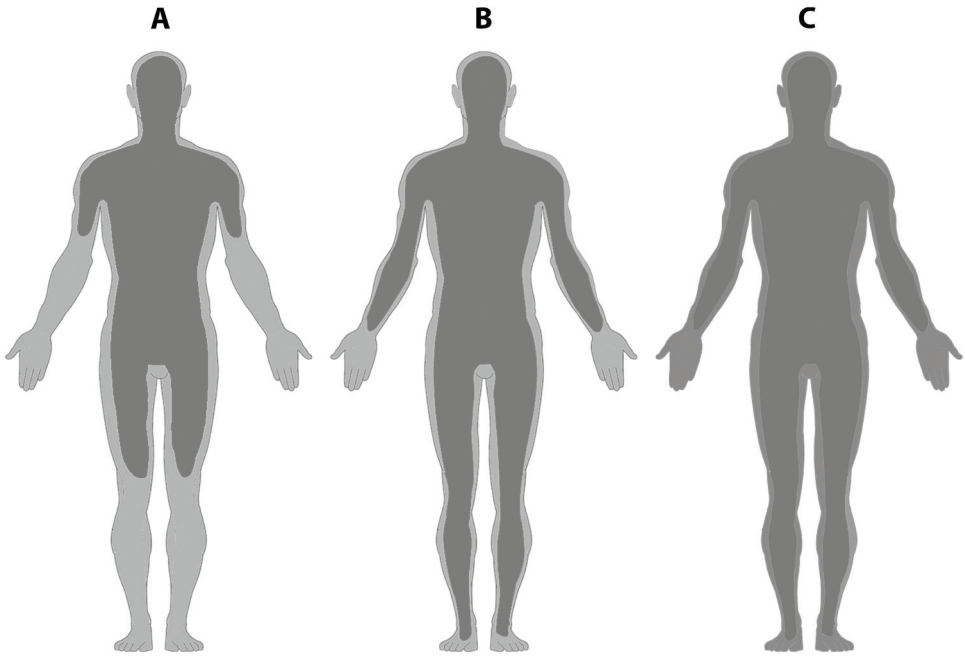

Figure 1-1: *Schematic overview of human temperature distribution. The dark area represents core body temperature, the light area represents the body shell. (A) Represents a euthermic state, (B) shows a situation where core body temperature rises and heat from the core is distributed to the periphery in order to expedite heat loss in the shell. (C) Represents a hyperthermic state where even the shell warms up to accommodate heat loss from the shell even further. Adapted from Hart et al.* [17]

Exercise: A Burden in Thermoregulation

A common cause for body temperature to be elevated is physical exercise. Muscle labour is generally very inefficient, producing approximately 20% kinetic energy and the remaining 80% of the total energy use is generated as heat.[8 21] With the thermoregulatory setpoint unaltered at 36.0 – 37.5°C, the rising core body temperature will induce heat dissipating mechanisms in order to maintain the core temperature as close to the normal levels as possible.[4 8 16] However, the increased metabolic heat production due to muscle activity will often surpass the body's heat dissipating capacity and core body temperature will rise as a result.[22 23] In fact, were no heat-dissipating mechanisms present, it is estimated that core body temperature would rise 1°C during every 5 minutes of exercise.[22] Several factors have been identified to influence body temperature during exercise, and can be divided into external factors and internal factors.

As external factors, environmental conditions play a paramount role in thermoregulation.[4 16 22] Since the body is subject to direct heat transfer between skin and air, a high ambient temperature increases heat load and results in a higher body temperature. Furthermore, with sweating being the body's principal heat dissipating mechanism, a high humidity negatively impacts on the speed of sweat evaporation and therefore also increases heat load. Lastly, a strong heat radiation (e.g. direct strong sunlight) can warm up the skin and impair heat dissipation.

In essence the human thermoregulatory system can be visualised according to the following schematic shown in Figure 1-2.

In addition, numerous internal factors are known to influence thermoregulation during exercise. For instance, high exercise intensity exercise such as running, cycling or dynamic sports such as football or rugby will cause a high metabolic rate. The consequential strongly increased metabolic heat production is known to increase the risk of heat illness.[24 25] Other thermoregulatory burdens are dehydration and low fluid intake prior to the exercise bout, as the reduced sweat rate lowers the body's capacity to dissipate heat by evaporation.[1 22 26] Male sex,[27] poor training,[28] advanced age[26] and a high body mass index[22 26 28] have all been reported to pose increased demand on the thermoregulatory system and hence increase the risk of heat-related problems such as heat exhaustion or heatstroke.

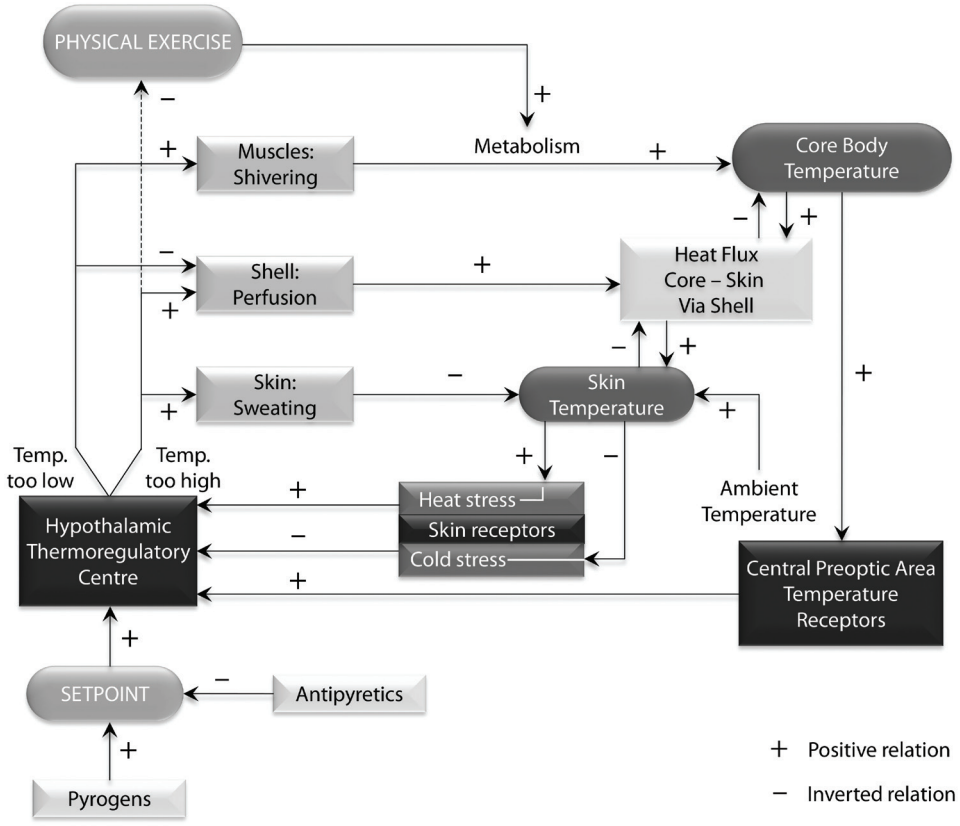

Figure 1-2: *Overview of human thermoregulation. Core body temperature is measured centrally in the preoptic area neurons and processed in the thermoregulatory centre located in the hypothalamus. The thermoregulatory centre compares the body's measured core body temperature to the hypothalamic setpoint, and uses different effector mechanisms to either increase core body temperature or lower it. This includes neurological effector mechanisms to the shell as well as behavioural mechanisms in the form of physical exercise. Adapted from Dr. P. Vis: Lecture Thermodynamics*

Rising Core Body Temperature: A Burden for Performance and Wellbeing

Whilst an increased core body temperature seems to be a normal physiological phenomenon during exercise, excessive rises can have a deleterious effect on the individual's wellbeing. A commonly observed problem in the heat is *heat syncope*. This is essentially caused by venous pooling of blood and vasodilatation in the skin, resulting in self-limiting syncope due to orthostatic hypotension.[22 27 29] A second commonly observed problem is *heat exhaustion*, causing symptoms including malaise, dizziness, nausea and headache. Heat exhaustion is thought to be caused by fluid and sodium deficiencies and precedes the onset of more serious heat-related problems if the individual is to continue

exercise.[22 27 30] Importantly, both heat syncope and heat exhaustion occur at core body temperatures <40.0°C, i.e. at relatively mildly elevated core body temperatures.

However, when thermoregulatory measures completely fail and core body temperature rises to values ≥40.0°C, *exertional hyperthermia* occurs.[22 31] Whilst exertional hyperthermia can exist on its own and may remain fully asymptomatic, it has been described to potentially have more profound health implications. The most extreme consequence of prolonged hyperthermia is *heatstroke*, which is defined as hyperthermia combined with neurological deficits such as confusion, convulsions or coma.[26 30 32 33] Whilst the exact aetiology of heatstroke is still unclear, it is thought to be based on an increased intestinal cell permeability which releases intestinal toxins into the circulation (endotoxaemia),[34] inducing an acute phase response with increased pro-inflammatory cytokine release (e.g. interleukin-1, interleukin-6).[10 30 35-38] In addition, this disease state induces prothrombotic changes that ultimately leads to diffuse intravascular coagulation, multi-organ failure, and ultimately death.[30 39-41] Whilst heatstroke is an extreme consequence of increased heat stress and increased body temperature, this disease state shows the profound derailment of several major body functions and demonstrates the importance of maintaining a normal body temperature at all times.

In addition to potentially endangering the individual's health, exertional hyperthermia has also been shown to potentially result in a decreased exercise performance.[42] The aetiology of hyperthermia-induced fatigue is thought to be related to several factors. First, several central (neurological) mechanism for fatigue has been suggested either as a result from the release of inflammatory cytokines or as a direct result of increased intracranial temperature. Both mechanisms induce a state of 'central fatigue' where motor neurons are innervated at a lower level than possible.[42-44] The postulated aim of this mechanism is an attenuated metabolic heat production resulting in a self-limiting exercise-induced thermogenesis, protecting the individual from heating up too much. The other mechanism causing hyperthermia-induced performance decrease is related to cardiovascular limitations, where the increased muscle blood flow and fluid and electrolyte losses result in a reduced stroke volume and lowered cardiac output.[15 21 23] Since blood volume depletion caused by sweating plays a prominent role in this mechanism, the medical and sports communities have produced detailed consensus statements underlining the importance of remaining adequate hydration at all times.[45-50]

Spinal Cord Injury: A Disruption in Thermoregulation

The nervous system plays an essential role in human thermoregulation, and can be divided into the efferent (or effector) system, and the afferent (or sensory) system. The earliest convincing evidence to show that sympathetic innervation results in cutaneous vasodilation or vasoconstriction was brought by Grant and Holling in 1938.[51] Effectively, they demonstrated that sympathetic ganglionectomy or nerve blockade on the sympathetic nerve fibres resulted in the inability to vasodilate when extremities were subjected to heat. Later research further detailed the importance of the sympathetic nervous system in thermoregulatory vasomotor function, including vasoconstriction during cold exposure.[11 52] Similarly, the nervous system also essentially induces cutaneous sweating to dissipate even more heat when needed.[9] Conversely, peripheral thermosensors submit their information to the central nervous system – specifically the hypothalamus – through the sensory afferents in the spinal cord.[9 53]

In spinal cord injured individuals, the interrupted neural connection prevents individuals to vasodilate or sweat below the level of the lesion, making them prone to thermoregulatory imbalance and heat problems.[9 53] The higher the spinal cord lesion, the greater the body surface area that is unable to elicit thermoregulatory responses.[53 54] Spinal cord injured individuals might therefore be at increased risk of developing heat problems during exercise, and can be considered a risk group.

Fever: A Physiological Deviation in Thermoregulation

The only exception when the body's temperature setpoint deviates from its normal value of 36.0-37.5°C is fever. During fever, the sight of infection causes the release of a multitude of inflammatory cytokines such as interleukin-1 and interleukin-6.[55-58] In turn, these cytokines stimulate the production of prostaglandins, thromboxane, leukotrienes and other acute phase proteins such as C-reactive protein, which all have their own specialized role in preparing the tissue for an immune reaction, attracting leukocytes, lymphocytes, inducing coagulation and starting tissue repair.[59 60] One of these substances, prostaglandin E_2, which is partly formed in the hypothalamus, increases the hypothalamic temperature setpoint and steers the body into elevating its temperature to temperatures up to ~41°C.[56 61] The higher temperature during fever helps certain enzyme reactions run faster than is normally the case, improving the immune response and thus improving the chance of survival.[62 63]

Outline of Thesis

This thesis explores thermoregulatory responses of athletes during exercise and aims to identify which factors influence the magnitude of the increases in core body temperature. In addition, this thesis aims to gain further knowledge in the mechanisms responsible for the development of hyperthermia, and to assess its effects on haemostasis.

Chapter 2 aims to assess what core body temperatures are commonly observed after a 15-km road race and assesses the incidence of exertional hyperthermia after the exercise bout. A second aim of *chapter 2* is to identify factors that significantly predict the rise in core body temperature during the exercise bout, and develop a general model to show which factors induce a higher core body temperature. Core body temperature is measured before the start of the race and immediately after finishing.

Chapter 3 aims to further elucidate to which extent individual thermoregulatory responses can be used to better predict an individual's core body temperature rise during exercise. To that end, thermoregulatory data from the same individuals who participated in two consecutive editions of a 15-km road race are correlated to each other. In addition, the predictive model devised in *Chapter 2* is expanded by supplementing it with the historical data of the first race edition to test if it can be used to better predict core body temperature responses in the second race edition.

Chapter 4 is aimed at identifying the role of the thermoregulatory setpoint during exercise. Previous literature suggests that exercise causes the release of several pro-inflammatory cytokines. In theory, these cytokines might stimulate the production of prostaglandin E_2 in the hypothalamus, which then increases the thermoregulatory setpoint. Whether an increased setpoint, and thus decreased thermoregulatory responses leading to a higher core body temperature during exercise exist, is still unknown. Therefore, this chapter examines subjects performing submaximal treadmill exercise and measures core body temperature whilst the production of prostaglandin E_2 is blocked. The hypothesis is that blocking prostaglandin E_2 production will result in a lower core body temperature during exercise compared to a control condition.

Chapter 5 is aimed at comparing the thermoregulatory responses in wheelchair tennis players with and without a spinal cord injury during an outdoor tennis match. Spinal cord injured athletes have been shown to be at increased risk of developing heat related problems, since they are unable to elicit thermoregulatory responses below the level of the injury. However, previous studies were predominantly performed under well controlled

laboratory conditions. As such, the thermoregulatory strain of wheelchair tennis players is currently unknown, in spite of many tennis tournaments being organized in hot and humid ambient conditions. This pilot study measures changes in core body temperature and skin temperature in a small group of elite international-level wheelchair tennis players with and without a spinal cord injury to compare responses across both groups.

Chapter 6 focuses on the potential effects of core body temperature on haemostasis. Literature involving research on both hypo- and hyperthermia suggests that temperature directly influences the activity coagulation proteins. To what extent an exercise-induced rise in core body temperature in asymptomatic subjects impacts on the activity of haemostasis has not been well described. Therefore, *chapter 6* is aimed at investigating whether an increased core body temperature during exercise induces prothrombotic changes. Furthermore, since haemostasis assays are routinely performed at a temperature of 37°C, whilst the *in vivo* temperature of the targeted population is expected to be higher, the assay results might not provide accurate information. A subgroup of subjects will therefore undergo further analyses in which the assay temperature will be corrected for the subjects' core body temperature during blood collection, to test whether adjusting the assay temperature provides more reliable information on haemostasis activity during exercise.

Chapter 7 provides a detailed general discussion on the main findings of the present thesis. The chapter closes with a future perspective and clinical implications of the main findings.

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Chapter 2

Incidence and Predictors of Exertional Hyperthermia After a 15-km Road Race in Cool Environmental Conditions

Matthijs T.W. Veltmeijer[#]
Thijs M.H. Eijsvogels[#]
Dick H.J. Thijssen
Maria T.E. Hopman

# Both authors contributed equally to the manuscript

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Abstract

Objectives: Current knowledge about the incidence and risk factors for exertional hyperthermia (core body temperature ≥40°C) is predominantly based on military populations or small-sized studies in athletes. We assessed the incidence of exertional hyperthermia in 227 participants of a 15-km running race, and identified predictors for exertional hyperthermia.

Design: Observational study.

Methods: We measured intestinal core body temperature before and immediately after the race. To identify predictive factors of maximum core body temperature, we entered sex, age, BMI, post-finish dehydration, number of training weeks, fluid intake before and during the race, finish time, and core body temperature change during warming-up into a backward linear regression analysis. Additionally, two subgroups of hyperthermic and non-hyperthermic participants were compared.

Results: In a WBGT of 11°C, core body temperature increased from 37.6±0.4°C at baseline to 37.8±0.4°C after warming-up, and 39.2±0.7°C at the finish. A total of 15% of all participants had exertional hyperthermia at the finish. Age, BMI, fluid intake before the race and the core body temperature change during warming-up significantly predicted maximal core body temperature ($p<0.001$). Participants with hyperthermia at the finish line had a significantly greater core body temperature rise ($p<0.01$) during the warming-up compared to non-hyperthermic peers, but similar race speeds ($p=0.34$).

Conclusion: 15% of the recreational runners developed exertional hyperthermia, whilst core body temperature change during the warming-up was identified as strongest predictor for core body temperature at the finish. This study emphasizes that exertional hyperthermia is a common phenomenon in recreational athletes, and can be partially predicted.

Introduction

Current knowledge about the incidence and risk factors for exertional hyperthermia (core body temperature (CBT) ≥40°C) and heat illness is largely based on retrospective studies investigating military populations during military exercises.[1-5] These studies involved well-trained soldiers performing continuous exercise (e.g. long-distance running) superimposed by bouts of high-intensity anaerobic exercise (e.g. heavy lifting). This type of exercise is substantially different from a typical athletic event popular in the general public, during which the athletes typically only perform continuous high-intensity exercise. Furthermore, paramount to the general public is that it is characterized by a wide range of individual traits, including a wide diversity in body mass, age and training and health status.[6] As all these factors may affect thermoregulatory responses differently, each individual may be subject to a different risk for developing heat-related problems, such as exertional hyperthermia or heat illness.[4 5 7] Previous studies that did focus on thermoregulation in participants of athletic events or outdoor time trials in cool to moderate conditions are based on relatively small to moderate sample sizes.[8-10] These studies reported exertional hyperthermia in 0-23% of their participants, mostly after performing marathon races, and this wide range makes it tenuous to draw any firm conclusions. In addition, no previous authors have confirmed whether this knowledge is applicable to the general public based on measurements in a large and heterogeneous sample size. Based on previous literature, risk factors for heat illness such as metabolic rate (i.e. running speed),[8 11 12] dehydration at the finish line and low fluid intake before and during the race,[13-15] increased body mass index,[3 13 16] poor training,[3] advanced age[14] and male sex[17] could significantly predict CBT at the finish line. However, it has never been confirmed whether these risk factors can be applied to identify athletes at risk of developing hyperthermia during large sports events.

Therefore, the main purpose of this study was to assess the CBT in a large (n=230) heterogeneous group of participants of a 15-km running race (Seven Hills Run, Nijmegen, the Netherlands). This race is one of the largest running events held in the Netherlands (>30.000 participants), and holds the men's and women's 15-km world records set in 2010 and 2009 respectively. As a secondary purpose, we identified factors that significantly predicted CBT at the finish line using a backward linear regression analysis. The third aim was to assess the differences between athletes finishing with a high CBT versus those with a low CBT, in order to identify key features that may explain the CBT rise during exercise. To that end, we compared body and race characteristics in athletes with a finish CBT ≥40°C (hyperthermic athletes) to an equally sized group of athletes that finished with the lowest CBT of all participants (non-hyperthermic athletes).

Methods

Five-hundred participants of the Seven Hills Run were randomly contacted and, if interested, were sent a study protocol. All volunteers were screened for the presence of any exclusion criteria for using the temperature pill: 1. a history of obstructive or inflammatory bowel disease, or any 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. Two hundred-thirty participants were included in the study: 111 men and 116 women, were aged 45±11 years and had a BMI of 22.7±2.7 kg/m^2 (Table 2-1). Study procedures were approved by the Radboud University Medical Centre Ethics Committee, accorded to the principles of the Declaration of Helsinki, and all participants provided written informed consent before participation.

Prior to the race, participants completed a questionnaire pertaining to their physical training. Participants self-reported their fluid intake from the time of getting out of bed on the day of the race and during the race. Body weight was measured before and after the race in a laboratory set up 50 meters from the finish line. CBT was measured at baseline in the laboratory about 2 hours before the start, 1 minute before the start (i.e. after warming-up), and within 15 seconds after finishing. Due to the large total number of participants in the race, runners started the race phased into in 9 separate 'waves' over a 1 hour period. Ten research assistants measured CBT in 25±1 participants per wave using 5 wireless receivers. Participants with a CBT ≥40°C upon finishing were compared to an equal number of participants that finished with the lowest CBT.

Participants 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). This measuring system 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. The change in CBT during warming-up was calculated by subtracting CBT at baseline from the CBT before the start of the race.

Body weight was measured to the nearest 0.1kg using an automatically calibrated balance (Seca 888; Hamburg, Germany) before and within 10 minutes after the race. The relative change in body weight was calculated and dehydration was defined as a body weight loss of ≥2%.[21] Participants were allowed to drink *ad libitum* before and during the race, whilst they self-reported the time and amount (standard sized cups, bottles, etc.) of their individual fluid intake before and during the race. No restrictions were imposed on the

Table 2-1: *Participant demographics, physical activity pattern, race characteristics, CBT, body weight, and fluid intake in the total group, a subgroup of hyperthermic participants at the finish line and a subgroup of non-hyperthermic participants.*

	Total group	Exertional Hyperthermia	Non-hyperthermia	P-Value
Characteristics				
Sex (male : female)	111:116	15 : 16	16 : 15	0.80
Age (years)	45 ± 11	43 ± 11	45 ± 11	0.49
Body mass index (kg/m^2)	22.7 ± 2.7	23.0 ± 2.4	22.1 ± 2.6	0.17
Physical activity pattern				
Number of previous participations in this event	5 ± 4	4 ± 3	5 ± 4	0.30
Training (weeks)	28 (range 0-52)	30 (range 3-52)	24 (range 0-52)	0.32
Running exercise (sessions/week)	2.5 ± 0.9	2.4 ± 0.8	2.7 ± 1.0	0.21
Race characteristics*				
Split time 0-5 km (min)	26.2 ± 3.7	25.8 ± 3.0	25.4 ± 4.0	ANOVA (split times):
Split time 5-10 km (min)	26.4 ± 3.9	26.0 ± 3.1	25.5 ± 4.0	Time / Group / Time x Group
Split time 10-15 km (min)	26.6 ± 6.7	26.4 ± 3.5	25.3 ± 4.1	p=0.16 / p=0.46 / p=0.10
Total race time (min)	79.1 ± 12.8	78.3 ± 9.4	76.2 ± 11.9	0.46
Total race speed (km/h)	11.7 ± 1.8	11.7 ± 1.5	12.1 ± 1.9	0.34
Core Body Temperature				
Baseline	37.6 ± 0.4	37.6 ± 0.4	37.4 ± 0.4	0.26
CBT change during warming-up	0.2 ± 0.5	0.5 ± 0.5	0.1 ± 0.4	<0.01
Start	37.8 ± 0.4	38.0 ± 0.5	37.5 ± 0.3	<0.001
Finish	39.2 ± 0.7	40.4 ± 0.4	38.1 ± 0.3	-
Body weight				
Baseline body weight (kg)	71.7 ± 11.9	72.3 ± 11.4	70.0 ± 12.8	0.46
Finish body weight (kg)	70.8 ± 11.7	71.3 ± 11.6	69.0 ± 12.6	0.48
Δ Body weight (%)	-1.5 ± 0.6	-1.7 ± 0.6	-1.5 ± 0.6	0.39
Classifying as dehydrated** (%)	21	29	24	0.67
Fluid intake				
Fluid intake before race (L)	1.18 ± 0.47	1.0 ± 0.4	1.3 ± 0.5	<0.05
Fluid intake during race (L)	0.06 ± 0.12	0.04 ± 0.06	0.07 ± 0.13	0.24

* Differences in split times were tested using a Two-Way Repeated Measures ANOVA.

** Participants classified as 'dehydrated' if body weight at the finish line was reduced ≥2%.

type of fluids consumed, though participants were requested to refrain from drinking between finishing and the second body weight measurement to avoid overestimating the post-race body weight. Furthermore, body weight change during the race (delta body weight) was corrected for fluid intake during the race by adding the fluid intake to the delta body weight.

Individual split times after 5-, 10- and 15-km were obtained from the organizational measuring system (ChampionChip®, MYLAPS, Nijmegen, the Netherlands).

Wet bulb globe temperature (WBGT) was measured every 30 minutes throughout the day using a portable climate monitoring device (Davis Instruments Inc., Hayward, U.S.A.) positioned in the start/finish area.

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 was reported as mean ± standard deviation unless otherwise indicated. A backward linear regression analysis was used to identify factors that significantly predicted finish CBT. Age, sex, BMI, finish time, fluid intake before and during the race, the presence of post-finish dehydration, the delta CBT between baseline and the start and the number of training weeks were included as potential factors that could predict finish CBT. Differences between the subgroups of hyperthermic and non-hyperthermic participants were tested using a Student's *t*-test for the continuous data, and a chi-square test for the nominal data (i.e. the presence of post-finish dehydration). The significance level was set at $p \leq 0.05$.

Results

The average race time was 79±13 min (range 55–165 min), with a mean running speed of 11.7±1.8 km/h (Table 2-1). Furthermore, the split times after every 5-km point were comparable (p=0.33) across the race. Under cool environmental conditions (WBGT was stable at 11°C throughout, $T_{DRY\text{-}BULB}$ 10.5°C, relative humidity 87%), CBT increased from 37.6±0.4°C at baseline to 37.8±0.4°C after the warming-up at the start, and was 39.2±0.7°C upon finishing (Figure 2-1). CBT could not be measured at the finish line in 18 participants (8%). Thirty-one participants(15%) showed a CBT ≥40°C and were hence classified as being hyperthermic. None of the participants reported any apparent heat-related physical complaints.

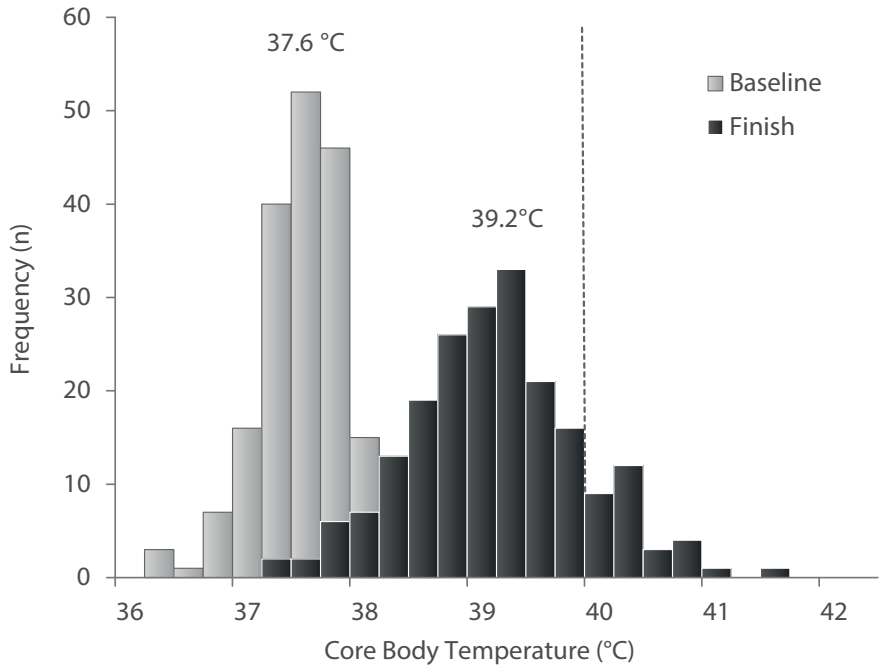

Figure 2-1: *Frequency distribution of core body temperature (CBT) at baseline (light bars) and finish line (dark bars). Fifteen percent of the participants had exertional hyperthermia at the finish line after completion of the race.*

Between baseline and finish, a 1.5±0.6% reduction in total body weight was observed, and 21% of all participants were classified as dehydrated (≥2% decrease in body weight). Self-reported fluid intake before the start of the race was 1.18±0.47L, whilst intake was 0.06±0.12L during the race.

The backward linear regression model ($r=0.41$, $p<0.001$) identified age ($B = -0.01$, $p=0.03$), BMI ($B=0.06$, $p<0.01$), self-reported fluid intake before the race ($B=-0.30$, $p=0.02$) and CBT change during warming-up ($\beta=0.56$, $p<0.001$) as parameters that significantly predicted CBT at the finish line (Table 2-2). Sex, finish time, self-reported fluid intake during the race, the presence of post-finish dehydration and number of training weeks were also entered into the regression analysis, but did not appear to influence finish CBT. These results remained unchanged when the analysis was repeated after replacing the total finish time for the split time in the last 5-km.

CBT change during warming-up was identified as the strongest predictor in our model. Accordingly, we were interested in the risk stratification of participants that demonstrated a CBT change during warming-up that was 2 times higher than the group average (0.2°C). Therefore, we created a new dichotomous variable in which participants were classified to a CBT rise ≤0.4°C or >0.4°C during warming-up. Exertional hyperthermia occurred significantly ($p<0.001$) more frequent in the participants with a CBT rise >0.4°C (33.3%) compared to participants with a CBT rise ≤0.4°C (9%) during warming-up (OR: 5.1, 95% CI: 2.2-11.7).

Two subgroups comprising 31 hyperthermic participants and 31 participants that finished with the lowest CBT of the total group (non-hyperthermic group) were selected for additional analyses with respect to body and race characteristics (Table 2-1). Within these groups, no differences were found for age, BMI, physical activity, race times, or body weight changes. The CBT change between baseline and the start of the race was significantly greater in the hyperthermic participants compared to the non-hyperthermic group ($p<0.01$). Furthermore, self-reported fluid intake before the race was significantly higher in the non-hyperthermic participants compared to the hyperthermic participants ($p<0.05$), whilst self-reported fluid intake during the race was similar in both groups.

Table 2-2: *Predictors for finish core body temperature.*

Variable	Univariate Analysis			Multivariate Analysis*		
	B	95% CI	β	B	95% CI	β
Constant				38.7	37.7 – 39.7	
Age	-0.01	-0.02 – -0.001	-0.19 [C]	-0.01	-0.02 - -0.001	-0.16 [C]
BMI	0.07	-0.02 – 0.12	0.25 [B]	0.06	0.02 – 0.10	0.21 [B]
CBT rise after warming-up	0.54	0.30 – 0.78	0.33 [A]	0.56	0.33 – 0.80	0.35 [A]
Fluid intake before race	-0.28	-0.52 - -0.03	-0.17 [C]	-0.30	-0.53 - -0.06	-0.18 [C]

* R^2 for model = 0.167; adjusted R^2 = 0.147
[A] $p<0.001$; [B] $p<0.01$; [C] $p<0.05$; [NS] not significant
CI = confidence interval; β = standardized B

Discussion

This study assessed the incidence of exertional hyperthermia in a large and heterogeneous group of athletes during a 15-km running event. We found that 15% of our participants passed the finish line with a CBT ≥40°C. Taking into consideration that approximately 30.000 participants entered the race, this would mean that as many as 4.200 participants may have developed exertional hyperthermia. We found that age, BMI, CBT change during warming-up and self-reported fluid intake before the race predicted CBT at the finish line, whereas sex, self-reported fluid intake during the race, the presence of post-finish dehydration, and the number of training weeks had no impact on finish CBT. Interestingly, we found no differences in body and race characteristics between the subgroups of hyperthermic athletes and their non-hyperthermic peers, apart from a higher (0.3L) fluid intake before the race by the non-hyperthermic athletes. However, the CBT change after the warming-up was significantly greater, and self-reported fluid intake before the race was significantly lower in the hyperthermic participants compared to the non-hyperthermic participants. Additionally, we found that athletes with a CBT rise after warming-up >0.4°C were significantly more likely (OR 5.1) to develop hyperthermia at the finish line. These results suggest that exertional hyperthermia is a common phenomenon in recreational athletes, does not necessarily result in physical complaints or a reduced exercise performance, and can be partially predicted.

To our knowledge, this is the first study to measure CBT at the finish line in a large and heterogeneous group of runners. We found that 15% of our participants developed a CBT ≥40°C after 15-km running in cool conditions. Previous smaller-sized studies demonstrated that exertional hyperthermia occurs frequently during military exercises and athletic events,[9 16 22] and our results are the first that confirm the relatively high incidence in a large sample-size from the general public. Interestingly, the reported incidence of hyperthermia in the present (15%) and previous studies (50% and 56%)[9 22] seems to be higher than the reported CBT in several other studies (0%, 3% and 11%).[10 11 23] The difference may relate to cooler conditions or longer exercise duration that was possibly performed at lower exercise intensity in the studies that found a smaller incidence. Another potential explanation might relate to the use of rectal probes in the three latter studies, which were inevitably inserted several minutes after finishing the race. As passive cooling may result in a CBT drop of 0.2-0.5°C within the first 5 minutes post-exercise,[24] the studies measuring rectal temperatures may have in fact underestimated the actual CBT in their participants.

Despite the high incidence of exertional hyperthermia, none of our participants reported any apparent heat-related complaints. Furthermore, we found no differences in race times

between the subgroups with and without exertional hyperthermia, nor any differences in split times at the 5-, 10- and 15-km points (i.e. a stable running pace throughout the race). These results suggest that hyperthermia *per se* does not necessarily result in a reduced exercise performance. Previous authors have questioned the presence of a 'critical' CBT threshold for a reduced exercise performance,[9 25] the latter of which is thought to be caused by a neurologically-mediated sustained decrease in muscle force production.[26] Based on a substantially larger sample-size, our results raise the same hesitations. Tolerance for hyperthermia is widely variable amongst athletes, but was demonstrated to be better in ambient conditions that favour low skin temperatures.[9 25 27] The relatively cool environmental conditions in the present study may therefore at least partially explain why both the hyperthermic and non-hyperthermic participants were able to preserve their race times throughout the race. It would be of great interest to further explore this by assessing CBT and skin temperature in a large group of athletes during a similar running event under cool, moderate and hot environmental conditions, and investigate whether hyperthermia only leads to reduced performance levels when skin temperatures are high.

Interestingly, participants who showed a CBT rise >0.4°C between baseline and the start of the race (i.e. after warming-up) had a significantly higher occurrence (OR 5.1) of exertional hyperthermia upon finishing than participants who had a lower CBT rise during warming-up. Furthermore, the hyperthermic participants had a significantly greater CBT rise during the warming-up compared to their non-hyperthermic peers. This finding raises the question as to whether a strong CBT rise during warming-up can help in identifying athletes at risk of developing hyperthermia during the race. To our knowledge, no such relationship has been reported previously. However, it has been reported that a previous episode of heat illness predisposes athletes for a repeat event,[13] suggesting that an intrinsic predisposition for heat illness may exist. This hypothesis is reinforced by our finding that athletes with the strongest CBT rise after the warming-up were significantly more likely to become hyperthermic at the finish line, which suggests that a similar intrinsic predisposition for developing hyperthermia may exist as well. The clinical implication of this finding is that measuring the CBT rise during warming-up may aid in identifying individuals who should be monitored more carefully, and may help initiate a more direct treatment strategy if problems do occur (i.e. physical complaints or performance detriments). Furthermore, it might also help to identify individuals who are most likely to benefit from any pre-cooling interventions.

By measuring a large number of possible predictive factors for the finish CBT, we were able to predict 16.7% of the total finish CBT. The finding that a higher self-reported fluid intake before the race was predictive for a lower finish CBT in both the total group (regression

analysis) and in the non-hyperthermic subgroup *versus* the hyperthermic subgroup could relate to a difference in hydration status prior to the exercise bout. Since we did not measure the participants' hydration status before the start of the race (e.g. through measuring urinary specific gravity at baseline), it is difficult draw any definitive conclusions about this. However, previous literature recommended that the consumption of ~0.4-0.5L of fluids 4 hours before the start of exercise ensures euhydration at the start of exercise.[28] Since the participants of the present study consumed 1.2±0.5L of fluids in the ~6 hour time span prior to the race, it is likely that they started the race in a euhydrated state. Apart from the potential role of fluid intake before the race, we found no other differences between both subgroups of hyperthermic and non-hyperthermic participants. This emphasizes the difficulty to predict CBT during exercise using traditional measures. Previous studies have shown that exercise intensity and physical fitness affect CBT during exercise.[3 12 13] As our study was performed under race conditions, most participants performed in the upper range of their possible exercise intensity levels. This leaves only a small range of exercise intensities to correlate with CBT, and may explain why we did not find a significant relationship between both parameters.

The strengths of this study are the inclusion of a large and heterogeneous group of participants, the real-life race setting in which participants reach peak performances that cannot be simulated in a laboratory situation, and the fact that we measured CBT within seconds after finishing instead of several minutes after finishing. However, this study was limited by the fact that no correction was applied for sweat entrapment in the participants' clothing after the race in regard to the body weight measurement after the race. Based on previous literature, it is known that not correcting for sweat entrapment in clothing may lead to a measurement error of 10%.[8] As subjects in the present study had an average weight loss of -1.0±0.5 kg, actual sweat losses might have been underestimated by ~0.10 kg on average. However, since the average change in body weight in the present study was already limited and remained below the recommended maximal weight change of 2%,[21] we believe that this small underestimation did not substantially influence our conclusions.

Conclusion

In cool environmental conditions (WBGT 11°C), 15% of the participants passed the finish line with exertional hyperthermia. The stable and similar race speeds in both the hyperthermic and non-hyperthermic participants suggest that hyperthermia *per se* does not necessarily result in performance detriments. The limited value of the predictors of the increase in CBT and the great similarities between both subgroups of hyperthermic and non-hyperthermic participants, emphasizes the difficulty to predict CBT during exercise. However, we did find that participants with a CBT rise after the warming-up >0.4°C were significantly more likely to develop hyperthermia at the finish line (OR 5.1), which may provide new prospects for predicting CBT and associated heat related problems during exercise.

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Chapter 5

Within-Subject Variation of Thermoregulatory Responses During Repeated Exercise Bouts

Matthijs T.W. Veltmeijer
Dick H.J. Thijssen
Maria T.E. Hopman
Thijs M.H. Eijsvogels

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Abstract

Aim: To assess the within-subject variation of thermoregulatory responses during two consecutive 15-km road races. Secondly, we explored whether gastrointestinal temperature (T_{GI}) data from the first race could improve our previously established predictive model for finish T_{GI} in the second race.

Methods: We measured T_{GI} before and immediately after both races in 58 participants, and determined correlation coefficients. Finish T_{GI} in the second race was predicted using a linear regression analysis including age, BMI, pre-race fluid intake, T_{GI} increase between baseline and the start of the race, and finish T_{GI} in the first race.

Results: Under cool conditions (WBGT 11-12°C), T_{GI} was comparable between both races at baseline (37.6±0.4°C *vs.* 37.9±0.4°C; p=0.24) and finish (39.4±0.6°C *vs.* 39.4±0.6°C; p=0.83). Finish T_{GI} correlated significantly between both races (r=0.50; p<0.001). The predictive model (p<0.001) could predict 32.2% of the finish T_{GI} in the second race (versus 17.1% without finish T_{GI} in race 1).

Conclusion: Our findings demonstrate that the use of previously obtained thermoregulatory responses results in higher predictability of finish core body temperatures in future races, enabling better risk assessment for those athletes that are most likely to benefit from preventive measures.

Introduction

An elevated core body temperature (CBT) is commonly observed in athletes performing exercise and does not typically affect health or performance.[1 2] The CBT rise is caused by the production of metabolic heat in the exercising muscle, which cannot be completely released to the environment.[3] If heat storage becomes uncompensable, athletes reduce their performance levels in anticipation of the ensuing CBT rise.[4] Interestingly, the maximal CBT that individuals reach during outdoor time trials in cool to moderate conditions varies widely, ranging from 37.3-41.5°C.[5-7] The variation in thermoregulatory responses has previously been linked to subject characteristics, (e.g. age, sex, exercise intensity, body weight, body mass index (BMI), muscle / fat mass [1 8-11]) and external factors (e.g. ambient temperature, wind speed, humidity).[1 5 12]

Predicting exercise-induced CBT rises can help athletes to estimate their maximal CBT during race conditions. We demonstrated in a previous study that age, BMI, fluid intake before the race and the core body temperature change during warming-up are the primary predictors for maximal gastrointestinal temperature (T_{GI}) in a 15-km road race under cool ambient conditions.[7] Nevertheless, the combination of these within-subject and external parameters could only predict 16.7% of finish T_{GI}. Previous studies revealed that a history of heat illness is an independent risk factor for a future repeated event.[1 9 10] These findings suggest that the magnitude of exercise-induced T_{GI} rises might be related to individually determined intrinsic factors. This would mean that, under exactly the same external conditions and with no changes in within-subject characteristics, one athlete would consistently demonstrate low CBT rises whereas another athlete will consistently demonstrate small CBT changes upon repeated equal bouts of exercise. Whether such consistent within individual thermoregulatory responses exist in the athletic populations, is currently unknown.

Therefore, the aim of this study was to assess the within-subject variation of thermoregulatory responses during two consecutive equal exercise bouts. Secondly, we explored whether including T_{GI} data from the first race edition could improve the predictability if the thermoregulatory responses during the second race edition. For these purposes, we performed T_{GI} measurements in 58 participants of a 15-km running event during two consecutive race editions, which were held under similar environmental conditions. We hypothesized that T_{GI} would strongly correlate between both exercise bouts and could improve the prediction of finish T_{GI} in a subsequent race.

Methods

We recruited 58 individuals (Table 3-1) that participated in two consecutive editions of a 15-km running event (Seven Hills Run, Nijmegen, the Netherlands; organized ~1 year apart). Before being included in the study, all subjects provided a written informed consent and all subjects were screened for the presence of any exclusion criteria for using the temperature capsule: 1. A history of obstructive or inflammatory bowel disease or prior abdominal surgery, 2. The presence of any implanted electric device, 3. A scheduled MRI scan within 1 week after the event, or 4. Pregnancy. Study procedures were approved by the Radboud University Medical Centre Ethics Committee and accorded to the principles of the Declaration of Helsinki. This study was conducted in agreement with the ethical standards according to Harriss *et al.*[13]

Table 3-1: *Subject characteristics in both race editions.*

Variable	Race Edition 1	Race Edition 2	P-Value
Sex (male : female)	31 : 28		-
Age (years)#	47 ± 10		-
Height (cm)	175 ± 8		-
Weight (kg)	73.0 ± 12.4	73.0 ± 12.3	0.71*
Body mass index (kg/m^2)	23.6 ± 2.7	23.7 ± 2.8	0.75
Body Surface Area (m^2)	1.88 ± 0.19	1.88 ± 0.19	1.00

Age during race edition 1 is reported.
* P-value refers to a Wilcoxon Signed Rank test.

Study procedures and measurements were identical in both race editions. Baseline measurements were performed 2 hours before the start of the race in a laboratory set up 50 meters from the finish line. T_{GI} was measured at baseline, 1 minute before the start (i.e. after warming-up), and within 15 seconds after finishing. No measurements were performed during exercise, and subjects were allowed to complete the race at a self-selected pace with *ad libitum* fluid intake.

Body height and weight (Seca 888 calibrated scale; Hamburg, Germany) were measured at baseline. Body mass index (BMI) and body surface area were calculated using the height and weight data. Body-surface area was calculated using the formula of DuBois *et al.*[14]

Subjects ingested an individually calibrated telemetric temperature capsule at least five hours (8 a.m.) before the race (start 1 p.m.) to prevent interaction of the T_{GI} measurements with fluid ingestion during testing.[15] T_{GI} was measured using a portable telemetry system

(CorTemp™ system, HQ Inc., Palmetto, USA), which has been demonstrated to safely and reliably measure T_{GI} as indicator of the subject's CBT.[16 17] The average of three consecutive measurements for each time point was used for further analyses. The T_{GI} rise between baseline and finish was calculated by subtracting the T_{GI} at baseline from the T_{GI} at the finish line.

Subjects self-reported the amount of fluid intake from the time of getting out of bed until the end of the race. Body weight was measured at baseline and within 10 minutes after the race, from which the relative change in body weight was calculated (expressed as percentage dehydration). Correction for fluid intake during the race was applied by adding the amount of fluids consumed to the baseline body weight and recalculating the body weight change. Subjects were allowed to drink *ad libitum* before as well as during the race. No restrictions were imposed on the type of fluids consumed, though subjects were requested to refrain from drinking between finishing and the second body weight measurement to avoid overestimating the post-race body weight.

Individual finish times after 15-km were obtained from the organizational measuring system (ChampionChip®, MYLAPS, Nijmegen, the Netherlands), and running speed was calculated accordingly.

Wet-bulb Globe Temperature (WBGT) was measured every 30 minutes throughout the day using a portable climate-monitoring device (Davis Instruments Inc., Hayward, USA) positioned in the start/finish area.

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 was reported as mean ± standard deviation, with the significance level was set at $p \leq 0.05$. Normality distribution was examined using a Kolmogorov-Smirnov test. In case of non-Gaussian distribution, log-transformation was performed and the data was re-examined for normality distribution. If normal distribution could not be attained, non-parametric tests were applied. Differences in subject and exercise characteristics between the race editions were analysed using a Student's *t*-tests. For study aim 1, a repeated measurements ANOVA was used to determine if thermoregulatory responses were comparable between race edition 1 and 2. Coefficients of variation expressed as percentage (CoV) were determined for each individual subject to gain more insight the individual variation of thermoregulatory responses and race speed between both race editions. Subsequently a Pearson correlation was used to determine the consistency of finish T_{GI} and the exercise-induced T_{GI} elevation. For study aim 2 we performed a linear regression analysis with finish

T_{GI} in race edition 2 as the dependent variable, and age, BMI, fluid intake before the race, T_{GI} change during warming-up (original model) and supplemented it with finish T_{GI} in race edition 1 as independent parameters.[7] To correct for large within-subject differences of metabolic heat production (e.g. race speed),[6 11] we performed additional analyses in which we excluded subjects that showed a >5% difference in race speed between both editions. Finally we created 3 dummy parameters (T_{GI} ≥39.0°C (yes/no), T_{GI} ≥39.5°C (yes/no), T_{GI} ≥40.0°C (yes/no)) for both race editions to explore the risk for exceeding these T_{GI} thresholds in the two consecutive road races. A Pearson's Chi Square test was used to calculate Relative Risks (RR) and their 95% confidence intervals (CI).

Results

Subject characteristics (i.e. baseline body weight, BMI and body surface area) did not differ between race 1 and 2 (Table 3-1). All subjects successfully completed both races at comparable running speeds (11.8±1.9 km/h *versus* 11.7±1.9 km/h, range 8.1-16.5 km/h; p=0.78; CoV 3±3%). Environmental conditions were cool and comparable between race edition 1 (WBGT 11°C, $T_{DRY\text{-}BULB}$ 10.5°C, relative humidity 87%, wind speed 3.4–5.4 m/s) and race edition 2 (WBGT 12.5°C, $T_{DRY\text{-}BULB}$ 11.5°C, relative humidity 88%, wind speed 3.4–7.9 m/s). Pre-race fluid intake was not different between both race editions (1147±448 mL *versus* 1095±444 mL; p=0.25), whereas fluid intake during the races was higher in race edition 2 *versus* 1 (129±146 mL *versus* 85±134 mL; p=0.02). Nevertheless, the percentage body weight loss was not different between both races (-1.6±0.6% *versus* -1.5±0.5%; p=0.25).

T_{GI} was not different at baseline (37.6±0.4°C *versus* 37.7±0.4°C; p=0.24; Cov 1±1%, Figure 3-1), before the start (37.8±0.4°C *versus* 37.9±0.5°C; p=0.28; CoV 1±1%) and immediately after finishing (39.4±0.6°C *versus* 39.4±0.6°C; p=0.83; CoV 1±1%), and demonstrated no difference in exercise-induced T_{GI} increase in both race editions (1.9±0.8°C *versus* 1.8±0.8°C; p=0.58). Finish T_{GI} (Pearson's r=0.50, p<0.001; Figure 3-2A) and the exercise-induced increase in T_{GI} (Spearman's r=0.40, p=0.002; Figure 3-2B) correlated significantly between both races. Correction for subjects with a >5% (n=14) difference in race speed between both race editions improved the correlation of finish T_{GI} between race edition 1 and 2 (Pearson's r=0.59, p<0.001). Lastly, a linear regression analysis revealed that the higher fluid intake in race 2 did not significantly influence T_{GI} at the finish line in race 2 (R^2 = 0.00; p=0.87). Excluding subjects that consumed <0.5L of fluids 4 hours prior to the exercise bout (n=6) and re-analysing the data did not affect the correlation of finish T_{GI} (r=0.48, p<0.001).

By applying our original linear regression model to the present subject population we were able to predict 17.1% (F-score 2.58, $p<0.05$) of the finish T_{GI} of race edition 2 (Table 3-2). Supplementing the model with finish T_{GI} of race edition 1 as an independent variable resulted in a higher predictive capacity of the regression model ($R^2=0.32$, F-score 4.66, $p=0.001$; Table 3-2). Interestingly, correction for subjects with a >5% difference in race speed resulted in an even stronger predictive model ($R^2=0.47$, $p<0.001$). Lastly, re-analysing our data after exclusion of subjects that consumed <0.5L of fluids 4 hours prior to exercise did not affect our predictive model ($R^2=0.31$, $p<0.01$).

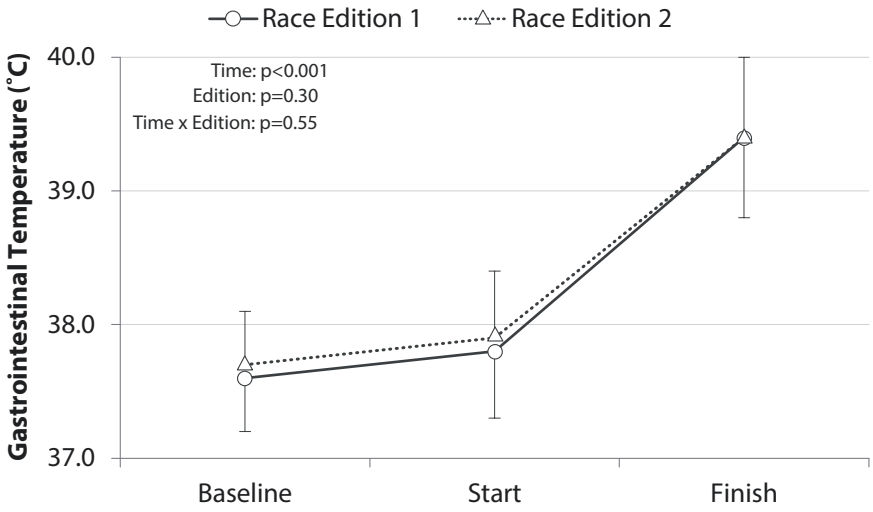

Figure 3-1: *Gastrointestinal temperature (T_{GI}) at baseline, 1 minute before the start and immediately after finishing in race edition 1 (solid line, circles) and race edition 2 (dotted line, triangles). T_{GI} was not different at all time points ($p=0.30$) and increased significantly over time ($p<0.001$).*

Lastly, runners that demonstrated a finish T_{GI} ≥39.0°C in race 1, had a 3.7 times larger chance (CI: 1.0 – 14.0) to exceed this T_{GI} threshold again in race edition 2 compared to athletes who had a finish T_{GI} lower than 39.0°C in race 1. Likewise, runners with a finish T_{GI}≥39.5°C and ≥40.0°C in race edition 1 had elevated risks to exceed these T_{GI} levels again in race edition 2 (RR: 6.5, CI: 2.0 – 21.0 and RR: 6.0, CI: 1.5 – 24.5 respectively).

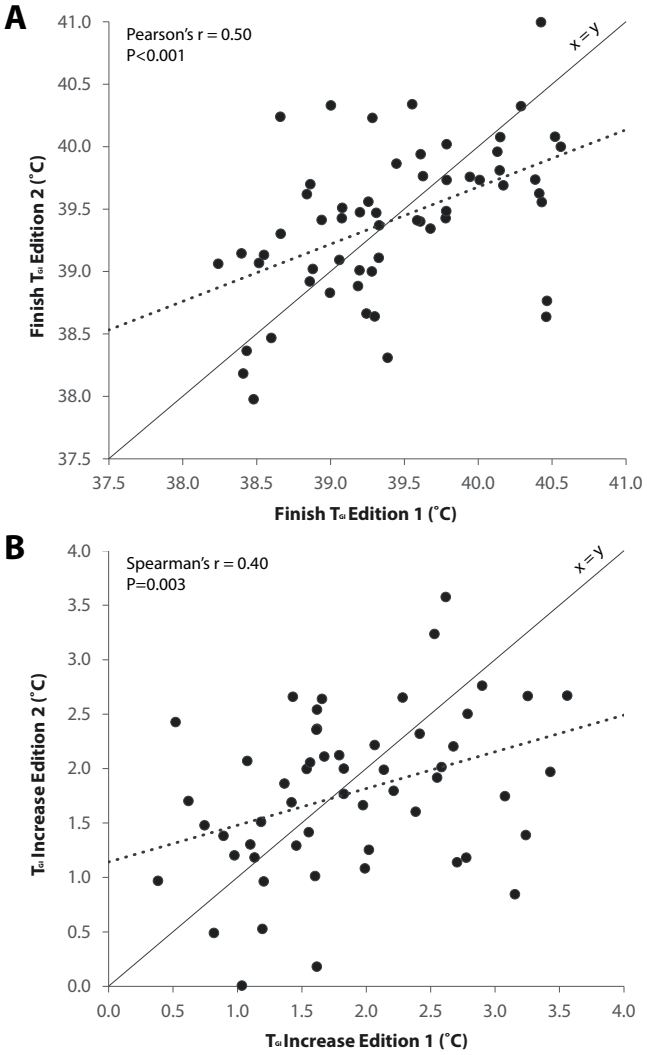

Figure 3-2: *Correlation between finish gastrointestinal temperature in race edition 1 (x-axis) and race edition 2 (y-axis; Figure 3-2A) and correlation between the gastrointestinal temperature increase (baseline to finish) between race edition 1 (x-axis) and race edition 2 (y-axis; Figure 3-2B). The regression analysis revealed that gastrointestinal temperature in race edition 1 accounted for 25% of the finish gastrointestinal temperature in race edition 2. The dotted lines refer to the correlation coefficients and the solid lines refer to the line of identity (x = y).*

Table 3-2: *Predictor characteristics for finish TGI of race edition 2 using our previously established predictive model (upper section) and our new model, which was supplemented with finish TGI in race edition 1 as potential predictive factor.*

Variable	Linear Regression Analysis		
Original Model*	**B**	**95% CI**	**β**
Constant	39.5	38.2 – 40.8	
Age	-0.02	-0.03 – 0.00	-0.29 ^{NS}
BMI	0.04	-0.02 – 0.10	0.18 ^{NS}
T_{GI} Rise After Warming-Up	0.30	0.05 – 0.54	0.31 ^{B}
Fluid Intake Before Race	0.00	-0.00 – 0.00	-0.18 ^{NS}
New Model**			
Constant	25.0	16.1 – 33.9	
Age	-0.01	-0.03 – 0.00	-0.21 ^{NS}
BMI	0.03	-0.03 – 0.08	0.14 ^{NS}
T_{GI} Rise After Warming-Up	0.19	-0.05 – 0.42	0.20 ^{NS}
Fluid Intake Before Race	0.00	-0.00 - 0.00	-0.17 ^{NS}
Finish T_{GI} Race 1	0.37	0.15 – 0.59	0.41 ^{A}

* R for original model = 0.41; R^2 = 0.17; F-score of change: 2.58, $p<0.05$ / ** R for new model = 0.57; R^2 = 0.32; F-score of change: 4.66, p=0.001
$^{A}p <0.005$; $^{B}p <0.05$; ^{NS} not significant
CI = confidence interval; β = standardized B

Discussion

This study assessed the within-subject variation of thermoregulatory responses in athletes participating in two consecutive editions of a 15-km road race in comparable environmental conditions. Our results demonstrate that T_{GI} was not different across both exercise bouts at baseline, start and finish, and show that both finish T_{GI} (r=0.50) as well as the exercise-induced T_{GI} increase (r=0.40) correlated significantly between the two race editions. Moreover, by supplementing our predictive model with the finish T_{GI} from the first race edition, we improved the predictive capacity of finish T_{GI} from 17.1% to 32.2%. Lastly, our results demonstrate that the chance of attaining a high T_{GI} was significantly larger if that subject demonstrated previous high exercise-induced thermoregulatory responses (relative risk varying from 3.7 – 6.5). These results suggest that CBT responses are not different within subjects over consecutive exercise bouts. Therefore, individual CBT data are valuable to improve the predictability of exercise-induced thermoregulatory responses and to identify which athletes are most likely to benefit from cooling strategies.

To our knowledge, this is the first study to directly compare and correlate T_{GI} in the same subjects performing two similar exercise bouts without applying any intervention. Previous studies that measured T_{GI} during repeated exercise bouts reported variable results, but are difficult to compare to the present study as they all imposed different kinds of potentially confounding interventions, including diurnal variation,[18] variable environmental conditions,[19] variable heat load[20] or variable exercise protocols.[21] By performing measurements in the same subjects who twice completed the same 15-km run under similar conditions, we were able to directly compare thermoregulatory responses whilst limiting the chance of confounders. Indeed, our results showed that BMI,[22] running speed[23] and hydration status,[24] which are known to influence CBT during exercise, were all similar across both exercise bouts and will therefore unlikely have influenced our results. Although fluid intake during the race was significantly higher in the second exercise bout (129 ± 146 mL *versus* 85 ± 134 mL), absolute differences between race editions were small (44 ± 150 mL), body weight changes were comparable (-1.6±0.6 *versus* -1.5±0.5% of total body weight), and regression analysis showed no impact of fluid intake on finish T_{GI}. To summarize, the significant correlations of finish T_{GI} (r=0.50) and T_{GI} increase (r=0.40) between both race editions suggest that the correlation of CBT at the finish line between two 15-km road races is moderate, whilst the coefficients of variation are low within subjects.

Our model that demonstrated a 17.1% predictive capacity for finish T_{GI} confirms previous findings (16.7% predictive capacity in a different study cohort).[7] By adding the finish T_{GI} from race 1 to this model to predict finish T_{GI} in race 2, we were able to improve the predictive capacity from 17.1% to 32.2%. Interestingly, correcting our model for changes in exercise intensity (<5% difference in finish time between race 1 and 2), further improved the predictability of finish T_{GI} (R^2=0.47). Furthermore, we demonstrated that individuals, who developed a finish T_{GI} ≥39.0°C during the first edition, were 3.7 times more likely to attain a similar or higher T_{GI} during a second exercise bout compared to subjects who finished with a T_{GI} ≤39.0°C. This chance was even greater if higher cut-off values were chosen; subjects finishing with a T_{GI} ≥40.0°C had a 6.0 times greater chance for exceeding this threshold again during a subsequent race. These findings may help to identify athletes that benefit from cooling interventions preceding and during exercise.[25]

The limited variation of exercise-induced T_{GI} responses within subjects, in combination with the large variation in thermoregulatory responses between subjects (T_{GI} increase ranging 0.4-3.6°C) raises questions regarding the underlying mechanisms that are responsible for this observation. In addition to anthropometric factors such as age,[10] sex,[9] and BMI[8] [10], inherited intrinsic factors might play an important role. For example, several genes have been linked to the development of heat illness.[10] Whether the genetic variation also

affects thermoregulatory responses and/or the capacity of heat dissipating mechanisms is currently unknown. Likewise, there is evidence that CBT responses are related to exercise-induced changes of the hypothalamic setpoint.[26 27] Potentially, the 'high-responders' in our study demonstrated a larger increase in the CBT setpoint compared to the 'low-responders'. Since our study did not include measurements of these intrinsic factors, future studies focussing on the potential underlying mechanisms are warranted.

This study was limited by the fact that we did not measure hydration status prior to the start of the exercise, which could mask differences in hydration status between both exercise bouts. However, previous literature recommended that the consumption of ~0.5L of fluids 4 hours prior to exercise should ensure euhydration at the start of the exercise.[28] Whilst 52 subjects met this criterion, 6 subjects did not. Re-analysis of our data without these subjects did not affect the correlation of finish T_{GI} or our predictive model. We therefore believe that differences in hydration status did not impact on our findings. Furthermore, this study was also limited by the fact that both race editions were separated by a ~1 year time span. This could have potentially lead to the occurrence of within-subject differences that could not have been accounted for (e.g. changes in health status, training status, etc.), possibly resulting in a suboptimal comparison between finish T_{GI} in both editions. Nevertheless, we still found a significant correlation of 0.50 in finish T_{GI} between both races. Therefore, given that a smaller time span between both exercise bouts might have resulted in a higher correlation, our results likely only underestimate the actual within-subject variation of thermoregulatory responses.

Conclusion

Exercise-induced thermoregulatory responses significantly correlated within subjects performing two consecutive conditions of a 15-km road race under cool environmental conditions, demonstrated a moderate within-subject variability and a low coefficient of variation. Athletes that showed a finish $T_{GI} \geq 40.0°C$ had a 6.0 times greater chance for exceeding this threshold again during a subsequent race. More importantly, the use of previously obtained thermoregulatory responses improves the predictability of finish core body temperatures in future races. Our findings enable identification of athletes that are the most likely to benefit from cooling interventions preceding and during exercise.

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Chapter 4

The Impact of Central and Peripheral Cyclooxygenase Enzyme Inhibition on Exercise-Induced Core Body Temperature Elevations

Matthijs T.W. Veltmeijer#
Dineke Veeneman#
Coen C.C.W. Bongers
Mihai G. Netea
Jos W. van der Meer
Thijs M.H. Eijsvogels
Maria T.E. Hopman

# Both authors contributed equally to the manuscript

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Abstract

Purpose: Exercise increases core body temperature (T_C) due to metabolic heat production. However, the exercise-induced release of inflammatory cytokines including interleukin-6 may also contribute to the rise in T_C by increasing the hypothalamic temperature setpoint. We aimed to investigate whether the exercise-induced increase in T_C is partly caused by an altered hypothalamic temperature setpoint.

Methods: 15 healthy, active male subjects aged 36±14 years were recruited. Subjects performed submaximal treadmill exercise in 3 randomized test conditions: (1) ibuprofen 400mg and acetaminophen 1000mg (IBU/APAP), (2) acetaminophen 1000mg (APAP) and (3) a control condition (CTRL). Acetaminophen and ibuprofen were used to block the effect of interleukin-6 at a central and peripheral level, respectively. T_C, skin temperature and heart rate were measured continuously during the submaximal exercise tests.

Results: Baseline values of T_C, skin temperature and heart rate did not differ across conditions. Serum interleukin-6 concentrations increased in all three conditions. A significantly lower peak T_C was observed in IBU/APAP (38.8±0.4°C) *versus* CTRL (39.2±0.5°C, p=0.02), but not in APAP (38.9±0.4°C) *versus* CTRL. Similarly, a lower ΔT_C was observed in IBU/APAP (1.7±0.3°C) *versus* CTRL (2.0±0.5°C, p<0.02), but not in APAP (1.7±0.5°C) *versus* CTRL. No differences were observed in skin temperature and heart rate responses across conditions.

Conclusions: The combined administration of acetaminophen and ibuprofen resulted in an attenuated increase in T_C during exercise when compared to a control condition. This observation suggests that a prostaglandin E_2 induced elevated hypothalamic temperature setpoint may contribute to the exercise-induced rise in T_C.

Introduction

Human core body temperature (CBT) is strictly regulated by the body's natural thermostat located in the hypothalamus. CBT is measured by preoptic area neurons and values are compared with the temperature setpoint, which is typically kept near 36.8±0.4°C.[1 2] When CBT increases beyond the setpoint temperature, several compensatory mechanisms are activated to release excess body heat and maintain a proper CBT.[3 4]

Exercise almost invariably causes CBT to rise, as a result of increased metabolic heat production due to muscle labor.[3 5 6] Since the hypothalamic temperature setpoint remains unchanged, a rise in CBT will activate heat loss mechanisms including skin vasodilatation and sweating.[3] These mechanisms are often insufficient and CBT will rise further.[7] Another cause for CBT to rise is infection- or inflammation-induced fever, which causes the hypothalamic temperature setpoint itself to rise. Multiple pro-inflammatory cytokines are released during infection, including interleukin-1β (IL-1β) and interleukin-6 (IL-6).[8] These cytokines stimulate the enzyme cyclooxygenase (COX) to synthesize prostaglandin E_2 (PGE_2), which in turn upregulates the temperature setpoint and via several mechanisms (e.g. vasoconstriction and shivering) may cause CBT to rise.[8-11] Antipyretic drugs mainly act by reducing PGE_2 synthesis by inhibiting COX enzyme activity.[9 10] COX can be inhibited either peripherally (non-steroidal anti-inflammatory drugs) or centrally in the hypothalamus (acetaminophen).[9 12]

Whilst current literature states that metabolic heat production is the sole cause for CBT to rise during exercise, previous authors have also reported that substantial amounts of pro-inflammatory cytokines are released during exercise.[13 14] It could therefore be hypothesized that the release of these cytokines during exercise can increase the hypothalamic setpoint, and may thus be partially responsible for the exercise-induced CBT rise.

Recent human studies suggested that drug-induced inhibition of PGE_2 synthesis may[15-17] or may not[18] attenuate the rise in CBT and skin temperature during exercise. These inconsistent findings may be explained by the different modes of exercise protocols (incremental *vs.* fixed intensity), but may also be caused by the fact that these studies inhibited COX-enzyme activity either centrally (acetaminophen) *or* peripherally (non-steroidal anti-inflammatory drugs). Since none of these studies used COX-inhibition via both pathways, to what extent inflammatory cytokines influence the CBT rise during exercise still needs to be elucidated.

The aim of this study was to investigate whether combined inhibition of central (acetaminophen) and peripheral (ibuprofen) PGE_2 synthesis can attenuate the rise in CBT during exercise. We hypothesized that the exercise-induced CBT elevations are attenuated in the combined COX inhibition conditions *versus* the control condition.

Methods

Fifteen healthy male volunteers unacclimatized to heat were included in this study (Table 4-1). Potential subjects were eligible to participate if they were aged between 18-60 years and performed regular running exercise for at least 1.5 hours per week. After providing written informed consent, potential subjects were screened for the presence of any exclusion criteria for using the COX-inhibitors or for using the temperature pill: I) a known hypersensitivity to acetaminophen or non-steroidal anti-inflammatory drugs II) a peptic ulcer in the medical history, III) a history of kidney disease, IV) a history of obstructive/inflammatory bowel disease or surgery (with exception of appendectomy and cholecystectomy), V) having an electrically implanted device, or VI) scheduled a MRI-scan within 5 days after the test-day. Study procedures were approved by the Radboud university medical center Ethics Committee and accorded to the principles of the declaration of Helsinki.

Table 4-1. *Subject characteristics and results of the maximal exercise test.*

	Mean ± SD	Range
Age (yrs)	36 ± 14	21 - 59
Body Mass Index (kg/m^2)	22.8 ± 1.9	19.2 - 27.4
Height (cm)	181 ± 8	170 - 190
Training time (hours / week)	4.4 ± 2.6	1.5 - 11
Maximal Heart Rate (bpm)	186 ± 11	159 - 197
VO_2max (mL/min/kg)	61.7 ± 9.9	43.1 - 78.0
Lactate pre-test (mmol/L)	1.5 ± 0.9	0.8 - 4.4
Lactate post-test (mmol/L)	13.9 ± 3.2	7.9 - 18.5

Each subject visited our laboratory four times. During the first visit, subjects performed a maximal treadmill exercise test to determine each subject's maximal heart rate. Visits 2 to 4 consisted of submaximal exercise tests on a treadmill where running speed was calibrated individually for each subject's maximal heart rate. Each submaximal exercise test comprised of 30 minutes continuous running at 85% of the subject's maximal heart rate, followed by 10 intervals with a 1 minute speed increase of 2km/h and 2 minute speed decrease of

2km/h compared to the continuous running speed of the first 30 minutes. This exercise protocol was selected based on pilot measurements within our own department to select the exercise protocol that elicits the strongest T_C rise within one hour. Running speeds of the second and third exercise test were kept identical to the first exercise test to ensure that stimuli for thermogenesis and cytokine release were identical across all exercise tests. Using a cross-over design with randomization of sequences, the following test medication was administered 45 minutes before the start of each submaximal exercise test.

1. IBU/APAP: Administration of 400mg ibuprofen (IBU) with 100mL of water and 1000mg acetaminophen (APAP) with 100mL of water.
2. APAP: Administration of 1000mg acetaminophen with 100mL of water. An extra 100mL of water was administered as a control substance for ibuprofen. This condition was added for comparison with previously performed studies using APAP only.[15 18 19]
3. CTRL: Control condition without inhibition of PGE_2 synthesis. Instead, 100mL of water was administered twice as control substances for acetaminophen and ibuprofen.

The use of the non-steroidal anti-inflammatory drug ibuprofen was chosen because of pharmacokinetics similar to acetaminophen. Since ibuprofen reaches its maximal plasma concentration 1-2 hours after ingestion, and acetaminophen reaches its maximal concentration 30 minutes to 2 hours after ingestion, administration of the test medication was timed such that maximal concentrations were attained 30 to 45 minutes into the exercise bout. The dosage of both APAP and IBU was based on the Dutch Guidelines for antipyretic treatment.[20] A minimum of 3 rest days was required between the submaximal exercise tests to enable full recovery, and subjects were not allowed to use acetaminophen or NSAIDs for at least 3 days preceding each measurement. All experiments were performed in the same room at the same temperature (21°C) and humidity (45%) and the same time of the day to prevent any interference of environmental conditions or circadian rhythm.[4] Also, subjects were instructed to consume 500mL of water 2-3 hours before the start of the exercise tests to ensure euhydration at the start of the exercise bouts.[21]

Each subject underwent a maximal exercise test on a treadmill (GTR-3.06, En-Bo Systems, Zwolle, Netherlands) using the Bruce protocol. Oxygen consumption was measured using a calibrated gas exchange analyser (Quark CPET, Cosmed, Italy) with a breathing mask. Heart rate was monitored using a Cosmed HR monitor (Cosmed, Italy). Capillary blood lactate levels were measured (Lactate Pro, Arkray, Kyoto, Japan) before and after the maximal exercise test as an indicator for achieving maximal exercise (>8 mmol/L). Other indicators for maximal exercise were a plateau in the VO_2-curve, a respiratory exchange ratio ≥1.1, and a maximal heart rate ≥95% of the age-predicted maximum. Subjects had

to meet 3 out of the 4 aforementioned criteria to achieve maximal exercise.

The subjects were instructed to ingest a telemetric temperature pill (CorTemp, HQ Inc, Palmetto Fl, USA) 6 hours prior to each submaximal exercise test to assure stomach passage and exclude interference from fluid or food ingestion.[22] Using an external recorder CBT was recorded every 20 seconds and averaged per minute. This method is known to be valid and safe, and was described in detail previously.[23]

The skin temperature (T_{SK}) was measured during each test using individual skin temperature sensors (iButtons, Maxim Integrated, San Jose, CA, USA). T_{SK} was measured every 30 seconds with a resolution of 0.0625°C. Using the ISO 9886 norm, 8 different iButtons were attached to the skin: on the forehead, right scapula, left thorax, right upper arm, left lower arm, left hand, right upper leg and left calf. Mean T_{SK} was calculated from a standard area weighing factors,[24] and averaged per minute.

To compare the exercise intensity during the submaximal exercise tests the heart rate was measured in beats per minute using a chest band system (Polar RS800, Oy, Kempele, Finland). The heart rate was measured every 15 seconds and averaged per minute.

To assess sweat losses, body weight was measured immediately before and after each exercise bout, after subjects towelled off sweat and with subjects wearing shorts and underwear only (Seca 888 scale, Hamburg, Germany). Relative body weight changes were calculated to assess the hydration status of subjects.

To compare the stimulus for PGE_2 synthesis in every test condition a venous blood sample was taken to measure the concentration of IL-6 at baseline (before taking the medication) and directly after completing the exercise test. A 10mL K3EDTA vacutainer tube was used to collect the blood sample and was immediately after collection centrifuged at 4°C and 3600 rpm for 12 minutes. All samples were subsequently stored at -80°C until further analysis. All blood samples were analysed on the same day after completing all experimental tests. A commercial IL-6 ELISA kit (Pelipair human IL-6 ELISA kit, Sanquin, Amsterdam, the Netherlands) was used for determining IL-6 concentrations. The detection limit of the IL-6 ELISA kits was 3 pg/ml.

Rate of perceived exertion (RPE) was measured every 6 minutes using the BORG-scale.[25] This scale ranges from 6-20 with 6 being very mild and 20 the most strenuous exercise. Furthermore we asked subjects to rate Thermal Sensation and Thermal Comfort every 6 minutes. Thermal Sensation measures the temperature perception of the subject with

a scale ranging from -3 being really cold to +3 being really hot. Thermal Comfort is a measure of how comfortable the temperature feels to the subject ranging from -4 being very comfortable to +4 being very uncomfortable.[26]

All data are presented as mean ± standard deviation unless indicated otherwise. Statistical analyses were conducted using SPSS version 20 (IBM SPSS version 20.0, Armonk, NY, USA). Changes over time (baseline *vs.* peak) and between conditions (IBU/APAP/CTRL) were analysed using a within-subject repeated-measures ANOVA. Delta (Δ) CBT and T_{SK} were determined as the difference between maximal value and baseline value. Group differences at the same time point (e.g. ambient temperature, baseline or peak CBT) were analysed using a within-subject one-way ANOVA. Due to the fact that some baseline values of the IL-6 concentrations were below the detection limit, a logistic regression analysis was performed to test whether more values were above the detection limit post-exercise compared to baseline. In case of a significant outcome a post-hoc Bonferroni test was applied. The level of significance was set at $p \leq 0.05$.

Results

All subjects successfully completed the maximal and submaximal exercise tests (Table 4-1). All subjects completed the entire study protocol within 4 weeks. Room temperature (IBU/APAP 21.1±0.9°C, APAP 21.3±0.6°C, CTRL 21.1±1.0°C, p=0.80) and humidity (IBU/APAP 42.3±9.1%, APAP 45.0±11.0%, CTRL 43.5±7.5%, p=0.60) were similar across the three test conditions. $VO_{2\ MAX}$ was 61.7 ± 9.9 mL/min/kg. Maximal heart rate was 186 ± 11 bpm. No adverse events occurred and all subjects met the criteria for achieving maximal exercise.

CBT was similar at baseline across the three conditions (IBU/APAP 37.1±0.2°C, APAP 37.3±0.2°C, CTRL 37.1±0.2°C; p=0.16). CBT increased significantly over time (p<0.001), and a significant time*condition interaction was found (p=0.048). Maximum CBT was significantly lower in the IBU/APAP condition compared to the CTRL condition (IBU/APAP 38.8±0.4°C versus CTRL 39.2±0.5°C; p=0.02) but not between APAP and CTRL (APAP 38.9±0.4°C). A lower ΔCBT was observed in the IBU/APAP condition *versus* the CTRL condition (IBU/APAP 1.7±0.3°C versus CTRL 2.0±0.5°C; p=0.042; Figure 4-1A). ΔCBT did not differ between APAP and CTRL (APAP 1.7±0.5°C).

T_{SK} was similar at baseline in all three conditions (IBU/APAP 31.8±0.4°C, APAP 32.0±0.4°C, CTRL 31.8±0.5°C; p=0.23). T_{SK} increased significantly over time in all conditions (p<0.001), though no differences across conditions (p=0.42) or time*condition (p=0.52) were found.

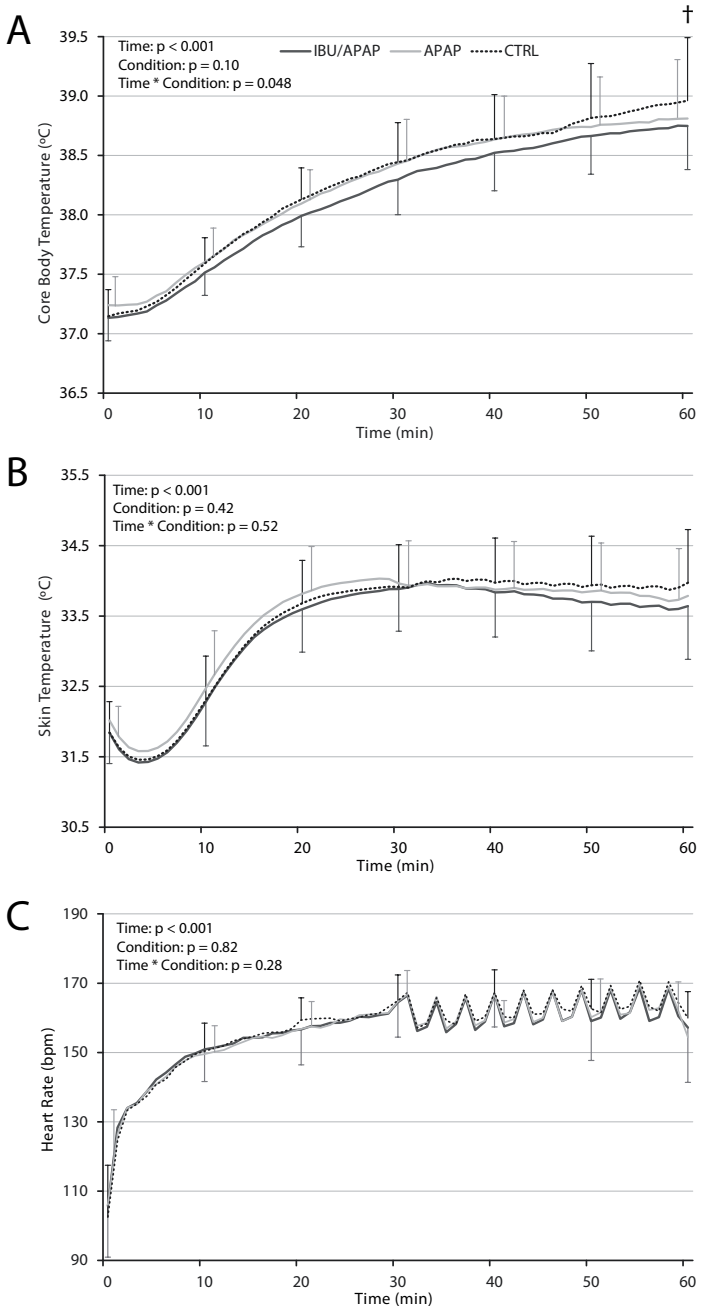

Figure 4-1A: *Core Body Temperature (CBT) during exercise during the exercise bouts.* **B:** *Skin Temperature (TSK) during the exercise bouts.* **C:** *Heart Rate (HR) during the exercise bouts. For readability purposes, the error bars are not visualized on the same time points.*

Also, there were no differences in maximum T_{SK} (IBU/APAP 34.2±0.6°C, APAP 34.3±0.6°C, CTRL 34.2±0.6°C; p=0.95) or ΔTsk (IBU/APAP 2.3±0.6°C, APAP 2.3±0.7°C, CTRL 2.4±0.6°C; p=0.53) across conditions (Figure 4-1B).

Before the start of the sub-maximal exercise tests, heart rate was similar across conditions (IBU/APAP 106±15 bpm, APAP 107±12 bpm, CTRL 102±15 bpm; p=0.51). Heart rate increased significantly over time in all conditions (p<0.001), though no significant condition*time interaction occurred (p=0.28). No differences in maximal heart rate (IBU/APAP 168±10 bpm, APAP 170±10 bpm, CTRL 172±6 bpm; p=0.35) or delta heart rate (IBU/APAP 64±16 bpm, APAP 64±19 bpm, CTRL 69±14 bpm; p=0.28) were observed across conditions (Figure 4-1C).

The prevalence of IL-6 concentration in serum exceeding the level of detection (>3mmol/L) was low across all conditions at baseline (IBU/APAP n=1 (7%; range 7), APAP n=3 (20%; range 3-24), CTRL n=1 (7%; range 4), whilst a substantially higher amount of samples post-exercise showed levels exceeding the level of detection (IBU/APAP n=10 (67%; range 3-8), APAP n=7 (47%; range 4-11), CTRL n=12 (80%; range 3-10)). No significance levels could be determined to compare pre- *versus* post-exercise values due to the low number of samples being below the level of detection pre-exercise.

Maximal RPE and average RPE were not different among conditions (Table 4-2). No significant differences were observed among conditions in maximal and average thermal comfort. There were no differences among conditions in maximal and average thermal sensation. Body weight change was similar across all conditions (Table 4-2).

Table 4-2: *Rate of perceived exertion, thermal comfort, thermal sensation scores and body weight change during the submaximal exercise tests.*

	IBU/APAP	APAP	CTRL	P-Value
RPE max (au)	13.9 ± 1.8	13.2 ± 2.2	13.2 ± 1.6	0.36
RPE average (au)	11.9 ± 1.0	11.6 ± 1.6	11.8 ± 1.2	0.57
Thermal Comfort max (au)	1.7 ± 1.2	1.7 ± 1.1	1.7 ± 1.1	0.97
Thermal Comfort average (au)	0.8 ± 1.1	0.7 ± 1.0	0.5 ± 0.9	0.16
Thermal Sensation max (au)	1.9 ± 0.8	1.9 ± 0.8	2.0 ± 0.6	0.86
Thermal Sensation average (au)	1.3 ± 0.5	1.3 ± 0.6	1.4 ± 0.6	0.88
Body weight change (%)	-1.6 ± 0.3	-1.5 ± 0.2	-1.5 ± 0.3	0.66

RPE: Rate of Perceived Exertion. IBU/APAP: peripheral and central COX inhibition by ibuprofen and acetaminophen. APAP: central COX inhibition by acetaminophen. CTRL: control condition. AU: arbitrary units

Discussion

The aim of this study was to investigate the effect of combined inhibition of central and peripheral PGE_2 synthesis on the rise in T_C during exercise. We found a significantly lower maximal T_C with combined inhibition (IBU/APAP) compared to CTRL, whilst central inhibition only (APAP) was not different from CTRL. No significant differences in maximal T_{SK}, heart rate, body weight change and RPE were observed across conditions. These results suggest that exercise-induced PGE_2 synthesis may impact on the thermoregulatory setpoint and may therefore contribute to the increase in T_C during exercise in humans.

The present study was performed under similar environmental circumstances in all three test conditions. Furthermore the intensity of exercise was identical across conditions, ensuring equal thermogenesis and release of pro-inflammatory cytokines was identical during each test. The measurements were performed in moderate temperatures since we wanted to replicate conditions similar to those typically encountered in recreational running. The randomization of all three conditions rules out a potential training effect. Although significance levels could not be determined, IL-6 levels showed a similarly low prevalence of values exceeding the level of detection pre-exercise, as well as a substantially higher prevalence exceeding the level of detection post-exercise. Whilst no significance levels could be determined for this difference, it does suggest that elevated IL-6 levels during exercise posed as stimulus for PGE_2 production across all conditions in line with previous literature[13 14]. Also, the lack of differences in body weight loss across conditions out rules any influence by differences sweat losses. Lastly, the study protocol was not blinded since humans are unable to (sub)consciously alter their body temperature and since the exercise protocols were kept identical (i.e. identical metabolic heat production during each exercise test). Blinding was therefore not expected to alter our results.

A significantly lower maximum T_C and ΔT_C were found in the IBU/APAP compared to the CTRL condition, but not between the APAP and CTRL condition. These observations support our hypothesis and suggest a superior effect of simultaneous central and peripheral COX inhibition, although a similar effect of central inhibition and combined inhibition cannot be completely ruled out given the similar delta T_C between IBU/APAP and APAP. Whilst we did not identify a significant effect of APAP alone, previous authors did[15 16]. Possible explanations for this may be differences in the exercise protocol[15 16], ambient conditions[15 16] or training status[16]. The primary site of action for acetaminophen is the inhibition of PGE_2 synthesis in the brain through the inhibition of the COX-1 and COX-2 enzyme[9 27]. Ibuprofen is a non-selective cyclooxygenase inhibitor in the NSAID group and the mechanism of action is lowering PGE_2 by directly inhibiting COX-1 and

COX-2 enzyme activity peripherally[9]. Because acetaminophen and ibuprofen act as COX-enzyme inhibitors on a central respectively a peripheral level they maximally inhibit PGE_2 synthesis produced by exercise-induced IL-6 release. Whilst COX has been shown to not affect forearm sweating[28], a clinical study suggested that the combined therapy of acetaminophen and ibuprofen is more effective in lowering T_C during fever[29]. The present study expands this observation to an exercise setting. Especially since previous human studies that used drug-induced inhibition of PGE_2 synthesis during exercise[15-18] used either central *or* peripheral COX inhibitors and reported conflicting results. Our study adds to this that the combination of central and peripheral COX inhibition is more effective than central COX inhibition only. This may also suggest that circulating prostaglandins from the periphery may also influence T_C, in addition to centrally synthesized prostaglandins. Moreover, exercise-induced PGE_2 synthesis impacts on the thermoregulatory setpoint and thus contributes to the increase in T_C. Whilst the difference of maximum T_C between all three conditions is small, we believe the difference is still relevant given the competitive nature of exercise in which even the smallest difference is important.

When T_C rises, mechanisms are activated to dissipate heat to the surroundings. One of these mechanisms is an elevated skin blood flow. Vasodilatation causes skin blood flow to increase so that warmer blood from the core is transported to the periphery and T_{SK} will rise[3]. One previous study showed a lower T_{SK} when acetaminophen was administered in comparison to a placebo[16]. Simultaneously they found a lower T_C in the acetaminophen group. Three other studies that investigated acetaminophen or a non-steroidal anti-inflammatory drug did not find a difference in T_{SK}[15 17 18]. We did not find any differences in T_{SK} across conditions either. Ambient temperatures were similar across conditions and have thus affected T_{SK} in a similar way. Whilst changes in T_C do affect T_{SK}, it has also been suggested that when T_C exceeds the value of 38°C the increase in skin blood flow during exercise is attenuated[30]. As all study participants demonstrated a maximum T_C >38°C, this might explain the observation that T_{SK} did not differ across conditions in the present study.

Our main goal was to investigate whether an altered setpoint plays a role in the rise in T_C during exercise for a better understanding of thermoregulation during exercise in humans. The combined central and peripheral COX blockade using acetaminophen and ibuprofen resulted in a slightly but significantly lower maximal T_C (0.3°C) compared to no COX blockade at all. This suggests that the exercise-induced rise in T_C may be partially explained by an elevated temperature setpoint. The limited T_C difference makes it uncertain whether the elevated setpoint impacts athletic performance, though further research into this is needed. Whilst we would not recommend chronic use of acetaminophen and ibuprofen during exercise to lower T_C given the potential adverse effects such as kidney damage

and gastro-intestinal problems, occasional use might be beneficial for athletes to slightly reduce their T_C at times of high thermal stress and improve exercise performance[16 31].

Conclusion

In conclusion, combined administration of acetaminophen and ibuprofen results in an attenuated maximal T_C during exercise compared to a control condition. This suggests that besides the production of metabolic heat, the release of pro-inflammatory cytokines contributes to an elevated hypothalamic thermoregulatory setpoint via increased levels of PGE_2. Our results suggest that an upregulated hypothalamic temperature setpoint might partially be responsible for the exercise-induced T_C rise.

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Chapter 5

Thermoregulatory Responses in Wheelchair Tennis Players: A Pilot Study

Matthijs T.W. Veltmeijer
Babette Pluim
Dick H.J. Thijssen
Maria T.E. Hopman
Thijs M.H. Eijsvogels

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Abstract

Study Design: Observational study.

Objectives: Spinal cord injured (SCI) individuals are thermoregulatory compromised due to an inability to vasodilate and sweat below the injury, increasing the risk, proportional to the injury level, for marked core body temperature (CBT) rises. We compared thermoregulatory responses between wheelchair tennis players with and without a SCI.

Setting: British Open 2013, Nottingham, UK.

Methods: Eight (3 SCI; 5 non-SCI) wheelchair tennis players played a 45-minute match whilst we continuously measured CBT, 8-point skin temperature (Mean-Tsk) and exercise intensity (METs). Thermal sensation and perceived exertion were measured before and after each set. Video-assisted logging of each serve, stroke and point duration was used to determine match intensity. No statistics were performed for CBT due to small sample-sizes.

Results: Wet-bulb Globe Temperature varied between 18-20°C. CBT increased stronger in the SCI players (+0.6±0.1°C; n=2) compared to the non-SCI players (+0.3±0.1°C; n=4), whilst Mean-Tsk was similar between groups (p=0.29). No Tsk differences were observed above (>T6) or below (≤T6) the lesion level. Thermal sensation, perceived exertion, exercise and match intensity were similar between groups (all p>0.05).

Conclusions: In this small, descriptive study, CBT increased slightly more in the SCI wheelchair tennis players compared to non-SCI players during a 45-minute match in moderate environmental conditions. Further research to investigate whether SCI players are more prone to heat illness is warranted.

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Introduction

The major tournaments in tennis, such as the Australian and US Open, are often played under highly demanding conditions, where ambient temperatures of >35°C have been reported. An elevated core body temperature (CBT) is known to reduce exercise performance or may even pose a threat to any athlete's wellbeing by progressing to heat illness, and in extreme cases multi-organ failure or even death.[1-3] Mediated by local and systemic (i.e. neural) pathways, the body's principal thermoregulatory mechanisms in response to an elevated CBT during exercise are cutaneous vasodilatation and active sweat secretion.[4 5]

In spinal cord injured (SCI) individuals the neural transmission of thermoregulatory signals is interrupted, leading to an inability to vasodilate and sweat below the level of the lesion.[6] Higher lesion levels therefore produce a larger body surface area that cannot actively regulate body temperature, resulting in a greater risk for heat-related problems. In laboratory settings, upper body exercise of similar intensity in SCI individuals and able-bodied controls was shown to result in a substantially higher increase in CBT in SCI subjects.[7-9] However, no previous study has assessed the thermal strain in wheelchair tennis players with and without a SCI during a match. This is of special importance since the major tournaments for elite wheelchair tennis players are frequently played under hot and humid ambient conditions, for instance in view of the 2016 Paralympics in Rio de Janeiro.

The aim of this pilot study was to compare the thermoregulatory responses in wheelchair tennis players with and without a SCI during an outdoor tennis match. We hypothesized that SCI players would exhibit a larger CBT rise during the match compared to the non-SCI players.

Materials and methods

Subjects

Eight elite, international-level wheelchair tennis players (3 SCI; 5 non-SCI) were included in the study (Table 1). All measurements were performed on-site during a single day at the British Open 2013 in Nottingham, United Kingdom. Due to the use of the telemetry system for measuring CBT, subjects with a history of (inflammatory) bowel disease or any abdominal surgery were excluded from this specific measurement. This study was approved by the ethics committee of the Radboud University Medical Centre, and all subjects provided a written informed consent before participation. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this research.

Table 5-1: *Individual subjects characteristics. SCI: Spinal cord injured; Non-SCI: Non-spinal cord injured*

Subject	Group	Sex	Age (yrs)	Height (m)	Weight (kg)	Nature of Disability
1	SCI	M	19	1.65	53.1	Brain stem infarction (ASIA B/C)
2	SCI	F	35	1.67	61.0	Traumatic SCI at T6 and L2 (ASIA C/D)
3	SCI	M	35	1.79	91.1	Traumatic SCI at T6 (ASIA A)
4	Non-SCI	M	34	1.23*	45.5*	Amputation of left leg just distal to hip joint and right leg just proximal to knee joint
5	Non-SCI	M	27	1.57	42.9	Diastrophic dysplasia
6	Non-SCI	M	20	1.50	47.5	Cerebral palsy
7	Non-SCI	F	18	1.60	41.8	Complete amputation of right leg
8	Non-SCI	F	32	1.60	53.1	Cerebral palsy; right-sided hemiplegia

* Measured without prosthesis

Experimental Procedures and Materials

All measurements were performed at outside courts. Subjects were instructed to warm-up for a fixed 5-minute period and subsequently play a simulated 45-minutes singles match against a similarly ranked opponent that also participated in the study. Measurements were started before the warming-up and continued throughout the match.

Core Body Temperature

Participants ingested an individually calibrated telemetric temperature pill, which measured (intestinal) CBT and transmitted the temperature via radio waves to an external recorder (CorTemp; HQ, Palmetto, FL, USA) that was attached underneath each participant's

wheelchair. This measuring system has been demonstrated to safely and reliably measure CBT during exercise and rest.[10 11] The wireless signal was recorded every 20 seconds and was post-hoc averaged for each minute.

Skin Temperature

We measured Skin Temperature (Tsk) every 30 seconds throughout the match by attaching wireless data loggers directly onto the skin (iButton; Maxim Integrated, San Jose, CA, USA) using Tegaderm transparent film dressing (Tegaderm™ Film 1624, Neuss, Germany). Use of these data loggers to measure Tsk has been validated and described in previous studies.[12] Tsk was measured at a resolution of 0.0625°C by attaching the data loggers on the forehead, right scapula, upper left thorax, right upper arm, left forearm, left hand, ventral side right upper leg and left calf. Mean-skin temperature (Mean-Tsk) was calculated according to ISO 9886 and averaged for each minute.[13] Tsk was further divided into an upper region above the lesion level (>T6) and a lower region below the lesion level (≤T6; i.e. right upper leg and left calf) to identify any differences in Tsk relating to the SCI. The level of T6 was chosen since all our subjects had their SCI distal to this level.

Thermal Sensation, Perceived Exertion and Exercise Intensity

Thermal sensation (7-point scale: -3 cold; +3 hot) and rating of perceived exertion (15-grade BORG scale) were assessed after each completed set.[14 15] Exercise intensity was determined using an accelerometer (SenseWear Pro3; Bodymedia, Pittsburg, PA, USA), which expressed exercise intensity in metabolic equivalent units (METs) for every minute played. The accelerometer was worn on the right upper arm throughout the match. Previous studies have demonstrated this method to validly and accurately measure energy expenditure including in wheelchair users.[16]

Video-assisted Logging of Match Intensity

In addition to the accelerometry data, alternative descriptive parameters concerning the intensity of the matches were obtained by video-recording all matches. A specifically developed tablet-app was used to manually tag the time point on which a point started (serve) and on which a point ended (ball was out, hit the net, hit the ground ≥3 times or hit the ground behind the player). This data was used to extract the average rally and rest duration. Stroke frequency was determined by manually counting the number of times that a ball was hit during each rally divided by the rally duration. These parameters were defined and determined in accordance with previous literature.[17]

Environmental Conditions

Wet-bulb Globe Temperature (WBGT) was measured using a portable weather station (Davis Instruments, Hayward, CA, USA) every 60 minutes throughout the day. This measurement allows for standardization of environmental conditions by accounting for outside temperature ($T_{DRY\text{-}BULB}$), outside temperature with correction for humidity ($T_{WET\text{-}BULB}$) and (solar) radiation (T_{GLOBE}). WBGT was calculated using the following formula: $WBGT = 0.1 \cdot (T_{DRY\text{-}BULB}) + 0.7 \cdot (T_{WET\text{-}BULB}) + 0.2 \cdot (T_{GLOBE})$.[3]

Statistics

Statistical analyses were performed using IBM SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Data was reported as mean ± standard deviation unless otherwise indicated. Due to the small sample-size no statistical analyses could be performed for the CBT data. Normality distribution was tested using a Shapiro-Wilk test. All normally distributed data was analysed using parametric tests (*t*-test and two-way repeated measurements ANOVA), and in case of non-Gaussian distribution non-parametric tests were applied (Mann-Whitney U test). The level of significance was set at $p \leq 0.05$.

Results

Two subjects (1 SCI, 1 non-SCI) were excluded from CBT measurements due to prior abdominal surgery. All measurements were performed in moderate environmental conditions (WBGT 17.9–20.0°C, $T_{DRY\text{-}BULB}$ 21.2-24.8°C, T_{GLOBE} 21.7-26.3°C, relative humidity 51.8-61.4%) with a complete cloud overcast.

The individual CBT for the SCI subjects and mean CBT for the non-SCI subjects are reported in Figure 1, and showed a slightly larger CBT increase in the SCI subjects (+0.6±0.1°C; n=2) compared to the non-SCI subjects (+0.3±0.1°C; n=4).

Before the match, we found no significant differences between groups in mean-Tsk ($p=0.58$). A significant change in mean-Tsk was reported over time ($p<0.001$; Figure 2A), whilst no differences were observed between SCI and non-SCI players ($p=0.29$). Tsk in the upper region changed significantly over time ($p<0.001$; Figure 2B), whilst no differences between both groups were reported ($p=0.66$). No differences were reported in the lower region Tsk over time ($p=0.42$) or between groups over time ($p=0.99$).

Thermal sensation increased similarly from 'cold' to 'warm' in both groups (Table 2). Rating of perceived exertion was similar at baseline (SCI 9.3±2.9, non-SCI 8.6±1.8; $p=0.54$) and

increased to similar levels at the end of the second set (SCI 13.7±1.2, non-SCI 13.2±2.5; p=0.61). Average exercise intensity levels (in METs) were similar throughout the match in both groups (4.9±0.7 *versus* 4.9±0.7; p=1.00). Match intensity parameters are reported in Table 2, and showed no differences between both groups.

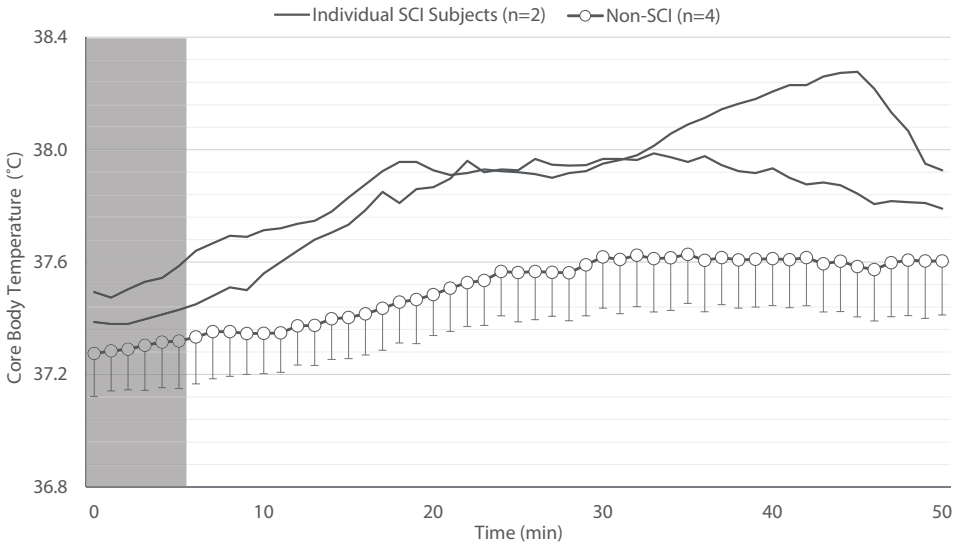

Figure 5-1: *Core body temperature throughout the match in the two individual SCI subjects (solid lines) and the non-SCI group (open circles). The grey area represents the warming-up period. Data are presented as individual values (SCI subjects) and mean ± SEM (non-SCI subjects).*

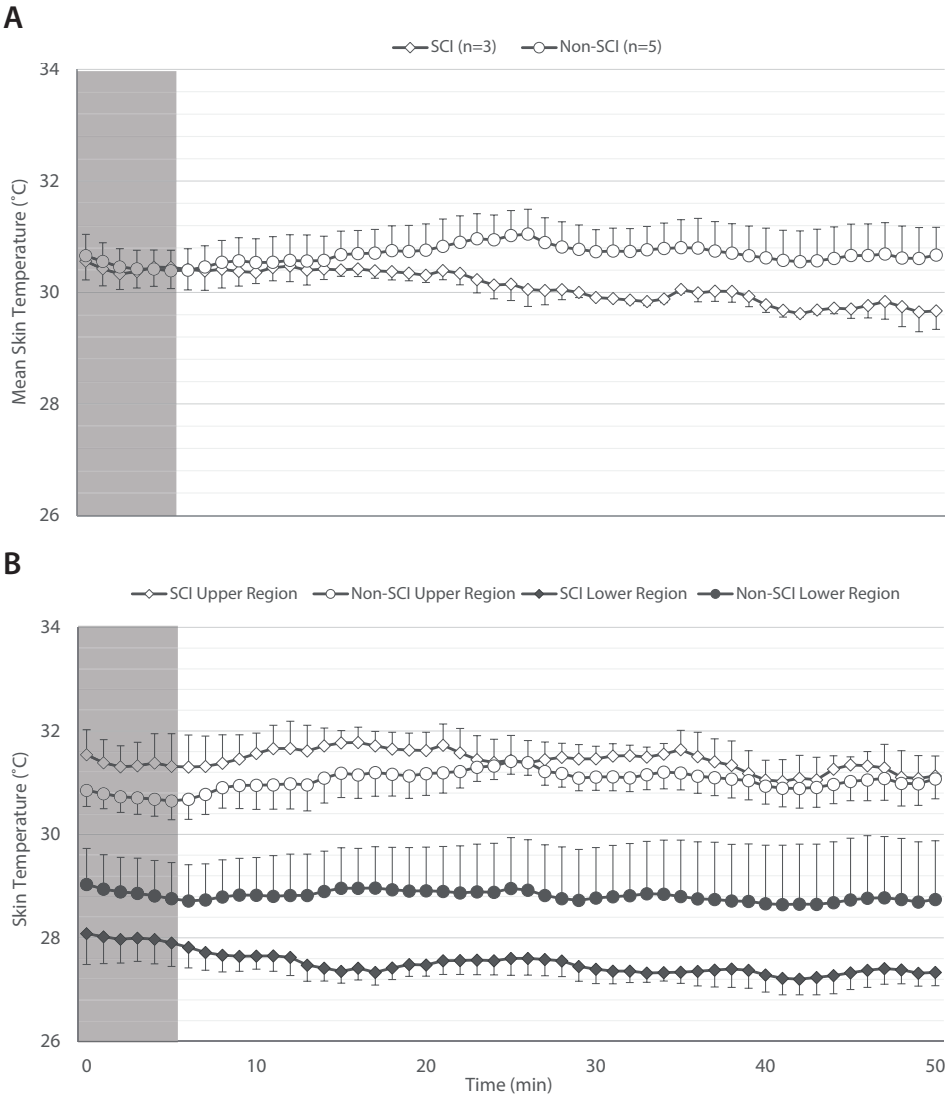

Figure 5-2: *Mean skin temperature* **(A)** *throughout the match in the SCI group (open squares) and the Non-SCI group (open circles). The grey area represents the warming-up period. Mean-Tsk changed significantly throughout the match in both groups over time (2W RM ANOVA: p<0.001), whilst no differences were reported observed between SCI and Non-SCI players (time x group: p=0.29). Skin temperatures in the upper skin region above (>T6) and in the lower skin region below (≤T6) the level of the spinal cord injury are shown* **(B)** *for the SCI group (upper region open squares; lower region closed squares) and the non-SCI group (upper region open circles; lower region closed circles). A significant change was observed in both groups in the upper region, however no differences were observed between the SCI and Non-SCI players. In the lower region, no differences were observed throughout the match (p=0.42) or between both groups of players (p=0.99). Data are presented as mean ± SEM.*

Table 5-2: *Match and exercise intensity parameters throughout the match, thermal sensation and rating of perceived exertion at baseline, after the first set and immediately after the match. P-values refer to a Mann-Whitney U test. SCI: Spinal cord injured; Non-SCI: Non-spinal cord injured.*

	SCI (n=3)	Non-SCI (n=5)	P-value
Match Intensity			
Duration of Rallies (sec)	8.4 ± 1.1	9.8 ± 2.1	0.36
Rest Time Between Points (sec)	21.7 ± 2.8	20.1 ± 3.0	0.36
Work : Rest Ratio	1 : 4.0 ± 1.5	1 : 3.3 ± 1.4	0.36
Effective Playing Time (% of total time)	21.3 ± 7.6	25.2 ± 7.5	0.36
Strokes per Rally (n)	3.7 ± 0.8	4.0 ± 0.7	0.76
Stroke Frequency (str/min)	26.1 ± 2.2	24.6 ± 2.6	0.36
Exercise Intensity (METs)	4.9 ± 0.7	4.9 ± 0.7	1.00
Thermal Sensation			
Baseline	-1.0 ± 0.0	-0.8 ± 1.3	0.33
After 1st Set	1.7 ± 1.2	1.2 ± 0.4	0.56
After 2nd Set	1.0 ± 1.7	1.6 ± 0.5	0.86
Rating of Perceived Exertion (BORG)			
Baseline	9.3 ± 2.9	8.6 ± 1.8	0.54
After 1st Set	12.3 ± 2.3	12.8 ± 1.8	0.76
After 2nd Set	13.7 ± 1.2	13.2 ± 2.5	0.61

Data are presented as mean ± SD

Discussion

The present study was the first to describe changes in CBT during exercise in wheelchair tennis players with and without a SCI. Our results are indicative that wheelchair tennis causes a rise in CBT under moderate environmental conditions, and that this rise may be stronger in SCI players compared to non-SCI players in spite of similar Tsk responses. These findings may have clinical and practical importance for SCI players, particularly whilst playing tennis matches that are organized in hot and humid conditions as can be expected during most Grand Slam tournaments or the 2016 Paralympics in Rio de Janeiro.

CBT showed a larger rise in the SCI subjects compared to the non-SCI subjects. Whilst it is difficult to draw any firm conclusions based on these descriptive data, these results are in support of our hypothesis and are in agreement with findings in previous laboratory-

based studies.[7-9 18] In able-bodied subjects, exercise intensity is strongly correlated to CBT increases.[3] The similar exercise intensity, match intensity and RPE between both groups suggest that these factors cannot explain the larger CBT rise in the SCI players.

Furthermore, is has been previously described that whilst paraplegic athletes show CBT responses similar to able-bodied athletes *during* exercise in moderate to hot conditions, the SCI athletes have a greater heat-storage in the lower body which results in a reduced ability to lower their CBT during recovery periods.[9] This observation is of particular importance in wheelchair tennis, since tennis is typically characterized by bouts of exercise (rallies) alternated by periods of relative rest (e.g. ball retrieval and preparation phase for next serve). This means that whilst the non-SCI players may be able to cool down during the rest periods in between rallies, the SCI players will not be able to do so as effectively. Therefore, an attenuated heat loss capacity in the SCI players, resulting from a severely reduced body surface area for active thermoregulation and increased lower-body heat storage, may be responsible for the observed CBT rise in our SCI subjects. As our measurements were performed under moderate conditions, differences between SCI and non-SCI wheelchair players may be exaggerated under hot conditions, placing SCI wheelchair players at potential risk for an impaired performance or developing heat illness during tournaments in the heat. Lastly, since the test matches in the present study were relatively short in duration compared to a typical wheelchair tennis match (~75 minutes), our results may have underestimated the CBT that would be attained towards the end of the matches.

Skin temperature responses were similar between SCI and non-SCI players. As Tsk is largely related to the ambient temperature, no group differences were expected.[19] Furthermore, any differences between both groups in upper and lower body Tsk responses would have likely resulted from the inability to vasodilate or sweat below the lesion level in the SCI players. Previous studies demonstrated that lower body Tsk increases during prolonged (90-minute) upper body exercise as a result of increased heat storage in that region.[9 20] Whilst both heat load as well as exercise duration during the present study were limited, this may explain lack of pronounced Tsk responses in the SCI and non-SCI group.

Our study had some limitations. An obvious limitation of our study is the low number of subjects, which makes it difficult to draw strong conclusions about the actual risk of heat stress that the SCI players are subjected to during a match. Further research with larger sample-sized should be performed to gain a better insight in the thermal stress that wheelchair players are exposed to and the associated risks for developing heat illness including collapse and heat stroke.

In conclusion, we provide some evidence for a larger increase in CBT in SCI wheelchair tennis players compared to non-SCI players whilst playing a 45-minute tennis match in moderate ambient conditions. This finding suggests that SCI players may have an elevated risk to develop heat illness, particularly under hot ambient conditions. We believe that our findings warrant future studies to specifically examine the thermoregulatory responses in SCI wheelchair tennis players, but also highlight the development of countermeasures to prevent hyperthermia.

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Chapter 9

The Impact of Exercise-Induced Core Body Temperature Elevations on Coagulation Responses

Matthijs T.W. Veltmeijer
Thijs M.H. Eijsvogels
Wideke Barteling
Kitty Verbeek – Knobbe
Waander L. van Heerde
Maria T.E. Hopman

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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.

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 (Figure 6-1). 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 enzyme-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

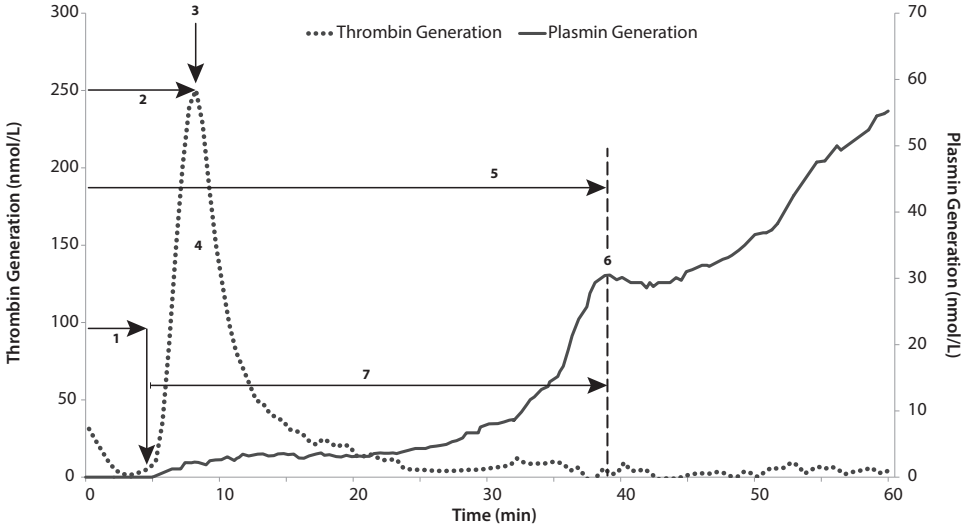

Figure 6-1: *Schematic overview of the Nijmegen Haemostasis Assay. The dotted line represents real-time measurement of thrombin generation, whilst the continuous line represents the simultaneous real-time measurement of plasmin generation in the same blood sample. For the purposes of the present study, 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]

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 6-1). 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 (Table 6-2). Lastly, neither finish-CBT nor ΔCBT correlated significantly with post-exercise haemostasis parameters, body weight loss or running speed (Table 6-3).

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 6-2A-D respectively). Plasmin peak time synchronized to actual CBT and plasmin peak height

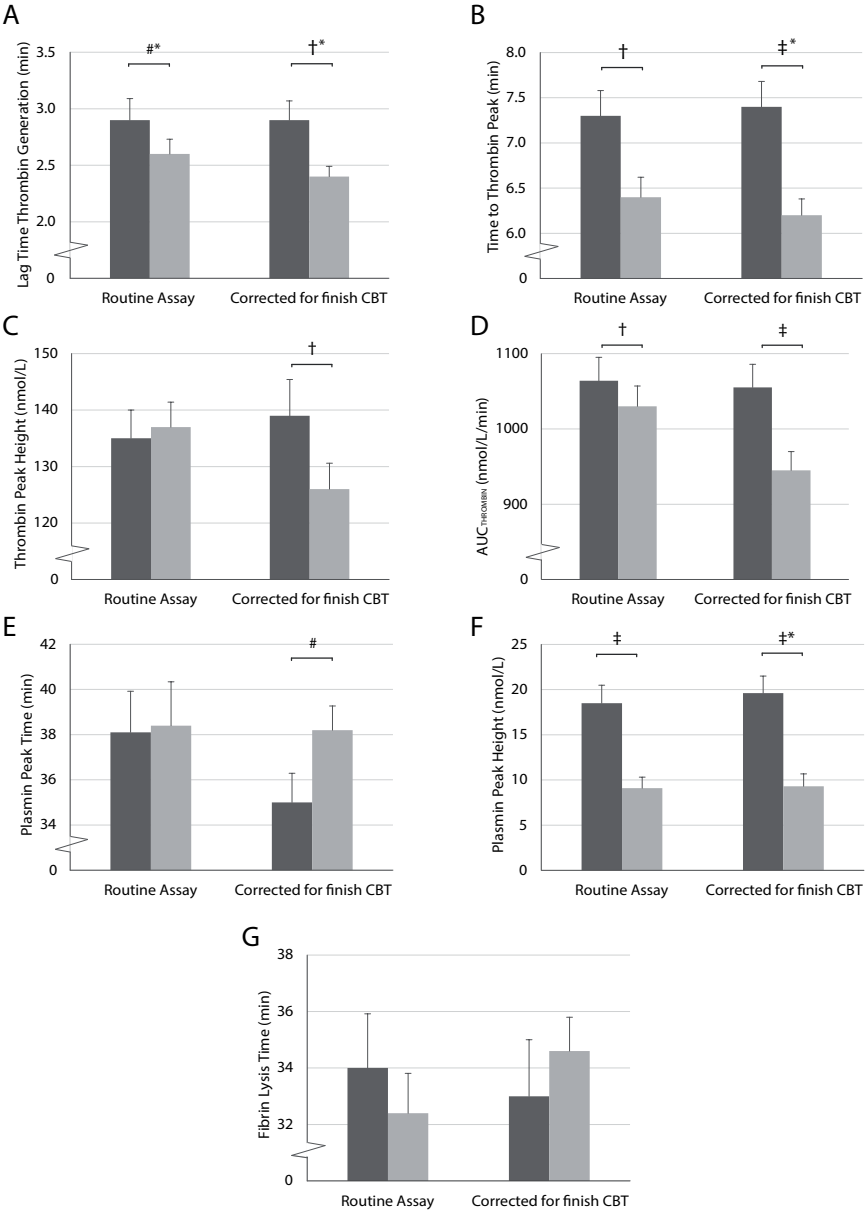

Figure 6-2: *Results of the routinely performed assays (left hand side of graph) versus the assay results corrected for the actual core body temperature (right hand side of graph) at baseline and post-finish (black and grey bars respectively). All corrected results for the baseline samples were corrected 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*

decreased significantly between baseline and finish (Figure 6-2E + F), whilst fibrin lysis time did not change significantly (Figure 6-2G). 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 6-4). 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 (Table 6-5).

Table 6-1: *The impact of finish core body temperature on haemostatic responses using routine assay analysis.*

Haemostasis Parameter	CBT <39.0°C (n=21)		39.0≤ CBT<40.0°C (n=24)		CBT ≥40.0°C (n=17)		2-way RM-ANOVA		
	Baseline	Post-Exercise	Baseline	Post-Exercise	Baseline	Post-Exercise	Exercise	CBT Groups	Exercise * CBT Groups
Lag Time Thrombin Generation (min)*	4.5 ± 0.9	3.7 ± 0.7	4.5 ± 1.3	4.0 ± 0.7	4.5 ± 1.1	3.7 ± 0.5	<0.001	0.92	0.32
Time to Thrombin Peak (min)*	8.8 ± 1.3	7.7 ± 1.0	9.0 ± 1.7	8.3 ± 1.0	9.0 ± 1.3	7.9 ± 0.7	<0.001	0.46	0.30
Thrombin Peak Height (nmol/L)	199 ± 25	201 ± 24	210 ± 30	209 ± 28	212 ± 30	214 ± 21	0.49	0.27	0.73
$AUC_{thrombin}$ (nmol/L/min)	1225 ± 141	1210 ± 151	1347 ± 169	1340 ± 188	1300 ± 159	1325 ± 143	0.93	0.02	0.40
Plasmin Peak Time (min)*	38.7 ± 10.3	35.2 ± 9.4	33.9 ± 8.5	34.6 ± 6.9	37.8 ± 7.7	36.7 ± 8.5	0.17	0.39	0.17
Fibrin Lysis Time (min)	32.2 ± 8.7	28.8 ± 9.3	28.2 ± 8.9	30.0 ± 7.2	32.2 ± 8.0	32.9 ± 8.4	0.084	0.35	0.06
							Non-Parametric Analysis		
							Baseline	Post-Exercise	ΔBaseline-Finish
Plasmin Peak Height (nmol/L)	19.9 ± 10.7	8.9 ± 7.3	19.3 ± 13.6	12.6 ± 11.8	18.0 ± 12.8	7.7 ± 4.7	0.62	0.46	0.40

Data are reported as mean ± standard deviation. CBT = Core Body Temperature *Log transformation was applied.

Table 6-2: *The impact of the magnitude of the exercise-induced increase in core body temperature on haemostatic responses.*

Haemostasis Parameter	ΔCBT <1.5°C (n = 24)		1.5≤ ΔCBT<2.5°C (n = 20)		ΔCBT ≥2.5°C (n = 18)		2-way RM-ANOVA		
	Baseline	Post-Exercise	Baseline	Post-Exercise	Baseline	Post-Exercise	Exercise	CBT Groups	Exercise * CBT Groups
Lag Time Thrombin Generation (min)*	4.5 ± 0.9	3.8 ± 0.7	4.6 ± 1.3	4.0 ± 0.7	4.4 ± 1.2	3.7 ± 0.5	<0.001	0.88	0.65
Time to Thrombin Peak (min)*	8.9 ± 1.4	7.8 ± 1.1	9.1 ± 1.6	8.2 ± 0.9	8.8 ± 1.4	7.9 ± 0.7	<0.001	0.65	0.75
Thrombin Peak Height (nmol/L)	198 ± 24	201 ± 24	212 ± 32	211 ± 30	214 ± 29	213 ± 18	0.78	0.17	0.66
$AUC_{thrombin}$ (nmol/L/min)	1236 ± 150	1232 ± 157	1330 ± 177	1331 ± 178	1328 ± 151	1328 ± 173	0.95	0.08	0.98
Plasmin Peak Time (min)*	37.2 ± 10.3	34.7 ± 8.9	36.3 ± 9.8	36.7 ± 7.4	36.1 ± 6.6	34.6 ± 8.1	0.25	0.92	0.31
Fibrin Lysis Time (min)	30.9 ± 8.8	28.8 ± 8.9	30.2 ± 10.4	32.3 ± 7.4	30.8 ± 6.3	30.4 ± 8.6	0.90	0.84	0.21
							Non-Parametric Analysis		
							Baseline	Post-Exercise	ΔBaseline-Finish
Plasmin Peak Height (nmol/L)#	17.0 ± 8.4	7.8 ± 6.7	20.1 ± 11.8	12.6 ± 11.3	21.0 ± 16.6	10.1 ± 8.4	0.90	0.24	0.72

Data are reported as mean ± standard deviation. CBT = Core Body Temperature * Log transformation was applied. #Due to a non-Gaussian distribution, a Kruskal-Wallis test was performed.

Table 6-3 *The correlation finish CBT and the exercise-induced increase in CBT (ΔCBT) with post-exercise haemostasis parameters.*

Haemostasis Parameter	Finish CBT		ΔCBT	
	R	P-value	R	P-value
Lag Time Thrombin Generation (min)*	0.62	0.38	0.11	0.06
Time to Thrombin Peak (min)*	0.21	0.15	0.19	0.16
Thrombin Peak Height (nmol/L)	0.19	0.17	0.18	0.17
$AUC_{thrombin}$ (nmol/L/min)	0.08	0.19	0.17	0.23
Plasmin Peak Time (min)*	0.85	0.62	0.06	0.02
Fibrin Lysis Time (min)	0.28	0.29	0.14	0.14
Plasmin Peak Height (nmol/L)#	0.96	0.94	0.01	0.01

*Log transformation was applied. #Spearman correlation was performed due to a non-Gaussian distribution.

Table 6-4: *The impact of finish core body temperature on haemostatic responses when assay temperature is corrected for actual baseline and finish CBT.*

Haemostasis Parameter	CBT <39.0°C (n=7)		39.0≤ CBT<40.0°C (n=8)		CBT ≥40.0°C (n=10)		2-way RM-ANOVA		
	Baseline	Post-Exercise	Baseline	Post-Exercise	Baseline	Post-Exercise	Exercise	CBT Groups	Exercise * CBT Groups
Thrombin Peak Height (nmol/L)	151 ± 31	138 ± 19	142 ± 37	122 ± 25	127 ± 26	122 ± 23	0.008	0.29	0.34
$AUC_{thrombin}$ (nmol/L/min)	1111 ± 169	1003 ± 110	1082 ± 134	940 ± 140	993 ± 151	908 ± 116	>0.001	0.24	0.57
Plasmin Peak Time (min)	38.2 ± 7.9	39.5 ± 3.9	31.9 ± 7.1	33.5 ± 3.4	35.6 ± 3.6	40.9 ± 5.3	0.040	0.020	0.33
Fibrin Lysis Time (min)	35.5 ± 8.4	34.8 ± 5.2	29.1 ± 7.9	29.4 ± 4.1	31.7 ± 4.4	38.5 ± 5.0	0.15	0.033	0.06
							Non-Parametric Analysis		
							Baseline	Post-Exercise	ΔBaseline-Finish
Lag Time Thrombin Generation (min)#	2.7 ± 0.8	2.4 ± 0.4	2.4 ± 0.4	2.4 ± 0.4	3.3 ± 0.9	2.4 ± 0.5	0.06	0.97	0.033
Time to Thrombin Peak (min)#	7.0 ± 1.1	6.3 ± 1.0	6.7 ± 1.3	6.1 ± 1.0	8.3 ± 1.4	6.3 ± 0.9	0.031	0.86	0.037
Plasmin Peak Height (nmol/L)#	17.4 ± 6.3	6.8 ± 2.9	25.4 ± 11.3	12.7 ± 8.4	16.4 ± 8.2	8.3 ± 7.0	0.17	0.29	0.80

Data are reported as mean ± standard deviation. CBT = Core Body Temperature; #Due to a non-Gaussian distribution, a Kruskal-Wallis test was performed.

Table 6-5: *The impact of the assay temperature on haemostatic responses in the subgroup of n=25.*

Haemostasis Parameter	Assay temperature 37°C		Assay temperature 38°C		Assay temperature 39°C		Assay temperature 40°C		2-way RM-ANOVA		
	Baseline	Post-Exercise	Baseline	Post-Exercise	Baseline	Post-Exercise	Baseline	Post-Exercise	Exercise	Assay temperature	Exercise * Assay temperature
Thrombin Peak Height (nmol/L)	135 ± 25	137 ± 22	140 ± 31	144 ± 26	128 ± 26	130 ± 22	119 ± 29	125 ± 22	0.10	<0.001	0.36
$AUC_{thrombin}$ (nmol/L/min)	1064 ± 156	1030 ± 134	1049 ± 144	1040 ± 135	972 ± 145	950 ± 126	961 ± 159	939 ± 125	0.05	<0.001	0.23
Plasmin Peak Time (min)	37.5 ± 9.1	37.8 ± 9.7	34.7 ± 4.4	36.2 ± 7.6	35.9 ± 5.7	37.3 ± 5.2	37.2 ± 6.2	38.3 ± 5.6	0.29	0.25	0.83
Fibrin Lysis Time (min)	34.5 ± 9.6	33.4 ± 10.0	31.7 ± 4.8	32.7 ± 8.4	33.0 ± 6.0	33.7 ± 5.4	33.8 ± 7.3	34.4 ± 6.6	0.77	0.49	0.64
Plasmin Peak Height (nmol/L)	18.5 ± 9.9	9.1 ± 6.1	20.6 ± 10.7	11.1 ± 7.1	17.7 ± 9.3	9.1 ± 6.9	19.1 ± 10.5	9.2 ± 7.0	<0.001	<0.03	0.57
									Non-Parametric Analysis		
									Baseline	Post-Exercise	ΔBaseline-Finish
Lag Time Thrombin Generation (min)#	2.9 ± 0.9	2.6 ± 0.6	2.7 ± 0.7	2.4 ± 0.5	2.8 ± 0.8	2.5 ± 0.5	2.8 ± 0.7	2.4 ± 0.5	0.26	0.11	0.79
Time to Thrombin Peak (min)#	7.3 ± 1.4	6.4 ± 1.1	7.2 ± 1.2	6.2 ± 0.9	7.2 ± 1.6	6.3 ± 1.0	7.2 ± 1.3	6.1 ± 1.0	0.30	0.09	0.51

Data are reported as mean ± standard deviation. CBT = Core Body Temperature; #Due to a non-Gaussian distribution, a Friedman test was performed.

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 individuals' 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 6-2). 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 (Table 6-5) 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 6-1 *versus* Table 6-5). 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.

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Chapter 7

General Discussion

The aim of this thesis was to further explore general thermoregulatory responses during exercise in a heterogeneous population and to identify which factors contribute to the magnitude of the core body temperature rise during exercise. This thesis was partly inspired by a personal experience during my medical training whilst performing a rotation in the Emergency Department in the Jeroen Bosch Hospital, Den Bosch. Whilst heatstroke is a rare phenomenon in the Netherlands, I encountered the following case.

Case Report

A 36-year old male army recruit was rushed into the emergency room following acute collapse during a training exercise. He was participating in a 'speed march', in which recruits had to complete a 7-km march as fast as possible whilst wearing full military outfit and gear comprising at least 10, but in practise 15kg of extra weight. The march was conducted during spring time on one of the first warm days of the year, with clear skies and an ambient temperature of 27°C. After marching 5-km, the recruit suddenly collapsed and the emergency services were called in.

Upon arrival of the paramedics, the patient was unresponsive, tachypneic (>30/min) and slightly hypoxic (O2 saturation 93%). In addition, he was tachycardic (170 BPM), hypotensive (80/40mmHg), and hyperthermic (auricular temperature 40.9°C). The patient was stabilized for transport by immediate administration of oxygen, fast infusion of unheated IV fluids and commencement of external body cooling using drenched towels and water. Upon arrival to the emergency ward, the patient was responsive to verbal stimulation, but was still tachypneic (>30/min), and showed slight improvement of haemodynamic functions (heart rate 150 BPM, blood pressure 97/50mmHg) with a rectal temperature of 40.6°C. Initial laboratory workup showed a slightly reduced kidney function (serum-urea: 9.9 µmol/L, creatinine: 146 µmol/L, glomerular filtration rate (MDRD): 47mL/min/1.73 m^2) but was otherwise unremarkable. Treatment with external cooling and IV fluids was continued until the patient's vital functions normalized and he had a rectal temperature of ≤38.0°C. The patient was observed for several days, and was discharged after complete resolution of all symptoms. He made a full recovery.

Hyperthermia: New Definitions Needed

This case report of a heatstroke patient demonstrates the fine line between clinically relevant and irrelevant hyperthermia. Our recruit got into severe physical problems at a core body temperature of 40.6 – 40.9°C during strenuous exercise – which was presumably even higher at the time of collapse given the delayed measurements. In contrast, 15% of our test subjects in *Chapter 2* also developed a core body temperature ≥40.0°C whilst performing strenuous high intensity exercise, however none of them had any physical symptoms. Whilst a multitude of factors (e.g. environmental conditions, clothing, acclimatization state, exercise intensity, etc.) distinguishes both cases, core body temperature was similar. These observations open up a new discussion on whether the term 'exertional hyperthermia' – i.e. a core body temperature above 40.0°C – is appropriate and clinically relevant.

Current literature defines 'exertional hyperthermia' as 'a core body temperature higher than 40.0°C during physical exercise'.[1] This definition implies that some degree of core body temperature rise during exercise falls within the normal physiological range, but that excessive rises will cause physical problems. The dichotomous and arbitrary threshold of 40.0°C is largely based on reports about heatstroke which underline that high core body temperatures can lead to heat illness.[2-6] However, as shown in *Chapter 2* of this thesis and other literature, many individuals sustain core body temperatures that approach or even exceed the threshold of exertional hyperthermia without developing any symptoms.[7-9] Hence, it can be questioned whether the term 'exertional hyperthermia' is clinically relevant in absence of symptoms. In contrast, subjects may already suffer symptoms such as syncope or heat exhaustion well before they develop core body temperatures higher than 40.0°C whilst other individuals might be better tolerant to the same conditions.[1] Importantly, 'hyperthermia' can also be defined as a 'core body temperature that is higher than the hypothalamic set-point', or in other words an increased core body temperature due to inadequate thermoregulatory measures.[2 10] Therefore, it would seem more prudent to speak of hyperthermia in any case where thermoregulatory measures fail and core body temperature rises above the assumed thermoregulatory setpoint. To account for the high individual variability of heat tolerance, further definition of hyperthermia as 'symptomatic' or 'asymptomatic hyperthermia' would be more meaningful to distinguish clinically relevant from irrelevant hyperthermia.

Symptomatic *versus* Asymptomatic Hyperthermia: A Role for Time of Exposure?

So why did none of the subjects in this thesis develop symptomatic hyperthermia, even though as much as 15% developed core body temperatures higher than 40°C? Part of the explanation might be due to the fact that it is unknown for how long our subjects were exposed to the extreme core body temperatures, but that the time of exposure was likely limited. Interestingly, a Middle East based research group previously performed several studies to investigate the pathogenesis and potential treatment of heatstroke using a baboon model.[11-16] In their model, the baboons needed to be heated in an incubator for several hours before heatstroke occurred. Although the mode of heating was different from our own subjects (passive external heating *versus* active heating through muscle labour), the results of Bouchama *et al.* show that prolonged exposure is necessary to induce health problems. Therefore, our subjects may not have been exposed to high core body temperatures long enough to develop any symptoms. In addition, tolerance to exercise in hot ambient conditions has been shown to be directly linked to both skin blood flow capacity and skin temperature irrespective of core body temperature.[17-19] Even more, skin temperature itself has been suggested to have a greater impact on thermoregulation than core body temperature.[19-22] Effectively, this means that so long as there is ample capacity to cool excess body heat via the skin – i.e. when skin temperature is low or there is still capacity to further perfuse the skin with core-heated blood – any heat-related symptoms or performance decrements are mitigated by the more efficient cooling. Although we did not measure skin temperature in our subjects, the cool ambient conditions in our studies have likely helped keep our subjects' skin temperature low. This would have allowed them to better tolerate their elevated core body temperatures and keep them from developing any heat-related symptoms. Other factors that might have prevented our subjects from developing heat-related symptoms likely include good training status and high fitness level[23-26] and no influence by a poor acclimatization state given the cool ambient conditions in which all measurements were performed.[23 24 26]

Predictability of Thermoregulatory Responses

Whilst previous literature agrees on several risk factors for developing heat illness, it is still difficult to predict which athletes will become severely hyperthermic and which athletes will only show modest increases of core body temperature. In *Chapters 2 and 3*, our predictive model for peak core body temperature was shown to have a predictive capacity of 32%.[27 28] A large part of the model includes previous data of the individuals'

thermoregulatory responses, accounting for as much as 25% of peak core body temperature during a 15-km road race.[28] These results suggest that a large part of the thermoregulatory responses during exercise are individually determined irrespective of external factors such as ambient conditions. This is further supported by evidence showing that a previous episode of heat illness increases the risk of a repeat event.[1 29 30] The individuality of thermoregulatory responses make a general risk assessment for extreme hyperthermia very difficult, and the findings of this thesis warrant a more individualistic approach. Factors to include into a predictive model for hyperthermia should include age, body mass index and fluid intake before commencement of exercise, but the model preferably also includes previous data from the individual including core body temperature rise during warming-up and a previous peak core body temperature. To make more accurate assessments, future studies should further investigate additional individual factors contributing to the thermoregulatory response during exercise. Whether hyperthermia becomes symptomatic or not likely depends on other (previously reported) risk factors including sleep deprivation, recent febrile illness, poor acclimatization state, medication use (diuretics and antidepressants amongst others), dehydration and clothing.[1 3 4 30 31]

Early identification of individuals who are at risk of developing extremely high core body temperatures (e.g. ≥40.0°C) can help identify those athletes who might benefit most from early (pre-) cooling interventions. Especially in hot environmental conditions, (continuous) monitoring of such individuals may help prevent the onset or limit the severity of symptomatic hyperthermia. When considering that a high core body temperature could attenuate exercise performance,[24 32] identifying the athletes who are most likely to develop the highest core body temperatures could help decide which athletes will benefit most from pre- or even precooling (i.e. cooling during exercise) to maintain or even boost their performance levels.[33-35]

Mechanism and Cytokines Involved in Exercise-Induced Hyperthermia

For several years it has been known that exercise induces the release of pro-inflammatory cytokines.[36-40] Whilst the exact mechanism between exercise and cytokine release is still unknown, it has been demonstrated that both prolongation of exercise as well as higher intensity of exercise induce a stronger cytokine release compared to relatively light and short-lasting exercise.[37-39 41-43] Hence, the mechanism of cytokine release is likely due to the sustained microtrauma in the exercising muscle, which causes local inflammation as part

of the tissue repair process.[44] Based on this information, we hypothesized that the release of inflammatory cytokines could upregulate the hypothalamic temperature setpoint in the same manner as during fever and we found evidence supporting this hypothesis. So which cytokines could be responsible for the upregulated hypothalamic setpoint during exercise?

As mentioned in *Chapter 4*, interleukin-6 and interleukin-1 play a central role in any inflammatory response, and are known to be released during exercise.[36-39] Whilst they stimulate the release of prostaglandin E_2, they have also been shown to directly upregulate the temperature setpoint itself, independent of prostaglandins.[45] Since only prostaglandin production was blocked in *Chapter 4*, the direct effect of the cytokines during exercise was not taken into consideration and has likely led to an underestimation of the true cytokine effect. Future studies should therefore further explore the direct impact of these cytokines on the hypothalamic temperature setpoint during exercise by applying interleukin-6 and interleukin-1 blockers. Another potential target cytokines could be tumor necrosis factor α, which is also known to be released during exercise.[39 42 43] Lastly, a potential but important other cause of pro-inflammatory cytokinaemia is endotoxaemia due to increased intestinal cellular permeability. Prolonged exercise leads to a dramatic redistribution of blood flow, with a restricted gastrointestinal blood flow of up to 80%. The resulting (mild) intestinal ischemic state causes cellular damage and induces endothelial leakage, resulting in the introduction of bacterial lipopolysaccharide (LPS) into the blood stream producing a response similar to systemic inflammatory response syndrome (SIRS).[24 46 47] Paradoxically, a potentially aggravating factor is the increased core body temperature caused by the exercise itself, which may lead to further intestinal cellular damage which could even further aggravate the cellular leakage.[24 47] Future studies investigating the interaction between the immune system and thermoregulation during exercise might therefore also consider endotoxaemia as an indirect causal factor for the increased hypothalamic setpoint.Taking these new insights into account, the schematic overview of human thermoregulation that was presented in *Chapter 1* can be expanded as shown in Figure 7-1. Based on our findings the release of pro-inflammatory cytokines during exercise leads to an upregulated hypothalamic temperature setpoint. This alternative mechanism is likely partially responsible for the increased core body temperature during exercise, with metabolic heat production due to muscle labour still being the major determining factor of core body temperature during exercise.

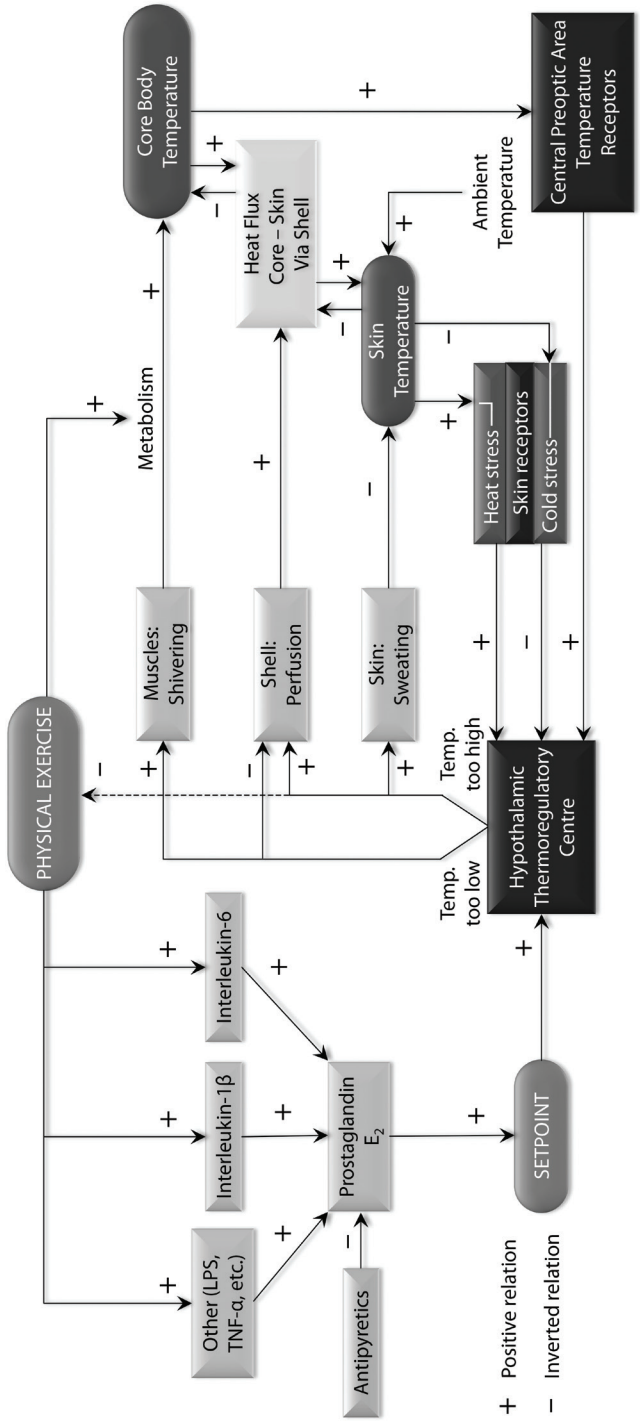

Figure 7-1: *Schematic overview of human thermoregulation, expanded with new knowledge gained in Chapter 4. We found evidence that pro-inflammatory cytokines, which are released during exercise, increase the hypothalamic setpoint and are hence partially responsible for the increased core body temperature during exercise. Legend: + = positive relation, - = negative relation. Adapted from P. Vis, Lecture Thermodynamics*

Why Does Hyperthermia Increase Haemostatic Activity?

As evidenced by previous literature, symptomatic hyperthermia in its most dramatic form of heatstroke can result in massive activation of the coagulation cascade.[2 11 15] *Chapter 5* demonstrated that exercise itself leads to procoagulant changes,[44 48-50] whereas a part of the procoagulant changes might be directly related to the increased core body temperature itself. Interestingly, we found these changes by modifying the environmental temperature of the haemostasis assay by analysing plasma samples that had already been frozen. The fact that we sill found a temperature effect even though the samples had previously been frozen suggests that the temperature effect is reversible. But why would the physiologic process of increased haemostasis under increased core body temperatures be beneficial for survival? From an evolutionary viewpoint, hunting for food or fleeing from danger carried a risk of being wounded and being subjected to potentially fatal bleeding. A haemostatic balance that is pushed toward a hypercoagulable state before being wounded would be favourable since a shortened bleeding time would reduce blood loss and thus increase survival.

In addition to limiting blood loss due to a potential injury, temperature-induced hypercoagulability is also favourable in everyday life when fighting infection. The myriad of pro-inflammatory cytokines that are released during infection play an intricate interaction with the coagulation cascade and also push the haemostatic balance towards hypercoagulability.[51-55] At the site of infection, the immune system tries to combat the pathogen by either destroying infected tissue or by directly attacking the pathogen itself. In this process, thrombi are formed at the site of infection to encapsulate the diseased tissue and pathogens, in order to limit further tissue damage and prevent further dissemination of the infection. By pushing the haemostatic balance towards hypercoagulability during hyperthermia, or in this case fever, augments the influence of the inflammatory cytokines to help contain and eliminate the infection.

Future Perspective and Clinical Implications

This thesis has produced new insights into the prevalence of hyperthermia during exercise and factors that are associated with the stronger rises in core body temperature. It was shown that risk assessment for strong core body temperature rises is very individual-specific but can be partially predicted. Hence, individuals who are most at risk of the strongest core body temperature rises can be identified beforehand and, if desired, be monitored more closely during exercise. With growing technological innovation over

the past years concerning ingestible temperature sensors,[56-58] future monitoring likely progresses to real-time monitoring of numerous body vitals including core body temperature. With growing interest in pre- and *per*cooling strategies (i.e. cooling *during* exercise), real-time monitoring may enable medical staff to better decide when to start cooling in order to maintain or even improve performance levels.[33-35] Importantly, this thesis has shown that hyperthermia during exercise is common and – at least in cool to moderate ambient conditions – does not necessarily lead to health problems. Medical staff should remain vigilant for the onset of heat-related symptoms, but treatment hyperthermia is not immediately necessary in absence of symptoms.

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Chapter 8

Summary

Chapter 1 introduces the general concepts of thermoregulation and maps out how exercise can be a burden for thermoregulation by producing heat in excess of heat dissipating capabilities. When core body temperature rises, it can cause several health problems such as heat exhaustion or syncope, but also lead to more serious health issues such as the occurrence of heatstroke. This is a disease state caused by a systemic inflammatory response mimicking sepsis and causing hyperactivation of the immune system and coagulation. Lastly, an elevated core body temperature can also limit exercise performance, either by inducing central fatigue or decreased cardiac output due to blood volume depletion, and usually a combination of both. The chapter ends by an outline of the thesis.

Chapter 2 aims to assess what core body temperatures are commonly observed after a 15-km road race and to assess how often exertional hyperthermia (a core body temperature ≥40.0°C) occurs. Secondly, chapter 2 aims to identify factors that significantly predict the rise in core body temperature during exercise and develop a predictive model. Core body temperature is measured before the start of the race and immediately after finishing in 227 volunteers. Core body temperature significantly increased from 37.6±0.4°C at baseline to 39.2±0.7°C after finishing. The temperature change between baseline and after warming up was identified as strongest predictor for core body temperature at the finish line. With as much as 15% of all subjects showing exertional hyperthermia at the finish line, the findings emphasize that it is a common phenomenon and can be partially predicted.

Chapter 3 aims at further elucidation of the predictability of individual thermoregulatory responses during exercise. Data from the same individuals participating in 2 consecutive editions of a 15-km road race are correlated. Addition of previously obtained thermoregulatory data from the first race edition to the predictive model as described in Chapter 2 proved to substantially better predictability of thermoregulatory responses for the second race edition, with a 32.2% predictability compared to a 17.1% predictability without the historical data.

In **Chapter 4** the role of the hypothalamic thermoregulatory setpoint during exercise is investigated. Previous literature demonstrated a substantial release of pro-inflammatory cytokines during exercise, theoretically causing a fever-like upregulation of the hypothalamic setpoint. In this chapter, subjects perform 3 bouts of identical treadmill exercise whilst ingesting medication that blocks prostaglandin E_2 (one of the main mediators of setpoint upregulation) production centrally and peripherally, only centrally

or not at all. The condition in which both central and peripheral prostaglandin E_2 blockade was applied showed a significantly lower peak core body temperature during the exercise bout compared to the control condition. This finding suggests that an upregulated hypothalamic setpoint might be partially involved in the core body temperature rise seen during exercise.

Chapter 5 comprises a pilot study, which investigates the differences in thermoregulatory responses between spinal cord injured and non-spinal cord injured elite wheelchair tennis players. A total of eight volunteers (3 spinal cord injured, 5 non-spinal cord injured wheelchair tennis players) played a 45-minute match whilst thermoregulatory responses including core body temperature and skin temperature were measured continuously. The main results show that core body temperature increased stronger in the spinal cord injured athletes compared to the non-spinal cord injured athletes, though no statistical significance could be calculated on this due to insufficient sample size. These descriptive data suggest that spinal cord injured wheelchair tennis players might be at increased risk of developing heat related problems during a match, and warrant further research into this subject.

Chapter 6 focusses on the potential effects of core body temperature on haemostasis. Haemostasis assays are performed in plasma samples obtained in volunteers before and immediately after finishing a 15-km road race. The main findings are that exercise induces a prothrombotic state, which might be partially dependent on the exercise-induced core body temperature rise. Adjusting the assay temperature to match the subject's actual core body temperature at the time of blood collection (which is often substantially higher than the standard 37°C which is assumed in routine assays) produced more pronounced haemostatic changes after exercise. These findings show that haemostasis is affected by exercise-induced core body temperature rise, but also demonstrate the importance of synchronizing the haemostasis assay temperature to the subject's core body temperature at the time of sample collection.

Chapter 7 summarizes, discusses and explains the findings of the present thesis. An attempt is made to provide future perspective on individual prediction and monitoring, and provides the clinical implications of the present findings.

Nederlandse Samenvatting

Hoofdstuk 1 introduceert de algemene concepten van thermoregulatie en zet uiteen hoe fysieke inspanning de thermoregulatie nadelig kan beïnvloeden doordat er meer warmte wordt geproduceerd dan de afkoelingsmechanismen kunnen afvoeren. Stijging van de kerntemperatuur kan leiden tot diverse gezondheidsproblemen, zoals warmte uitputting of warmte syncope, maar het kan ook leiden tot ernstigere gezondheidsproblemen zoals hitteberoerte. Hitteberoerte is een aandoening welke wordt veroorzaakt door een systemische inflammatoire respons die lijkt op sepsis en massale activatie van het immuunsysteem en de stollingscascade kan veroorzaken. Ten slotte wordt besproken hoe een verhoogde kerntemperatuur ook het prestatievermogen tijdens inspanning kan verlagen door enerzijds 'centrale vermoeidheid', anderzijds een verlaagde cardiac output door bloedvolume depletie, maar meestal een combinatie van beide. Het hoofdstuk eindigt met een uiteenzetting van de inhoud van dit proefschrift.

Hoofdstuk 2 heeft als doel te onderzoeken welke kerntemperaturen veelal voorkomen na het lopen van een 15-km hardloopwedstrijd, en maakt een inschatting hoe vaak inspanningsgebonden hyperthermie (een kerntemperatuur ≥40.0°C) voorkomt. Ten tweede heeft dit hoofdstuk als doel factoren te identificeren die de stijging van de kerntemperatuur tijdens inspanning significant kunnen voorspellen. Bovendien wordt gepoogd hiervoor een voorspellend model te ontwikkelen. Bij 227 vrijwilligers wordt de kerntemperatuur gemeten voor de start van de race en direct na het finishen. De kerntemperatuur steeg significant van 37.6±0.4°C in rust vóór warming-up naar 39.2±0.7°C direct na het finishen. De temperatuurstijging tussen de rustmeting en na de warming-up bleek de sterkste voorspeller te zijn van de kerntemperatuur op de finish lijn. Van alle proefpersonen bleek 15% op de finish lijn te voldoen aan de criteria voor inspanningsgebonden hyperthermie. Deze bevindingen tonen aan dat hyperthermie een veel voorkomend fenomeen is, en deels kan worden voorspeld.

Hoofdstuk 3 heeft als doel de voorspelbaarheid van de individuele thermoregulatoire respons tijdens inspanning verder in kaart te brengen. Gegevens van een groep vrijwilligers die aan twee opvolgende edities van hetzelfde 15-km hardloopevenement meedoen worden met elkaar gecorreleerd. Het toevoegen van de thermoregulatoire gegevens uit de eerste editie aan het voorspellend model zoals beschreven in hoofdstuk 2 blijkt de voorspelbaarheid van de kerntemperatuur op de finish lijn substantieel te verbeteren. Het oorspronkelijke model heeft een voorspelbare waarde van 17.1%, terwijl het toevoegen van de historische gegevens de voorspelbaarheid verbetert naar 32.2%.

In **hoofdstuk 4** wordt de rol van het hypothalame temperatuur setpoint tijdens inspanning onderzocht. Voorgaande literatuur beschreef eerder dat er een aanzienlijke hoeveelheid pro-inflammatoire cytokines vrijkomt tijdens fysieke inspanning. In theorie kan dit een respons veroorzaken welke lijkt op een koortsreactie en hierdoor het hypothalame setpoint verhoogt zoals ook het geval is tijdens koorts. Proefpersonen in dit hoofdstuk voeren driemaal identieke inspanning uit op een loopband waarbij ze voorafgaand aan de inspanning medicatie slikken die de prostaglandine E_2 productie (een van de belangrijkste mediatoren die het setpoint verhogen) blokkeert op centraal en perifeer niveau, op alleen centraal niveau of helemaal niet blokkeert. Wanneer zowel centrale als perifere blokkade van de prostaglandine productie plaats vindt blijkt de maximale kerntemperatuur tijdens inspanning significant lager te liggen vergeleken met de controle conditie (geen blokkade). Deze bevinding suggereert dat een verhoogd temperatuur setpoint deels verantwoordelijk zou kunnen zijn voor de stijging in kerntemperatuur tijdens inspanning.

Hoofdstuk 5 omvat een pilot studie gericht op het onderzoeken of er verschillen in thermoregulatoire reacties zijn tussen elite rolstoeltennissers met en zonder dwarslaesie. In totaal acht vrijwilligers (waarvan 3 met een dwarslaesie en 5 zonder dwarslaesie) speelden een 45-minuten durende rolstoeltennis wedstrijd terwijl verschillende thermoregulatoire parameters continue gemeten werden, waaronder kerntemperatuur en huidtemperatuur. Het belangrijkste resultaat laat zien dat de rolstoeltennissers met dwarslaesie een sterkere stijging van de kerntemperatuur lieten zien dan de rolstoeltennissers zonder dwarslaesie, hoewel deze data door een te kleine steekproef niet op significantie getoetst kon worden. Deze beschrijvende data suggereren dat rolstoeltennissers met een dwarslaesie mogelijk een verhoogd risico hebben op het ontwikkelen van hittegerelateerde gezondheidsproblematiek. Deze resultaten maken vervolgonderzoek om deze relatie verder te exploreren noodzakelijk.

Hoofdstuk 6 richt zich op de potentiële effecten van een verhoogde kerntemperatuur op het stollingssysteem. De stollingsactiviteit wordt bepaald in plasma monsters welke verkregen zijn direct voorafgaand aan en na afloop van het lopen van een 15-km hardloopwedstrijd. De belangrijkste bevindingen tonen dat fysieke inspanning een verhoogde stollingsactiviteit veroorzaakt, welke mogelijk deels veroorzaakt wordt door de verhoogde kerntemperatuur tijdens inspanning. Wanneer de temperatuur van de stollingsassay zodanig wordt aangepast dat deze de kerntemperatuur van de proefpersoon tijdens bloedafname benadert (deze ligt vaak hoger dan de 37 C waar vanuit wordt gegaan bij de assay), dan blijkt de verhoogde stollingsactiviteit aanzienlijk meer uitgesproken te zijn na inspanning. Deze bevindingen tonen dat de bloedstolling wordt beïnvloed door de inspanningsgebonden stijging van de kerntemperatuur, maar

toont bovendien het belang aan van het synchroniseren van de assay temperatuur met de daadwerkelijke kerntemperatuur van de proefpersoon ten tijde van bloedafname.

Hoofdstuk 7 geeft een samenvatting van de belangrijkste bevindingen van dit proefschrift, en bediscussieert deze. Tevens wordt gepoogd een toekomstperspectief te schetsen van de individuele voorspelbaarheid en monitoring van de kerntemperatuur. Bovendien worden de klinische implicaties van de bevindingen bediscussieerd.

Dankwoord

Hoewel ik in dit proefschrift met de eer mag strijken, zijn alle studies in dit werk natuurlijk tot stand gekomen dankzij de inzet van velen. Zonder iemand tekort te willen doen, wil ik graag een aantal mensen wie een bijzondere rol hebben gespeeld in mijn onderzoeksperiode specifiek benoemen.

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Maria, al in het eerste jaar van mijn Geneeskunde opleiding kwamen wij in contact. Eerst als vrijwilliger bij de eerste editie van het Vierdaagse onderzoek, en het jaar erop was ik al welkom om als student tijdens de zomervakantie een kleine onderzoeksstage te komen doen. Elk jaar breidden we mijn betrokkenheid bij het Vierdaagse onderzoek en het Zevenheuvelenloop onderzoek verder uit, en al snel werd ik onderdeel van het meubilair. Ik was altijd welkom om mee te helpen, en hier profiteerden wij allebei van. Nog steeds ben ik erg jaloers op jouw eindeloze nieuwsgierigheid, interesse, manier van out-of-the-box denken, en op jouw open houding. We waren het lang niet altijd met elkaar eens, en dit heeft nogal eens tot stevige discussies en impasses geleid. Maar nooit was jouw wil wet; het sterkste argument won altijd. Maria, dank voor alle kansen die je me hebt gegeven, dank voor al jouw wijsheden, kritische noten en de mooie onderzoeksjaren dit ik in grote zelfstandigheid op jouw afdeling heb mogen beleven.

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Martijn, wij leerden elkaar kennen toen jij nog als student rondliep op onze afdeling. Echt student was je eigenlijk niet, want al vanaf het begin was het iedereen duidelijk dat we snel collega's zouden worden. Jouw grote statistische kennis kwam mij meer dan eens goed van pas, en onze 'spar' sessies waarin we ideeën wisselden over de analyse en interpretatie van onze studies heb ik altijd bijzonder goed gewaardeerd. Bovenal waardeer ik onze interesse in fotografie en onze vriendschap het meest, en ben ik blij dat jij ook nog wel eens aan mij vraagt: "hoe heb je dat gedaan?" in plaats van alleen andersom.

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List of Publications

1. Eijsvogels TM, **Veltmeijer MT**, Schreuder TH, et al. The impact of obesity on physiological responses during prolonged exercise. Int J Obes (Lond) 2011;35(11):1404-12.
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11. **Veltmeijer MT**, Veeneman D, Bongers CC, et al. The Impact of Central and Peripheral Cyclooxygenase Enzyme Inhibition on Exercise-Induced Core Body Temperature Elevations. *Accepted* - Int J Sports Phys Perform 2016

Curriculum Vitae

Matthijs Veltmeijer werd op 26 januari 1988 geboren te UMC St. Radboud, Nijmegen. Na het Voorbereidend Wetenschappelijk Onderwijs op het Titus Brandsma College te Oss succesvol te hebben voltooid, startte hij in 2006 met de opleiding Geneeskunde aan de Radboud Universiteit Nijmegen. Tijdens zijn tweede studiejaar nam kwam hij voor het eerst in aanraking met wetenschappelijk onderzoek als lid van het Nijmeegse Vierdaagse onderzoeksteam. Het jaar erop volgde de eerste kleine wetenschappelijke stage op de afdeling Integratieve Fysiologie, en het volgende jaar een grote wetenschappelijke stage als onderdeel van de Master Geneeskunde. Ook tijdens zijn coschappen bleef Matthijs in de avond- en weekenduren betrokken bij het Vierdaagse maar ook Zevenheuvelenloop onderzoeksteam als Student Assistent en voerde diverse metingen en analyses uit in het kader van deze studies. Na afronding van zijn Geneeskunde studie in 2012 werden deze activiteiten voortgezet als fulltime promotieonderzoek. Na 2 jaar fulltime te hebben gewerkt als promovendus op de afdeling Fysiologie in het Radboudumc, vervolgde Matthijs zijn medische carrière als arts op de Spoedeisende Hulp en Eerste Hart Hulp in Gelre Ziekenhuizen Zutphen en later op de Spoedeisende Hulp in Viecuri Medisch Centrum Venlo.

Dit proefschrift richt zich op thermoregulatie tijdens hardlopen. Na het behandelen van de algemene principes van thermoregulatie tijdens inspanning wordt onderzocht hoe vaak hyperthermie (een kerntemperatuur hoger dan 40°C) voorkomt na het lopen van een 15-km hardloopwedstrijd. Tevens worden factoren geïdentificeerd die significant kunnen voorspellen in hoeverre de kerntemperatuur tijdens inspanning zal stijgen. In de opvolgende hoofdstukken wordt de rol onderzocht van afzonderlijke systemen die invloed hebben op, of beïnvloed worden door de kerntemperatuur tijdens inspanning. Allereerst wordt onderzocht in hoeverre pro-inflammatoire cytokines welke aangrijpen op het hypothalame temperatuursetpoint een rol spelen in de stijging van de kerntemperatuur tijdens inspanning. Voorts wordt in een beschrijvende pilotstudie onderzocht of elite rolstoeltennis atleten met een dwarslaesie een grotere kans hebben op warmte-gerelateerde gezondheidsproblemen vergeleken met elite rolstoeltennis atleten zonder dwarslaesie. Als laatste worden de potentiële effecten van een verhoogde kerntemperatuur op het stollingssysteem beschreven.