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

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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.

Key words: Thermoregulation, Exercise, Setpoint, Running, Hyperthermia

INTRODUCTION

Human core body temperature (T_C) is strictly regulated by the body's natural thermostat located in the hypothalamus. T_C 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 T_C increases beyond the setpoint temperature, several compensatory mechanisms are activated to release excess body heat and maintain a proper T_C[3,4].

Exercise almost invariably causes T_C 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 T_C will activate heat loss mechanisms including skin vasodilatation and sweating[3]. These mechanisms are often insufficient and T_C will rise further[7]. Another cause for T_C 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 T_C 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 T_C 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 T_C rise.

Recent human studies suggested that drug-induced inhibition of PGE_2 synthesis may[15-17] or may not[18] attenuate the rise in T_C 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 T_C 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 T_C during exercise. We hypothesized that the exercise-induced T_C 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 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.

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 $\geq$1.1, and a maximal heart rate $\geq$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 T_C 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) T_C 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 T_c) 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 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.

T_C 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). T_C increased significantly over time (p<0.001), and a significant time*condition interaction was found (p=0.048). Maximum T_C 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 ΔT_C 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 1A). ΔT_C 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. 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 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 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 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 2).

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].

Practical Applications:

- Combined blockade of prostaglandin E_2 production both centrally (acetaminophen) and peripherally (ibuprofen) resulted in a slightly but significantly lower maximal core body temperature during one hour of strenuous running exercise.
- This finding suggests that part of the rise in core body temperature during exercise might be caused by an altered hypothalamic temperature setpoint. The remainder of the temperature rise is still attributable to metabolic heat production.
- Whilst chronic use of COX inhibitors is not recommended, occasional use of 1000mg acetaminophen and/or 400mg ibuprofen might be beneficial for athletes to reduce the exercise-induced T_C at times of high thermal stress.

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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Figure legend

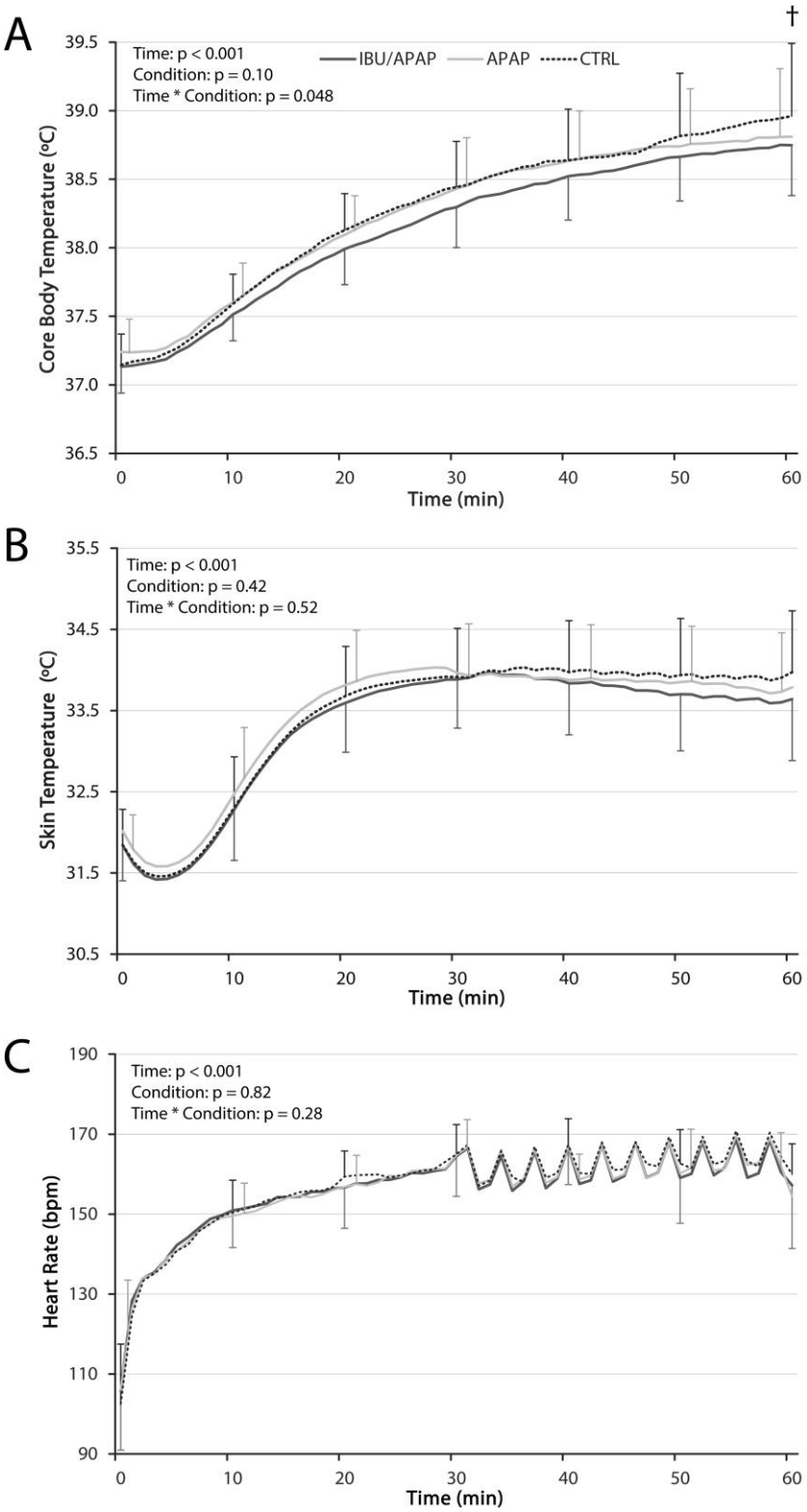

Figure 1 A: Core Body Temperature (T_C) during exercise during the exercise bouts. Maximum T_C and ΔT_C are significantly lower in the IBU/APAP condition compared to the CTRL condition. **B:** Skin Temperature (T_{SK}) during the exercise bouts. No significant differences were observed in maximum T_{SK} and ΔT_{SK}. There was a significant interaction effect, but no effect

for condition. **C:** Heart Rate (HR) during the exercise bouts. No significant differences were observed in maximum HR and ΔHR. There was a significant interaction effect, but no effect for condition. For readability purposes, the error bars are not visualized on the same time points. † = $p<0.05$.

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

Characteristic		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

Table 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.