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

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33

1 **ABSTRACT**

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

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

13 **Results:** Baseline values of T_C , skin temperature and heart rate did not differ across conditions.
14 Serum interleukin-6 concentrations increased in all three conditions. A significantly lower peak
15 T_C was observed in IBU/APAP ($38.8\pm 0.4^\circ\text{C}$) versus CTRL ($39.2\pm 0.5^\circ\text{C}$, $p=0.02$), but not in
16 APAP ($38.9\pm 0.4^\circ\text{C}$) versus CTRL. Similarly, a lower ΔT_C was observed in IBU/APAP
17 ($1.7\pm 0.3^\circ\text{C}$) versus CTRL ($2.0\pm 0.5^\circ\text{C}$, $p<0.02$), but not in APAP ($1.7\pm 0.5^\circ\text{C}$) versus CTRL. No
18 differences were observed in skin temperature and heart rate responses across conditions.

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

23
24 **Key words:** Thermoregulation, Exercise, Setpoint, Running, Hyperthermia

1 INTRODUCTION

2 Human core body temperature (T_C) is strictly regulated by the body's natural thermostat located
3 in the hypothalamus. T_C is measured by preoptic area neurons and values are compared with
4 the temperature setpoint, which is typically kept near $36.8 \pm 0.4^\circ\text{C}^{1,2}$. When T_C increases beyond
5 the setpoint temperature, several compensatory mechanisms are activated to release excess
6 body heat and maintain a proper $T_C^{3,4}$.

7 Exercise almost invariably causes T_C to rise, as a result of increased metabolic heat
8 production due to muscle labor^{3,5,6}. Since the hypothalamic temperature setpoint remains
9 unchanged, a rise in T_C will activate heat loss mechanisms including skin vasodilatation and
10 sweating³. These mechanisms are often insufficient and T_C will rise further⁷. Another cause for
11 T_C to rise is infection- or inflammation-induced fever, which causes the hypothalamic
12 temperature setpoint itself to rise. Multiple pro-inflammatory cytokines are released during
13 infection, including interleukin- 1β (IL- 1β) and interleukin-6 (IL-6)⁸. These cytokines stimulate
14 the enzyme cyclooxygenase (COX) to synthesize prostaglandin E_2 (PGE_2), which in turn
15 upregulates the temperature setpoint and via several mechanisms (e.g. vasoconstriction and
16 shivering) may cause T_C to rise⁸⁻¹¹. Antipyretic drugs mainly act by reducing PGE_2 synthesis
17 by inhibiting COX enzyme activity^{9,10}. COX can be inhibited either peripherally (non-steroidal
18 anti-inflammatory drugs) or centrally in the hypothalamus (acetaminophen)^{9,12}.

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

24 Recent human studies suggested that drug-induced inhibition of PGE_2 synthesis may¹⁵⁻
25 ¹⁷ or may not¹⁸ attenuate the rise in T_C and skin temperature during exercise. These inconsistent
26 findings may be explained by the different modes of exercise protocols (incremental vs. fixed
27 intensity), but may also be caused by the fact that these studies inhibited COX-enzyme activity
28 either centrally (acetaminophen) or peripherally (non-steroidal anti-inflammatory drugs). Since
29 none of these studies used COX-inhibition via both pathways, to what extent inflammatory
30 cytokines influence the T_C rise during exercise still needs to be elucidated.

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

37 METHODS

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

49

1 Each subject visited our laboratory four times. During the first visit, subjects performed a
2 maximal treadmill exercise test to determine each subject's maximal heart rate. Visits 2 to 4
3 consisted of submaximal exercise tests on a treadmill where running speed was calibrated
4 individually for each subject's maximal heart rate. Each submaximal exercise test comprised of
5 30 minutes continuous running at 85% of the subject's maximal heart rate, followed by 10
6 intervals with a 1 minute speed increase of 2km/h and 2 minute speed decrease of 2km/h
7 compared to the continuous running speed of the first 30 minutes. This exercise protocol was
8 selected based on pilot measurements within our own department to select the exercise protocol
9 that elicits the strongest T_C rise within one hour. Running speeds of the second and third exercise
10 test were kept identical to the first exercise test to ensure that stimuli for thermogenesis and
11 cytokine release were identical across all exercise tests. Using a cross-over design with
12 randomization of sequences, the following test medication was administered 45 minutes before
13 the start of each submaximal exercise test.

- 14 1. IBU/APAP: Administration of 400mg ibuprofen (IBU) with 100mL of water and
15 1000mg acetaminophen (APAP) with 100mL of water.
- 16 2. APAP: Administration of 1000mg acetaminophen with 100mL of water. An extra
17 100mL of water was administered as a control substance for ibuprofen. This condition
18 was added for comparison with previously performed studies using APAP only^{15,18,19}.
- 19 3. CTRL: Control condition without inhibition of PGE₂ synthesis. Instead, 100mL of water
20 was administered twice as control substances for acetaminophen and ibuprofen.

21 The use of the non-steroidal anti-inflammatory drug ibuprofen was chosen because of
22 pharmacokinetics similar to acetaminophen. Since ibuprofen reaches its maximal plasma
23 concentration 1-2 hours after ingestion, and acetaminophen reaches its maximal concentration
24 30 minutes to 2 hours after ingestion, administration of the test medication was timed such that
25 maximal concentrations were attained 30 to 45 minutes into the exercise bout. The dosage of
26 both APAP and IBU was based on the Dutch Guidelines for antipyretic treatment²⁰. A minimum
27 of 3 rest days was required between the submaximal exercise tests to enable full recovery, and
28 subjects were not allowed to use acetaminophen or NSAIDs for at least 3 days preceding each
29 measurement. All experiments were performed in the same room at the same temperature
30 (21°C) and humidity (45%) and the same time of the day to prevent any interference of
31 environmental conditions or circadian rhythm⁴. Also, subjects were instructed to consume
32 500mL of water 2-3 hours before the start of the exercise tests to ensure euhydration at the start
33 of the exercise bouts²¹.

34
35 Each subject underwent a maximal exercise test on a treadmill (GTR-3.06, En-Bo
36 Systems, Zwolle, Netherlands) using the Bruce protocol. Oxygen consumption was measured
37 using a calibrated gas exchange analyser (Quark CPET, Cosmed, Italy) with a breathing mask.
38 Heart rate was monitored using a Cosmed HR monitor (Cosmed, Italy). Capillary blood lactate
39 levels were measured (Lactate Pro, Arkray, Kyoto, Japan) before and after the maximal exercise
40 test as an indicator for achieving maximal exercise (>8 mmol/L). Other indicators for maximal
41 exercise were a plateau in the VO_2 -curve, a respiratory exchange ratio ≥ 1.1 , and a maximal
42 heart rate $\geq 95\%$ of the age-predicted maximum. Subjects had to meet 3 out of the 4
43 aforementioned criteria to achieve maximal exercise.

44 The subjects were instructed to ingest a telemetric temperature pill (CorTemp, HQ Inc,
45 Palmetto FL, USA) 6 hours prior to each submaximal exercise test to assure stomach passage
46 and exclude interference from fluid or food ingestion²². Using an external recorder T_C was
47 recorded every 20 seconds and averaged per minute. This method is known to be valid and safe,
48 and was described in detail previously²³.

49 The skin temperature (T_{SK}) was measured during each test using individual skin
50 temperature sensors (iButtons, Maxim Integrated, San Jose, CA, USA). T_{SK} was measured

1 every 30 seconds with a resolution of 0.0625°C. Using the ISO 9886 norm, 8 different iButtons
2 were attached to the skin: on the forehead, right scapula, left thorax, right upper arm, left lower
3 arm, left hand, right upper leg and left calf. Mean T_{SK} was calculated from a standard area
4 weighing factors²⁴, and averaged per minute.

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

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

12 To compare the stimulus for PGE₂ synthesis in every test condition a venous blood
13 sample was taken to measure the concentration of IL-6 at baseline (before taking the
14 medication) and directly after completing the exercise test. A 10mL K3EDTA vacutainer tube
15 was used to collect the blood sample and was immediately after collection centrifuged at 4°C
16 and 3600 rpm for 12 minutes. All samples were subsequently stored at -80°C until further
17 analysis. All blood samples were analysed on the same day after completing all experimental
18 tests. A commercial IL-6 ELISA kit (Pelipair human IL-6 ELISA kit, Sanquin, Amsterdam, the
19 Netherlands) was used for determining IL-6 concentrations. The detection limit of the IL-6
20 ELISA kits was 3 pg/ml.

21 Rate of perceived exertion (RPE) was measured every 6 minutes using the BORG-
22 scale²⁵. This scale ranges from 6-20 with 6 being very mild and 20 the most strenuous exercise.
23 Furthermore we asked subjects to rate Thermal Sensation and Thermal Comfort every 6
24 minutes. Thermal Sensation measures the temperature perception of the subject with a scale
25 ranging from -3 being really cold to +3 being really hot. Thermal Comfort is a measure of how
26 comfortable the temperature feels to the subject ranging from -4 being very comfortable to +4
27 being very uncomfortable²⁶.

28
29 All data are presented as mean \pm standard deviation unless indicated otherwise.
30 Statistical analyses were conducted using SPSS version 20 (IBM SPSS version 20.0, Armonk,
31 NY, USA). Changes over time (baseline vs. peak) and between conditions (IBU/APAP/CTRL)
32 were analysed using a within-subject repeated-measures ANOVA. Delta (Δ) T_C and T_{SK} were
33 determined as the difference between maximal value and baseline value. Group differences at
34 the same time point (e.g. ambient temperature, baseline or peak T_C) were analysed using a
35 within-subject one-way ANOVA. Due to the fact that some baseline values of the IL-6
36 concentrations were below the detection limit, a logistic regression analysis was performed to
37 test whether more values were above the detection limit post-exercise compared to baseline. In
38 case of a significant outcome a post-hoc Bonferroni test was applied. The level of significance
39 was set at $p \leq 0.05$.

42 RESULTS

43 All subjects successfully completed the maximal and submaximal exercise tests (Table 1). All
44 subjects completed the entire study protocol within 4 weeks. Room temperature (IBU/APAP
45 21.1 \pm 0.9°C, APAP 21.3 \pm 0.6°C, CTRL 21.1 \pm 1.0°C, $p=0.80$) and humidity (IBU/APAP
46 42.3 \pm 9.1%, APAP 45.0 \pm 11.0%, CTRL 43.5 \pm 7.5%, $p=0.60$) were similar across the three test
47 conditions. $VO_{2\text{ MAX}}$ was 61.7 \pm 9.9 mL/min/kg. Maximal heart rate was 186 \pm 11 bpm. No
48 adverse events occurred and all subjects met the criteria for achieving maximal exercise.

49

1 T_C was similar at baseline across the three conditions (IBU/APAP $37.1 \pm 0.2^\circ\text{C}$, APAP
2 $37.3 \pm 0.2^\circ\text{C}$, CTRL $37.1 \pm 0.2^\circ\text{C}$; $p=0.16$). T_C increased significantly over time ($p<0.001$), and a
3 significant time*condition interaction was found ($p=0.048$). Maximum T_C was significantly
4 lower in the IBU/APAP condition compared to the CTRL condition (IBU/APAP $38.8 \pm 0.4^\circ\text{C}$
5 versus CTRL $39.2 \pm 0.5^\circ\text{C}$; $p=0.02$) but not between APAP and CTRL (APAP $38.9 \pm 0.4^\circ\text{C}$). A
6 lower ΔT_C was observed in the IBU/APAP condition versus the CTRL condition (IBU/APAP
7 $1.7 \pm 0.3^\circ\text{C}$ versus CTRL $2.0 \pm 0.5^\circ\text{C}$; $p=0.042$; Figure 1A). ΔT_C did not differ between APAP
8 and CTRL (APAP $1.7 \pm 0.5^\circ\text{C}$).

9
10 T_{SK} was similar at baseline in all three conditions (IBU/APAP $31.8 \pm 0.4^\circ\text{C}$, APAP
11 $32.0 \pm 0.4^\circ\text{C}$, CTRL $31.8 \pm 0.5^\circ\text{C}$; $p=0.23$). T_{SK} increased significantly over time in all conditions
12 ($p<0.001$), though no differences across conditions ($p=0.42$) or time*condition ($p=0.52$) were
13 found. Also, there were no differences in maximum T_{SK} (IBU/APAP $34.2 \pm 0.6^\circ\text{C}$, APAP
14 $34.3 \pm 0.6^\circ\text{C}$, CTRL $34.2 \pm 0.6^\circ\text{C}$; $p=0.95$) or ΔT_{sk} (IBU/APAP $2.3 \pm 0.6^\circ\text{C}$, APAP $2.3 \pm 0.7^\circ\text{C}$,
15 CTRL $2.4 \pm 0.6^\circ\text{C}$; $p=0.53$) across conditions (Figure 1B).

16
17 Before the start of the sub-maximal exercise tests, heart rate was similar across
18 conditions (IBU/APAP 106 ± 15 bpm, APAP 107 ± 12 bpm, CTRL 102 ± 15 bpm; $p=0.51$). Heart
19 rate increased significantly over time in all conditions ($p<0.001$), though no significant
20 condition*time interaction occurred ($p=0.28$). No differences in maximal heart rate (IBU/APAP
21 168 ± 10 bpm, APAP 170 ± 10 bpm, CTRL 172 ± 6 bpm; $p=0.35$) or delta heart rate (IBU/APAP
22 64 ± 16 bpm, APAP 64 ± 19 bpm, CTRL 69 ± 14 bpm; $p=0.28$) were observed across conditions
23 (Figure 1C).

24
25 The prevalence of IL-6 concentration in serum exceeding the level of detection
26 ($>3\text{mmol/L}$) was low across all conditions at baseline (IBU/APAP $n=1$ (7%; range 7), APAP
27 $n=3$ (20%; range 3-24), CTRL $n=1$ (7%; range 4), whilst a substantially higher amount of
28 samples post-exercise showed levels exceeding the level of detection (IBU/APAP $n=10$ (67%;
29 range 3-8), APAP $n=7$ (47%; range 4-11), CTRL $n=12$ (80%; range 3-10)). No significance
30 levels could be determined to compare pre- versus post-exercise values due to the low number
31 of samples being below the level of detection pre-exercise.

32
33 Maximal RPE and average RPE were not different among conditions (Table 2). No
34 significant differences were observed among conditions in maximal and average thermal
35 comfort. There were no differences among conditions in maximal and average thermal
36 sensation. Body weight change was similar across all conditions (Table 2).

37 38 39 **DISCUSSION**

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

47 The present study was performed under similar environmental circumstances in all three test
48 conditions. Furthermore the intensity of exercise was identical across conditions, ensuring equal
49 thermogenesis and release of pro-inflammatory cytokines was identical during each test. The
50 measurements were performed in moderate temperatures since we wanted to replicate

1 conditions similar to those typically encountered in recreational running. The randomization of
2 all three conditions rules out a potential training effect. Although significance levels could not
3 be determined, IL-6 levels showed a similarly low prevalence of values exceeding the level of
4 detection pre-exercise, as well as a substantially higher prevalence exceeding the level of
5 detection post-exercise. Whilst no significance levels could be determined for this difference,
6 it does suggest that elevated IL-6 levels during exercise posed as stimulus for PGE₂ production
7 across all conditions in line with previous literature^{13,14}. Also, the lack of differences in body
8 weight loss across conditions out rules any influence by differences sweat losses. Lastly, the
9 study protocol was not blinded since humans are unable to (sub)consciously alter their body
10 temperature and since the exercise protocols were kept identical (i.e. identical metabolic heat
11 production during each exercise test). Blinding was therefore not expected to alter our results.
12

13 A significantly lower maximum T_C and ΔT_C were found in the IBU/APAP compared to the
14 CTRL condition, but not between the APAP and CTRL condition. These observations support
15 our hypothesis and suggest a superior effect of simultaneous central and peripheral COX
16 inhibition, although a similar effect of central inhibition and combined inhibition cannot be
17 completely ruled out given the similar delta T_C between IBU/APAP and APAP. Whilst we did
18 not identify a significant effect of APAP alone, previous authors did^{15,16}. Possible explanations
19 for this may be differences in the exercise protocol^{15,16}, ambient conditions^{15,16} or training
20 status¹⁶. The primary site of action for acetaminophen is the inhibition of PGE₂ synthesis in the
21 brain through the inhibition of the COX-1 and COX-2 enzyme^{9,27}. Ibuprofen is a non-selective
22 cyclooxygenase inhibitor in the NSAID group and the mechanism of action is lowering PGE₂
23 by directly inhibiting COX-1 and COX-2 enzyme activity peripherally⁹. Because
24 acetaminophen and ibuprofen act as COX-enzyme inhibitors on a central respectively a
25 peripheral level they maximally inhibit PGE₂ synthesis produced by exercise-induced IL-6
26 release. Whilst COX has been shown to not affect forearm sweating²⁸, a clinical study
27 suggested that the combined therapy of acetaminophen and ibuprofen is more effective in
28 lowering T_C during fever²⁹. The present study expands this observation to an exercise setting.
29 Especially since previous human studies that used drug-induced inhibition of PGE₂ synthesis
30 during exercise¹⁵⁻¹⁸ used either central or peripheral COX inhibitors and reported conflicting
31 results. Our study adds to this that the combination of central and peripheral COX inhibition is
32 more effective than central COX inhibition only. This may also suggest that circulating
33 prostaglandins from the periphery may also influence T_C, in addition to centrally synthesized
34 prostaglandins. Moreover, exercise-induced PGE₂ synthesis impacts on the thermoregulatory
35 setpoint and thus contributes to the increase in T_C. Whilst the difference of maximum T_C
36 between all three conditions is small, we believe the difference is still relevant given the
37 competitive nature of exercise in which even the smallest difference is important.
38

39 When T_C rises, mechanisms are activated to dissipate heat to the surroundings. One of these
40 mechanisms is an elevated skin blood flow. Vasodilatation causes skin blood flow to increase
41 so that warmer blood from the core is transported to the periphery and T_{SK} will rise³. One
42 previous study showed a lower T_{SK} when acetaminophen was administered in comparison to a
43 placebo¹⁶. Simultaneously they found a lower T_C in the acetaminophen group. Three other
44 studies that investigated acetaminophen or a non-steroidal anti-inflammatory drug did not find
45 a difference in T_{SK}^{15,17,18}. We did not find any differences in T_{SK} across conditions either.
46 Ambient temperatures were similar across conditions and have thus affected T_{SK} in a similar
47 way. Whilst changes in T_C do affect T_{SK}, it has also been suggested that when T_C exceeds the
48 value of 38°C the increase in skin blood flow during exercise is attenuated³⁰. As all study
49 participants demonstrated a maximum T_C >38°C, this might explain the observation that T_{SK}
50 did not differ across conditions in the present study.

1
2 Our main goal was to investigate whether an altered setpoint plays a role in the rise in T_C during
3 exercise for a better understanding of thermoregulation during exercise in humans. The
4 combined central and peripheral COX blockade using acetaminophen and ibuprofen resulted in
5 a slightly but significantly lower maximal T_C (0.3°C) compared to no COX blockade at all. This
6 suggests that the exercise-induced rise in T_C may be partially explained by an elevated
7 temperature setpoint. The limited T_C difference makes it uncertain whether the elevated setpoint
8 impacts athletic performance, though further research into this is needed. Whilst we would not
9 recommend chronic use of acetaminophen and ibuprofen during exercise to lower T_C given the
10 potential adverse effects such as kidney damage and gastro-intestinal problems, occasional use
11 might be beneficial for athletes to slightly reduce their T_C at times of high thermal stress and
12 improve exercise performance^{16,31}.
13
14

15 **Practical Applications:**

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

25 26 **Conclusion**

27 In conclusion, combined administration of acetaminophen and ibuprofen results in an
28 attenuated maximal T_C during exercise compared to a control condition. This suggests that
29 besides the production of metabolic heat, the release of pro-inflammatory cytokines contributes
30 to an elevated hypothalamic thermoregulatory setpoint via increased levels of PGE_2 . Our results
31 suggest that an upregulated hypothalamic temperature setpoint might partially be responsible
32 for the exercise-induced T_C rise.
33
34

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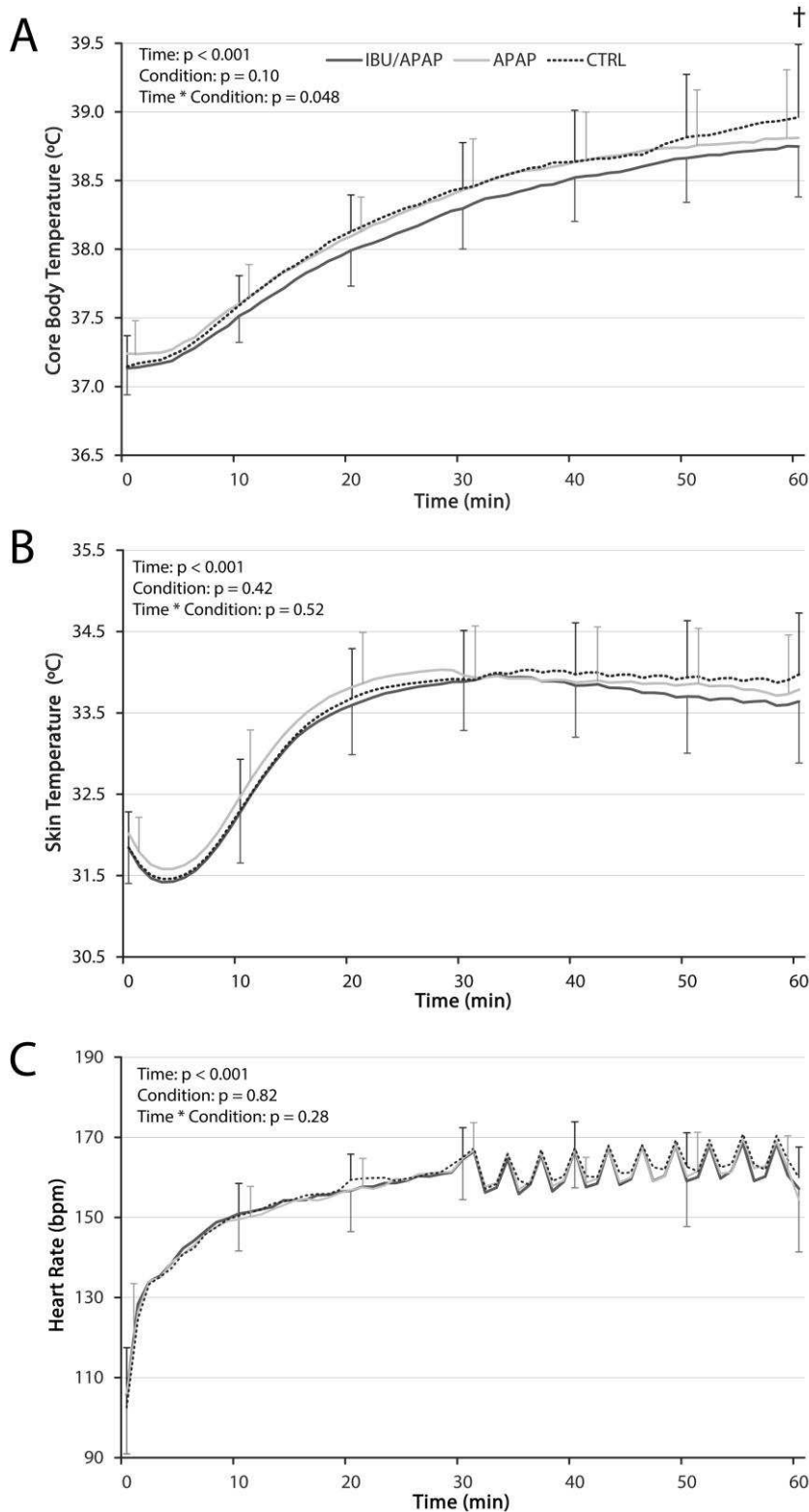
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- 23

1 **Figure legend**

2



3

4

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

1 for condition. **C:** Heart Rate (HR) during the exercise bouts. No significant differences were
2 observed in maximum HR and Δ HR. There was a significant interaction effect, but no effect
3 for condition. For readability purposes, the error bars are not visualized on the same time points.
4 † = $p < 0.05$.
5

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

2

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 ₂ max (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

3

4

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

3

	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

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

6