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Acetaminophen (Paracetamol) induces hypothermia during acute cold stress

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https://doi.org/10.1007/s40261-017-0560-x

PUBLISHER

© Springer

VERSION

AM (Accepted Manuscript)

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Foster, Josh, Alexis R. Mauger, Andrew Govus, David Hewson, and Lee Taylor. 2017. “Acetaminophen (paracetamol) Induces Hypothermia During Acute Cold Stress”. figshare. https://hdl.handle.net/2134/28351.

Acetaminophen (paracetamol) induces hypothermia during acute cold stress.

Article Type: Original Research

Running Title: Acetaminophen administration and hypothermia

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Word Count: 3,498

Reference Count: 42

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Abbreviations: APAP, acetaminophen, T_c, core temperature, T_{sk}, skin temperature, COX, cyclooxygenase

KEY POINTS

Accidental hypothermia was the primary or secondary diagnosis in over 100,000 hospital admissions from 2005 to 2015 in the United Kingdom. In this study we sought to determine whether acetaminophen, a non-prescription drug used to manage mild pain and fever, reduced core temperature stability during a 2-hour passive cold or thermoneutral exposure.

Acetaminophen had no effect on core temperature in thermoneutral conditions compared with a placebo, but reduced core temperature by up to 0.57°C after 2-hours cold exposure. The present results improve our knowledge about the side-effects of acetaminophen and provides important information of relevance for hypothermia pathology.

ABSTRACT

Background: Acetaminophen is an over-the-counter drug used to treat pain and fever, but it has also been shown to reduce core temperature (T_c) in the absence of fever. However, this side-effect is not well examined in humans, and it is unknown if the hypothermic response to acetaminophen is exacerbated with cold exposure.

Objective: To address this question, we mapped the thermoregulatory responses to acetaminophen and placebo administration during exposure to acute cold (10°C) and thermal neutrality (25°C).

Methods: Nine healthy Caucasian males (age: 20 to 24 years) participated in the experiment. In a double-blind, randomised, repeated measures design, participants were passively exposed to a thermo-neutral or cold environment for 120-minutes, with administration of 20 mg/kg lean body mass acetaminophen or a placebo 5-minutes prior to exposure. T_c, skin temperature (T_{sk}), heart rate, and thermal sensation were measured every 10-minutes, and mean arterial pressure was recorded every 30-minutes. Data were analysed using linear mixed effects models. Differences in thermal sensation were analysed using a cumulative link mixed model.

Results: Acetaminophen had no effect on T_c in a thermo-neutral environment, but significantly reduced T_c during cold exposure, compared with a placebo. T_c was lower in the acetaminophen compared with the placebo condition at each 10-minute interval from 80 to 120-minutes into the trial (all $p < 0.05$). On average, T_c decreased by 0.42 ± 0.13°C from baseline after 120 minutes of cold exposure (range 0.16 to 0.57°C), whereas there was no change in the placebo group (0.01 ± 0.1°C). T_{sk}, heart rate, thermal sensation, and mean arterial pressure were not different between conditions ($p > 0.05$).

Conclusion: This preliminary trial suggests that acetaminophen-induced hypothermia is exacerbated during cold stress. Larger scale trials seem warranted to determine if acetaminophen administration is associated with an increased risk of accidental hypothermia, particularly in vulnerable populations such as frail elderly individuals.

Key Words: acetaminophen, paracetamol, thermoregulation, cold exposure, thermogenesis, hypothermia

1.1 INTRODUCTION

Accidental hypothermia is characterised by an unintended core temperature (T_c) reduction to 35°C or lower. Such a fall in T_c can induce ventricular fibrillation and ultimately cardiac arrest if T_c declines to < 28ºC [1, 2]. In the United States, hypothermia was the cause or contributing cause of death in over 5500 cases between 2006 and 2010 [3], but this is likely underestimated since T_c needs to be measured at or near the time of death. Nonetheless, data from United Kingdom hospital episode statistics indicate that hypothermia was the primary or secondary diagnosis in over 100,000 hospital admissions from 2005 to 2015 [4]. Although death from hypothermia is rare, it remains a significant health risk in elderly and very young individuals, particularly during winter months and unaccustomed cold spells [1]. Interestingly, there is a growing body of evidence demonstrating that acetaminophen could reduce T_c stability during cold exposure (discussed below), placing users at an increased risk of accidental hypothermia.

Acetaminophen is an over-the-counter drug marketed as paracetamol in Europe and Tylenol in the United States. It is best known for its ability to decrease pain perception and reduce T_c during a fever; each of these actions are in part mediated through an inhibition of cyclooxygenase (COX) enzyme activity [5]. However, there is evidence of a 'hypothermic' action of acetaminophen, which refers specifically to an acetaminophen-induced reduction in T_c independent of febrile status. In mice, high doses (150 to 300 mg/kg body mass) administered intravenously reduced T_c by 2 to 4°C [6–8]. In humans, there have been 246 reports in Vigibase© (the World Health Organisation international database of adverse drug reactions) specific to acetaminophen-induced accidental hypothermia [9]. In addition, several case studies report profound hypothermia following therapeutic doses [10] and high doses of acetaminophen when ingested orally [11, 12]. Finally, oral acetaminophen administration (20 mg/kg lean body mass) reduced T_c in young adults by ~0.2°C (range, 0.10 to 0.39°C) during exposure to mild cold [13]. Although the T_c reductions were small, this hypothermic side-effect of acetaminophen occurred in all thirteen participants. Despite this data, additional criteria, such as the environmental temperature, are needed to accurately predict when acetaminophen poses the greatest risk for hypothermia development. Since the COX pathway could be involved in non-febrile thermogenesis [14, 15], inhibition of this enzyme by acetaminophen might cause T_c to fall during cold exposure, while exerting negligible effects on T_c while exposed to a warm environment.

If acetaminophen-induced hypothermia is a risk during cold exposure, this can have major implications for public health recommendations. Each year in the United States, ~6% of adults are prescribed acetaminophen at doses of more than 4 g/day [16], while it is also available over-the-counter without prescription. Acetaminophen is recommended as the first line analgesic for the elderly because it has minimal drug interactions and is well tolerated when taken at recommended doses [17]. It is also recommended for use in neonatal intensive care units following minor procedures and circumcision [18, 19]. Each of these age groups have a high incidence of accidental hypothermia due to a decreased ability to produce heat and make perceptually driven behavioural changes [20, 21]. Due to its hypothermic effects, use of acetaminophen in these populations could decrease their T_c to the point in which they are clinically hypothermic. However, the question remains as to whether acetaminophen exerts its hypothermic effect by increasing heat loss, or decreasing heat production. If the COX pathway is required for full heat production, inhibition of its activity by acetaminophen would cause T_c to fall during cold exposure while exerting no hypothermic action during a thermo-neutral exposure (in which no heat production is required).

The aim of this trial was to examine the thermoregulatory response to acetaminophen administration (20 mg/kg of lean body mass) during a 120-minute exposure to a thermo-neutral and cold environment in healthy adult humans. Due to a potential role of COX in non-febrile thermogenesis [14, 15], it was hypothesised that acetaminophen would reduce T_c in cold conditions, but have no effect on T_c in thermo-neutral conditions relative to a placebo.

1.2 METHODS

1.2.1 Ethical Approval

Experimental procedures were approved by the University Research Ethics committee (approval code 2014ISPAR011). All experimental procedures conformed to the standards set by the World Association Declaration of Helsinki 'Ethical Principles for Research Involving Human Subjects'.

1.2.2 Sample Size Calculation

Power analyses were conducted with GPower software version 3.1 (Heinrich University, Düsseldorf, Germany) to determine the sample necessary to achieve two-tailed statistical significance ($\alpha = 0.05$), with a power of 0.90 and a partial eta-squared (η^2) of 0.42. Using T_c data from a previous experiment where acetaminophen was tested as a hypothermic agent [22], it was determined that nine participants were required to reach the statistical power. If acetaminophen exerts the hypothesised hypothermic response, a larger project within vulnerable populations may be warranted to determine if acetaminophen contributes to accidental hypothermia admissions.

1.2.3 Participants

Nine Caucasian males [age: 22 ± 1 years, height: 179 ± 5 cm, body mass: 80.7 ± 11.9 kg, body fat (20 ± 5%)] volunteered to take part in this study. Participants were provided with written information regarding the experimental procedures, with supporting oral explanations from the principal investigator. All participants subsequently provided written informed consent. The participants were non-smokers, non-febrile (resting $T_c < 38°C$), and free from musculoskeletal injury.

1.2.4 Inclusion & Exclusion Criteria

Prior to each laboratory visit, participants completed an alcohol use disorder identification test [AUDIT; [23]], a breathalyser test (AlcoSense, One, Berkshire, UK), and an acetaminophen risk assessment questionnaire. To avoid any risk of liver damage inflicted by acetaminophen, participants were not able to participate in the research if they scored above ten on the AUDIT

questionnaire or alcohol was present in their bloodstream (i.e. > 0% blood alcohol content). In addition, the acetaminophen dose was relative to lean body mass, as it is a closer indicator of liver volume than total body mass [24]. No participants presented with any pre-existing medical conditions that may have put them at an increased risk of acetaminophen toxicity. Due to potential thermoregulatory adaptations [25, 26], individuals were not permitted to take part in any experimental procedures if they were heat/cold acclimated or acclimatised. Thus, those who had travelled to a hot/cold climate or participated in a laboratory based heat/cold acclimation protocol less than three weeks before the experiment were not permitted to take part. All participants presented to the laboratory with a stable resting T_c of 36.5-37.5°C.

1.2.5 Experimental Design

A schematic of the experimental design is displayed in Figure 1. To determine if acetaminophen reduces T_c stability during cold stress compared to a placebo, the participants visited the laboratory on five occasions, each separated by at least seven days. On visit 1, participants arrived fasted (overnight) and their body fat was assessed via air displacement plethysmography (Bod Pod, 2000A, Birmingham, UK). The body fat reading from this test was used to determine the participant's dose of acetaminophen received in the experimental trials. Visits 2-5 (experimental trials) were randomised (SPSS Inc., Chicago, USA), double blinded (drug only), and followed a repeated measures design. On these visits, participants were exposed to either cold [10°C, 40% relative humidity (r.h)] or thermo-neutral (25°C, 40% r.h.) environmental temperatures for 120 minutes, having been administered acetaminophen (20 mg/kg of lean body mass) or a placebo (dextrose). Acetaminophen (Paracetamol, Aspar Pharmaceuticals, London, UK) was administered via the oral route. The placebo was matched in terms of appearance i.e. the same number of capsules were provided to the participants. The average dose of acetaminophen administered in the present work was 1,287 ± 173 mg (range, 1,082 to 1,486 mg).

*****please insert Figure 1 near here*****

1.2.6 Experimental Protocol

All participants arrived at the laboratory at 10:00. Upon arrival, participants were instrumented for the measurement of T_c, skin temperature (T_{sk}), and heart rate (see "Instrumentation and Equations" for details). Thirty minutes after arrival participants consumed a standardised breakfast [cornflakes (50 g), milk (250 ml) and 1 litre of tap water] and ingested acetaminophen or a placebo one hour after the meal was consumed. Participants remained rested in an upright, seated position between meal consumption and acetaminophen or placebo ingestion to ensure resting physiological status was attained. Participants were wheeled into the environmental chamber immediately following drug administration, and remained in the seated position for the duration of the protocol. Clothing was shorts and calf length socks, representing a Clo value of ~0.1. Resting measurements of T_c, T_{sk}, heart rate and thermal sensation were collected five minutes prior to acetaminophen and placebo ingestion, and subsequently every 10 minutes for 120 minutes' post-ingestion. Blood pressure was measured prior to chamber entry and every 30 minutes (pre-ingestion, 30, 60, 90, 120 minutes post-ingestion) until the end of the trial. Data in Tables 1 and 2 provide the mean and range for each variable (T_c, T_{sk}, heart rate, and MAP) at 30-minute intervals.

1.2.7 Instrumentation and Equations

T_c was measured via insertion of a rectal thermistor (Henleys Medical Supplies, 400H/4491H, Hertfordshire, UK) 10 cm beyond the anal sphincter. The thermistor was connected via cable to a portable data logger (Libra Medical, ET402, Birmingham, UK), in which T_c was continuously displayed throughout each experimental protocol. This was only visible to the researchers, not the participants.

Copper based thermocouples (Grant, EUS-U-VS5-0, Dorset, UK) connected to a wireless data logger (Grant, Squirrel Series, Dorset, UK) recorded T_{sk} at four sites: calf, thigh, chest, and triceps [27]. Thermocouples were securely attached to the belly of each muscle by hypafix surgical adhesive tape (BSN medical, D-22771, Hamburg, Germany). The weighted T_{sk} of four sites was subsequently calculated using the equation below [27]:

$$\text{Mean } T_{sk} = 0.3\times(T_{arm} + T_{chest}) + 0.2\times(T_{calf} + T_{thigh})$$

Thermal sensation was obtained using a 0 to 8 scale ranging from unbearably cold (0) to unbearably hot (8). Heart rate was measured during all tests using short-range telemetry (Polar, FS1, Warwick, UK), and was expressed as beats per minute (b/min).

Blood pressure was measured using a portable blood pressure monitor (Omron M5-1, Omron, Milton Keynes, UK). Measurements were taken at baseline (pre), and every 30 minutes of the 120-minute exposure period. Mean arterial pressure (MAP) was later calculated as [(2 × DBP) + SBP]/3 mmHg.

1.2.8 Statistical Analysis

All statistical analyses were performed using the *'nlme', 'ordinal', 'ez', 'sjPlot'* and *'stats'* packages in *R* (version 3.3.2). Normality assumptions were checked using quantile-quantile plots [28] and were plausible in all instances. Central tendency and dispersion are reported as means ± standard deviation (SD). The Akaike information criteria (AIC) was used to determine model fit [29]. The correlation structure with the lowest AIC was chosen based on this procedure. A linear mixed model with fixed ('drug', 'time') and random ('subject i.d') effects was fitted with an autoregressive correlation structure (to account for autocorrelation) to examine the effect of acetaminophen on T_c, T_{sk}, and heart rate in thermo-neutral and cold conditions [Time (13 levels): pre, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120 minutes × Drug (2 levels): placebo, acetaminophen]. The same model with different levels of time [Time (5 levels): pre, 30, 60, 90, 120 minutes) × Drug (2 levels): placebo, acetaminophen] was fitted to determine the effect of acetaminophen on MAP in thermo-neutral and cold conditions. A cumulative link model was used to compare thermal sensation scores between placebo and acetaminophen in the thermo-neutral and cold conditions. The two-tailed alpha level of significance testing was set as $p \leq 0.05$. 95% confidence intervals (CI) are presented to denote the imprecision of the point estimate.

1.3 RESULTS

1.3.1 Thermo-neutral

There was no main effect for drug or interaction effect (drug × time) for T_c, T_{sk}, heart rate, TSS, or MAP. A main effect for time was present in each of these variables apart from MAP, showing that T_c, T_{sk}, heart rate and TSS changed ($p < 0.05$) over time with no differences observed between acetaminophen and placebo. Descriptive (mean ± SD) data for each 30-minute interval is shown in Table 1.

1.3.2 Cold

The T_c response during cold exposure differed between the acetaminophen and placebo conditions. An interaction effect ($F_{1,12} = 2.25$, $p = 0.01$), main effect for drug ($F_{1,2} = 2.25$, $p < 0.01$), and main effect for time ($F_{1,12} = 8.33$, $p < 0.01$) was found between placebo (37.06 ± 0.20°C; 95% CI = 36.99 to 37.12°C) and acetaminophen (36.90 ± 0.32°C; 95% CI = 36.79 to 37.01°C). Specifically, T_c was 0.18, 0.19, 0.22, 0.27, 0.29 and 0.35°C lower in the acetaminophen trial at time points 70 to 120 minutes compared with the placebo. The peak T_c reduction in the nine participants (120 minute compared with baseline) was 0.16 to 0.57°C (mean = 0.40 ± 0.15°C). Mean and individual T_c responses over the 120-minute exposure period are displayed in Figures 2 and 3 respectively.

There were no main effects for drug or interaction effect between drug and time for T_{sk}, heart rate, TSS, or MAP. A main effect for time was present in each of these variables excluding MAP. All descriptive data for each30 minute interval is shown in Table 2. For T_c, Table 3 displays the model's fixed effects coefficients and random effect variances.

*****please insert Table 1 & 2 near here*****

*****please insert Figure 2 near here*****

*****please insert Figure 3 near here*****

*****please insert Table 3 near here*****

1.4 DISCUSSION

It was hypothesised that acetaminophen would reduce T_c in cold conditions, but have no effect on T_c in thermo-neutral conditions relative to a placebo. The experimental hypothesis was accepted. The major finding of the present study was that, compared with a placebo, acetaminophen administration reduced T_c (0.16 to 0.57°C decrease after 120 minutes exposure) during an acute cold stress (10°C), while it appeared to have no effect on thermoregulation at a thermo-neutral ambient temperature (25°C). During cold exposure, acetaminophen caused T_c to fall by ~0.40°C compared with the baseline value at 120 minutes, while it did not decline in the placebo trial. The variability in the response may be due to between subject differences in the rate of acetaminophen absorption, but unfortunately this was not analysed in this trial. The hypothermic response to acetaminophen ingestion observed in the current study corroborates our prior work in humans, in which acetaminophen reduced T_c by ~0.19°C in humans exposed to mild cooling [13]. Furthermore, this is the first study to demonstrate that the ambient temperature can dictate the degree of hypothermia induced by acetaminophen. During cold exposure, this trial shows that healthy young adults could not defend their T_c following acetaminophen administration (Figure 2). Given that elderly individuals already struggle to defend their T_c without prior drug ingestion [20], it is reasonable to suspect that acetaminophen would cause T_c to decline at a faster rate, increasing the risk of accidental hypothermia.

The notion that ambient and skin temperature dictates the magnitude of acetaminophen's hypothermic action is in line with previous research. In a recent experiment, acetaminophen (20 mg/kg) had no effect on sweat output and T_c during 1-hour exercise in hot conditions (34°C, 52% r.h.) at a fixed rate of heat production (8 W/kg) [30]. In that study, the mean skin temperature increased by 1°C during the trial (up to ~35°C), a condition in which no heat producing mechanisms will be active [31, 32]. Because the mean skin temperature during cold stress was ~24°C at the end of the trial (Figure 2), cutaneous vasoconstriction and active thermogenesis were required for T_c to remain stable [33, 34]. The presence of thermogenesis and vasoconstriction indicates that acetaminophen may reduce T_c through inhibition of at least one of these mechanisms, but the precise mechanism needs to be confirmed in future work. Previous data demonstrated that acetaminophen reduced T_c by 0.10 to 0.39°C (mean ± SD, 0.19 ± 0.09°C) at rest when the mean skin temperature was ~27°C [13]. Similar reductions in skin temperature

induce shivering thermogenesis [33], which, if inhibited by acetaminophen, may explain the small reduction in T_c seen previously [13].

Studies in mice have shown T_c fell by 0.40, 0.80, and 2°C following 1-hour acetaminophen infusion of 100, 200, and 300 mg/kg body mass respectively [14]. Thus, acetaminophen-induced hypothermia is not only dependent on ambient temperature, but also on the dose administered. It is important to note here that mice are often housed in environments of 18 to 20°C, which is 8 to 10°C beneath their normal thermo-neutral zone [35]. These housing conditions are consistent in experiments concerning acetaminophen-induced hypothermia in rodents [6, 8, 14], such that these animals constantly produce heat to maintain their T_c. Inhibition of this heat production through acetaminophen may explain its hypothermic action, a notion that should be confirmed through the administration of high dose acetaminophen in mice housed within and below their thermo-neutral zone (i.e. 30°C and 20°C, respectively).

It is possible that the acetaminophen-induced reduction in T_c observed in the present study was due to inhibition of cyclooxygenase (COX). There are two COX isoforms (COX-1 and -2), and their function is to convert arachidonic acid to prostaglandin (PG) H_2 [36], which cell-specific isomerases and synthases then convert to prostanoids [(PGE_2, PGF_2, PGD_2, PGI_2, and thromboxane A_2 (TXA_2)]. The strongest evidence that acetaminophen-induced hypothermia is mediated through COX inhibition was provided by Ayoub and colleagues [14], who demonstrated that acetaminophen reduced T_c by 4°C in wild-type mice, but by only 1.5°C in a COX-$1^{-/-}$ strain. In addition, they showed a strong relationship between brain PGE_2 concentrations and T_c, where the maximum reduction in T_c was met with a 96% reduction in brain PGE_2. Data supporting a role for a COX-1 splice variant (COX-1b) in the hypothermic effect of acetaminophen is equivocal. While infusion of putative COX-1b inhibitors aminopyrene and antipyrene exert a similar hypothermic effect to acetaminophen [14, 37], genetic studies suggest that the human *COX-1b* gene produces a non-functional protein because it retains intron-1 [38]. Even when this was corrected via site-directed mutagenesis, acetaminophen did not inhibit COX-1b activity [39]. Taken together, these studies suggest that COX-1 mediated PGE_2 production may be required for normal T_c maintenance in mammals housed in sub-neutral ambient temperatures, while COX-1b is unlikely to be involved. If this were true, similar hypothermic responses would be expected with non-selective COX inhibitors Ibuprofen and

Aspirin, or SC-560, a COX-1 specific inhibitor. Whether these drugs initiate a loss of T_c control during cold exposure has not yet been determined.

Given acetaminophen reduced T_c stability in healthy adult males (Figure 2), its hypothermic effect is likely to be larger in populations already considered vulnerable in sub-neutral ambient temperatures (i.e. the very young and the elderly). Accidental hypothermia is a rising global health concern. In the USA, the Centre for Disease Control and Prevention report that hypothermia was the cause of nearly 17,000 deaths from 1999 to 2011 [40]. In the UK, hospital episode statistics show that there were over 108,000 admissions to NHS hospitals from 2005 to 2014, where hypothermia was the primary or secondary cause [4]. This database also shows that the very young (0-4 years; 43,868 admissions) and the elderly (≥ 65 years; 48,477 admissions) make up 85% of the total admissions. This is concerning for two reasons. Firstly, acetaminophen is the most frequently administered analgesic among frail and pre-frail elderly individuals [41], with no age-related delay in drug absorption [42]. Secondly, acetaminophen is commonly used for neonatal pain management [43]. In the perioperative setting, T_c monitoring after acetaminophen administration in these vulnerable groups is recommended. A 2011 study showed that intravenous acetaminophen (~20 mg/kg) did not cause hypothermia in 93 neonates [44]. However, the ambient temperature was not reported (presumably 23-25°C), and only the skin temperature was measured. This is problematic since our work showed a clear reduction in T_c without a change in skin temperature between acetaminophen and placebo [13]. Moreover, neonates are exposed to cold stress when wet with amniotic fluid, during transportation, or during surgery. Based on our data, we propose that acetaminophen may increase the risk of neonatal hypothermia only when coupled with one of these cold stressors, and not in a thermo-neutral environment.

1.4.1 Limitations

This study has limitations that should be considered in future work. Firstly, we did not measure metabolic heat production or cutaneous blood flow, key parameters that control T_c during cold stress. Although a reduction in T_c from resting value is the primary variable of interest from a medical standpoint, it is still unknown what aspect of the thermoregulatory system acetaminophen targets to exert this effect. Measuring metabolic heat production and changes in cutaneous blood flow in future studies of a similar design may help to elucidate the mechanism

that regulates acetaminophen's hypothermic action. Secondly, no pharmacokinetic parameters are reported in this experiment. Disparity in the plasma concentration of acetaminophen throughout each trial may have explained the between subject variability in the hypothermic response elicited by acetaminophen i.e. a low plasma concentration may result in a reduced hypothermic response. We administered a dose relative to lean body mass to reduce the variability in acetaminophen absorption, and our previous experiment showed that a 20 mg/kg lean body mass dose was appropriate for therapeutic plasma concentrations to be reached within the 120-minute exposure period [13].

1.4.2 Conclusions

In conclusion, this preliminary trial demonstrated that acute acetaminophen ingestion (20 mg/kg lean body mass) reduced T_c maintenance during acute cold exposure in healthy young adults. We are the first to show that the hypothermic action of acetaminophen is strongly influenced by the ambient temperature in which it is administered. Future research should determine if this effect is amplified in new-borns and in elderly individuals, placing them at risk of accidental hypothermia. It should also be determined if hypothermic effects are limited to acetaminophen, or are present in COX inhibitors such as Ibuprofen and Aspirin (non-selective COX inhibitors), or COXIBs (COX-2 selective inhibitors). If all COX inhibitors induce hypothermia during cold exposure, the prescription of these medications to individuals vulnerable to hypothermia should be carefully considered during cold spells and in the perioperative period.

Additional information

Competing interests

The authors declare they have no competing interests.

Author contributions

JF, LT, and ARM contributed to the conception and design of the study. JF, LT, DH, AG and JH contributed to data interpretation and manuscript revision. JF collected the data. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors approved the final version of the manuscript and all authors qualifying for authorship are listed.

Acknowledgements

The authors thank Miss Katie Thomasson and Mr Jack Field for their assistance with data collection. The authors also thank those who participated in this experiment.

Funding

None declared

Figure Captions:

Fig 1. A flowchart of the study design. APAP = Acetaminophen. Visits 2-5 completed in a randomised order for each participant. Visits separated by 1-week and drug administration double blinded.

Fig 2. Mean and SD of the T_c (A, C) and T_{sk} (B, D) response during the 120-minute exposure to 25°C (left panel i.e. A, B) and 10°C (right panel i.e. C, D). The triangles and squares represent the placebo and acetaminophen trials, respectively. ∗ Main effect for condition. # Main effect for time. † Overall interaction effect. 'a' Interaction effect at a specific time point. Significance set at $p < 0.05$.

Fig 3. Change in T_c during cold exposure in each participant following administration of a placebo (A) or acetaminophen (B).

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Table 1. Descriptive data for each of the five response variables in the thermo-neutral condition (25°C). Descriptive data are the mean values (± standard deviation) during the 120-minute exposure period. The range is provided in parentheses.

		Time-point (minutes)				
		Pre	**30**	**60**	**90**	**120**
T_c (°C)	**Placebo**	37.00 ± 0.13 (36.80 - 37.15)	36.93 ± 0.15 (36.72 - 37.13)	36.95 ± 0.15 (36.73 - 37.15)	36.94 ± 0.14 (36.75 - 37.15)	36.94 ± 0.16 (36.74 - 37.21)
	Acetaminophen	37.04 ± 0.20 (36.78 - 37.25)	36.95 ± 0.22 (36.78 - 37.14)	36.93 ± 0.21 (36.77 - 37.05)	36.91 ± 0.23 (36.68 - 37.10)	36.89 ± 0.19 (36.62 - 37.10)
T_{sk} (°C)	**Placebo**	30.6 ± 0.9 (28.7 - 31.8)	30.9 ± 0.7 (29.9 - 31.9)	30.8 ± 0.7 (29.8 - 31.7)	30.7 ± 0.7 (29.5 - 31.8)	30.7 ± 0.7 (29.3 - 31.7)
	Acetaminophen	30.3 ± 0.6 (29.0 - 31.1)	30.8 ± 0.5 (29.9 - 31.4)	30.7 ± 0.4 (29.9 - 31.2)	30.7 ± 0.5 (29.9 - 31.5)	30.6 ± 0.6 (29.6 - 31.6)
HR (b/min)	**Placebo**	65 ± 8 (53 - 79)	59 ± 8 (50 - 76)	58 ± 10 (46 - 79)	58 ± 9 (48 - 74)	60 ± 10 (49 - 86)
	Acetaminophen	68 ± 8 (53 - 81)	62 ± 10 (49 - 80)	65 ± 10 (50 - 84)	59 ± 7 (49 – 68)	59 ± 10 (42 - 71)
TS (0 to 8 scale)	**Placebo**	4.0 ± 0.1 (4.0 - 4.5)	4.1 ± 0.3 (4.0 - 5.0)	4.2 ± 0.4 (4.0 - 5.0)	4.3 ± 0.4 (4.0 - 5.0)	4.4 ± 0.6 (4.0 - 5.5)
	Acetaminophen	4.0 ± 0.2 (3.5 - 4.5)	4.2 ± 0.3 (4.0 - 5.0)	4.3 ± 0.4 (4.0 - 5.0)	4.4 ± 0.4 (4.0 - 5.0)	4.6 ± 0.5 (4.0 - 5.0)
MAP (mmHg)	**Placebo**	91 ± 7 (83 - 103)	91 ± 9 (73 - 101)	91 ± 10 (81 - 113)	92 ± 4 (88 - 99)	90 ± 6 (82 - 99)
	Acetaminophen	88 ± 6 (80 - 97)	91 ± 6 (82 - 100)	88 ± 9 (78 - 111)	88 ± 5 (83 - 97)	91 ± 6 (85 - 104)

Core temperature (T_c), Skin temperature (T_{sk}), Heart rate (HR), Thermal sensation (TS), Mean arterial pressure (MAP)

Table 2. Descriptive data for each of the five response variables in the cold condition (10°C). Descriptive data are the mean values (± standard deviation) during the 120-minute exposure period. The range is provided in parentheses.

		Time-point (minutes)				
		Pre	**30**	**60**	**90**	**120**
T_c (°C)	**Placebo**	36.98 ± 0.20 (36.70 - 37.13)	37.09 ± 0.19 (36.79 - 37.38)	37.03 ± 0.22 (36.72 - 37.34)	36.97 ± 0.23 (36.71 - 37.29)	36.96 ± 0.25 (36.64 - 37.19)
	Acetaminophen	36.97 ± 0.21 (36.61 - 37.36)	37.05 ± 0.26 (36.59 - 37.49)	36.94 ± 0.31 (36.52 - 37.45)	36.76 ± 0.30* (36.33 - 37.29)	36.58 ± 0.23* (36.11 - 36.87)
T_{sk} (°C)	**Placebo**	30.5 ± 0.5 (29.6 - 31.3)	25.8 ± 1.0 (24.7 - 27.6)	24.9 ± 1.0 (23.8 - 26.9)	24.4 ± 1.0 (23.2 - 26.5)	24.2 ± 1.0 (22.8 - 26.6)
	Acetaminophen	30.7 ± 0.7 (29.6 - 31.8)	26.1 ± 1.0 (24.7 - 28.2)	25.1 ± 1.0 (23.7 - 26.6)	24.5 ± 1.2 (23.0 - 26.3)	24.3 ± 1.3 (22.4 - 26.5)
HR (b/min)	**Placebo**	68 ± 7 (54 - 79)	62 ± 9 (48 - 74)	61 ± 4 (55 - 67)	57 ± 8 (48 - 68)	60 ± 9 (51 - 75)
	Acetaminophen	66 ± 11 (50 - 79)	59 ± 9 (41 - 70)	58 ± 10 (39 - 73)	54 ± 7 (42 - 64)	57 ± 9 (41 - 70)
TS (0 to 8 scale)	**Placebo**	4.1 ± 0.2 (4.0 - 4.5)	2.8 ± 0.4 (2.0 - 3.0)	2.3 ± 0.5 (1.5 - 3.0)	1.9 ± 0.2 (1.5 - 2.0)	1.8 ± 0.4 (1.0 - 2.0)
	Acetaminophen	3.9 ± 0.2 (3.5 - 4.0)	2.3 ± 0.4 (2.0 - 3.0)	2.2 ± 0.4 (1.5 - 3.0)	1.8 ± 0.6 (1.0 - 3.0)	1.7 ± 0.5 (1.0 - 2.5)
MAP (mmHg)	**Placebo**	92 ± 10 (78 - 104)	97 ± 9 (86 - 112)	99 ± 8 (90 - 110)	97 ± 7 (88 - 111)	105 ± 8 (92 - 117)
	Acetaminophen	93 ± 6 (78 - 102)	94 ± 9 (74 - 102)	103 ± 7 (91 - 111)	96 ± 6 (88 - 104)	99 ± 6 (77 - 104)

Core temperature (T_c), Skin temperature (T_{sk}), Heart rate (HR), Thermal sensation (TS), Mean arterial pressure (MAP). * denotes significant difference between the APAP and placebo condition ($p < 0.05$).

Table 3. Beta coefficients (*B*), 95 % confidence intervals (CI), alpha values (*p)*, and the Phi coefficient are reported for the fixed components (drug & time) during exposure to cold stress (10ºC). The standard deviation of the intercept and residual are reported for the random effect (subject ID). Bold values indicate a significant interaction effect between DRUG and TIME ($p < 0.05$).

	Core Temperature (ºC)		
	B	*CI*	*p*
Fixed Parts			
Intercept	36.95	36.71 to 37.13	**<.001**
Drug×Time Interaction			
DRUG:TIME10	0.03	-0.11 to 0.17	.694
DRUG:TIME20	-0.03	-0.17 to 0.11	.672
DRUG:TIME30	-0.06	-0.20 to 0.09	.442
DRUG:TIME40	-0.10	-0.24 to 0.04	.179
DRUG:TIME50	-0.12	-0.26 to 0.02	.109
DRUG:TIME60	-0.13	-0.28 to 0.01	.076
DRUG:TIME70	-0.18	-0.32 to -0.03	**.021**
DRUG:TIME80	-0.21	-0.36 to -0.07	**.006**
DRUG:TIME90	-0.24	-0.38 to -0.10	**.002**
DRUG:TIME100	-0.29	-0.43 to -0.15	**<.001**
DRUG:TIME110	-0.31	-0.45 to -0.17	**<.001**
DRUG:TIME120	-0.36	-0.50 to -0.22	**<.001**
Phi Coefficient			
0.938			
Random Parts (Subject ID)			
		Standard Deviation	
Intercept		0.13	
Residual		0.16	