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Foster, Josh and Mauger, Alexis R. and Govus, Andrew and Hewson, David and Taylor, Lee (2017) Acetaminophen (Paracetamol) Induces Hypothermia During Acute Cold Stress. *Clinical drug investigation* . pp. 1-11. ISSN 1179-1918.

DOI

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

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3 [x/fulltext.html](https://link.springer.com/article/10.1007/s40261-017-0560-x/fulltext.html)

4

5 For citations:

6 Foster J, Mauger AR, Govus A, Hewson D, Taylor L. Acetaminophen (Paracetamol) Induces
7 Hypothermia During Acute Cold Stress. Clin Drug Investig. 2017, doi: 10.1007/s40261-017-0560-x.

8

9 **Acetaminophen (paracetamol) induces hypothermia during** 10 **acute cold stress.**

11

12 **Article Type:** Original Research

13 **Running Title:** Acetaminophen administration and hypothermia

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26 **Word Count:** 3,498

27 **Reference Count:** 42

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

38 **KEY POINTS**

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

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

47

48 **ABSTRACT**

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

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

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

63 Results: Acetaminophen had no effect on T_c in a thermo-neutral environment, but significantly
64 reduced T_c during cold exposure, compared with a placebo. T_c was lower in the acetaminophen
65 compared with the placebo condition at each 10-minute interval from 80 to 120-minutes into the
66 trial (all $p < 0.05$). On average, T_c decreased by $0.42 \pm 0.13^\circ\text{C}$ from baseline after 120 minutes of
67 cold exposure (range 0.16 to 0.57°C), whereas there was no change in the placebo group ($0.01 \pm$
68 0.1°C). T_{sk} , heart rate, thermal sensation, and mean arterial pressure were not different between
69 conditions ($p > 0.05$).

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

74

75 **Key Words:** acetaminophen, paracetamol, thermoregulation, cold exposure, thermogenesis,
76 hypothermia

77

78 **1.1 INTRODUCTION**

79 Accidental hypothermia is characterised by an unintended core temperature (T_c) reduction to
80 35°C or lower. Such a fall in T_c can induce ventricular fibrillation and ultimately cardiac arrest if
81 T_c declines to $< 28^\circ\text{C}$ [1, 2]. In the United States, hypothermia was the cause or contributing
82 cause of death in over 5500 cases between 2006 and 2010 [3], but this is likely underestimated
83 since T_c needs to be measured at or near the time of death. Nonetheless, data from United
84 Kingdom hospital episode statistics indicate that hypothermia was the primary or secondary
85 diagnosis in over 100,000 hospital admissions from 2005 to 2015 [4]. Although death from
86 hypothermia is rare, it remains a significant health risk in elderly and very young individuals,
87 particularly during winter months and unaccustomed cold spells [1]. Interestingly, there is a
88 growing body of evidence demonstrating that acetaminophen could reduce T_c stability during
89 cold exposure (discussed below), placing users at an increased risk of accidental hypothermia.

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

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

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

128 **1.2 METHODS**

129 **1.2.1 Ethical Approval**

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

133 **1.2.2 Sample Size Calculation**

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

142 **1.2.3 Participants**

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

149 **1.2.4 Inclusion & Exclusion Criteria**

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

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

163 **1.2.5 Experimental Design**

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

178

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

180

181 **1.2.6 Experimental Protocol**

182 All participants arrived at the laboratory at 10:00. Upon arrival, participants were instrumented
183 for the measurement of T_c , skin temperature (T_{sk}), and heart rate (see “Instrumentation and
184 Equations” for details). Thirty minutes after arrival participants consumed a standardised
185 breakfast [cornflakes (50 g), milk (250 ml) and 1 litre of tap water] and ingested acetaminophen
186 or a placebo one hour after the meal was consumed. Participants remained rested in an upright,
187 seated position between meal consumption and acetaminophen or placebo ingestion to ensure
188 resting physiological status was attained. Participants were wheeled into the environmental
189 chamber immediately following drug administration, and remained in the seated position for the
190 duration of the protocol. Clothing was shorts and calf length socks, representing a Clo value of
191 ~ 0.1 . Resting measurements of T_c , T_{sk} , heart rate and thermal sensation were collected five
192 minutes prior to acetaminophen and placebo ingestion, and subsequently every 10 minutes for
193 120 minutes’ post-ingestion. Blood pressure was measured prior to chamber entry and every 30
194 minutes (pre-ingestion, 30, 60, 90, 120 minutes post-ingestion) until the end of the trial. Data in
195 Tables 1 and 2 provide the mean and range for each variable (T_c , T_{sk} , heart rate, and MAP) at 30-
196 minute intervals.

197 **1.2.7 Instrumentation and Equations**

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

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

208

$$209 \text{ Mean } T_{sk} = 0.3 \times (T_{\text{arm}} + T_{\text{chest}}) + 0.2 \times (T_{\text{calf}} + T_{\text{thigh}})$$

210

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

214 Blood pressure was measured using a portable blood pressure monitor (Omron M5-1, Omron,
215 Milton Keynes, UK). Measurements were taken at baseline (pre), and every 30 minutes of the
216 120-minute exposure period. Mean arterial pressure (MAP) was later calculated as $[(2 \times \text{DBP}) +$
217 $\text{SBP}]/3$ mmHg.

218 **1.2.8 Statistical Analysis**

219 All statistical analyses were performed using the ‘*nlme*’, ‘*ordinal*’, ‘*ez*’, ‘*sjPlot*’ and ‘*stats*’
220 packages in R version 3.3.2 (R Core Development Team 2014). Normality assumptions were
221 checked using quantile-quantile plots [28] and were plausible in all instances. Central tendency
222 and dispersion are reported as means \pm standard deviation (SD). The Akaike information criteria
223 (AIC) was used to determine model fit [29]. The correlation structure with the lowest AIC was
224 chosen based on this procedure. A linear mixed model with fixed (‘drug’, ‘time’) and random
225 (‘subject i.d’) effects was fitted with an autoregressive correlation structure (to account for
226 autocorrelation) to examine the effect of acetaminophen on T_c , T_{sk} , and heart rate in thermo-
227 neutral and cold conditions [Time (13 levels): pre, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110,
228 120 minutes \times Drug (2 levels): placebo, acetaminophen]. The same model with different levels
229 of time [Time (5 levels): pre, 30, 60, 90, 120 minutes) \times Drug (2 levels): placebo,
230 acetaminophen] was fitted to determine the effect of acetaminophen on MAP in thermo-neutral
231 and cold conditions. A cumulative link model was used to compare thermal sensation scores
232 between placebo and acetaminophen in the thermo-neutral and cold conditions. The two-tailed
233 alpha level of significance testing was set as $p \leq 0.05$. 95% confidence intervals (CI) are
234 presented to denote the imprecision of the point estimate.

235 1.3 RESULTS

236 1.3.1 Thermo-neutral

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

242 1.3.2 Cold

243 The T_c response during cold exposure differed between the acetaminophen and placebo
244 conditions. An interaction effect ($F_{1,12} = 2.25$, $p = 0.01$), main effect for drug ($F_{1,2} = 2.25$, $p <$
245 0.01), and main effect for time ($F_{1,12} = 8.33$, $p < 0.01$) was found between placebo ($37.06 \pm$
246 0.20°C ; 95% CI = 36.99 to 37.12°C) and acetaminophen ($36.90 \pm 0.32^\circ\text{C}$; 95% CI = 36.79 to
247 37.01°C). Specifically, T_c was 0.18, 0.19, 0.22, 0.27, 0.29 and 0.35°C lower in the
248 acetaminophen trial at time points 70 to 120 minutes compared with the placebo. The peak T_c
249 reduction in the nine participants (120 minute compared with baseline) was 0.16 to 0.57°C
250 (mean = $0.40 \pm 0.15^\circ\text{C}$). Mean and individual T_c responses over the 120-minute exposure period
251 are displayed in Figures 2 and 3 respectively.

252 There were no main effects for drug or interaction effect between drug and time for T_{sk} , heart
253 rate, TSS, or MAP. A main effect for time was present in each of these variables excluding
254 MAP. All descriptive data for each 30 minute interval is shown in Table 2. For T_c , Table 3
255 displays the model's fixed effects coefficients and random effect variances.

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

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

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

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

260

261 1.4 DISCUSSION

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

279 The notion that ambient and skin temperature dictates the magnitude of acetaminophen's
280 hypothermic action is in line with previous research. In a recent experiment, acetaminophen (20
281 mg/kg) had no effect on sweat output and T_c during 1-hour exercise in hot conditions (34°C, 52%
282 r.h.) at a fixed rate of heat production (8 W/kg) [30]. In that study, the mean skin temperature
283 increased by 1°C during the trial (up to ~35°C), a condition in which no heat producing
284 mechanisms will be active [31, 32]. Because the mean skin temperature during cold stress was
285 ~24°C at the end of the trial (Figure 2), cutaneous vasoconstriction and active thermogenesis
286 were required for T_c to remain stable [33, 34]. The presence of thermogenesis and
287 vasoconstriction indicates that acetaminophen may reduce T_c through inhibition of at least one of
288 these mechanisms, but the precise mechanism needs to be confirmed in future work. Previous
289 data demonstrated that acetaminophen reduced T_c by 0.10 to 0.39°C (mean \pm SD, 0.19 \pm 0.09°C)
290 at rest when the mean skin temperature was ~27°C [13]. Similar reductions in skin temperature

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

293 Studies in mice have shown T_c fell by 0.40, 0.80, and 2°C following 1-hour acetaminophen
294 infusion of 100, 200, and 300 mg/kg body mass respectively [14]. Thus, acetaminophen-induced
295 hypothermia is not only dependent on ambient temperature, but also on the dose administered. It
296 is important to note here that mice are often housed in environments of 18 to 20°C, which is 8 to
297 10°C beneath their normal thermo-neutral zone [35]. These housing conditions are consistent in
298 experiments concerning acetaminophen-induced hypothermia in rodents [6, 8, 14], such that
299 these animals constantly produce heat to maintain their T_c . Inhibition of this heat production
300 through acetaminophen may explain its hypothermic action, a notion that should be confirmed
301 through the administration of high dose acetaminophen in mice housed within and below their
302 thermo-neutral zone (i.e. 30°C and 20°C, respectively).

303 It is possible that the acetaminophen-induced reduction in T_c observed in the present study was
304 due to inhibition of cyclooxygenase (COX). There are two COX isoforms (COX-1 and -2), and
305 their function is to convert arachidonic acid to prostaglandin (PG) H_2 [36], which cell-specific
306 isomerases and synthases then convert to prostanoids [(PGE₂, PGF₂, PGD₂, PGI₂, and
307 thromboxane A₂ (TXA₂)]. The strongest evidence that acetaminophen-induced hypothermia is
308 mediated through COX inhibition was provided by Ayoub and colleagues [14], who
309 demonstrated that acetaminophen reduced T_c by 4°C in wild-type mice, but by only 1.5°C in a
310 COX-1^{-/-} strain. In addition, they showed a strong relationship between brain PGE₂
311 concentrations and T_c , where the maximum reduction in T_c was met with a 96% reduction in
312 brain PGE₂. Data supporting a role for a COX-1 splice variant (COX-1b) in the hypothermic
313 effect of acetaminophen is equivocal. While infusion of putative COX-1b inhibitors aminopyrene
314 and antipyrene exert a similar hypothermic effect to acetaminophen [14, 37], genetic studies
315 suggest that the human COX-1b gene produces a non-functional protein because it retains intron-
316 1 [38]. Even when this was corrected via site-directed mutagenesis, acetaminophen did not
317 inhibit COX-1b activity [39]. Taken together, these studies suggest that COX-1 mediated PGE₂
318 production may be required for normal T_c maintenance in mammals housed in sub-neutral
319 ambient temperatures, while COX-1b is unlikely to be involved. If this were true, similar
320 hypothermic responses would be expected with non-selective COX inhibitors Ibuprofen and

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

323 Given acetaminophen reduced T_c stability in healthy adult males (Figure 2), its hypothermic
324 effect is likely to be larger in populations already considered vulnerable in sub-neutral ambient
325 temperatures (i.e. the very young and the elderly). Accidental hypothermia is a rising global
326 health concern. In the USA, the Centre for Disease Control and Prevention report that
327 hypothermia was the cause of nearly 17,000 deaths from 1999 to 2011 [40]. In the UK, hospital
328 episode statistics show that there were over 108,000 admissions to NHS hospitals from 2005 to
329 2014, where hypothermia was the primary or secondary cause [4]. This database also shows that
330 the very young (0-4 years; 43,868 admissions) and the elderly (≥ 65 years; 48,477 admissions)
331 make up 85% of the total admissions. This is concerning for two reasons. Firstly, acetaminophen
332 is the most frequently administered analgesic among frail and pre-frail elderly individuals [41],
333 with no age-related delay in drug absorption [42]. Secondly, acetaminophen is commonly used
334 for neonatal pain management [43]. In the perioperative setting, T_c monitoring after
335 acetaminophen administration in these vulnerable groups is recommended. A 2011 study showed
336 that intravenous acetaminophen (~ 20 mg/kg) did not cause hypothermia in 93 neonates [44].
337 However, the ambient temperature was not reported (presumably 23-25°C), and only the skin
338 temperature was measured. This is problematic since our work showed a clear reduction in T_c
339 without a change in skin temperature between acetaminophen and placebo [13]. Moreover,
340 neonates are exposed to cold stress when wet with amniotic fluid, during transportation, or
341 during surgery. Based on our data, we propose that acetaminophen may increase the risk of
342 neonatal hypothermia only when coupled with one of these cold stressors, and not in a thermo-
343 neutral environment.

344 **1.4.1 Limitations**

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

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

359 **1.4.2 Conclusions**

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

370

371 **Additional information**

372 **Competing interests**

373 The authors declare they have no competing interests.

374 **Author contributions**

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

381 **Acknowledgements**

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

384 **Funding**

385 None declared

386

387 **Figure Captions:**

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

391 **Fig 2.** Mean and SD of the T_c (A, C) and T_{sk} (B, D) response during the 120-minute exposure to
392 25°C (left panel i.e. A, B) and 10°C (right panel i.e. C, D). The triangles and squares represent the
393 placebo and acetaminophen trials, respectively. * Main effect for condition. # Main effect for time.
394 † Interaction effect. Significance set at $p < 0.05$.

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

397

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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 (\pm 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 \pm 0.13 (36.80 - 37.15)	36.93 \pm 0.15 (36.72 - 37.13)	36.95 \pm 0.15 (36.73 - 37.15)	36.94 \pm 0.14 (36.75 - 37.15)	36.94 \pm 0.16 (36.74 - 37.21)
	Acetaminophen	37.04 \pm 0.20 (36.78 - 37.25)	36.95 \pm 0.22 (36.78 - 37.14)	36.93 \pm 0.21 (36.77 - 37.05)	36.91 \pm 0.23 (36.68 - 37.10)	36.89 \pm 0.19 (36.62 - 37.10)
T_{sk} (°C)	Placebo	30.6 \pm 0.9 (28.7 - 31.8)	30.9 \pm 0.7 (29.9 - 31.9)	30.8 \pm 0.7 (29.8 - 31.7)	30.7 \pm 0.7 (29.5 - 31.8)	30.7 \pm 0.7 (29.3 - 31.7)
	Acetaminophen	30.3 \pm 0.6 (29.0 - 31.1)	30.8 \pm 0.5 (29.9 - 31.4)	30.7 \pm 0.4 (29.9 - 31.2)	30.7 \pm 0.5 (29.9 - 31.5)	30.6 \pm 0.6 (29.6 - 31.6)
HR (b/min)	Placebo	65 \pm 8 (53 - 79)	59 \pm 8 (50 - 76)	58 \pm 10 (46 - 79)	58 \pm 9 (48 - 74)	60 \pm 10 (49 - 86)
	Acetaminophen	68 \pm 8 (53 - 81)	62 \pm 10 (49 - 80)	65 \pm 10 (50 - 84)	59 \pm 7 (49 - 68)	59 \pm 10 (42 - 71)
TS (0 to 8 scale)	Placebo	4.0 \pm 0.1 (4.0 - 4.5)	4.1 \pm 0.3 (4.0 - 5.0)	4.2 \pm 0.4 (4.0 - 5.0)	4.3 \pm 0.4 (4.0 - 5.0)	4.4 \pm 0.6 (4.0 - 5.5)
	Acetaminophen	4.0 \pm 0.2 (3.5 - 4.5)	4.2 \pm 0.3 (4.0 - 5.0)	4.3 \pm 0.4 (4.0 - 5.0)	4.4 \pm 0.4 (4.0 - 5.0)	4.6 \pm 0.5 (4.0 - 5.0)
MAP (mmHg)	Placebo	91 \pm 7 (83 - 103)	91 \pm 9 (73 - 101)	91 \pm 10 (81 - 113)	92 \pm 4 (88 - 99)	90 \pm 6 (82 - 99)
	Acetaminophen	88 \pm 6 (80 - 97)	91 \pm 6 (82 - 100)	88 \pm 9 (78 - 111)	88 \pm 5 (83 - 97)	91 \pm 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 (\pm 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 \pm 0.20 (36.70 - 37.13)	37.09 \pm 0.19 (36.79 - 37.38)	37.03 \pm 0.22 (36.72 - 37.34)	36.97 \pm 0.23 (36.71 - 37.29)	36.96 \pm 0.25 (36.64 - 37.19)
	Acetaminophen	36.97 \pm 0.21 (36.61 - 37.36)	37.05 \pm 0.26 (36.59 - 37.49)	36.94 \pm 0.31 (36.52 - 37.45)	36.76 \pm 0.30* (36.33 - 37.29)	36.58 \pm 0.23* (36.11 - 36.87)
T_{sk} (°C)	Placebo	30.5 \pm 0.5 (29.6 - 31.3)	25.8 \pm 1.0 (24.7 - 27.6)	24.9 \pm 1.0 (23.8 - 26.9)	24.4 \pm 1.0 (23.2 - 26.5)	24.2 \pm 1.0 (22.8 - 26.6)
	Acetaminophen	30.7 \pm 0.7 (29.6 - 31.8)	26.1 \pm 1.0 (24.7 - 28.2)	25.1 \pm 1.0 (23.7 - 26.6)	24.5 \pm 1.2 (23.0 - 26.3)	24.3 \pm 1.3 (22.4 - 26.5)
HR (b/min)	Placebo	68 \pm 7 (54 - 79)	62 \pm 9 (48 - 74)	61 \pm 4 (55 - 67)	57 \pm 8 (48 - 68)	60 \pm 9 (51 - 75)
	Acetaminophen	66 \pm 11 (50 - 79)	59 \pm 9 (41 - 70)	58 \pm 10 (39 - 73)	54 \pm 7 (42 - 64)	57 \pm 9 (41 - 70)
TS (0 to 8 scale)	Placebo	4.1 \pm 0.2 (4.0 - 4.5)	2.8 \pm 0.4 (2.0 - 3.0)	2.3 \pm 0.5 (1.5 - 3.0)	1.9 \pm 0.2 (1.5 - 2.0)	1.8 \pm 0.4 (1.0 - 2.0)
	Acetaminophen	3.9 \pm 0.2 (3.5 - 4.0)	2.3 \pm 0.4 (2.0 - 3.0)	2.2 \pm 0.4 (1.5 - 3.0)	1.8 \pm 0.6 (1.0 - 3.0)	1.7 \pm 0.5 (1.0 - 2.5)
MAP (mmHg)	Placebo	92 \pm 10 (78 - 104)	97 \pm 9 (86 - 112)	99 \pm 8 (90 - 110)	97 \pm 7 (88 - 111)	105 \pm 8 (92 - 117)
	Acetaminophen	93 \pm 6 (78 - 102)	94 \pm 9 (74 - 102)	103 \pm 7 (91 - 111)	96 \pm 6 (88 - 104)	99 \pm 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 (10°C). The standard deviation of the intercept and residual are reported for the random effect (subject ID).

	Core Temperature (°C)		
	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		