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1 ColdZyme® Mouth Spray reduces duration of upper respiratory tract infection symptoms in  
2 endurance athletes under free living conditions.

3  
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17  
18  
19 **Author contributions**

20 GD conceived and designed the research. GD, EP, AWJ, GMS, ARJ, HR, and KD conducted the  
21 research. GD wrote the manuscript and all authors read, edited and/or approved the final  
22 manuscript.

23  
24  
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26 This study was funded by Enzymatica AB (Sweden), manufacturer of ColdZyme® Mouth Spray.

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28

29

30 **Abstract**

31 Upper respiratory tract infection (URTI) can compromise athlete preparation and performance, so  
32 countermeasures are desirable. The aim of this study was to assess the effects of ColdZyme®  
33 Mouth Spray (ColdZyme) on self-reported upper respiratory tract infection in competitive  
34 endurance athletes under free-living conditions.

35 One hundred and twenty-three endurance-trained, competitive athletes (recruited across 4 sites in  
36 England, UK) were randomised to control (no treatment, n = 61) or ColdZyme (n = 62) for a 3-  
37 month study period (between December 2017 – February 2018; or December 2018 – April 2019).  
38 They recorded daily training and illness symptoms (Jackson common cold questionnaire) during the  
39 study period.

40 A total of 130 illness episodes were reported during the study with no difference in incidence  
41 between groups (episodes per person:  $1.1 \pm 0.9$  Control,  $1.0 \pm 0.8$  ColdZyme,  $P = 0.290$ ). Episode  
42 duration was significantly shorter in ColdZyme compared to Control: Control  $10.4 \pm 8.5$  days vs  
43 ColdZyme  $7.7 \pm 4.0$  days,  $P = 0.016$ ). Further analysis to compare episodes with poor vs good  
44 compliance with ColdZyme instructions for use (IFU) within the ColdZyme group showed a further  
45 reduction in duration of URTI when compliance was good ( $9.3 \pm 4.5$  days in ColdZyme poor IFU  
46 compliance vs  $6.9 \pm 3.5$  days in ColdZyme good IFU compliance,  $P = 0.040$ ).

47 ColdZyme may be an effective countermeasure to reduce URTI duration, which was significantly  
48 lower (by 26-34%) in the ColdZyme treatment group (with no influence on incidence). This may  
49 have implications for athlete performance.

50

51 **Key words**

52 Common Cold, Illness, Training, Exercise, Immunology, Countermeasure

53

54

55

56

57 **Abbreviations**

|    |       |                                      |
|----|-------|--------------------------------------|
| 58 | ANOVA | analysis of variance                 |
| 59 | IFU   | instructions for use                 |
| 60 | OTC   | over-the-counter                     |
| 61 | PCR   | polymerase chain reaction            |
| 62 | sRPE  | session rating of perceived exertion |
| 63 | URS   | upper respiratory symptoms           |
| 64 | URT   | upper respiratory tract              |
| 65 | URTI  | upper respiratory tract infection    |

66

67 **Introduction**

68

69 The incidence of upper respiratory illnesses is higher than normal in some groups of athletes, and  
70 such infections can compromise training and/or competition performance.<sup>1</sup> Endurance athletes are  
71 often associated with a higher than normal incidence of infections, especially of the upper  
72 respiratory tract (URTI).<sup>2,3</sup> This is typically related to a high training load and/or heavy competition  
73 schedule. More recent debates have questioned whether athletes do experience a higher incidence,  
74 compared to the general adult population (i.e. one to three individual episodes of upper respiratory  
75 tract infection per year<sup>4,5</sup>). However, it is clear that reporting of upper respiratory symptoms (URS)  
76 in athletes cluster around periods of intensive training and/or competition.<sup>6,7</sup> Experiencing URTI or  
77 illness symptoms can result in a loss of training days and a performance decrement (<sup>6,8,9</sup>) so  
78 strategies to reduce the risk of contracting these illnesses may be of direct benefit to athletes. This  
79 may also limit the risks of spreading infection to others (i.e. teammates). The possible links between  
80 URTI incidence and athletic performance is highlighted by research showing World Championship  
81 and Olympic medal winning athletes reported fewer URS than less successful athletes.<sup>6,10,11</sup> This is  
82 likely related, at least in part, to the ability (and resource) to successfully implement strategies that  
83 reduce URTI risk.<sup>12,13</sup>

84

85 Strategies to minimise the risk of contracting a URTI and/or reduce time taken to clear an infection  
86 have focussed on avoidance of exposure and minimising the controllable risk factors that are  
87 associated with lowered immune defence (e.g. intensified training, life stressors), but these may be  
88 difficult to avoid for many athletes.<sup>6,14</sup> Other strategies have focussed on nutritional interventions  
89 purported to reduce the immune perturbations caused by strenuous exercise and training.  
90 Unfortunately, many such strategies have limited success.<sup>11-15</sup> An alternative strategy that has  
91 received little attention in athletic populations, is the use of products that may inhibit viral  
92 infectivity (for example, via limiting viral entry or replication/propagation after initial exposure).  
93 Most URTIs are caused by viral infection, with over 200 known viruses, the most common being  
94 rhinoviruses, coronaviruses, influenza viruses, adenoviruses, parainfluenza viruses, respiratory  
95 syncytial viruses and enteroviruses.<sup>16</sup> Infection is initially established in the mucosa of the  
96 nasopharynx before spreading anteriorly, through the nasal region (<sup>17</sup>), with local symptoms  
97 typically beginning in the throat before nasal congestion, rhinorrhoea, sneezing and cough tend to  
98 develop.<sup>18</sup>

99

100 It is possible that inhibiting viral propagation in this area during the incubation period, may prevent  
101 or shorten the duration of viral URT infection. ColdZyme® Mouth Spray (ColdZyme) consists of a  
102 hyperosmotic glycerol solution containing cold-adapted trypsin from the Atlantic cod (*Gadus*

103 *morhua*) and has been shown to reduce URTI duration in a number of studies in healthy and clinical  
104 (i.e. non-athletic) populations.<sup>19,20</sup> It is suggested that orally spraying of the solution forms a  
105 temporary barrier on the pharynx that prevents viral binding and entry. The idea that local effects in  
106 this part of the URT can successfully reduce URTI duration is also supported by other studies  
107 showing effective protection against the common cold with substances administered orally (e.g.  
108 zinc lozenges<sup>21</sup>). ColdZyme spray solution has demonstrated broad antiviral activity *in vitro*,  
109 deactivating 64-100% of virus activity for common URTI-causing pathogens (influenza virus,  
110 rhinovirus, adenovirus and coronavirus).<sup>22</sup> Clarsund et al. (19) found that ColdZyme treatment was  
111 effective against the common cold in healthy adults inoculated with rhinovirus-16: most notably the  
112 duration of illness was reduced by 54% in those who were infected. Also, Clarsund et al. (20)  
113 reported a case study of a 12-year old boy with common variable immunodeficiency, and found a  
114 reduction in reported common cold infection and a 3-fold decrease in missed school days when  
115 using ColdZyme. However, no randomised controlled trials have examined whether such products  
116 can reduce URTI/URS incidence or duration in athletic populations. One recent study (23) did  
117 examine ColdZyme in athletes, but it lacked a control group and made comparisons with  
118 retrospective historical data from athlete's own training diaries, which has obvious limitations for  
119 establishing efficacy. The aims of this study were, therefore, to assess the efficacy of ColdZyme on  
120 URTI incidence, symptom ratings, and missed (or reduced) training in competitive endurance  
121 athletes under free-living conditions, in a prospective randomised controlled trial.

122

123

## 124 **Methods**

125

126 Type of Study:

127 Prospective, open label, parallel groups, randomised controlled trial.

128

129 Participants:

130 Endurance-trained; competitive athletes (e.g. long-distance runners; triathletes; cyclists) were  
131 recruited. Participants were excluded if on long term medication; currently smoking; allergic to  
132 any of the ingredients in ColdZyme; had any other current medical conditions that may be  
133 aggravated by use of the product; were currently using any medication (except for  
134 contraceptives), or food supplements; were currently using any other relevant products or  
135 supplements (nutritional or otherwise) that may influence the common cold; were currently  
136 taking part in another study that may compromise results of this study; or were pregnant, breast-  
137 feeding or planning to become pregnant during the study.

138

139 Ethical approval:

140 The study was conducted in accordance with the Declaration of Helsinki and approved by the  
141 ethics committees of the authors' Universities. All participants were informed, both verbally and  
142 in writing, of the nature and risks of the study before giving their written consent to take part.

143

144 Design:

145 Athletes were monitored over a 3-month period of using ColdZyme product (or control). During  
146 this period they completed self-report training logs, and the Jackson common cold questionnaire  
147 (<sup>24</sup>). The ColdZyme group were also required to keep a personal record of product usage. The  
148 study period was in UK winter months. Tranche 1 took place between December 2017 – March  
149 2018 and Tranche 2 took place between December 2018 and April 2019. Tranche 1 took place  
150 in Southeast England (East Kent and Medway areas). Tranche 2 took place in Southeast  
151 England, as in Tranche 1, plus at 3 additional sites: Tonbridge, UK; Merseyside, UK, and  
152 Lincolnshire, UK. Participants were stratified by sport type, sex, age, and usual training volume  
153 and randomised to either control or ColdZyme group by random number generation  
154 ([www.randomization.com](http://www.randomization.com)). Randomisation was also applied at each site independently so that  
155 groups were matched within each location. The allocation schedule was concealed from  
156 investigators involved in recruiting the participants, hence group allocations were only provided  
157 one at a time.

158

159 Treatment:

160 Participants in the treatment (ColdZyme) group were asked to use the ColdZyme product in  
161 accordance with manufacturer instructions for treatment of suspected common cold/URTI (at  
162 first self-perceived signs of URTI). Briefly, this included instruction to spray 2 times (1 dose)  
163 every second hour up to 6 times daily.

164

165 Participants in the control group were asked to continue with their normal training, to log all  
166 activities and URTI (Jackson questionnaire) but were not provided with ColdZyme. Participants  
167 were not restricted from using over-the-counter (OTC) medication if they felt it necessary, but  
168 were required to record any usage in their illness log.

169

170 Training monitoring:

171 Participants were required to log s every exercise training session. They were required to  
172 provide details on exercise type, duration and a single overall rating of their perceived exertion  
173 for the session (using the session rating of perceived exertion method, sRPE <sup>25</sup>).

174

175 Monitoring of upper respiratory illness:

176 Participants were required to complete the Jackson questionnaire daily (<sup>24</sup>). Completion of this  
177 prospective questionnaire first requires participants to indicate if they believe they are suffering  
178 from a common cold/URTI. If they answer yes to the initial question, they must then rate which  
179 of the 8 Jackson score symptoms they are suffering (headache, chilliness, sneezing, sore throat,  
180 malaise, cough, nasal discharge, nasal obstruction) and give a rating of the severity of each  
181 symptom experienced (0, none; 1, mild; 2, moderate; 3, severe). An episode of URTI was  
182 defined using the Jackson criteria (as applied by Martineau et al.<sup>26</sup>): scores for each of 8  
183 symptoms were summed for each day to generate a total Jackson score, and an episode was  
184 defined as those lasting  $\geq 3$  days and with either i) a total Jackson symptom score of  $\geq 14$  +  
185 subjective impression of having a cold (question 1), or ii) a total Jackson symptom score of  $\geq 14$   
186 + nasal discharge for at least 3 days, or iii) a total Jackson symptom score  $< 14$  + subjective  
187 impression of having a cold + nasal discharge for at least 3 days.

188

189 Data analysis:

190 All data analysis was conducted using IBM SPSS statistics version 25 (IBM, Armonk, NY).  
191 Data were checked for normal distribution prior to analysis. Data that did not have a normal  
192 distribution (sRPE-based training load) were normalised via log transformation prior to analysis.  
193 Data that could not be normalised by log or square root transformation (missed and reduced  
194 training) were analysed with non-parametric tests. Group comparisons were made using  
195 independent samples t-test (or for missed and reduce training data, Mann-Whitney U test; and  
196 for OTC medication use, chi-squared analysis). Training load variables were compared between  
197 groups, and across repeated time-points (weekly over study period, and weeks before, during  
198 and after URTI episodes) with 2-way mixed ANOVA (between factor: group, and within  
199 group/repeated factor: time point). Post hoc paired t-tests were used, where necessary, to  
200 compare within/repeated time-points following significant main effects in this factor.

201

202

## 203 **Results**

204

205 A total of 130 subjects were enrolled, but 7 were lost to follow-up (n = 3 [2 Control, 1 ColdZyme]  
206 due to injury meaning no regular training during study period; n = 2 [1 Control, 1 ColdZyme]  
207 voluntarily withdrew before completion; n = 2 [1 Control, 1 ColdZyme] did not return logs and  
208 could no longer be contacted/did not reply to communications). Analysis was completed on n = 123  
209 (n = 61, age  $39.3 \pm 11.5$  years, control and n = 62, age  $39.5 \pm 12.1$  years, ColdZyme; male n = 60,  
210 female n = 63). Athletes were all competitive endurance athletes in current training, ranging from



211 club level athletes to age-group international level, with comparable average training load in each  
212 group (see below). The majority of athletes were runners (n = 38 control, n = 44 ColdZyme), then  
213 triathletes (n = 13 control, n = 13 ColdZyme) and cyclists (n = 8 control, n = 4 ColdZyme) with a  
214 small number from other sports (swimming n = 1 control, n = 1 ColdZyme; and rowing n = 1  
215 ColdZyme).

216

217 At least one URTI episode was recorded during the study period in 76.4% of all participants (77.0%  
218 control, 75.8% ColdZyme). In total 130 episodes were recorded by all participants over the study  
219 period with no difference between groups in the incidence rate (mean incidence rate per person over  
220 study period was:  $1.1 \pm 0.9$  Control,  $1.0 \pm 0.8$  ColdZyme,  $P = 0.290$ ). Symptom duration and  
221 severity ratings were also lower in the ColdZyme group (see Table 1).

222

223 \*\*\*Please insert Table 1 near here\*\*\*

224

### 225 **Symptoms duration**

226 Overall Control vs ColdZyme mean episode duration was  $10.4 \pm 8.5$  days in Control and  $7.7 \pm 4.0$   
227 days in ColdZyme ( $P = 0.016$ , see Table 1). On further examination of usage records and diaries  
228 from Tranche 1 (December 2017-February 2018) it became apparent that, despite being instructed  
229 to follow the manufacturer's IFU (i.e. to use 1 dose every second hour up to 6 times daily), not all  
230 of the participants in the ColdZyme group followed the recommendations for use (~37% of  
231 recorded episodes were not treated according to the ColdZyme IFU). For Tranche 2 (December  
232 2018-March 2019) we provided additional information and regular reminders to participants to  
233 overcome this. These procedures appear to have been effective as poor compliance with IFU was  
234 only evident in 14% of reported episodes. Nevertheless, these subjects/episodes (with poor IFU  
235 compliance) from both Tranches do provide a useful comparator group for some statistical  
236 comparisons, which may overcome some of the limitations that arise in open label trials (i.e.  
237 potential placebo effects in treatment group). Poor compliance with IFU was considered as not  
238 using the ColdZyme product in accordance with guidelines (i.e. less than 4 doses per day). When  
239 those randomised to ColdZyme but with poor compliance (Poor IFU comp) were separated from  
240 those with good compliance (Good IFU comp) the observed effect between Control and ColdZyme  
241 groups becomes even more evident (episode duration  $10.4 \pm 8.5$  days in Control vs  $6.9 \pm 3.5$  days in  
242 ColdZyme Good IFU comp,  $P = 0.004$ ). Direct comparison between compliance groups also shows  
243 a significantly shorter episode duration with good compliance (episode duration  $9.3 \pm 4.5$  days in  
244 ColdZyme poor IFU comp vs  $6.9 \pm 3.5$  days in ColdZyme Good IFU comp,  $P = 0.040$ ).

245

### 246 **Jackson Symptom Score**

247 **Total symptom score during episode:** This parameter shows the overall symptom impact (product  
248 of number of Jackson symptoms and symptom severity ratings, and accounting for episode  
249 duration). Overall Control vs ColdZyme mean Jackson symptom score was  $74.9 \pm 72.0$  in Control  
250 and  $43.6 \pm 30.1$  in ColdZyme ( $P = 0.003$ , see Table 1). When comparing episodes with good and  
251 poor IFU compliance, the ColdZyme Good IFU comp group had a significantly lower symptom  
252 score ( $40.0 \pm 26.5$ ) than the Control group ( $P = 0.002$ ), whereas the Poor IFU comp group ( $52.6 \pm$   
253  $35.7$ ) were not significantly different from control ( $P = 0.100$ ). Direct comparison between the  
254 episodes with bad and good IFU compliance did not show any statistically significant difference  
255 however ( $P = 0.152$ ).

256  
257 **Average symptom score per day during episode:** This parameter is directly related to the severity  
258 rating and number of symptoms experienced on average (per day) in each episode. Overall Control  
259 vs ColdZyme mean Jackson symptom score was  $6.9 \pm 2.8$  in Control and  $5.5 \pm 2.4$  in ColdZyme ( $P$   
260  $= 0.006$ , see Table 1). When comparing episodes with good and poor IFU compliance, the  
261 ColdZyme Good IFU comp group had a significantly lower symptom score ( $5.6 \pm 2.7$ ) than the  
262 Control group ( $P = 0.014$ ), and so did the ColdZyme Poor IFU comp group ( $5.3 \pm 1.7$ ) ( $P = 0.033$ ).  
263 Direct comparison between the episodes with bad and good IFU compliance did not show any  
264 statistically significant difference ( $P = 0.481$ ).

### 265 266 **Daily training logs**

267 Participants typically trained between 4 and 10 h per week. Participants were asked to record every  
268 training session (duration and sRPE) in their training logs. Training 'load' was quantified as the  
269 product of sRPE and duration for each session (and summed each week). Sufficient detail to allow  
270 full analysis of training data was provided by 93 participants (whereas 19 participants [ $n = 9$   
271 Control,  $n = 10$  ColdZyme] failed to record sRPE but recorded details on training type and duration,  
272 and 11 participants [ $n = 9$  Control,  $n = 10$  ColdZyme] failed to record either sRPE or duration).

273  
274 For the 93 subjects with complete training logs, there were no significant between-group differences  
275 in training load or the profile of training load across the study period (2-way mixed ANOVA: group  
276  $P = 0.925$ ; time  $P = 0.055$ ; group  $\times$  time  $P = 0.626$ ). For the  $n = 19$  who did record duration only  
277 there was no difference in average weekly training time (Control  $6.0 \pm 1.5$  h; ColdZyme  $5.9 \pm 1.8$  h,  
278  $P = 0.851$ ).

279  
280 For analysis of training load data (see Figure 1) each subject's average healthy (i.e. when not  
281 experiencing or recovering from a URTI episode or injury) value was calculated and training load  
282 profile across the 12-week period expressed as a percentage of this. There was a trend for training

283 load to increase across the study period although this did not reach statistical significance, but  
284 importantly this pattern did not differ between groups. There was a significant reduction in training  
285 load during the weeks in which URTI episodes were experienced ( $P < 0.01$  compared to other, none  
286 URTI weeks, see Figure 2), however, this did not differ between groups (2-way mixed ANOVA:  
287 group  $P = 0.424$ ; time  $P < 0.001$ ; group  $\times$  time  $P = 0.269$ ). There was also no difference in the rate  
288 of return to normal training load, following reported URTIs, between groups.

289

290 \*\*\*Please insert Figure 1 near here\*\*\*

291

292 \*\*\*Please insert Figure 2 near here\*\*\*

293

294

### 295 **Missed and reduced training days**

296

297 \*\*\*Please insert Table 2 near here\*\*\*

298

### 299 **Missed training**

300 The number of missed training days (caused by URTI episode/symptoms etc) was significantly  
301 lower in the ColdZyme compared to Control group ( $P = 0.013$ , see Table 2). When considering IFU  
302 compliance, there were significant differences between Control and ColdZyme Good IFU comp ( $P$   
303  $= 0.021$ ) and ColdZyme Poor IFU comp ( $P = 0.045$ ). There was no significant difference between  
304 ColdZyme Good and Poor IFU comp however ( $P = 0.406$ ).

305

### 306 **Reduced training**

307 The number of days on which training was reduced, as a consequence of an episode, was not  
308 significantly different between the ColdZyme and Control groups ( $P = 0.475$  see Table 2). When  
309 considering IFU compliance, there were no significant differences between Control and ColdZyme  
310 Good IFU comp ( $P = 0.288$ ) or ColdZyme Poor IFU comp ( $P = 0.336$ ). There was also no  
311 significant difference between ColdZyme Good and Poor IFU comp ( $P = 0.269$ ).

312

### 313 **Use of OTC medication**

314 Participants felt the need to use OTC medication for 48% of cases (32/67 episodes) in Control and  
315 38% of cases (24/63 episodes) in ColdZyme, with no difference between groups ( $\chi^2 P = 0.266$ ). The  
316 OTC medications used were most commonly cold and flu formulations (e.g. Lemsip; Control = 14,  
317 ColdZyme = 11), followed by analgesics (e.g. paracetamol (acetaminophen); Control = 12,

318 ColdZyme = 12), with others used rarely (throat lozenges or cough mixture, Control = 5, ColdZyme  
319 = 0; and decongestants, Control = 1, ColdZyme = 1).

320

### 321 **Adverse events reporting**

322 One participant reported a potential adverse event (unsettled stomach) during the study, in the  
323 ColdZyme (treatment) group. It was not serious and no special treatment was necessary. No other  
324 adverse events were reported by any participant.

325

### 326 **Discussion**

327 This is the first randomised controlled trial to examine the efficacy of ColdZyme mouth spray on  
328 URTI outcomes in athletes (and the first to study any intervention of a product purported to act via  
329 local mechanisms of inhibiting viral infectivity and propagation in the URT). The main finding  
330 from this study is that ColdZyme did not alter the chances of contracting a URTI (no effect on  
331 URTI incidence) but it was able to reduce the duration for which URTI symptoms persisted, and  
332 reduce mean daily ‘severity’ ratings, in competitive endurance athletes. This benefit was evident in  
333 the ColdZyme group overall, with further analysis showing that the benefit was significantly more  
334 apparent when compliance was good (i.e. ~26% vs ~34% shorter episode duration) but was lost for  
335 duration if compliance with the IFU was poor. The ColdZyme group also reported fewer missed  
336 training days as a consequence of URTI episodes. The average episode duration was ~10.4 days in  
337 the control group, 7 days of which (~66%) resulted in compromised training (~3.4 d reduced  
338 training and 3.5 d missed training per episode). In the ColdZyme group episodes of URTI had less  
339 of an effect on compromising training (4.6 of 7.7 d [~60%]), with the difference resulting from  
340 significantly less missed training days (Control 3.5 of 10.4 d [~34%] missed training days per  
341 episode, and ColdZyme 1.6 of 7.7 d [~21%] missed training days per episode). These results  
342 suggest that ColdZyme mouth spray can reduce symptom duration and severity ratings (during a  
343 self-reported URTI) in endurance athletes, consequently reducing the number of missed training  
344 days. This may help to reduce the negative impact of an illness episode on athlete performance,  
345 since a loss of training days is associated with performance decrement in athletes (<sup>6,8,9</sup>). ColdZyme  
346 is a potential countermeasure to reduce the negative impact caused by such illness.

347

348 ColdZyme has been shown to have broad-range antiviral activity against common URTI-causing  
349 viruses *in vitro*, deactivating 64-100% of virus activity for influenza virus, rhinovirus, adenovirus,  
350 and coronavirus (<sup>22</sup>). A possible mechanism for the present results, therefore, is that the oral  
351 application via spraying forms a temporary barrier on the oropharynx that prevents viral binding  
352 and entry, and that the regular reapplication (i.e. up to 6 times per day, in line with manufacturer  
353 IFU) can inhibit viral propagation to a sufficient extent so as to reduce viral load and allow a more

354 rapid clearance of infection in endurance athletes. The fact that the spray is applied to the throat  
355 suggests that the oropharynx is an important area for propagation and target for treatment. This is  
356 further supported, for example, by the reduction in common cold duration that has been observed  
357 with the use of zinc gluconate lozenges <sup>(21)</sup>. Indeed, this is in line with previous research on  
358 ColdZyme using the quarantine human viral challenge model with rhinovirus-16 <sup>(19)</sup>. The present  
359 study, however, is the first to examine ColdZyme in free living/real-world conditions in a  
360 randomised controlled trial with athletes.

361

362 It is possible that some findings of this study may be influenced by the open label nature of the trial.  
363 For the primary outcomes (URTI reporting) previous research <sup>(27)</sup> has shown that the magnitude of  
364 the placebo effect has a small influence on URTI reporting (see further discussion in limitations  
365 below). For the other (secondary) parameters, such as missed or reduced training, we are not aware  
366 of any research on how the placebo effect may influence these. Ultimately, the decision to deviate  
367 from planned training is a choice made by the athlete so it is possible that the placebo effect  
368 influences this to a greater extent. However, a possible explanation for our findings for missed  
369 training days could also be that both poor IFU compliance and good IFU compliance groups  
370 reported significantly lower average daily severity ratings than the control group. Indeed, it would  
371 seem logical that symptom severity would be the most important factor influencing athletes'  
372 decisions about whether to train as normal, reduce training or miss training altogether. We also  
373 cannot exclude the possibility that those in the treatment group felt more willing to continue with  
374 training as they knew they were receiving an intervention that may help.

375

### 376 **Limitations**

377 The main limitation is the open label nature of this study, which presents the possibility of a placebo  
378 effect in the treatment group and/or a nocebo effect in the control group. At the time of  
379 commencing the study, the manufacturer was not able to provide an appropriate placebo. Future  
380 research would be enhanced with a full placebo so that a fully placebo-controlled, double-blind  
381 design could be implemented. Although previous research has shown that knowledge of the  
382 treatment does not influence objective biomarkers of immune function in response to exercise (e.g.  
383 immune cell functions <sup>29,30</sup>), self-report results for URTI parameters are likely more prone to  
384 influence. Research on the placebo effect has shown only modest placebo effects for common cold  
385 symptoms, with an episode duration comparable to the present study <sup>(27)</sup>. In this study they  
386 observed an effect size below the minimal threshold for a small effect (for common cold duration  
387 <sup>27</sup>), whereby the placebo effect influenced average reported duration by less than 0.7 days (i.e.  
388 <10%) and severity ratings by 8-17%. However, the effect was larger for individuals who had a  
389 higher belief in the possible beneficial effects (i.e. expectation) at the point of enrolment. It is

390 possible therefore that some individuals in the ColdZyme group had positive expectations, which  
391 could have influenced their URTI scores. Unfortunately we did not capture data on expectations in  
392 the treatment group, and this would be beneficial to include in future studies (although an  
393 appropriate placebo is preferable).

394

395 On examination of usage records and diaries from Tranche 1 (December 2017-February 2018) it  
396 became apparent that, despite being instructed to follow the manufacturer IFU (i.e. 1 dose every  
397 second hour up to 6 times daily), not all of the participants in the ColdZyme group followed the  
398 recommendations for use (~37% of recorded episodes were not treated according to the ColdZyme  
399 IFU). For Tranche 2 (December 2018-April 2019) we provided additional information and regular  
400 reminders to participants to overcome this. These procedures appear to have been effective as poor  
401 compliance with IFU was only evident in 14% of reported episodes, but compliance was still not  
402 entirely optimal (a common problem with free-living human trials). On the one hand, this is a  
403 limitation - possibly reducing the magnitude of effect in the ColdZyme group. However, on the  
404 other hand, separate analysis of the good and bad compliance episodes does provide a useful  
405 comparator condition which may overcome some of the limitations that arise in open label trials  
406 (i.e. potential placebo effects in treatment group), as can be seen in the ColdZyme Good IFU comp  
407 vs ColdZyme Poor IFU comp and ColdZyme Good IFU comp vs Control comparisons.

408

409 The use of self-report methods and illness questionnaires has the limitation of being subjective and  
410 presents the possibility of athletes reporting symptoms/self-reporting illness in the absence of a true  
411 infection. Some studies have reported an increased occurrence of allergy-type symptoms that are  
412 often mistaken by athletes as URTI, although this tends to be more common in spring (Northern  
413 hemisphere), when responses to environmental allergens such as pollen are more common <sup>(28)</sup>. The  
414 current study was conducted in the winter months when URTI-incidence is known to be at a peak.  
415 In addition, the validated Jackson questionnaire and scoring criteria were used, which help to  
416 protect against false positive episode counts (although these can never be completely prevented). It  
417 was not feasible in the present study to confirm infection via laboratory-based diagnostic methods  
418 (e.g. polymerase chain reaction (PCR) detection of URTI-causing pathogens from throat and nasal  
419 swabs and/or biological fluids). It would be beneficial for future studies to include these  
420 measurements to 1) confirm the presence of URTI during self-reported episodes, and 2) monitor  
421 viral load during the course of an episode to provide insight into the mechanisms of action (for  
422 example, this would allow greater insight into the pattern of propagation, spread and infection/virus  
423 clearance rate at different sites within the URT).

424

425 **Conclusions**

426 It is clear that reporting of upper respiratory illness symptoms in athletes cluster around periods of  
427 intensive training and/or competition (6,7). This can have a direct and significant impact on athletes'  
428 performance in competition, preparation and training and general wellbeing (6,8,9). In this study we  
429 provide the first evidence from a randomised trial in athletes under free-living conditions on the  
430 effects of a proposed non-nutritional countermeasure (ColdZyme Mouth Spray) to self-reported  
431 URTI in athletes, which may have implications for athletes' absence from training and athlete  
432 performance. We show that ColdZyme mouth spray used in accordance with manufacturer  
433 instructions can reduce self-reported URTI episode duration (by 3.5 days: ~34% reduction in  
434 episode duration). This was also associated with a reduction in lost training days (~54% reduction,  
435 from 3.5 days lost per episode in Control to 1.6 days lost per episode in ColdZyme). This may  
436 provide an effective strategy to reduce the impact of upper respiratory illness on training and  
437 competition.  
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529 **Table headings**

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531

532 **Table 1:** Overview of self-report URTI data

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534 **Table 2:** Days that training was affected by URTI episodes/symptoms

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541 **Figure titles and captions**

542

543 **Figure 1.** Pattern of training load change across study period (% of healthy average).

544

545 **Figure 2.** Pattern of training load in 2 weeks before URTI onset, during URTI and 3 weeks after

546 URTI subsided.

547 *Significantly different to 'healthy' weeks (\*\*  $P < 0.01$ )*

548

549 **Table 1:** Overview of self-report URTI data

|  | Control     | ColdZyme<br>(overall) | ColdZyme<br>(Poor IFU comp) | ColdZyme<br>(Good IFU comp) |
|--|-------------|-----------------------|-----------------------------|-----------------------------|
| URT I episodes<br>(per person)                   | 1.1 ± 0.9   | 1.0 ± 0.8             | #n/a                        | #n/a                        |
| URT I episode duration<br>(d)                    | 10.4 ± 8.5  | *7.7 ± 4.0            | 9.3 ± 4.5                   | **†6.9 ± 3.5                |
| Jackson Symptom Score<br>(episode total)         | 74.9 ± 72.0 | **43.6 ± 30.1         | 52.6 ± 35.7                 | **40.0 ± 26.5               |
| Jackson Symptom Score<br>(daily episode average) | 6.9 ± 2.8   | **5.5 ± 2.4           | *5.3 ± 1.7                  | *5.6 ± 2.7                  |

550 *NOTE: #n/a = Not relevant or calculated since compliance can only be analysed when an episode exists*

551 ColdZyme Poor IFU comp = Poor compliance with ColdZyme IFU (e.g. less than 4 doses per day);

552 ColdZyme Good IFU comp = Good compliance with ColdZyme IFU.

553 *Significantly different to Control: \* P < 0.05; \*\* P < 0.01*

554 *Significant difference between poor and good IFU compliance groups † P < 0.05; ‡ P < 0.01*

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558 **Table 2:** Days that training was affected by URTI episodes/symptoms

|                       | Control   | ColdZyme<br>(overall) | ColdZyme<br>(poor IFU comp) | ColdZyme<br>(good IFU comp) |
|-----------------------|-----------|-----------------------|-----------------------------|-----------------------------|
| Days missed           | 3.5 ± 5.0 | *1.6 ± 2.5            | *1.6 ± 2.9                  | *1.6 ± 2.3                  |
| Days reduced training | 3.4 ± 5.1 | 3.0 ± 3.4             | 3.1 ± 3.1                   | 2.9 ± 3.7                   |

559 *Average days missed/reduced per URTI episode*

560 ColdZyme Poor IFU comp = Poor compliance with ColdZyme IFU (e.g. less than 4 doses per day);

561 ColdZyme Good IFU comp = Good compliance with ColdZyme IFU.

562 *Significantly different to Control: \* P < 0.05*

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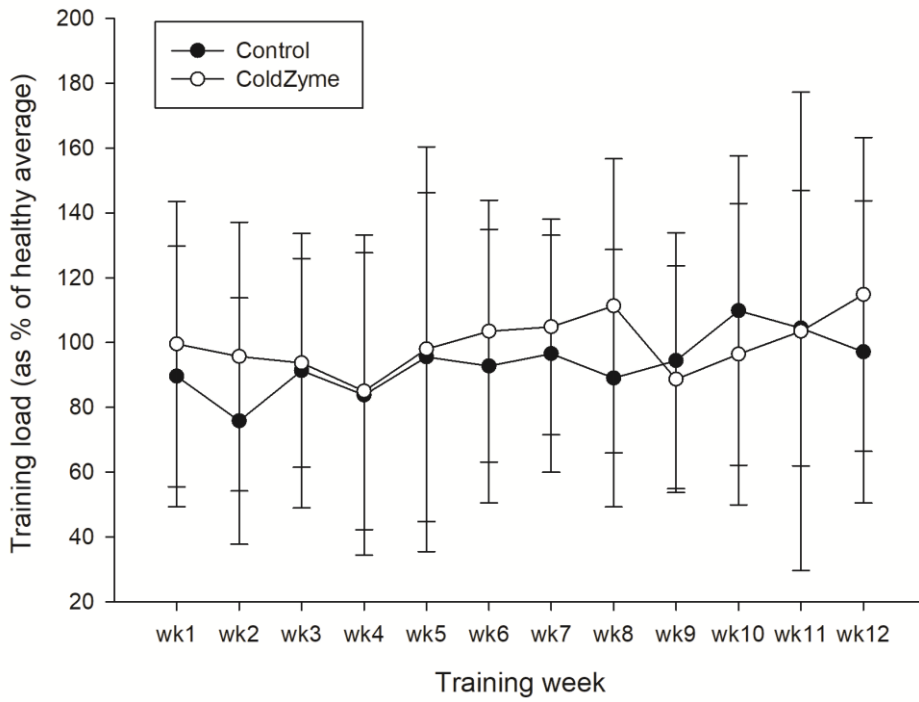


Figure 1

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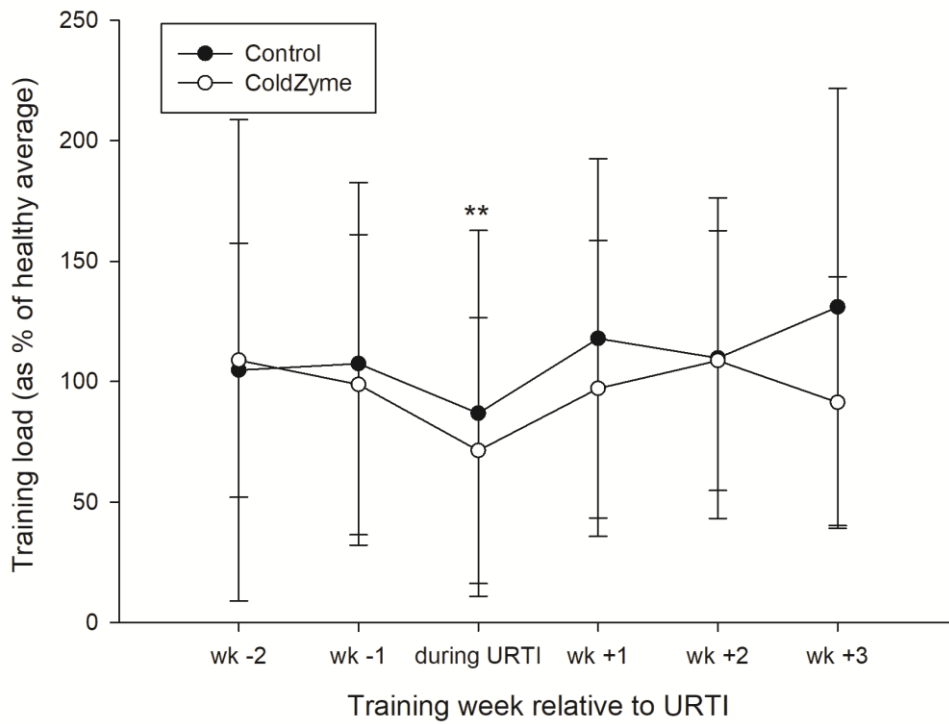


Figure 2