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Elevated Arousal following Acute Ammonia Inhalation is not Associated with Increased Neuromuscular Performance

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Brief running head: Ammonia Inhalation, Arousal and Neuromuscular

Performance

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Many athletes seek to enhance their performance using legal ergogenic aids, including 2 ammonia inhalants (AIs). AIs trigger the inhalation reflex and increase blood pressure, 3 respiration and heart rate; but, despite their widespread use, there is little evidence for the 4 benefits of AI on exercise performance. We aimed to determine the psychological and 5 6 neuromuscular impact of acute ammonia inhalation. Fourteen non-resistance trained males 7 completed three trials: control, experimental (AI), and sham. The order of the sham and experimental trials was randomized. Participants completed handgrip and knee extension 8 maximal voluntary contractions (MVC), and countermovement jump (CMJ). Heart rate and 9 alertness were recorded at rest and immediately following control, experimental or sham 10 11 treatment, followed by functional performance measurements. Reaction time, electromechanical delay, rate of force development and peak force were calculated from 12 MVCs, and peak power from CMJ. On completion of trials, perceived performance was 13 14 recorded. Statistical significance was accepted at P < 0.05. Heart rate (P < 0.001), alertness (P=0.009) and perceived performance (P=0.036) were elevated by AIs. Markers of functional 15 performance were unaltered by AIs. Alertness was moderately correlated with perceived 16 17 performance in control (r=0.61) and sham conditions (r=0.54), and very-highly correlated in the experimental condition (r=0.90). AI elevates alertness and perceived physical 18 performance, but not peak strength, power, or neuromuscular drive. Als may be a useful 19 psychological stimulant to increase focus and mental preparation, however it is unlikely that 20 this will improve functional performance in an untrained population. Our data suggest 21 however, that ammonia inhalants may improve the perception of an individual's performance. 22

23 Keywords: Smelling Salts, Ergogenic Aid, Stimulant, Alertness, Performance

24 Introduction

25 Muscle force and power are key determinants of physical health and sporting performance (1, 2). While chronic adaptations in such functional components are influenced by factors 26 including muscle mass, morphology, and neural activation (3); psychological arousal may also 27 have an acute role in influencing functional performance (4). As such, maximising both 28 physical and psychological capacity will enhance performance (5). 29 While mass and 30 morphology can only effectively be enhanced through continual exercise training, neural activation and arousal can be altered instantaneously (6). Because of the potential 31 32 instantaneous performance benefits associated with enhanced arousal state (4) many athletes 33 use ergogenic aids in pursuit of superior performance during competition (7). For example, 34 various ergogenic aids have been shown to increase central nervous system (CNS) activation, improving performance outcomes (7). While some such aids are banned within sport because 35 of potential health risks (8), there remain many legal aids, but most of these lack rigorous 36 evidence to support their application (9, 10). 37

38

Legal supplementation is commonplace throughout competitive sport. In a survey of American 39 40 athletes in the collegiate system, 89% reported using nutritional supplements (11), with ~50% of powerlifters reportedly making use of non-nutritional ergogenic aids during competition, 41 including ammonia inhalants (AIs) (12). AIs are a common example of stimulant-based 42 ergogenic aid used within competitive sport; yet, despite their popularity, there has been little 43 research into their effect on sporting performance. Greater anaerobic power has been observed 44 45 with AI use in a fatigued state (13), but they have been shown not to impact lower limb or full body static strength (14, 15), dynamic strength (16, 17), or number of repetitions to fatigue 46 (16). However, no previous study has assessed the influence of AIs on isolated upper body 47

48 strength only. Meanwhile, rate of force development (RFD) may (14) or may not (15) be 49 influenced by AI use. While the above-mentioned findings all relate to experienced resistance-50 or anaerobically-trained individuals, it has been proposed that untrained individuals may 51 benefit from psychological arousal to a greater extent than those who are trained (4). Arousal 52 has been widely assessed by measuring alertness (18, 19); however, to date no study has 53 assessed alertness specifically following AI use.

54

Despite the scarcity of evidence for their efficacy, AI use remains widely popular among those 55 competing in sports such as powerlifting, weightlifting, track and field, boxing, American 56 football, hockey and mixed martial arts (20). To help understand their popularity, we can 57 explore the physiological responses induced by AI use. It is believed that ammonia inhalation 58 triggers the trigeminal nerve via chemoreceptors within the nasal, oral and pulmonary mucosa 59 (21). The respiratory and vasomotor centres within the medulla oblongata respond to this 60 irritation, promoting inhalation reflex and increased blood pressure leading to elevated 61 respiration and heart rate (HR) (22). Perry et al. (2016) evidenced this response, demonstrating 62 increased middle cerebral artery blood flow velocity and HR immediately following AI use 63 (15). Furthermore, improved cerebral delivery of oxygenated blood and CNS excitation, result 64 in increased consciousness and enhanced sympathetic activity. In terms of an advantageous 65 effect for sporting performance, these responses could potentially elicit cognitive enhancement 66 and increase central drive (13). Equivocal findings of an effect of AIs on RFD (14, 15) compel 67 further investigation; if there is, as suggested, an increase in central drive associated with AI 68 69 use, elevated RFD would be expected, accompanied by a shortening of electromechanical delay 70 (EMD), and therefore, overall reaction time (RT). These enhancements could be expected to promote an increase in peak power production, although to date the only study to report an AI-71

induced increase in peak power, investigated performance in already-fatigued athletes (13).
Therefore, it remains unclear whether AIs can acutely enhance neuromuscular processes
sufficiently to impact functional performance.

75

76 The aim of this study was to determine the effect of AI use on peak power production, and on RT and RFD during maximal isometric strength assessment, among non-resistance trained 77 individuals. Additionally, we assessed the effect of AI use on HR and cognitive alertness and 78 examined the relationship between these parameters and functional performance. As arousal 79 may acutely benefit performance and has a greater impact on untrained than trained individuals, 80 we believe that studying an exclusively untrained cohort may provide the best opportunity to 81 82 explain the popularity of AI use via its effect on arousal and enhanced psychological readiness to perform. Furthermore, enhanced arousal and subsequent psychological readiness to perform 83 may translate to an overall perception of improved performance compared to when not using 84 the stimulant and thus, may potentially help to further explain the popularity of AI use. We 85 therefore hypothesised that alertness would increase in line with HR following AI use, and that 86 87 this would be associated with decreased RT and increased RFD, which would result in elevated 88 peak power, but not peak strength. It was also hypothesised that participants would perceive a sense of improved overall performance after AI use. 89

90

91 Methods

92 Participants

Fourteen non-resistance trained male participants with no history of neuromuscular or
musculoskeletal disorders were recruited (age 20 (SD 1) years, height 179.9 (SD 5.4) cm, body

mass 76.2 (SD 12.7) kg, weekly physical activity 3.5 (SD 1) days). Participants were required 95 96 to read a study information sheet and complete a pre-participation questionnaire to determine 97 eligibility. Eligible volunteers provided written informed consent after being supplied with information about any benefits and potential risks involved with their participation. The study 98 was approved by the local Research Ethics Committee (SSREC No. 701/705), and all 99 procedures performed were in accordance with the ethical standards of the 1964 Helsinki 100 101 declaration and its later amendments. Participants were eligible if they were over the age of 102 18, had not previously used AIs, and had no known respiratory or cardiovascular illnesses.

103

104 Design

Following full familiarisation of the testing procedures, participants completed 3 trials 105 separated by 7-days, each at the same time of day. The three trials consisted of control (CN), 106 experimental (AI) and sham conditions, where the order of the latter two was chosen by 107 participant block randomisation. Trial 1 (CN) also acted as a participant familiarisation and 108 109 followed an identical protocol to trials 2 and 3, except that no inhalant was provided (Figure 1). During trials 2 and 3 participants inhaled either AI within the experimental condition or 110 water within the sham condition prior to all measurements of functional performance. A sham 111 condition of water was chosen over a scented alternative (as has been adopted elsewhere (16)) 112 as evidence shows that other strong smelling substances can promote muscle activity (23). 113 After the control trial, participants were informed that they would inhale the AI during one 114 115 trial, and water (sham) during the other, and that these trials would be randomised in order. The order of the AI and sham trials was not revealed to participants prior to inhalation. 116 Functional performance was determined via knee extension (KE) and handgrip (HG) maximal 117 voluntary isometric contraction (MVC) using dominant limbs, and bilateral countermovement 118

jump (CMJ); each incorporating 3 repetitions. Each participant identified their dominant upper
and lower limbs by indicating their handedness, and the leg they would use to kick a ball (24),
respectively. Participants rested for 20-minutes between tasks, and 5-minutes between
repetitions. Finally, participants self-rated their perceived performance across the 3 tasks (KE
and HG MVCs, and CMJ). The order of measurements was consistent across all visits (Figure
1).

- 125
- 126

[Figure 1]

127

128 Procedures

Participants refrained from strenuous physical activity, alcohol, and caffeine for >24h 129 preceding laboratory visits, and maintained similar diet for 48h, incorporating 1h fast 130 immediately prior to each trial. Upon arrival to the laboratory, participant height and mass 131 were recorded before resting in a semi-reclined, supine position for 20-minutes. Next, resting 132 HR was recorded (FT1 Polar monitor, FT7 sensor. Warwick, UK); three 5s stable readings 133 were noted, and the mean value was recorded for analysis; intraclass correlation coefficient 134 (ICC) of resting HR between trials 1, 2 and 3 was 0.85 with no significant differences in resting 135 HR between trials. Participants then indicated their alertness on a 200mm visual analogue 136 scale (VAS), which ranged from 'not alert' at the extreme left to 'highest possible alertness' at 137 the extreme right, which is believed to be similar to an alertness VAS which have been used 138 previously (25, 26). The two ends of the VAS were anchored by perpendicular lines, but there 139 were no increments between the end markers. This method was chosen as VAS has been shown 140 to be a reliable and valid assessment of alertness (25, 26). The ICC of pre-inhalation alertness 141

between trials 1-3 was 0.94, and there were no significant pre-inhalation differences in alertness 142 between trials (P>0.05). During trials 2 and 3, participants inhaled from an unmarked flask 143 containing either 35.99g of AI (Nose Tork, Crystals of Ammonium Carbonate, Oklahoma, US) 144 or water and cotton wool before all MVC and CMJ repetitions. Both flasks were matched 145 exactly in volume and appearance, although the scent and physical reaction of participants after 146 inhalation could not be controlled for between the experimental and sham trials. Participants 147 148 were instructed to inhale sharply, while the flask was positioned ~150mm below the columella. HR and alertness were recorded as before, immediately following inhalation prior to one of the 149 150 three repetitions, allocated at random. During CN (trial 1), participants followed the same procedure, but no flask was positioned below the columella. 151

152

153 For assessment of MVC, participants were coupled to an isokinetic dynamometer (Biodex System 3, Medical Systems, Shirley, New York, USA). In accordance with the manufacturers' 154 instructions, seat positions were adjusted to suit each participant's anthropometric 155 156 characteristics, and straps (across the chest and pelvis) were used to secure the participant in the required position. The final positioning of each participant was recorded on the initial visit 157 and replicated throughout the experimental period to ensure constancy between trials. Two 158 pairs of Ag/AgCl self-adhesive electrodes (PNS Dual Element Electrode, Vermed, Vermont, 159 USA) were affixed to pre-prepared skin, 1 pair over vastus lateralis and 1 pair over 160 brachioradialis, in accordance with SENIAM guidelines. Ground electrodes were affixed over 161 patella and olecranon, respectively. Surface electromyogram (sEMG) was captured during all 162 MVCs, at a sampling rate of 2kHz, via Acknowledge[®] 3.9.1.6 software integrated with Biopac 163 MP100 hardware (Biopac Systems Inc., Goleta, California, USA). For KE MVC a Velcro cuff 164 was secured proximal to the medial malleolus. Gravitational corrections were performed, in 165

accordance with existing recommendations, in order to account for the effect of limb weight. 166 The lateral femoral epicondyle of the dominant leg was visually aligned with the dynamometer 167 axis of rotation, and a knee joint angle of 60° was set (0° = full extension) (27). Participants 168 performed a standardised submaximal warm-up, consisting 2 sets of 3 x 5s isometric KE 169 contractions at 50% and 75% of perceived maximum, respectively. During all contractions 170 participants were instructed to cross their arms in front of their chest. For HG MVC the 171 shoulder of the dominant arm was flexed to 90° (0° = neutral), and secured to a support, with 172 an elbow joint angle of 90° (28). A similar warm-up protocol was performed, using a HG 173 174 dynamometer (MLT003/D, AD Instruments Inc. Colorado Springs, Colorado, USA), and the contralateral arm remained in a neutral position. 175

176

Following the respective warm-ups, MVC was assessed via 3 x 5s maximal contractions. 177 During trials 2 and 3 participants inhaled either AI or water prior to each contraction. The time 178 between inhalation and contraction varied by small durations between each test due to the 179 randomised audio prompt which was used to measure RT. However, time between inhalation 180 and contraction did not exceed 30 seconds. Dynamometer outputs were sampled 181 synchronously with sEMG via Acknowledge® 3.9.1.6 (Biopac Systems Inc., Goleta, 182 California, USA). Participants were instructed not to hold back any effort for subsequent 183 contractions; the same investigator provided standardized verbal commands and 184 encouragement, to assist the participants in achieving maximal effort for every contraction. 185 Participants reacted to an audio prompt and were instructed to exert as much force as possible, 186 as quickly as possible, in response to the prompt, allowing RT and RFD to be calculated (29). 187 The contraction containing the highest peak was designated MVC. The ICC for HG and lower 188 limb MVCs between trials was 0.96 and 0.94, respectively. From these contractions, RT 189

(latency between audio stimulus and onset of force production) and EMD (latency between 190 onset of sEMG and onset of force production) were calculated. ICCs for RT and EMD were 191 calculated as 0.81 and 0.13 for HG, and 0.88 and 0.14 for the lower limb, respectively. 192 Additionally, RFD was analysed over 0-20, 0-50, 0-100 and 0-200ms from the onset of force 193 production. In all cases, onsets were considered as >2SD from baseline. ICCs between trials 194 for HG and lower limb, respectively, are as follows: 0-20ms (0.63 and 0.58), 0-50ms (0.74 and 195 196 0.81), 0-100ms (0.75 and 0.88), and 0-200ms (0.77 and 0.94). Analysis was performed using MATLAB version 7.11.0.584 (R2010b) software (The MathWorks, Inc. Natick, 197 198 Massachusetts, USA) and force and sEMG onsets were visually confirmed post hoc (29).

199

Peak power was assessed via CMJ (JUMP-MD, Vertical Jump Meter T.K.K.5406) according 200 to the formula: W = (78.6 * d) + (60.3 * m) - (15.3 * h) - 1308 (where: W = peak power, 201 d = displacement, m = body mass, h = height) (30). First, participants warmed-up by 202 completing 3 CMJs at 50% of perceived maximum effort. Three maximal effort CMJs were 203 then performed, with 5 minutes rest between attempts; during trials 2 and 3 participants inhaled 204 either AI or water prior to each attempt. The greatest displacement achieved across the 3 205 attempts was used to calculate peak power. The ICC of all trials was calculated as 0.97. 206 Following assessment of all functional measures, participants self-rated their perceived 207 performance level using a 200mm VAS which ranged from 'worst performance' at the extreme 208 left to 'best possible performance' at the extreme right (31). The two ends of the VAS were 209 210 anchored by perpendicular lines, but there were no increments between the end markers. Perception of performance ICC between the three trials was calculated as 0.85. 211

213 Statistical Analyses

214 Data were assessed for normal distribution and equal variances using Shapiro-Wilk and Levene's Test for Homogeneity of Variances, respectively. The percentage change (Δ %) in 215 pre- to post-inhalation HR and alertness was firstly calculated for all three conditions. These 216 data were subsequently used to calculate the percentage change from the CN during the AI and 217 Sham conditions. MVCs, CMJ power, EMD, RT and perceived overall performance were also 218 calculated as the percentage change from the CN. Heart rate, alertness, MVCs, CMJ, EMD, 219 220 RT and perceived overall performance were then analysed using a paired sample Student's ttest to identify significant differences between AI and sham conditions. Wilcoxon W rank test 221 was used when normality assumptions were violated. Cohen's D (d) effect size was 222 subsequently calculated for t-tests, where values of 0.2, 0.5 and 0.8 represented a small, medium and 223 large effect, respectively. Additionally, RFD (0-20, 0-50, 0-100 and 0-200ms) from each 224 condition were analysed using two-way repeated measures ANOVA. Where significant and 225 non-significant effects were observed, partial η^2 effect sizes (η^2_p) were calculated by 226 η^2_p =SS_{conditions}/ (SS_{conditions}+SS_{error}). Association between pairs of variables was assessed using 227 Pearson's correlation coefficient. Correlation coefficients were interpreted as < 0.30 =228 negligible, 0.30 - 0.49 = low, 0.50 - 0.69 = moderate, 0.70 - 0.89 = high, 0.90 - 1.00 = very229 high (32). Statistical significance was accepted at P<0.05. All statistical analysis was 230 performed using Jamovi (Version 0.9), and figures were created using Microsoft Excel 231 (Version 16.31) and Jamovi. 232

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236 **Results**

When measured relative to the CN condition, the percentage difference in heart rate ($t_{(13)}$ =4.76, d=1.27, *P*<0.001) and alertness ($t_{(13)}$ =3.1, d=0.837, *P*=0.009) were elevated by 11.5% [95% CI: 6.3, 16.7] and 20.1% [6.1, 34.2] respectively following AI use compared to the sham condition (Figure 2). The change in perception of performance from the CN condition was also higher after AI use by 8.6% [0.66, 16.6] when compared to the sham condition ($t_{(13)}$ =2.34, d=0.63, *P*=0.036; Figure 2C).

- 243
- 244

[Figure 2]

245

KE MVC ($W_{(13)}=72$, d=0.31, P=0.24), HG MVC ($t_{(13)}=-0.264$, d=-0.07, P=0.80), and peak 246 247 power ($t_{(13)}=0.625$, d=0.17, P=0.54) were all unaffected by AI use. The percentage change in RT and EMD from the CN also did not differ between conditions during KE MVC ($t_{(13)}=1.72$, 248 d =0.46, P=0.11; $t_{(13)}$ =0.13, d=0.03, P=0.9, respectively), or during HG MVC ($W_{(13)}$ =46, 249 250 d=0.08, P=0.72; t₍₁₃₎=1.34, d=0.36, P=0.2, respectively) (Table 1). RFD during KE MVC $(F_{(2,13)}=3.1, \eta^2_p=0.04, P=0.064)$ and HG MVC $(F_{(2,13)}=3.27, \eta^2_p=0.2, P=0.54)$ did not differ 251 between the three conditions. However, a significant main time effect for RFD development 252 between 20 – 200ms was observed during the KE and HG MVC ($F_{(2,13)}=9.7$, $\eta^2_p=0.08$, 253 P < 0.001; $F_{(2,13)} = 50.2$, $\eta^2_p = 0.79$, P < 0.001, respectively), and the results of post-hoc analysis 254 are displayed in Table 1). 255

256

There were positive correlations between alertness and perceived performance post-AI $(r_{(12)}=0.90, P<0.001)$, post-sham $(r_{(12)}=0.54, P=0.046)$, and post-CN $(r_{(12)}=0.61, P=0.02)$ (Figure 3). There was no relationship between HR and alertness post-AI $(r_{(12)}=-0.33, P=0.25)$, post-sham $(r_{(12)}=-0.20, P=0.50)$, or post-CN $(r_{(12)}=0.07, P=0.81)$; nor between HR and perceived performance post-AI $(r_{(12)}=-0.25, P=0.38)$, post-sham $(r_{(12)}=-0.15, P=0.62)$, or post-CN $(r_{(12)}=0.06, P=0.84)$.

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266

[Figure 3]

267

268 **Discussion**

The aim of this study was to examine the effects of ammonia inhalant use on functional performance and psychological alertness, in non-resistance trained males. Despite being commonly used as a performance-enhancing stimulant, we observed no improvement in any aspect of functional performance following AI use. Interestingly however, use of the stimulant did elicit an increase in individual perception of overall performance, which was very highly associated with elevated rating of alertness.

275

Despite a lack of research existing to support their beneficial effects, AIs are commonly used
in resistance-based exercise as they are believed to temporarily increase consciousness and
arousal of an individual during training and competition (20). The current study is the first to

assess arousal, via an alertness scale, alongside markers of functional performance, following 279 AI use; specifically, we observed an increase in alertness ratings after stimulant inhalation. It 280 281 is possible that alertness was elevated as a result of a sympathetic nervous response, as AIs have previously been reported to increase breathing rate and HR following inhalation (15, 20, 282 21). However, despite an acute elevation in HR (Figure 2A), there was no correlation between 283 HR and alertness, which suggests that sympathetic nervous response was not exclusively 284 285 responsible for increases in alertness with AI use. Elsewhere, Perry et al. (15) evidenced an acute increase in blood flow to the brain, as measured by middle cerebral artery blood velocity, 286 287 immediately after AI use. Consequently, our finding that alertness was elevated following AI use may be attributable to greater delivery of oxygenated blood to the brain (33). It is known 288 that a reduction in cerebral blood flow impairs cognitive function (34), which may well be 289 290 linked to performance decrements associated with inadequate levels of arousal during training and competition (35). With this in mind, AI-induced enhancement of alertness may help to 291 explain the popularity of this particular stimulant within performance sport. However, such 292 interpretations are beyond the scope of this study. 293

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The current study was also the first to investigate the effects of AIs on perception of overall 295 performance, with participants self-rating that a superior performance was achieved during the 296 AI trial (Figure 2C). Interestingly, moderate-very high positive correlations were observed 297 between perceived performance and alertness ratings within all three conditions, with the 298 highest correlation observed in the AI trial (Figure 3). It is possible that elevated alertness 299 induced by AI use could have improved participant's perception of their performance by 300 enhancing focus and ensuring optimal psychological state was achieved. Furthermore, AIs are 301 believed to increase not only the focus, but also the effort exerted by athletes (20). 302 Subsequently, an individual's reflection of their performance with increased alertness, reduced 303

distraction, and potentially higher levels of effort may have translated into higher self-rating of perceived performance. An improved psychological readiness to perform and consequently, enhanced perception of performance may provide further evidence as to why AI use is prevalent during competition (11, 12). Furthermore, limited data exist regarding the use of AI within different sporting performances. Therefore, when uninformed on physical performance, individuals may argue that AI use are important as from a perceived performance perspective, they believe to perform better after using the stimulant.

311

Although psychological factors were immediately improved by AI use, there were no 312 improvements in any of the functional performance variables measured when compared to the 313 CN or sham conditions. These findings support previous studies which have attempted to 314 investigate the effects of AIs on functional performance such as maximal strength and power 315 Resistance trained individuals (> 2 y training experience) have previously 316 output. demonstrated no improvement in isometric or dynamic contractions of submaximal or maximal 317 318 intensity, following AI inhalation (14–17). These previous studies assessed strength using closed-chain exercises involving multiple muscle groups; in such cases participant familiarity 319 with the exercise test is critical, even among well-trained individuals (36). The current study 320 recruited healthy, recreationally active participants who were unfamiliar with resistance 321 training techniques, as these individuals could be expected to receive a greater beneficial effect 322 from increasing arousal (4). By adopting open-chain exercises, we were able to isolate single 323 muscle groups to assess maximum isometric contraction with minimal participant learning 324 effects (37) and greater sensitivity. Additionally, internal joint angles of 60° (KE) and 90° 325 (HG) were selected to optimise isometric force (27, 28). Nonetheless, MVC, expressed relative 326 to CN, did not differ between AI and sham conditions (KE: [-1.36, 7.04], HG: [-6.22, 4.87]). 327

328

329 Similar to previous research, we recorded no improvement in absolute maximal force production with AIs. However, aspects of force generation, such as RFD and EMD have 330 previously been shown to improve following AI use (14), as well as with other stimulants, such 331 as caffeine, both in the presence (38) and absence (39) of enhanced performance. Interestingly, 332 Bartolomei et al. (2018) reported an association between RFD and CMJ performance, despite 333 no effect of AI use on CMJ peak power (14). However, in contrast to the previous study we 334 observed no effect of AIs on RFD, and further, no effect on EMD or RT. Similar RFD results 335 have been reported previously elsewhere, as Perry et al. (2016) also reported no difference 336 following AI use compared to a control (15). The inconsistency in RFD outcomes with AI use 337 could stem from the type of exercise used to assess RFD – while both Perry et al. (2016) and 338 Bartolomei et al. (2018) utilised maximal isometric mid-thigh pull, participants in the latter 339 study sustained 6 s contractions (compared to 2 s contractions in the former) (14, 15). While 340 our present study adopted 5 s contractions, these involved fewer muscle groups, and were 341 separated by 5 min rest periods (compared to 3 min (14)). As such, and similar to during the 342 study by Perry et al. (2016), participants in the present study may have been less subject to 343 fatigue than were those involved in the study by Bartolomei et al. (2018). As previous findings 344 345 have shown AIs to improve peak and mean power in fatigued individuals (13), so too it is possible that improvements in RFD may also be observable only in individuals exhibiting 346 greater levels of fatigue. It is well established that RFD is impaired by fatigue, indeed such 347 impairment is more pronounced than is impairment in strength (40). Fatigue-induced decline 348 in RFD presents a greater potential for a stimulant-derived increase, therefore future research 349 should explore the relative impact of AI use on aspects of force production, including RFD, in 350 the presence of fatigue-induced decrements. 351

Contrary to our hypothesis, we observed no effect of AI use on peak power. Previously, peak 353 and mean anaerobic power have been seen to increase post-AI use (13). In that study 354 355 participants completed a 30 s Wingate anaerobic cycling test - given the large muscle mass used in such a test, and the benefits of increased blood flow to working muscles in the latter 356 half of the test (41), elevated mean power could be explained by an AI-induced sympathetic 357 response. However, peak power would not be enhanced by such an increase in oxidative 358 359 metabolism. Unlike the present study, where participants were well rested prior to performing CMJ, participants in the aforementioned study by Secrest et al. (2015) were required to reach 360 361 fatigue before the effects of AI use on anaerobic power was assessed (13). Similar to RFD; AI use may only produce substantive effects on power performance when peak power has 362 previously been depleted by individuals reaching a state of fatigue, such as may be experienced 363 in the latter stages of sporting competition. Indeed, based on the results of their survey of 364 powerlifters, Pritchard et al. (2014) reported that use of AIs is most common near the end of 365 competition (12). Although existing evidence permits us to speculate that AI use may be able 366 to offset some of the decrement in RFD and peak power that is experienced alongside fatigue, 367 our findings demonstrate that in a well-rested state, there is no performance benefit to AI use, 368 despite elevated arousal and an associated perception of performance enhancement. 369

370

There are limitations which must be considered when interpreting our findings. Despite AIs being held at a distance of 150 mm (in accordance with manufacture suggestions), it is possible that participants did not inhale a consistent volume throughout the trial, however this closely reflects real world practice. To the best of our knowledge, no research provides data regarding the volume of substance inhaled from set distances. Whilst one similar study held AI 10cm below the nose (14), other studies in this area did not identify a distance. As inhalation volumes for each participant and test cannot be controlled for, we incorporated a distance of 15cm and

asked participants to sharply inhale when instructed to aid with intra- and inter-visit 378 consistency. In addition, rapid rating of alertness was required to ensure that the observations 379 380 represented participant alertness immediately after AI use, precluding the use of an extensive questionnaire, as such alertness was reported using VAS. VASs have been shown to be an 381 effective method of collecting data efficiently, however, the limited time available to record 382 alertness prevented us from collecting a rating from participants prior to every repetition (i.e., 383 384 following every inhalation). A pragmatic decision was taken to record alertness during one repetition (from three) of each assessment, randomly selected. Furthermore, whilst we 385 386 attempted to reduce potential learning effects by familiarising participants during the control visit and randomising the order of the experimental and sham conditions, it is possible that 387 randomising the order of the strength and power performances during each visit may have 388 reduced this risk further. Lastly, it much be acknowledged that EMD displayed poor reliability 389 within this study. Therefore, the EMD results within this study should be interpreted with 390 caution. 391

392

In conclusion, we have demonstrated for the first time that ammonia inhalant use elevates levels of alertness and perception of overall performance in active, non-resistance trained males. However, peak force and rate of force development, reaction time, and peak power all remain unaffected by ammonia inhalant use. Ammonia inhalants remain a popular sporting supplement, although perceived benefits may outweigh any true ergogenic effect; further research is required to explore the potential of acute ammonia inhalation to counter the performance-damaging effects of fatigue.

400

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408 **Disclosure Statement**

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523 Tables

524

Table 1. Mean (SD) rate of force development over 0-20, 0-50, 0-100 and 0-200 ms from onset of force production, electromechanical delay (EMD), and reaction time (RT), within the control condition (no inhalant) and post-inhalation within the experimental (ammonia) and sham conditions (water).

		Control	Ammonia	Sham
	0-20	222 70 (260 22)	206.06 (424.00)	206.07 (205.75)
	ms	252.19 (200.25)	380.80 (434.09)	390.97 (395.75)
HG RFD (N∙s⁻¹)	0-50 ms	475.23 (445.69) *	683.29 (545.07) *	806.15 (638.21) *
	0-100 ms	958.43 (596.87) **†	1195.76 (639.31) ^{**†}	1445.52 (893.24) **†
	0-200 ms	1315.20 (464.45) ^{**†}	1442.78 (612.09) **†	1398.89 (481.44) **†
	EMD	55.14 (36.56)	58.54 (17.27)	49.57 (21.70)
	(ms)			
HG	RT (ms)	339.86 (134.83)	297.36 (116.36)	280.86 (60.41)
KE	0-20	294 10 (333 12)	537.14 (647.69)	312.91 (277.81)
RFD	ms	274.10 (555.12)		
(N·s ⁻	0-50	570.95 (510.52) *	730.35 (612.35) *	579.35(453.77) *
1)	ms	570.85 (510.52)		

	0-100 ms	676.03 (461.36) **	813.37 (487.33) **	652.94 (414.91) **
0-20 ms	0-200 ms	556.96 (298.20) ¶	452.77 (200.95) ¶	604.34 (281.76) ¶
KE	EMD (ms)	84.89 (30.88)	88.75 (46.45)	87.64 (27.22)
	RT (ms)	380.79 (129.58)	402.54 (150.68)	357.18 (84.99)

530 531

HG = handgrip; KE = knee extension.

532 Significant main effect for time during rate of force development is represented by the following

533 symbols: * = P < 0.05 from 20ms; ** = P < 0.001 from 20ms; $\dagger = P < 0.001$ from 50ms; $\P = P < 0.05$ 534 from 100ms.

535

537 Figures



- 542 Figure 1. Timeline of measures during the 3 laboratory visits.
- CN = control, AI = ammonia inhalant.
- *MVC* = maximal isometric voluntary contraction, *CMJ* = countermovement jump.
- 545 Participants inhaled AI or water prior to each contraction and CMJ during trials 2 and 3. Heart rate
- 546 and alertness were measured immediately post-inhalation prior to repetition 1, 2 or 3 (assigned
- *at random) of knee extension MVC, handgrip MVC and CMJ.*



Figure 2. Mean (SD) of heart rate (A), alertness (B), and perceived performance (C) presented
as the percentage difference from the control during the experimental (ammonia) and sham
(water) conditions.

VAS = visual analogue scale.





560 Figure 3. Relationship between alertness and perceived performance including 95%

561 confidence intervals: (A) post-inhalation of ammonia (AI), (B) post-inhalation of water, (C)

562 during control condition (no inhalant).

VAS = visual analogue scale.