Northumbria Research Link

1   **Nutritional Modulation of Sleep Latency, Duration, and Efficiency: A**

2   **Randomised, Repeated-Measures, Double-Blind Deception Study**

3

4   Carl Langan-Evans[1], Mark A. Hearris[1], Chloe Gallagher[1], Stephen Long[1], Craig Thomas[2],

5   Andrew D. Moss[1], William Cheung[3], Glyn Howatson[3][4], and James P. Morton[1]

6

7   [1]Research Institute for Sport and Exercise Sciences, Liverpool John Moores University,

8   Liverpool, UNITED KINGDOM; [2]School of Sport, Exercise and Health Sciences (SSEHS)

9   Loughborough University, Loughborough, UNITED KINGDOM; [3]Department of Sport,

10   Exercise and Rehabilitation & Applied Sciences, Northumbria University, Newcastle upon Tyne,

11   UNITED KINGDOM; [4]Water Research Group, School of Environmental Sciences and

12   Development, North West University, Potchefstroom, SOUTH AFRICA

13

14   **Running Title:** NUTRITIONAL BLEND IMPROVES MEASURES OF SLEEP

15

16   **Address for Correspondence:**

17   Dr Carl Langan-Evans, Research Institute for Sport and Exercise Sciences, Faculty of Science,

18   Liverpool John Moores University, Tom Reilly Building, Liverpool, Merseyside L3 2EX, United

19   Kingdom; Phone: +44 0151 231 4344; E-mail: C.LanganEvans@ljmu.ac.uk

ABSTRACT

27

28 **Purpose:** To test the hypothesis that a novel nutritional blend comprised of tryptophan, glycine,

29 magnesium, tart cherry powder and L-theanine, enhances subjective and objective measures of

30 sleep during free living conditions. **Methods:** In a randomised, repeated measures crossover and

31 double blind deception design, participants (n = 9 male and 7 female; age: 24 ± 3 years; body

32 mass: 69.8 ± 11.6 kg; stature: 170.8 ± 9.1 cm) completed a 3 day familiarisation period, followed

33 by 3 day intervention and placebo trials. Subjective Pittsburgh Quality Sleep Index, Core

34 Consensus Sleep Diary and Karolinska Sleepiness Scale survey tools, alongside objective

35 actigraphy measures of sleep were assessed, with daily nutritional intake, activity and light

36 exposure standardised between trials. Participants provided daily urine samples for assessment of

37 targeted and untargeted metabolomes. **Results:** The intervention trial reduced sleep onset latency

38 (-24 ± 25 mins; p = 0.002), increased total sleep time (22 ± 32 mins; p = 0.01) and sleep

39 efficiency (2.4 ± 3.9 %; p = 0.03), whilst also reducing morning sleepiness (p = 0.02).

40 Throughout the study, 75 % of participants remained blinded to sleep assessment as a primary

41 outcome measure, with 56 % subjectively indicating improved sleep during the intervention trial.

42 Metabolomic analysis highlighted several significantly altered metabolomes related to sleep

43 regulation between trials, inclusive of 6-sulfatoxymelatonin, D-serine and L-glutamic acid.

44 **Conclusions:** Data demonstrate that employing the proposed blend of novel nutritional

45 ingredients during free living conditions reduced sleep onset latency, increased total sleep

46 duration and increased sleep efficiency, leading to reduced perceptions of morning sleepiness.

47 These effects may be mediated by the upregulation of key metabolites involved in the

48 neurophysiological modulation of the sleep/wake cycle. **Key Words:** ACTIGRAPHY,

49 NUTRITION, ERGOGENIC DIETARY SUPPLEMENTS, METABOLOMICS

INTRODUCTION

Sleep is an essential process in aiding the recovery of several psycho-physiological functions, including learning and memory, the central nervous and metabolic cost of daytime activities and the restoration of the immune and endocrine systems (1). In order to maintain optimal health and daytime functioning, it is recommended that adults achieve at least 7 hours of total sleep time with ≥85 % sleep efficiency (2, 3). However, a large scale global survey of participants aged 15 to 65 years, has highlighted that 56 % of respondents from the United States of America, alongside 31 % in Western Europe and 23 % in Japan have experienced some form of sleep disturbance, such as poor sleep induction (55-69 %), maintenance (78-63 %) and overall sleep quality (52-31 %) (4). The impact of these sleep disruptions often results in deleterious effects on daytime functioning, therefore causing negative repercussions to both personal and professional activities. Indeed, there is accumulating evidence demonstrating how sleep disturbance may cause reductions in both athletic performance and physical activity levels (5, 6). Moreover, it has been reported that sleep disturbance is a well understood factor in all cause morbidity and mortality across a range of populations (7). To that end, changes in lifestyle factors, improved sleep hygiene and nutrition have all been proposed as potential modulators for sleep disturbances in order to offset these issues (8, 9).

The nutritional regulation of sleep has been of consistent research interest for many years, most notably in athletic populations, as has been recently addressed in a number of narrative reviews and an expert consensus statement (1, 10, 11). Several studies have examined various nutritional ingredients with proposed sleep enhancing qualities across a range of participant groups with and without sleep disorders, inclusive of high glycaemic index carbohydrates (12, 13), tryptophan (14), tart cherries (15, 16), glycine (17), magnesium (18) and L-theanine (19).

2

73 Within these investigations there have been numerous suggested regulatory mechanisms, with

74 metabolomic interactions of specific neurotransmitters involved in the sleep/wake cycle,

75 purported to enhance regulation of the circadian rhythm of sleep (20). However, despite

76 promising outcomes in the capacity to improve sleep, many of these studies have been conducted

77 with predominantly male participants and with limited samples sizes in order to make definitive

78 conclusions (21). Furthermore, these ingredients have principally been studied in isolation, with

79 only one investigation to date examining their combined efficacy on various sleep outcome

80 measures (22). On this basis, there is considerable scope to further investigate the combined

81 benefit of these sleep enhancing agents, particularly given their reported individual effectiveness.

82 A recent critical review (23) has suggested that to sufficiently investigate and determine

83 the true efficacy of any intervention treatment on sleep mechanisms, participants should be

84 appropriately blinded to their sleeping patterns being monitored. To examine sleep as a primary

85 variable there are several available methods, with the criterion standard of polysomnography

86 affording an in-depth view across numerous outcome measures, inclusive of sleep stages (24).

87 However, despite the validity of this technique, measurement needs to be conducted within a

88 laboratory setting, leading to indirect disturbances in participants normal sleeping patterns and

89 reducing ecological validity as assessments cannot be conducted during free living conditions

90 (23). Consequently, given the complexity of configuration prior to and during polysomnography

91 utilisation, this makes the capacity to deceive participants difficult, thereby not fulfilling the

92 potential to truly blind experiments. Despite being limited in the capability to assess sleep stages,

93 the use of actigraphy has been proposed as a method that is less invasive than polysomnography

94 and has been suggested as less likely to interrupt participants regular sleeping patterns (11, 25).

95 As such, given actigraphy monitors can be easily administered and also utilised to measure

96 daytime activities, this facilitates the opportunity to blind participants and provide a more

97 accurate overview of sleep assessment in free living conditions (26).

98   With this in mind, the aim of the present study was to test the hypothesis that a novel

99 nutritional blend comprised of tryptophan, glycine, magnesium, tart cherry powder and L-

100 theanine, would improve subjective and objective measures of sleep in male and female

101 participants during free living conditions. To this end, we adopted an experimental design

102 inclusive of an initial familiarisation period for the assessment of habitual sleeping patterns, light

103 exposures, physical activity levels and daily energy intakes, prior to participating in a

104 randomised crossover trial whereby participants ingested either an intervention or placebo

105 treatment. Importantly, the intervention was administered double blind, with participants being

106 deceived as to the true aim of the study.

107

108 **METHODS**

109 **Participants**

110   The study was approved by the Liverpool John Moores University Research Ethics

111 Committee (Protocol code: 21/SPS/003) and all procedures were conducted according to the

112 manual of the Declaration of Helsinki 1964 and its later amendments. Sample size was

113 determined a priori according to an effect size of $d = 1.00$, where a sample size of 16 would

114 provide an α-value of 0.05 and a power of 0.96 (G*Power, version 3.1.9.6). Sixteen participants

115 (9 males and 7 females; age: 24 ± 3 years; body mass: 69.8 ± 11.6 kg; stature: 170.8 ± 9.1 cm)

116 were recruited for the study and prior to commencement, were provided with an information

117 sheet and gave written informed consent. Inclusion criteria stipulated participants must be a.)

118 aged between 18-40 years, b.) non-smokers, c.) not experiencing any medically diagnosed sleep

119 disorders, environmental sleep disturbances and/or taking sleep aid medications, d.) had not

120 travelled across different time zones in the month prior to study, e.) have no history of

121 neurological, cardiovascular, metabolic and/or psychiatric illnesses or diseases and f.) not be

122 taking any medications or supplements, which may contraindicate the study.

123

124 **Study Design**

125   Initially, participants were familiarised to the study measures and procedures, whilst also

126 establishing a baseline for nocturnal sleeping patterns, daily energy/macronutrient intakes and

127 activity profiles, which enabled duplication during the main intervention and placebo trials (see

128 Figure 1a & b). In a randomised, repeated measures crossover and double blind deception

129 design, participants were then assigned to either the intervention or placebo trial determined by

130 an online randomisation system (See Figure 1c & d). Both the familiarisation and the main trial

131 assessments were 3 days in length, all of which commenced on the same days of the week for all

132 participants and with a 4 day period between conditions to minimise any crossover effects. The

133 intervention treatment comprised of 1000 mg of tryptophan, 3000 mg of glycine, 300 mg of

134 magnesium, 220 mg of tart cherry powder and 200 mg of L-theanine, with the placebo treatment

135 containing 4720 mg of cellulose, both of which were administered across all 3 nights of each

136 respective main trial in opaque pill format. Additionally, the participants were also informed that

137 the study aim was to assess the effect of two supplemental treatments on resting fat oxidation

138 measured on the final day of main trial periods (see Figure 1c) and with all subsequent

139 assessments being administered for standardisation between conditions. The intention of this

140 deception blinding, was to make participants unaware of the true aim of the study (27).

141 **Study Measures**

142 *Body Mass and Stature:* Body mass was measured to the nearest 0.01 kg on a calibrated digital

143 scale, with measures of stature established to the nearest 0.1 cm using a free standing stadiometer

144 (Seca 702 & 123; Seca GmbH, Hamburg, Germany).

145

146 *Subjective Sleep Assessments:* To examine subjective assessments of nocturnal sleep patterns,

147 alongside evening and morning alertness and sleepiness, participants recorded responses within

148 electronic versions of the Pittsburgh Quality Sleep Index (PSQI) (28), Core Consensus Sleep

149 Diary (CSD) (29) and Karolinska Sleepiness Scale (KSS) (30). The PSQI is a 9-unit

150 questionnaire, which retrospectively examines sleep across a one month period and is rated on 7

151 components of sleep quality, latency, duration, efficiency, disturbance, medication and day time

152 dysfunction, to generate a global score. The core CSD is a 10-unit survey, assessing a range of

153 information related to sleep patterns, whereas the KSS is a 9-point Likert scale utilised to

154 examine levels of alertness or sleepiness, both prospectively captured at the time of response.

155 Upon bed time, each participant recorded the clock time for the first and second units within the

156 core CSD and the following morning upon getting out of bed, recorded the information for the

157 remainder of the units.

158

159 *Objective Sleep, Activity and Light Assessments:* To objectively assess nocturnal sleep patterns,

160 daytime activity profiles and light luminous intensity (lux) exposure, each participant was

161 equipped with a lightweight, waterproof, wrist worn actigraphy monitor (MotionWatch 8,

162 CamNtech Ltd, Cambridgeshire, United Kingdom). The tri-axial accelerometer within the

163 monitor detects acceleration ranging from 0.01 to 8 *G* in magnitude, with a frequency of 3-

164 11 Hz. Additionally, the monitor contains a digital human eye response optimized light sensor,

165 capable of capturing 0 to 64,000 lux at a resolution of 0.25 to 16.0 lux in one sample per second.

166 For sleep and activity measurement, the monitor has been assessed for validity and reliability of

167 its algorithms against both polysomnography and indirect calorimetry across a range of

168 demographics and is effective when set above medium sensitivity and epoch lengths of 30

169 seconds (31, 32). Each monitor was configured in these modes and participants were requested to

170 press the marker button during specific morning and night time periods (see *Study Procedures*).

171 The recorded markers from the monitor and the collated information from the core CSD, were

172 then used to determine bed time, sleep onset, sleep offset and get up time, so that nocturnal sleep

173 behaviours could be automatically calculated using the appropriate software (MotionWare

174 version 1.2.5, CamNtech Ltd, Cambridgeshire, United Kingdom). From the analysis, the

175 following sleep characteristics were established: sleep onset latency (SOL), wake after sleep

176 onset (WASO), total sleep time (TST), time in bed (TIB), and sleep efficiency (SE). After

177 excluding sleep measurement periods, day time activity profiles were automatically calculated by

178 the software into vigorous, moderate, low and sedentary phases, with light exposure averaged

179 across each of the night and day time periods.

180

181 *Daily Energy and Macronutrient Intakes:* Within the familiarisation period, all *ad libitum* food

182 and fluid intake were prospectively recorded by participants using both weighed food inventory

183 and the remote food photography method (RFPM) (33). In brief, participants were guided on

184 how to measure all food and fluids on an electronic digital scale (AccuWeight 201, Nanlgood

185 Network Technology Co. Ltd., Shenzhen, China) and record the details within the MyFitnessPal

186 application (Francisco Partners, San Francisco, California, USA). Additionally, participants were

187 also instructed to capture photographs at 45 and 90° angles before and after each eating and/or

188 drinking occasion, which was recorded and timestamped via the WhatsApp application

189 (WhatsApp Inc, Mountain View, California, USA) as previously described (34). These data were

190 then assessed by two accredited sport nutrition practitioners utilising dietary analysis software

191 (Nutritics V5. Nutritics Ltd., Swords, Co. Dublin, Ireland) to establish respective daily energy

192 and macronutrient values (see Table 1), with systematic bias of measurements via independent t-

193 tests highlighting no differences between analyses ($p < 0.05$). Within both main intervention and

194 placebo trials, participants were instructed to repeat the eating and drinking patterns at the same

195 times of day as in the familiarisation period, with reminders inclusive of previous recorded

196 images, descriptions and MyFitnessPal data sent by members of the research team via the

197 WhatsApp application. The participants were also instructed to follow the same procedures of

198 weighed food inventory and RFPM as described above, for confirmation of adherence.

199 Compliance to these procedures was 100% across all participants, which ensured daily energy

200 and macronutrient values were duplicated across main trials.

201

202 *Urine Collection, Storage and Metabolomic Analysis:* Urinary voids were collected by

203 participants into sterilised measuring cylinders and immediately stored at -20 °C within a home

204 freezer unit. The samples were then returned to the research team, thawed at room temperature,

205 centrifuged at 12,000 G for 1 min and subsequently transferred to a 1.5 mL Eppendorf tubes for

206 immediate storage at −80 °C. A random sub group of $n = 8$ participants samples were then

207 examined for targeted and untargeted metabolomic profiles. For sample preparation, 500 µL of

208 urine was desalted using Zeba™ spin columns (Thermo Scientific, Hemel Hempstead, United

209 Kingdom), flash frozen in liquid nitrogen and lyophilized overnight. The samples were then

210   reconstituted in 100 µL (90/10 v/v) LC/MS grade water acetonitrile and spin filtered with

211   Costar® Spin-X® 0·22 micron cellulose filters (Corning, Glendale, Arizona, USA) at 2000 *g* for

212   5 mins. The resulting concentrated urine samples were then transferred to 1.5 mL amber vials

213   with 150 µL micro inserts. For the targeted analysis of 6-Sulfatoxymelatonin, conventional

214   standard addition methodology was applied, whereby 1.0 mg of chemical standard (Biosynth®

215   Carbosynth Ltd, Berkshire, United Kingdom) was reconstituted in 100 µL of LC/MS grade water

216   to generate a 30 mM stock and then serial diluted down to a 10 µM working solution. The RT

217   and accurate mass were confirmed using hydrophilic liquid interaction chromatography (HILIC)

218   and the mass spectrometry signal acquisition was performed and quantified using a single ion

219   monitoring mode with 10 ppm mass channel tolerance. The HILIC profiling of the samples was

220   performed on a Vanquish^{TM} Ultra High Pressure Liquid Chromatography (UHPLC) separation

221   system connected to an IDX high-resolution mass spectrometer system (Thermo Scientific,

222   Hemel Hempstead, United Kingdom).

223   Mass spectrometry data were captured using the AcquieX acquisition workflow (data

224   dependent analysis methodology) and orbitrap detector operating parameters were MS1 mass

225   resolution 60K, for MS2 30K collisional stepped energy (HCD) 20, 35, 50 in step collision

226   mode. The mass scan range was 100-1000 m/z, RF lens was 35%, AGC target mode custom and

227   normalised AGC target was set to 25 % (100 % = 3e6) with a maximum injection time of 50 ms.

228   The intensity threshold was set to 2e4, with all data acquired in profile mode. A corresponding

229   extraction blank was used to created background exclusion list and a pooled QC were used to

230   create the inclusion list.

231   HILIC separation was achieved using a Waters^{TM} Acquity UPLC BEH amide column

232   (2.1 x 150mm with particle size of 1.7 µm), operating at 65°C with a flow rate of 200 µL·min^{-1}

233   (Waters Ltd., Wilmslow, UK). The LC gradient consisted of a binary buffer system, of which

234   buffer A was 95/5 % (LC/MS grade water/ACN) and Buffer B was 90/10 % (ACN/water), with

235   both containing 10 mM ammonium formate additives. Independent buffer systems were used for

236   positive and negative modes, respectively. For positive modes, the pH of buffers was adjusted

237   using 0.1 % formic acid and for negative modes 0.1 % ammonia solution. The LC gradient were

238   the same for both polarities, with 95 % B at T0 hold for 2 min and a linearly decrease to 50 % B

239   at 11 min hold for 4.5 min and return to the starting condition and hold for further 4.5 min

240   (column stabilization). The total run was approximately 21.5 min per injection, with each volume

241   and applied voltage being 3 µL/3.5 kV and 5 µL/2.5 kV for positive and negative modes,

242   respectively. The HESI conditions for 200 µL·min^{-1} were sheath gas 35, aux gas 7 and sweep gas

243   of 0, with an ion transfer tube temperature of 300°C and vaporizer temperature of 275°C.

244   Positive and negative data sets were processed via Thermo Scientific Compound Discoverer 3.2

245   with untargeted metabolomic workflow, alignment model adaptive curve, minimum intensity

246   1e^6, S/N threshold 3:1, compound consolidation, mass tolerance 10 ppm and retention time

247   tolerance 0.3 min settings. Database matching was performed using Thermo Scientific m/z

248   Cloud^{TM} spectral library for targets with a similar index of >70% MS2 spectra. Those

249   metabolites that could be matched ($n = 134$) and had a relative standard deviation of 30% or less

250   within the quality controls were retained for analysis.

251

252   *Deception Blinding Protocol:* Given the status of COVID-19 at the time of the study assessment

253   period and with governmental restrictions on certain testing procedures in place, the research

254   team were unable to conduct valid assessments of resting fat oxidation via indirect calorimetry.

255   To compensate, a *mock* fat oxidation assessment was conceived, whereby participants were

256   provided with an exetainer collection vial equipped with a bespoke breathing tube. Following the

257   final assessment day of both intervention and placebo main trials and upon waking, participants

258   were instructed to blow into the breathing tube for a period of 60 seconds, so their subsequent

259   breath sample could be assessed for endogenous fat utilisation utilising indirect calorimetry.

260   However once samples were collected, they were discarded, given they were not required for

261   analysis within the primary examinations of the deception design.

262

263   **Study Procedures**

264   *Familiarisation:* On the day prior to the beginning of the familiarisation assessment period,

265   participants were measured for body mass and stature, completed the PSQI and then provided

266   with the subjective and objective sleep measurement apparatus and urine cylinders as described

267   in the *Study Measures*. Participants were then subsequently categorised based on their global

268   PSQI score, with < 5 regarded as *good sleep quality* and > 5 as *poor sleep quality* types (28).

269   During this assessment period, all communication was recorded and timestamped via the

270   WhatsApp application. On the evening prior to the first day of assessment, participants were

271   instructed to wear the actigraphy monitor on their non-dominant wrist, with recording

272   programmed to begin at 6.00am on the following morning. Participants were requested to

273   continuously wear the monitor at all times, other than if there was a need to remove it for safety

274   reasons i.e., contact exercise. On the morning of each assessment day, upon sleep offset and

275   getting out of bed, participants were instructed to press the marker button of the monitor, provide

276   an initial urine sample and then complete the electronic core CSD and KSS survey tools.

277   Throughout the remainder of each assessment day, participants provided weighed food inventory

278   and RFPM for each subsequent individual food and fluid intake occasion (see *Study Measures*),

279   whilst also indicating the details of any exercise bouts, inclusive of modality and load (i.e.,

280   duration, distance, sets, repetitions etc.). A further two urine samples were collected at regular

281   spaced intervals, based on the estimated get up and bed times derived from individual participant

282   PSQI responses. Finally, participants were directed to complete the relevant section within the

283   core CSD and KSS, press the wristwatch actigraphy monitor marker button when getting into

284   bed and again when they intended to go to sleep. The monitor and urine samples were then

285   returned to the research team on the day after the final assessment day (see Figure 1a & b).

286

287   *Main Trials:* Following the familiarisation period, sleep measures were analysed from the

288   actigraphy monitor and if SE was $\geq$ 85 % and TST $\geq$ 7 hours, then participants were further

289   classified as *normal* and below both of these values as *deficient* sleep types (2, 3). During main

290   trials, participants continued to be contacted via the WhatsApp online application, with

291   collection of measures, morning procedures, energy/macronutrient/fluid intakes, exercise

292   occasions and urine sampling repeated in the same manner and time of day as established in the

293   familiarisation period. The placebo and intervention treatments were administered two hours

294   prior to each participant's respective bed time, which was replicated at the same time within the

295   familiarisation period. The sleep offset and get up times of participants were not standardised, to

296   examine if any of the conditions resulted in an extended time in bed (see Figure 1c & d). Upon

297   completion of the study, all participants were interviewed to assess the efficacy of the deception

298   blinding procedures. Initially, participants were queried as to if they could detect any differences

299   between the two main trials. Participants were then informed that the deception was not the true

300   aim of the study and asked if they could speculate as to the real aim. Finally, participants were

301   informed of the study aim and asked if they could identify the intervention and placebo

302   conditions.

303

304   **Statistical Analyses**

305   Descriptive statistics inclusive of mean $\pm$ *SD*, 95 % confidence intervals (95 % CI) and

306   frequency are provided for all data where appropriate, with analyses performed using SPSS

307   version 26 (PASW, Chicago, Illinois, USA) and the α-level set at $p<0.05$. Residuals of the data

308   were explored for normality utilising histograms and Shapiro-Wilk tests, with box plots

309   examined for outliers. All data are presented as the mean of each 3 day period for the

310   intervention and placebo trials; therefore, comparisons were assessed via parametric paired

311   samples *t*-tests or non-parametric Wilcoxon tests for normally and non-normally distributed data,

312   respectively. Additionally, effect sizes (ES) were calculated utilising Hedges *g* with the

313   following quantitative criteria to explain the practical significance of the findings: trivial <0.2,

314   small 0.2 – 0.59, moderate 0.6 – 1.19, large 1.20 – 1.99, and very large >2.0 (35). Given the

315   ordinal nature of the KSS data, Pearson's Chi Squared test was employed to cross tabulate

316   frequency percentages between participant responses. Metabolomics data was auto scaled and

317   cube root transformed using MetaboAnalyst 5.0 software (36) before performing detailed partial

318   least squares discriminant analysis (PLS-DA) between intervention and placebo trial conditions.

319   The relative abundance of the metabolites from the intervention with variable importance in

320   projection (VIP) factor >1 was then compared with the placebo trial.

321 **RESULTS**

322 **Sleep Classifications and Bed Times**

323   Prior to starting the familiarisation assessment period, $n = 8$ participants were

324 subjectively classified as having *poor sleep quality* (50 %) with the remaining $n = 8$ classified as

325 having *good sleep quality* (50 %) based on global PSQI scores. Following the familiarisation

326 period, $n = 14$ participants were objectively classified as *deficient* (87.5 %) and $n = 2$ classified

327 as *normal* (12.5 %) sleep types based on actigraphy monitoring. Mean bed time during the

328 familiarisation assessment period was 11.47 pm $\pm$ 26 mins, which was consistently replicated at

329 11.48 pm $\pm$ 26 mins and 11.50 pm $\pm$ 26 mins in both the intervention and placebo trials,

330 respectively.

331

332 **Activity Profiles and Light Exposure**

333   There were no differences in mean night time light exposure between intervention (1.06 $\pm$

334 0.91 lux) and placebo (1.29 $\pm$ 0.95 lux) main trials, with a -0.24 $\pm$ 0.53 lux variance of a small

335 effect between conditions ($p = 0.10$; 95 % CI = -0.53 to 0.55 lux; ES = 0.24). Additionally, there

336 were also no differences in mean day time light exposure between intervention (351.66 $\pm$ 289.58

337 lux) and placebo (352.48 $\pm$ 361.74 lux) main trials, with a -0.80 $\pm$ 295.19 lux difference of a

338 trivial effect between conditions ($p = 0.99$; 95 % CI = -158.10 to 156.50 lux; ES = 0.01).

339 Measurements of mean participant activity are highlighted in Figure 2, demonstrating no

340 differences in vigorous (Figure 2a, 9 $\pm$ 1 vs. 8 $\pm$ 11 mins·day^{-1}, $p = 0.83$; 95 % CI = -4 to 5

341 mins·day^{-1}; ES = 0.09), moderate (Figure 2b, 25 $\pm$ 23 vs. 35 $\pm$ 29 mins·day^{-1}, $p = 0.22$; 95 % CI

342 = -25 to 6 mins·day^{-1}; ES = 0.37), low (Figure 2c, 473 $\pm$ 93 vs. 453 $\pm$ 80 mins·day^{-1}, $p = 0.15$; 95

343 % CI = -7 to 47 mins·day^{-1}; ES = 0.22) or sedentary (Figure 2d, 360 $\pm$ 117 vs. 378 $\pm$ 109

344   mins·day^{-1}, $p = 0.13$; 95 % CI = -40 to 5 mins·day^{-1}; ES = 0.16) profiles, resulting in trivial to

345   small effects between the intervention and placebo main trials, respectively.

346

347   **Actigraphy Sleep Assessments**

348   For measures of SOL, there were differences of a large effect between the intervention

349   (16 ± 6 mins) and placebo (40 ± 25 mins) main trials, with a mean reduction of -24 ± 25 mins (p

350   = 0.002; 95 % CI = -38 to -10 mins; ES = 1.29) between conditions (see Figure 3a). There were

351   no differences in WASO between the intervention (49 ± 15 mins) and placebo (51 ± 17 mins)

352   main trials, resulting in a trivial effect based on the mean difference of -2 ± 11 mins ($p = 0.36$; 95

353   % CI = -8 to 3 mins; ES = 0.12) as highlighted in Figure 3b. For measures of TST there were

354   differences of a small effect between the intervention (422 ± 34 mins) and placebo (400 ± 44

355   mins) main trials, due to a mean increase of 22 ± 32 mins ($p = 0.01$; 95 % CI = 6 to 39 mins; ES

356   = 0.55) between conditions (see Figure 3c). There were no differences for overall TIB between

357   the intervention (506 ± 38 mins) and placebo (495 ± 42 mins) main trials, with a small effect

358   given the mean difference of 11 ± 26 mins ($p = 0.13$; 95 % CI = -4 to 25 mins; ES = 0.27) as

359   shown in Figure 3d. Finally, there were differences of a small effect in measures of SE between

360   the intervention (83.3 ± 3.5 %) and placebo (80.9 ± 5.9 %) main trials, based on an increase in

361   efficiency of 2.4 ± 3.9 % ($p = 0.03$; 95 % CI = 0.3 to 4.5 %; ES = 0.48) between conditions (see

362   Figure 3e).

363

364   **Subjective Sleep Assessments & Deception Blinding**

365   There were no differences evident in subjective scores of evening sleepiness between the

366   intervention and placebo main trials (see Figure 4a). However, there were differences in morning

15

367   sleepiness (see Figure 4b), whereby in the intervention trial participants reported a lower level of

368   some signs of sleepiness when compared to the placebo trial ($p = 0.02$). During post testing exit

369   interviews, $n = 12$ (75 %) participants were unable to identify that the true outcome of the study

370   was to assess sleep, with the remaining $n = 4$ (25 %) being aware. Of the $n = 16$ participants, $n =$

371   9 (56 %) identified the intervention trial as the assessment period where they perceived they had

372   their best sleep, $n = 1$ (6 %) stated the placebo trial and $n = 6$ (38 %) were unable to identify any

373   difference between conditions.

374

375   **Targeted and Untargeted Urinary Metabolomic Analysis**

376   Results of the PLS-DA visualization for both treatments is presented in Figure 5,

377   demonstrating a distinct phenotypic change in the urinary metabolome between the intervention

378   and placebo treatments. Twenty five database matched metabolites were highlighted to be

379   statistically different between the intervention and placebo trials based on VIP score factor > 1.

380   Figure 6 highlights those metabolites identified as proxy markers of sleep regulation inclusive of

381   the targeted metabolite 6-sulfatoxymelatonin (a.), alongside untargeted amino acids D-serine (b.)

382   and L-glutamic acid (c.), both of which were up and downregulated between the intervention and

383   placebo trials, respectively.

384

385   **DISCUSSION**

386   In a randomised, repeated measures crossover and double blind deception design, the aim

387   of the present study was to test the hypothesis that a novel nutritional blend comprised of

388   tryptophan, glycine, magnesium, tart cherry extract and L-theanine, would improve subjective

389   and objective measures of sleep in male and female participants during free living conditions.

390 The data from this study demonstrates that a blend of sleep enhancing agents, shortened time to

391 fall asleep and extended time asleep, therefore resulting in greater sleep efficiency and leading to

392 reduced feelings of morning sleepiness. Evaluation of the urinary metabolome also suggests that

393 such alterations to sleeping patterns may be mediated by the upregulation of 6-

394 sulfatoxymelatonin and glycine co-agonist D-serine, in parallel to a downregulation of the

395 excitatory neurotransmitter L-glutamic acid, which are modulators of the sleep/wake cycle,

396 respectively. Taken together, these results indicate the efficacy of this novel blend of nutritional

397 ingredients to improve outcome measures of sleep during free living conditions, in both male and

398 females without medically diagnosed sleep disorders.

399 A key strength of this investigation was in deception blinding participants to sleep

400 assessment as the true aim of the study. On this basis, 75 % of participants were unaware that

401 sleep was being assessed as a primary outcome measure and over 50 % identified they had a

402 perceived sense of improved sleep during the intervention trial. Whilst this method has been

403 utilised in other nutritional investigations (27), to the authors' knowledge this study represents

404 the first application of this approach when examining the potential for nutritional modulation of

405 subjective and objective sleep outcomes and therefore enhances the applicability of the findings

406 (23). Additionally, participants were also exposed to an initial assessment period with the dual

407 objective of familiarisation to outcome measures, whilst also establishing a baseline for

408 standardisation of bed times, daily nutritional intake, activity profiles and light exposure within

409 the main intervention and placebo trials. This has been previously highlighted as an important

410 consideration in deciphering the translational potential of nutrition intervention studies (37) and

411 serves to further establish the ecological validity of the outcome results.

412   To date, only one other study by Halson and colleagues (22) has examined the effect of a

413   combined blend of sleep active ingredients, inclusive of high glycaemic carbohydrates, tart

414   cherry, tryptophan (in the form of α-lactalbumin), adenosine-5-monophosphate, valerian and L-

415   theanine in a double blind, placebo controlled crossover design. Whilst utilising

416   polysomnography in contrast to the actigraphy measures within this study, Halson et al.

417   highlighted a 9 minute reduction in SOL between the intervention and placebo treatments, yet

418   contrary to our findings, found no other differences in subjective or objective measures of sleep

419   outcomes between conditions. It should be noted that a limitation within the present study, is the

420   inability of a reductionist approach to understand the individual or combined effects of the agents

421   within the intervention treatment, on the capacity to improve the subjective and objective

422   measures of sleep. However, given the strength of the study design, the nutritional blend did

423   indeed impact some of these measures and to that end, the subsequent sections will serve to

424   discuss the potential sleep enhancing mechanisms of each ingredient contained within the

425   intervention treatment.

426   Dietary tryptophan (Trp) is a well-established sleep active ingredient, which has over

427   four decades of research within this area (14). Trp crosses the blood brain barrier by active

428   transport, therefore acting to release the monoamine neurotransmitter serotonin, a precursor of

429   melatonin (38). However, given other large neutral amino acids (LNAAs) also compete to cross

430   the blood brain active barrier system, it is the addition of an adequate dose of dietary Trp (1000-

431   3000 mg), alongside an optimal Trp:LNAA ratio which increases brain bioavailability and can

432   subsequently lead to enhanced sleep through the upregulation of melatonin (14, 39). In

433   comparison to our study, Hartmann and Spinweber (40) found that a dose of 1000 mg of

434   tryptophan resulted in significantly reducing subjective assessments of SOL in those with mild

435   insomnia (over 30 minutes SOL). Additionally, Chauffard-Alboucq and colleagues (41) also

436   highlighted that a dose of 1000 mg of tryptophan was effective in quadrupling the Trp:LNAA

437   ratio, whilst also leading to enhanced feelings of sleepiness after 90 minutes of ingestion. Based

438   on these investigations, it could be postulated that the dosage of tryptophan included in the

439   presented study increased brain Trp levels, thereby upregulating serotonin and melatonin

440   pathways. Despite not directly assessing the Trp:LNAA ratio, this notion is supported by an

441   enhanced metabolomic abundance of urinary 6-sulfatoxymelatonin (see Figure 6a), which could

442   further explain the exhibited improvements in sleep during the intervention trial. In parallel to

443   Trp, tart cherries are known to contain endogenous melatonin (42), which can also directly

444   regulate sleep mechanisms via the aforementioned pathways. Howatson and colleagues (15)

445   demonstrated that a 7 day administration of tart cherry juice resulted in significant improvements

446   in sleep time and quality. Indeed, whilst the provision of the intervention treatment within the

447   present study was for a 3 day period, this resulted in similar favourable increases in both TST (22

448   vs. 34 mins) and enhancements in SE (2.4 vs. 5.5 %). Furthermore, Howatson et al. also

449   highlighted significant increases in urinary melatonin during tart cherry supplementation, also

450   corresponding to the amplified metabolomic abundance of urinary 6-sulfatoxymelatonin within

451   this study.

452   Glycine and L-theanine are amino acids, which are both endogenously synthesised/stored

453   and can be ingested through diet. In a similar manner to Trp, glycine is also able to cross the

454   blood brain barrier, which alongside co-agonist D-serine, targets the *N*-methyl-D-aspartate

455   (NMDA) receptors within the suprachiasmatic nucleus, further acting to upregulate whole body

456   vasodilation and therefore reduce core temperature leading to subsequent sleep enhancement

457   (17). Additionally, L-theanine also crosses the blood brain barrier and acts on the ionotropic

458   glutamate receptors and partially on the NMDA receptors to upregulate both serotonin and

459   glycine (19). A study by Yamadera and colleagues (43) examined the effects of glycine on

460   subjective sleep parameters and objective sleep via polysomnography. Whilst utilising different

461   measures compared to those within this study, a comparable dose of 3000 mg of glycine

462   improved objective assessments of SOL and subjective assessments of sleep quality and daytime

463   sleepiness. Although no assessments of core temperature were conducted within the present

464   study, given the known effects of core temperature reduction on upregulating sleep (44), it is

465   tempting to speculate that the glycine modulation of this mechanism may have also contributed

466   to the improvements in sleep outcome measures, particularly given the increased metabolomic

467   abundance of D-serine (see Figure 6b). However, to date this has only been conducted in animal

468   model studies, with more conclusive evidence needed within human populations (45). In a

469   similar manner to the present study, Ozeki and colleagues also examined the efficacy of L-

470   theanine on sleep via both subjective sleep scores and objective measurements of actigraphy

471   (19). Utilising a 200 mg dose and across a 3 day measurement period, the intervention

472   significantly improved subjective assessments of sleep, alongside an actigraphy derived 2.8 %

473   improvement in sleep efficiency when compared to a placebo condition, in direct parallel to the

474   results of this study.

475   An additional interesting finding within this investigation was a downregulated

476   metabolomic abundance of L-glutamic acid within the intervention compared to the placebo

477   trials (see Figure 6c). L-glutamic acid has been highlighted as a potent excitatory

478   neurotransmitter, resulting in stimulation of the orexinergic neurons, which in turn promotes

479   arousal and inhibition of both non-rapid eye and rapid eye movement sleep stages (46).

480   Conversely, both endogenous melatonin and D-serine have been demonstrated to attenuate the

481 excitatory neurotransmitter action of L-glutamic acid via inhibition of specific NMDA receptor

482 binding sites (47, 48). To that end, it is tempting to speculate that the upregulated metabolomic

483 abundance of urinary 6-sulfatoxymelatonin and D-serine in the intervention trial may have

484 resulted in the downregulated metabolomic abundance of L-glutamic acid, therefore leading to

485 greater subjective and objective sleep enhancement in comparison to the placebo trial. However,

486 much of the former evidence in this area has principally been conducted in rodent models and

487 more research is needed in human trials to substantiate this hypothesis.

488 Finally, magnesium is a key micronutrient, which also acts to regulate the conductivity of

489 the NMDA receptors, whilst aiding the binding of monoamines such as serotonin to their

490 respective sites (49), presenting the assumption that magnesium deficiency may lead to

491 disturbances in sleep, which can be enhanced with exogenous supplementation (50). A recent

492 systematic review and meta-analysis highlighted that administration of 320-729 mg·day^{-1}

493 magnesium, resulted in improvements in subjective and objective sleep, that included 17 minute

494 reductions in SOL and 16 minute increases in TST when compared to placebo conditions (50).

495 However, it should be noted that these treatments were delivered across various dosing

496 protocols, in predominantly elderly populations with magnesium deficiencies and across a range

497 of sleep disturbances and disorders. Consequently, given magnesium status was not assessed in

498 the present study participant cohort, it is difficult to consider the interaction of this ingredient

499 within the intervention treatment and with more research on the efficacy of magnesium

500 supplementation in diverse populations needed.

501 Finally, the present data may be considered of practical relevance for those individuals

502 who partake in regular exercise and physical activities. Indeed, in athletic populations, it has

503 been established that sleep disturbances can result in negative consequences on physical

504 performance, exercise recovery and exercise induced injuries/diseases, with these outcomes

505 being mediated by an enhanced perception of exertion via increased psycho-physiological strain

506 (5). Furthermore, reductions in sleep duration and quality are likely to reduce participation in

507 physical activity, which contributes to a wide range of health related outcomes within general

508 populations (6). Accordingly, improvements in sleep have been shown to enhance exercise

509 performance and increase the likelihood of participation of physical activities in athletic and

510 general populations, respectively (5, 6). On this basis, further investigation implementing the

511 nutritional blend proposed within this study and the potential effects of ameliorated sleep

512 outcomes on exercise performance and physical activity is certainly warranted.

513

514 **CONCLUSIONS**

515   In conclusion, this study highlights the efficacy of a novel blend of sleep enhancing

516 nutritional agents on subjective and objective measurements of sleep in both male and female

517 participants without medically diagnosed sleep disorders and during free living conditions.

518 Additionally, a key strength of this investigation included 75 % of participants being unaware

519 that sleep was being measured as a primary outcome, therefore enhancing the ecological validity

520 and applicability of the findings. The combination of ingredients contained within the blend, led

521 to significant decreases in sleep onset latency and increases in total sleep time and sleep

522 efficiency, alongside subjective reductions in morning sleepiness, as has been demonstrated

523 within the previous research literature. Furthermore, potential mechanisms for these effects may

524 have been as the result of metabolomes related to the sleep/wake cycle. The replication of this

525 study to examine potential efficacy across longer time periods and in those with medically

526 diagnosed sleep disorders is also warranted.

533   **Conflicts of Interest**

534   James P. Morton is a consultant for SiS plc. The other authors disclose no conflicts of interest. SiS plc

535   had no role in the design, execution, interpretation, or writing of the study. The results of the study are

536   presented clearly, honestly, without fabrication, falsification, or inappropriate data manipulation. The

537   results of the present study do not constitute endorsement by the American College of Sports Medicine.

REFERENCES

1.   Doherty R, Madigan S, Warrington G, Ellis J. Sleep and nutrition interactions: implications for athletes. *Nutrients*. 2019;11(4):822.

2.   Hirshkowitz M, Whiton K, Albert SM, et al. National Sleep Foundation's sleep time duration recommendations: methodology and results summary. *Sleep Health.* 2015;1(1):40-3.

3.   Ohayon M, Wickwire EM, Hirshkowitz M, et al. National Sleep Foundation's sleep quality recommendations: first report. *Sleep Health.* 2017;3(1):6-19.

4.   Léger D, Poursain B, Neubauer D, Uchiyama M. An international survey of sleeping problems in the general population. *Curr Med Res Opin.* 2008;24(1):307-17.

5.   Chennaoui M, Arnal PJ, Sauvet F, Léger D. Sleep and exercise: a reciprocal issue? *Sleep Med Rev.* 2015;20:59-72.

6.   Kline CE. The bidirectional relationship between exercise and sleep: implications for exercise adherence and sleep improvement. *Am J Lifestyle Med.* 2014;8(6):375-9.

7.   Saltychev M, Juhola J, Ervasti J, et al. Association of changes in lifestyle with changes in sleep difficulties: an analysis of 38 400 participants over a 16-year follow-up. *BMJ Open.* 2021;11(10):e050046.

8.   Albakri U, Drotos E, Meertens R. Sleep health promotion interventions and their effectiveness: an umbrella review. *Int J Environ Res Public Health.* 2021;18(11):5533.

9.   Burrows T, Fenton S, Duncan M. Diet and sleep health: a scoping review of intervention studies in adults. *J Hum Nutr Diet.* 2020;33(3):308-29.

10.   Halson SL. Sleep in elite athletes and nutritional interventions to enhance sleep. *Sports Med.* 2014;44 Suppl 1(Suppl 1):S13-23.

11.   Walsh NP, Halson SL, Sargent C, et al. Sleep and the athlete: narrative review and 2021 expert consensus recommendations. *Br J Sports Med.* 2020;bjsports-2020-102025. PubMed PMID: 33144349.

12.   Vlahoyiannis A, Aphamis G, Andreou E, Samoutis G, Sakkas GK, Giannaki CD. Effects of high vs. low glycemic index of post-exercise meals on sleep and exercise performance: a

565 randomized, double-blind, counterbalanced polysomnographic study. *Nutrients*.

566 2018;10(11):1795.

567 13.   Afaghi A, O'Connor H, Chow CM. High-glycemic-index carbohydrate meals shorten

568 sleep onset. *Am J Clin Nutr.* 2007;85(2):426-30.

569 14.   Silber BY, Schmitt JA. Effects of tryptophan loading on human cognition, mood, and

570 sleep. *Neurosci Biobehav Rev.* 2010;34(3):387-407.

571 15.   Howatson G, Bell PG, Tallent J, Middleton B, McHugh MP, Ellis J. Effect of tart cherry

572 juice (Prunus cerasus) on melatonin levels and enhanced sleep quality. *Eur J Nutr.*

573 2012;51(8):909-16.

574 16.   Losso JN, Finley JW, Karki N, et al. Pilot study of the tart cherry juice for the treatment

575 of insomnia and investigation of mechanisms. *Am J Ther.* 2018;25(2):e194-e201.

576 17.   Bannai M, Kawai N. New therapeutic strategy for amino acid medicine: glycine improves

577 the quality of sleep. *J Pharmacol Sci.* 2012;118(2):145-8.

578 18.   Held K, Antonijevic IA, Künzel H, et al. Oral Mg(2+) supplementation reverses age-

579 related neuroendocrine and sleep EEG changes in humans. *Pharmacopsychiatry*.

580 2002;35(4):135-43.

581 19.   Rao TP, Ozeki M, Juneja LR. In search of a safe natural sleep aid. *J Am Coll Nutr.*

582 2015;34(5):436-47.

583 20.   Jenkins TA, Nguyen JC, Polglaze KE, Bertrand PP. Influence of tryptophan and

584 serotonin on mood and cognition with a possible role of the gut-brain axis. *Nutrients*.

585 2016;8(1):56.

586 21.   St-Onge MP, Mikic A, Pietrolungo CE. Effects of diet on sleep quality. *Adv Nutr.*

587 2016;7(5):938-49.

588 22.   Halson SL, Shaw G, Versey N, et al. Optimisation and validation of a nutritional

589 intervention to enhance sleep quality and quantity. *Nutrients*. 2020;12(9):2579.

590 23.   Vlahoyiannis A, Sakkas GK, Manconi M, Aphamis G, Giannaki CD. A critical review on

591 sleep assessment methodologies in athletic populations: factors to be considered. *Sleep Med.*

592 2020;74:211-23.

593   24.   Depner CM, Cheng PC, Devine JK, et al. Wearable technologies for developing sleep
594   and circadian biomarkers: a summary of workshop discussions. *Sleep*. 2020;43(2):zsz254.

595   25.   Lujan MR, Perez-Pozuelo I, Grandner MA. Past, present, and future of multisensory
596   wearable technology to monitor sleep and circadian rhythms. *Front Digit Health*.
597   2021;3:721919.

598   26.   Kirk U, Ngnoumen C, Clausel A, Purvis CK. Using actigraphy and heart rate variability
599   (HRV) to assess sleep quality and sleep arousal of three app-based interventions: sleep music,
600   sleepcasts, and guided mindfulness. *J Cogn Enhanc*. 2022;6(2):216-31.

601   27.   James LJ, Moss J, Henry J, Papadopoulou C, Mears SA. Hypohydration impairs
602   endurance performance: a blinded study. *Physiol Rep*. 2017;5(12):e13315.

603   28.   Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep
604   Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res*.
605   1989;28(2):193-213.

606   29.   Carney CE, Buysse DJ, Ancoli-Israel S, et al. The consensus sleep diary: standardizing
607   prospective sleep self-monitoring. *Sleep*. 2012;35(2):287-302.

608   30.   Akerstedt T, Gillberg M. Subjective and objective sleepiness in the active individual. *Int
609 J Neurosci*. 1990;52(1-2):29-37.

610   31.   Elbaz M, Yauy K, Metlaine A, Martoni M, Leger D. Validation of a new actigraph
611   motion watch versus polysomnography on 70 healthy and suspected sleep-disordered subjects. *J
612 Sleep Res*. 2012;21(Suppl 1):218.

613   32.   Landry GJ, Falck RS, Beets MW, Liu-Ambrose T. Measuring physical activity in older
614   adults: calibrating cut-points for the MotionWatch 8©. *Front Aging Neurosci*. 2015;7:165.

615   33.   Martin CK, Han H, Coulon SM, Allen HR, Champagne CM, Anton SD. A novel method
616   to remotely measure food intake of free-living individuals in real time: the remote food
617   photography method. *Br J Nutr*. 2009;101(3):446-56.

618   34.   Hannon MP, Parker LJF, Carney DJ, et al. Energy requirements of male academy soccer
619   players from the English Premier League. *Med Sci Sports Exerc*. 2021;53(1):200-10.

620 35.   Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in
621 sports medicine and exercise science. *Med Sci Sports Exerc.* 2009;41(1):3-13.

622 36.   Pang Z, Chong J, Zhou G, et al. MetaboAnalyst 5.0: narrowing the gap between raw
623 spectra and functional insights. *Nucleic Acids Res.* 2021;49(W1):W388-W96.

624 37.   Close GL, Kasper AM, Morton JP. From paper to podium: quantifying the translational
625 potential of performance nutrition research. *Sports Med.* 2019;49(Suppl 1):25-37.

626 38.   Richardson GS. The human circadian system in normal and disordered sleep. *J Clin
627 Psychiatry.* 2005;66 Suppl 9:3-9; quiz 42-3.

628 39.   Hajak G, Huether G, Blanke J, et al. The influence of intravenous L-tryptophan on
629 plasma melatonin and sleep in men. *Pharmacopsychiatry*. 1991;24(1):17-20.

630 40.   Hartmann E, Spinweber CL. Sleep induced by L-tryptophan. Effect of dosages within the
631 normal dietary intake. *J Nerv Ment Dis.* 1979;167(8):497-9.

632 41.   Chauffard-Alboucq FA, Leathwood PD, Dormond CA. Changes in plasma amino acid
633 and subjective sleepiness ratings in humans after consumingL-tryptophan/maltodextrin mixes.
634 *Amino Acids.* 1991;1(1):37-45.

635 42.   Burkhardt S, Tan DX, Manchester LC, Hardeland R, Reiter RJ. Detection and
636 quantification of the antioxidant melatonin in Montmorency and Balaton tart cherries (Prunus
637 cerasus). *J Agric Food Chem.* 2001;49(10):4898-902.

638 43.   Yamadera W, Inagawa K, Chiba S, Bannai M, Takahashi M, Nakayama K. Glycine
639 ingestion improves subjective sleep quality in human volunteers, correlating with
640 polysomnographic changes. *Sleep Biol Rhythms.* 2007;5(2):126-31.

641 44.   Barrett J, Lack L, Morris M. The sleep-evoked decrease of body temperature. *Sleep*.
642 1993;16(2):93-9.

643 45.   Kawai N, Sakai N, Okuro M, et al. The sleep-promoting and hypothermic effects of
644 glycine are mediated by NMDA receptors in the suprachiasmatic nucleus.
645 *Neuropsychopharmacology*. 2015;40(6):1405-16.

646 46.   Alam MA, Mallick BN. Glutamic acid stimulation of the perifornical-lateral
647 hypothalamic area promotes arousal and inhibits non-REM/REM sleep. *Neurosci Lett.*
648 2008;439(3):281-6.

649 47.   Bavithra S, Sugantha Priya E, Selvakumar K, Krishnamoorthy G, Arunakaran J. Effect of
650 melatonin on glutamate: BDNF signaling in the cerebral cortex of polychlorinated biphenyls
651 (PCBs)-exposed adult male rats. *Neurochem Res.* 2015;40(9):1858-69.

652 48.   Yovanno RA, Chou TH, Brantley SJ, Furukawa H, Lau AY. Excitatory and inhibitory D-
653 serine binding to the NMDA receptor. *bioRxiv*. 2022.03.07.483247.

654 49.   Chollet D, Franken P, Raffin Y, Malafosse A, Widmer J, Tafti M. Blood and brain
655 magnesium in inbred mice and their correlation with sleep quality. *Am J Physiol Regul Integr
656 Comp Physiol.* 2000;279(6):R2173-8.

657 50.   Mah J, Pitre T. Oral magnesium supplementation for insomnia in older adults: a
658 systematic review & meta-analysis. *BMC Complement Med Ther.* 2021;21(1):125.

659

FIGURE LEGENDS

661

662   **Figure 1** Overview of study design, inclusive of (a.) familiarisation assessment period, (b.)

663   familiarisation assessment methods and procedures, (c.) intervention and placebo assessment

664   periods and (d.) intervention and placebo assessment methods and procedures

665

666   **Figure 2** Comparison of mean and individual responses for (a). vigorous, (b.) moderate, (c.) low

667   and (d.) sedentary activity profiles, between the intervention (grey bars) and placebo (white bars)

668   trials

669

670   **Figure 3** Comparison of mean and individual responses for (a.) sleep onset latency, (b.) wake

671   after sleep onset, (c.) total sleep time, (d.) time in bed and (E.) sleep efficiency outcomes,

672   between the intervention (grey bars) and placebo (white bars) trials. *denotes significant

673   difference between conditions ($p < 0.05$)

674

675   **Figure 4** The frequency of subjective Karolinska Sleepiness Scale scores across (a.) evening and

676   (b.) morning measurement periods between intervention and placebo trials. *denotes significant

677   difference between equitable scoring measure ($p < 0.05$)

678

679   **Figure 5** Partial least squares discriminant analysis of key identified urinary metabolomes

680   between the intervention (red) and placebo (green) trials

681

682   **Figure 6** Original and normalised concentration of key urinary metabolites (a.) 6-

683   sulfatoxymelatonin, (b.) D-serine and (c.) L-glutamic acid between the intervention (red bars)

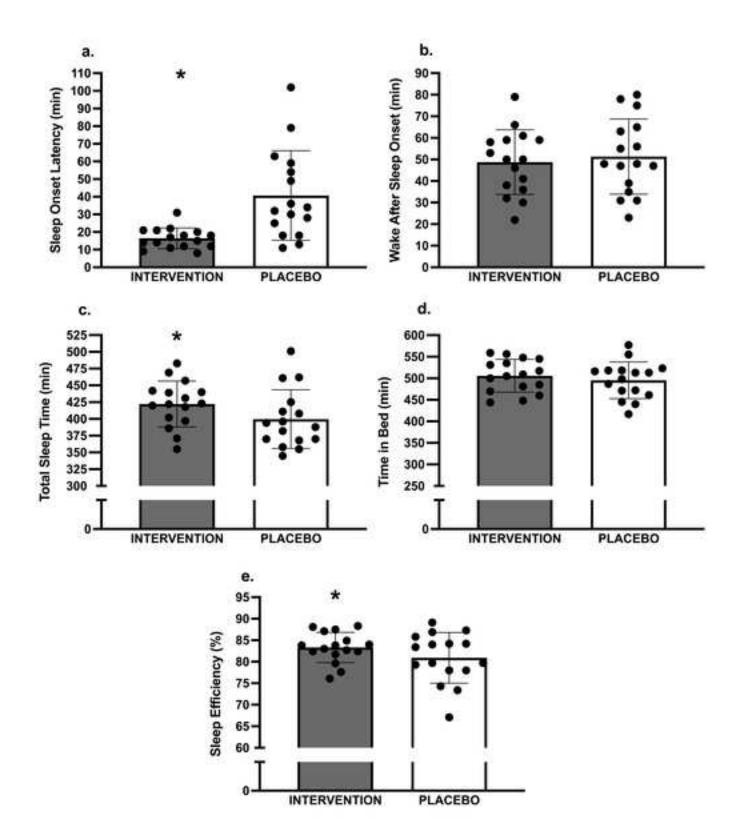684   and placebo (green bars) trials

Table 1

Table 1 Estimated daily energy and macronutrient intakes assessed during the familiarisation period, which were duplicated during main intervention and placebo trials

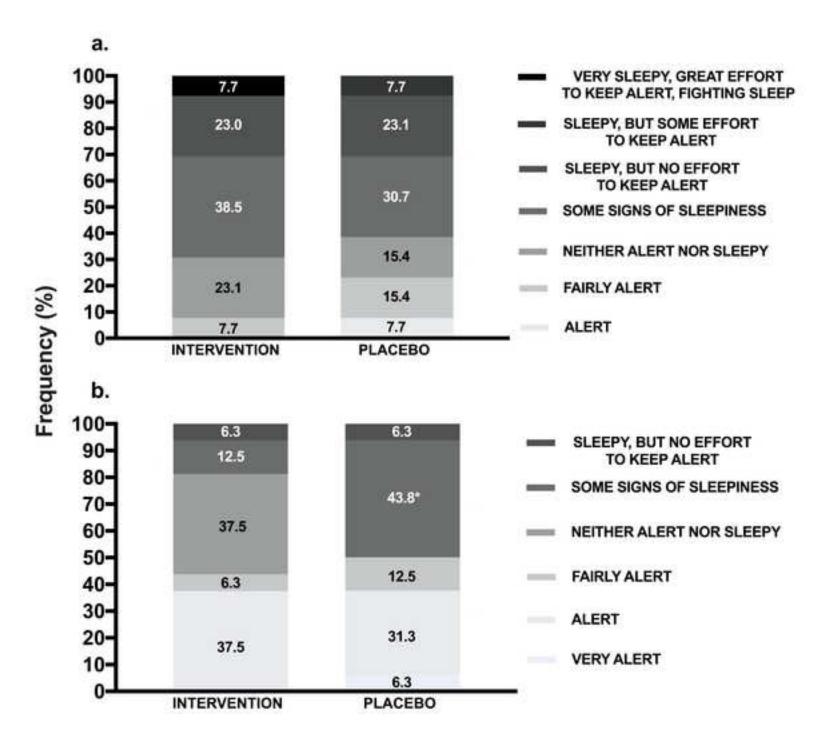| INTAKE	Energy (kcal·day^{-1})	CHO g·day^{-1} (g·kg^{-1})	Protein g·day^{-1} (g·kg^{-1})	Fat g·day^{-1} (g·kg^{-1})
Day 1	2010 ± 567	257 ± 79 *(3.5 ± 1.0)*	101 ± 38 *(1.6 ± 0.5)*	62 ± 27 *(0.9 ± 0.3)*
Day 2	2114 ± 769	273 ± 90 *(3.7 ± 1.1)*	104 ± 49 *(1.6 ± 0.8)*	65 ± 39 *(1.0 ± 0.5)*
Day 3	2036 ± 782	264 ± 110 *(3.8 ± 1.2)*	100 ± 50 *(1.6 ± 0.7)*	63 ± 35 *(0.9 ± 0.4)*
TOTAL	**2053 ± 640**	**265 ± 82** *(3.7 ± 1.0)*	**101 ± 39** *(1.6 ± 0.6)*	**63 ± 28** *(0.9 ± 0.3)*

Relative values are presented in italics within parenthesis. CHO = carbohydrate

Figure 1

Figure 2

Figure 3

Click here to access/download;Figure;Fig3.tiff ⬇

Figure 4   Click here to access/download;Figure;Fig4.tiff ⬥

Figure 5

Figure 6