Title: Acute glutamine supplementation does not improve 20-km self-paced cycling performance in the heat.

Running heading: Glutamine does not improve cycling in the heat.

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Abbreviations

½ RT	half relaxation time
20TT	20 km time trial
CD	contraction duration
CI	credible interval
CON	control
CNS	central nervous system
CV	coefficient of variation
ELISA	enzyme-linked immunosorbent assay
EMG	electromyography
GI	gastrointestinal
GLUT	glutamine
HR	heart rate
ICC	intraclass correlation
I-FABP	intestinal fatty acid binding protein
IL-6	interleukin 6
MCMC	Markov chain Monte Carlo
MD	mean difference
MVC	maximum voluntary contraction
POMS	profile of mood states
Pt	peak torque
Pr	probabilty
RPE	Rating of Perceived Exertion
RR	rate of relaxation
RTD	rate of torque development
SESOI	smallest effect size of interest
T_{re}	core (rectal) temperature
TNF-α	tumour necrosis factor alpha
TPt	time to peak torque
T_{sk}	mean skin temperature

54	USG	urine specific gravity
55	VA	voluntary activation
56	VL	vastus lateralis
57	VM	vastus medialis
58	VO_{2max}	maximal aerobic capacity

ABSTRACT

Introduction: The premise of this study was to investigate the effect of acute glutamine supplementation on 20 km time trial cycling performance in the heat, neuromuscular function, inflammation and endotoxemia.

Methods: Twelve cyclists completed two, 20-km time trials (20TT) in 35 °C (50% relative humidity). Participants ingested either glutamine (GLUT; 0.9 $g \cdot kg^{-1}$ fat-free mass) or a placebo (CON) 60 min before each 20TT. Physiological and perceptual measures were recorded during each 20TT, and neuromuscular function assessed pre- and post-exercise. Venous blood was analysed for endotoxins, markers of gut damage (inflammatory fatty acid binding protein; I-FABP) and inflammatory cytokines (interleukin-6, IL-6; tumour necrosis factor-alpha, TNF-α). Data were analysed using linear mixed models in a Bayesian framework.

Results: 20TT in the heat increased I-FABP and elevated inflammatory cytokines (IL-6 and TNF-α) compared to pre-exercise values but did not result in endotoxemia. Completion time was not statistically different between conditions (mean difference [95% credible interval] = 11 s [-23, 44]). Relative to CON, GLUT did not alter any physiological or perceptual measures during the 20TT.

Conclusion: Glutamine supplementation does not improve 20TT performance in the heat or preserve neuromuscular function when compared to a placebo. These findings suggest that glutamine is not an ergogenic aid or prophylactic intervention for heat-induced gut damage during short-duration self-paced exercise in hot environments.

KEYWORDS

Glutamine, Exercise, Endotoxemia, Hyperthermia, Inflammation

1. INTRODUCTION

Hot environmental conditions hinder endurance exercise performance (Galloway & Maughan, 1997; Tatterson, Hahn, Martini, & Febbraio, 2000) and multiple causative factors may contribute to the premature development of fatigue (Nybo, Rasmussen, & Sawka, 2014). One proposed pathway, the 'neuroinflammatory model', links hyperthermic-impairments in gastrointestinal (GI) blood flow and death of GI cells (Lambert, 2008) with the translocation of endotoxins and release of pro-inflammatory cytokines into systemic circulation (e.g., interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) (Lim & Mackinnon, 2006). Elevated circulating levels of these inflammatory cytokines have been connected to transient fatigue-like symptoms and altered motivational states (Dantzer, 2004). These cytokines are also theorised to contribute to a downregulation of neural drive to the skeletal muscle, impairing exercise performance in the heat (Vargas & Marino, 2014).

Elevated markers of GI damage, endotoxins and inflammatory cytokines have been repeatedly detected in athletes following prolonged (~3-7 h) endurance competition in thermally stressful environments (Camus et al., 1997; Gill et al., 2015; Øktedalen, Lunde, Opstad, Aabakken, & Kvernebo, 1992). Similarly, increases in some, or all, of these variables have also been reported after short-duration (~20-134 min), fixed-intensity exercise in simulated hot environments (Marchbank et al., 2011; Osborne, Stewart, Beagley, & Minett, 2019; Pugh, Impey, et al., 2017; Selkirk, McLellan, Wright, & Rhind, 2008). Based on this previous research, it is plausible that short-duration self-paced exercise in the heat may also result in GI damage, exertional-endotoxemia and impaired performance. However, the external validity of fixed-paced tasks in the heat has been previously criticised, as constant work rates prelude the anticipatory behavioural regulation from afferent feedback and/or pacing strategy in self-paced exercise trials (Tucker, 2008). As such, the effect of short-duration time-trial tasks in the heat on GI damage, endotoxemia, inflammation and central fatigue remains to be elucidated.

If short-duration self-paced exercise in the heat does result in heat-induced GI damage, preventative interventions that protect the GI barrier have the potential to mitigate this negative feed-forward loop and improve exercise performance (Guy & Vincent, 2018; Lambert, Broussard, Mason, Mauermann, & Gisolfi, 2001). Glutamine, a non-essential amino acid, has been found to augment the intestinal barrier and protect against septic shock in clinical populations (Castell, 2003; De-Souza & Greene, 2005), and reduce intestinal damage and permeability during exertional heat-stress (Pugh, Sage, et al., 2017; Zuhl et al., 2015; Zuhl et al., 2014). Glutamine's role in stabilising the intestinal lining appears multifactorial, with two distinct pathways proposed by which it may

regulate barrier function: providing an essential energy source for gut mucosal epithelial cells; and enhanced expression of transmembrane proteins which form tight junctions along the intestinal barrier (Rao & Samak, 2012).

Previous studies have primarily focused on the mechanistic application of glutamine on GI function during fixed-pace exercise tasks, rather than the potentially ergogenic effect on self-paced performance (Lambert et al., 2001; Pugh, Sage, et al., 2017; Zuhl et al., 2015; Zuhl et al., 2014). While the occurrence of GI damage and exertional endotoxemia during short-duration, self-paced exercise in the heat is currently unknown, it could be postulated that glutamine supplementation may protect against possible GI damage and pro-inflammatory cytokine release during exercise in the heat. As elevated levels of inflammatory cytokines have been implicated in fatigue-like sensations (i.e., reduced motivation) and impaired efferent drive (Vargas & Marino, 2017; Vargas & Marino, 2014), a glutamine-mediated reduction in these cytokines may preserve voluntary activation of skeletal muscle and, therefore, positively influence exercise performance.

The current study aimed to investigate the effect of acute glutamine supplementation on 20 km time trial cycling performance in the heat. A secondary aim was to investigate the effect of glutamine supplementation on neuromuscular function, inflammation and endotoxemia. It was hypothesised that glutamine would improve exercise performance (i.e., faster time trial completion) and preserve neuromuscular function in association with an attenuation of the development of central fatigue. Further, glutamine supplementation would maintain intestinal barrier integrity, observable via a diminished level of circulating endotoxins, inflammatory cytokines, markers of GI damage and subjective GI symptoms.

2. METHODS

2.1 Participants

A convenience sample of twelve male cyclists (mean ± standard deviation); age: 32 ± 6 y, body mass: 78 ± 8 kg, fat-free mass: 65 ± 6 kg, VO_{2max}: 61.0 ± 6.2 mL·kg^{-1}·min^{-1}, $Power_{max}$: 430 ± 48 W; HR_{max}: 189 ± 8 beats·min^{-1}) volunteered to participate in this study. All participants cycled at least twice per week (distance: 225 ± 93 km·wk^{-1}) and were classified as trained or well-trained athletes (performance level 3 or 4) (De Pauw et al., 2013). Participants were non-smokers, free of any injury or illnesses, reported no history of GI or kidney diseases and were consuming no other supplements. All participants were informed of the study requirements and procedures

before obtaining written and verbal consent. The University Human Research Ethics Committee approved this project before the commencement of any testing (Approval #: 1700000620).

2.2 Experimental overview

Participants visited the laboratory on three separate occasions; an initial familiarisation trial followed by two experimental trials, involving glutamine supplementation (GLUT) or a placebo control (CON). The familiarisation trial involved an incremental maximal aerobic capacity test (VO_{2max}), followed by neuromuscular testing practice and a 20 km time trial (20TT). The experimental trials were completed in a double-blind, randomised, crossover manner, each separated by ≥7 days. All testing sessions involved a 20TT undertaken in environmental conditions of 35.1 ± 0.5 °C and 51 ± 4% relative humidity. Participants were asked to diarise their food and fluid intake for 24 h, and their physical activity for 48 h, before the first experimental trial and to replicate this for the subsequent trial. Participants were also asked to abstain from caffeine and alcohol for 12 h, and strenuous physical activity for 48 h before each experimental trial. Compliance to these requests was verbally assessed before each trial. A schematic for the experimental trials is shown in Fig. 1.

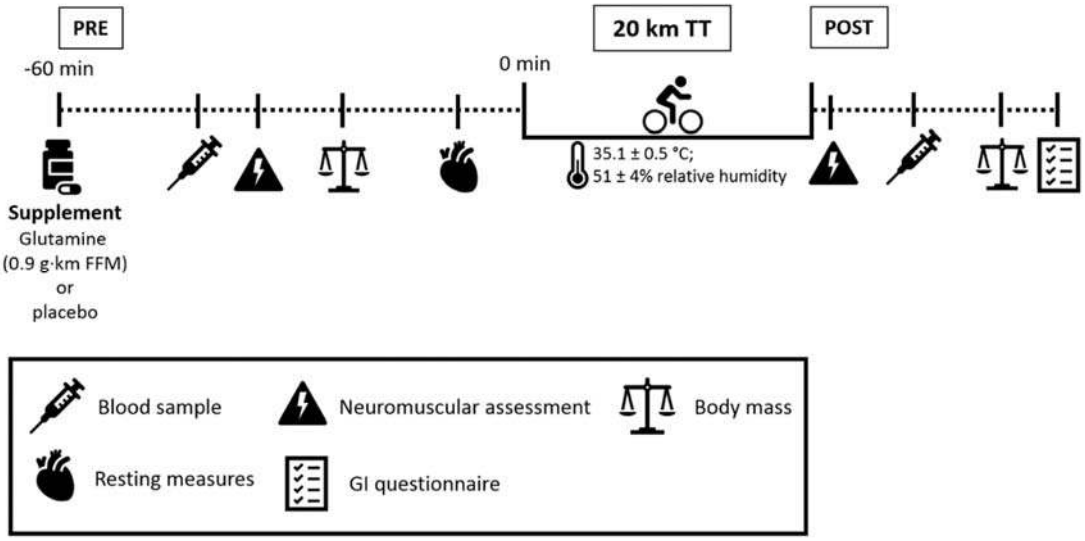


Figure 1. Experimental trial schematic. Participants completed both conditions in a double-blind, randomised, crossover manner, each separated by ≥7 days.

164 **2.3 Experimental trials**

165 Each experimental trial was matched for the time of day (±2 h), and upon arrival, participants ingested a GLUT
166 or CON solution from an opaque drink bottle. The GLUT solution compromised of powdered glutamine (0.9 $g \cdot kg^{-1}$
167 of fat-free mass; L-Glutamine; Bulk Nutrients, Grove, Australia) mixed with 450 mL of room-temperature water
168 and 50 mL of sugar-free lemon cordial (Diet Rite, Tru Blu Beverages, Bundamba, Australia). This dosage has
169 been previously shown to attenuate GI permeability when compared to a placebo (Pugh, Sage, et al., 2017; Zuhl
170 et al., 2015). The CON beverage contained matched fluid volume (i.e., water and cordial) but was void of any
171 supplement. Body mass, fat mass and fat-free mass were measured during the familiarisation trial to calculate the
172 supplement dose using multi-frequency bioelectrical impedance analysis (MC-780MA; Tanita Corp., Tokyo,
173 Japan). All solutions were independently mixed immediately before ingestion, to limit glutamine degradation in
174 the aqueous solution.
175
176 Participants provided urine and blood samples and consumed the supplement drink before undertaking a pre-
177 exercise neuromuscular test. This was followed by nude body mass weigh, instrumentation and completion of
178 pre-trial forms (i.e., 24-h dietary recall, 48-h physical activity recall, profile of mood states questionnaire
179 (POMS)). All participants wore cycling apparel (i.e., bib, socks and cycling shoes). Participants were seated for 5
180 min in a climate-controlled laboratory (24.2 ± 1.0 °C, 60 ± 4% relative humidity) and baseline physiological (i.e.,
181 resting T_c, T_{skin}, HR) and perceptual (i.e., thermal sensation, thermal comfort) data collected. Participants then
182 entered a climate-controlled chamber (35.1 ± 0.5 °C, 51 ± 4% relative humidity) and completed a 20TT. All 20TTs
183 were completed on a Velotron Pro cycle ergometer (RacerMate Inc., Washington, USA), commencing from a
184 seated, stationary position. Velotron 3D software (Version NB04.1.0.2101, RacerMate Inc., Washington, USA)
185 provided elapsed distance feedback over a computer-simulated flat cycling course. A constant background scene
186 was shown, and no simulated wind resistance or computer opponents were displayed. Participants were instructed
187 to complete the 20 km distance as quickly as possible and could freely alter gearing using a hood-mounted toggle-
188 switch. Performance feedback was withheld during both 20TTs, and provided only after study completion. Highly
189 reliable 20TT performance results (intra-class correlation; ICC = .93; coefficient of variation; CV = 0.9%) are
190 reported using familiarised, trained cyclists in our laboratory (Borg et al., 2018). The time delay between
191 completion of the supplement consumption and commencement of the 20TT was 61 ± 7 min. This lead-in time
192 was chosen to align with peak plasma glutamine concentrations as reported in previous research (Ziegler et al.,
193 1990). All fluid was withheld during 20TT and until after the collection of post-exercise measures.

194

195 **2.4 Thermophysiological measures**

196 Heart rate (HR) was recorded using a chest strap (Polar Electro Oy, Kempele Finland) and software (Polar Team^2,
197 Kempele Finland). Core (rectal) temperature (T_{re}) was assessed via a thermistor (449H; Henleys Medical,
198 Hertfordshire, UK) inserted 12 cm beyond the anal sphincter and connected to a wireless data logger (T-Tec 7 RF
199 7-3E) set to record every 2 s. No participant reached the T_{re} termination threshold of 40 °C during the exercise
200 bout.

201

202 Skin temperature was recorded at 5 s intervals with wireless iButton thermocrons (DS1922L-F50 iButtons, Maxim
203 Integrated, San Jose, USA) attached with adhesive tape (Leuko Sportstape Premium; Beiersdorf, Hamburg,
204 Germany) to four sites: posterior neck, right scapula, posterior left hand and mid-anterior shin. Mean skin
205 temperature (T_{sk}) was calculated according to the published four-site formula (ISO 9886, 2004).

206

207 A mid-stream urine sample was collected at the start of each experimental trial to assess specific gravity (PAL-
208 10S; Atago Co. Ltd, Tokyo, Japan) and urine colour (scale: 1-8 AU) (Armstrong et al., 2010). Body fluid loss was
209 calculated via pre- to post-exercise nude weight change, using calibrated scales (WB-110AZ; Tanita Corp., Tokyo,
210 Japan).

211 Perceptual measures were recorded every 2 km during each 20TT. These included Borg's 15-point rating of
212 perceived exertion (RPE) scale (Borg, 1998), a 16-point thermal sensation scale ranging from 0 'unbearably cold'
213 to 8 'unbearably hot' (Young, Sawka, Epstein, Decristofano, & Pandolf, 1987), and a modified 10-point thermal
214 comfort scale ranging from 1 'comfortable' to 5 'extremely uncomfortable' (Gagge, Stolwijk, & Hardy, 1967).
215 After the post-exercise blood draw, participants completed a post-trial GI distress form (adapted from Pfeiffer,
216 Cotterill, Grathwohl, Stellingwerff, and Jeukendrup (2009) to assess the incidence of GI symptoms and severity
217 on a 100 point scale (0 = none; 100 = severe), and provided a session RPE (sRPE) (Foster et al., 2001).

218

219 **2.5 Neuromuscular function**

220 The neuromuscular function of the right knee extensor muscles was assessed pre- and immediately post-exercise
221 using a Biodex System 3 dynamometer (Biodex Medical Systems, Shirley, New York, USA). Participants were
222 seated in an upright position with the back of the chair adjusted to 95° from horizontal and the lever fulcrum
223 aligned with the right lateral epicondyle of the femur. Participants were secured using chest, waist, right thigh and

ankle straps. Voluntary activation (VA) of the knee extensors was assessed using the twitch interpolation technique on the right femoral nerve. Self-adhesive gel electrodes (Pals; Axelgaard Manufacturing Co. Ltd., Fallbrook, CA) were positioned on the right femoral triangle and the border of the right gluteal fold. The current was applied to the nerve by a Digitimer DS7AH stimulator (Digitimer Ltd., Welwyn Garden City, Hertfordshire, England) using a single, 100 μs, square-wave pulse. A twitch ramp procedure was completed at the start of each trial with a stimulus of increasing current delivered to a resting muscle until a plateau in twitch torque was observed, and then increased by an additional 10% to ensure supramaximal stimulation.

Participants undertook a standardised warm-up before completing a set of five, 5 s maximal voluntary isometric knee extensions (MVC) at 90° knee flexion, with a 30 s rest between each repetition. Participants received loud verbal encouragement to maximally contract and visual feedback of their torque. The primary investigator manually triggered a femoral nerve stimulation when the participant demonstrated a plateau in MVC torque during each repetition. An additional resting stimulation was triggered upon the completion of each MVC to assess peripheral contractile properties. Another set of five, 5 s MVCs with stimulation were completed immediately post-exercise.

The twitch interpolation formula used to calculate VA was: VA (%) = (1 – amplitude/resting control twitch torque) * 100 (Allen, Gandevia, & McKenzie, 1995). Only MVC repetitions that demonstrated a torque plateau were included in VA calculations. MVC torque was considered the mean torque value of 25 ms preceding the interpolated twitch, and the peak torque recorded in the 100 ms following the stimulus was considered the superimposed torque. The difference between the MVC torque and the superimposed torque was considered the amplitude. Participants were extensively familiarised with all neuromuscular testing procedures during the initial laboratory visit and were able to reproduce similar MVC torque values consistently with a CV of 5.6%. These neuromuscular assessments of MVC torque and VA are highly reliable by our laboratory, with calculated ICC's of .94 and .94, respectively. Evoked twitch contractile data were averaged for each time point and analysed for peak twitch torque (Pt; maximum evoked twitch torque); time to peak torque (TPt; time from torque onset to Pt); half relaxation time (½ RT; time for torque to decrease by half of Pt); contraction duration (CD; TPt plus ½ RT); rate of torque development (RTD; slope of twitch-torque curve from onset to Pt); rate of relaxation (RR; slope of twitch-torque curve from Pt to ½ RT).

2.6 Surface electromyography (EMG)

Muscle activation of the right vastus medialis (VM) and vastus lateralis (VL) were measured using surface EMG during the neuromuscular assessments. All sites were shaved, abraded and swabbed with alcohol before electrode placement. Electrodes (Ambu Blue Sensor N-00-S; Ambu A/S, Ballerup, Denmark) were positioned parallel with the muscle fibres, and an earth electrode was attached to the lateral femoral epicondyle. Electrodes remained attached during the 20TT to ensure consistency between the pre- and post-neuromuscular assessments. EMG data were sampled at 1 kHz through a 16-bit PowerLab 26T AD unit (AD Instruments, Sydney, Australia) (amplification=1000; common mode rejection ratio=110 dB) and band-pass filtered (20-500 Hz). Raw EMG data were smoothed using the RMS method (100 ms window) through LabChart 8.1.5 software (AD Instruments, New South Wales, Australia). Voluntary muscle activation was considered the mean smoothed EMG value of a 500 ms period preceding each interpolated stimulus. Post-exercise EMG amplitudes were compared as a relative change to the mean values obtained during pre-exercise MVCs and presented as a percentage.

2.7 Blood markers

Pre- and post-exercise blood samples were drawn from an antecubital venipuncture using a butterfly needle (21G, BD, North Ryde, Australia) into EDTA vacutainer tubes (BD, North Ryde, Australia) and immediately centrifuged at 3500 RPM for 15 min at 4 °C. Following centrifugation, pyrogen-free aliquots of plasma were frozen at -80˚C for a maximum of 6 months before analysis.

Circulating IL-6 (HS600B; R&D Systems, Minneapolis, USA), TNF-α (EK-0001; elisakit.com, Melbourne, Australia), and inflammatory fatty acid binding protein (I-FABP) (EHFABP2; Thermo Scientific, Fredrick, USA) concentrations were determined using quantitative sandwich enzyme-linked immunoassay assays (ELISA), prepared in accordance with the manufacturer's protocols. All samples were diluted to reduce interference and avoid a matrix effect. Absorbance was quantified using a SpectroStar Nano (BMG Labtech, Offenburg, Germany) with wavelength subtraction to correct for optical imperfections in the plates. Intra-assay CV was calculated as 8.0% (IL-6), 8.0% (TNF-α) and 7.1% (I-FABP), respectively.

Endotoxin concentrations were quantified using a kinetic chromogenic *Limulus* amebocyte lysate (LAL) assay kit (50-650U), as instructed by the manufacturer (Lonza, Walkersville, USA). In short, the *E. coli* 055:B5 endotoxin standard was reconstituted with LAL reagent water and vortexed for 15 min to produce a 50 $EU \cdot mL^{-1}$ stock

284 solution. Serial dilutions of this stock standard (5, 0.5, 0.05, 0.005 $EU \cdot mL^{-1}$) were prepared in duplicate, with each
285 solution vortexed for at least 2 min between dilutions. Plasma samples were heated for 45 min at 75 °C to
286 inactivate endotoxin-neutralising agents and diluted at 1:10 with magnesium chloride (MgCl) to overcome
287 chelation from EDTA. Plates were incubated for 20 min at 37 °C in a SpectroStar Nano (BMG Labtech,
288 Offenburg, Germany) before the addition of 100 µL of reconstituted Kinetic-QCL™ Reagent to each well. Plates
289 were then read at 405 nm, and this was repeated every 61 s for 118 cycles. A log/log (time for the sample to
290 increase 0.2 absorbance units/concentration) linear correlation of each standard was calculated (r = -0.999) to
291 determine the sample concentration. Sterile tips (Biopur epTIPS; Eppendorf AG, Germany) were used for all
292 pipetting to reduce endotoxin contamination.
293
294 **2.8 Data Analysis**
295 Data were analysed using linear mixed models in a Bayesian framework (Mengersen, Drovandi, Robert, Pyne, &
296 Gore, 2016). Exploratory plots were inspected for normality before Markov chain Monte Carlo (MCMC, 50k
297 iterations, 1k burn-in, thinned by a factor of 10) procedures generated posterior predicted values using the 'rjags'
298 and 'R2jags' packages in R (Mengersen et al., 2016). Models utilised vague prior distributions for each regression
299 coefficient (mean 0, precision 0.001) and each variance parameter (shape 0.01, scale 0.01). Models included *time*,
300 *condition* and *time* x *condition* as fixed factors. When there was no evidence of a *time* x *condition* intervention,
301 the term was removed from the final model.
302
303 Posterior estimates of interest were: mean and 95% credible interval [CI]; mean difference (MD, and associated
304 95% CI) between conditions or time points of interest; and Cohen's d, where $\sqrt{\text{Var}(k - l)}$ was the denominator
305 for d when comparing conditions or time points k and l (Cohen, 1988). Where the 95% CI (of a regression
306 coefficient or MD) did not include zero, there was sufficient evidence of a statistical effect or difference. Cohens
307 d values were interpreted as small (0.20 – 0.49), moderate (0.50 – 0.79) and large (≥0.80) (Cohen, 1988). Primary
308 outcomes of interest in this study were considered: MVC torque, VA, I-FABP and TNF-α. For these variables,
309 we also calculated the probability that the within-condition difference exceeded the smallest effect size of interest
310 (SESOI), denoted as d < -SESOI or d > SESOI, depending on the direction of the difference. The SESOI for MVC
311 and VA was: -1.33 and -0.51 (Périard, Christian, Knez, & Racinais, 2014; Périard, Cramer, Chapman, Caillaud,
312 & Thompson, 2011); for I-FABP: 2.66 (Pugh, Impey, et al., 2017; Pugh, Sage, et al., 2017; van Wijck et al., 2011);

313 and for TNF-α: 0.44 (Lim et al., 2009; Zuhl et al., 2015). The convergence of MCMC to the posterior was visually
314 assessed via trace plots, and posterior predictive checks were performed for all models.
315
316 **3. RESULTS**
317 No statistical differences in mood (i.e., POMS) upon arrival or baseline hydration measures (USG and urine
318 colour) were observed between conditions (Table 1). Pre-exercise body mass, resting T_{re} and T_{sk} were also similar
319 between conditions (Table 1). The absence of any statistical difference in baseline measures indicates that
320 participants commenced each 20TT in a similar psychological and physiological state.
321
322 Post-exercise fluid loss, both absolute and relative, were not statistically different between conditions (GLUT =
323 1.3 kg loss [1.0, 1.3] and 1.5% loss [1.3, 1.7]; CON = 1.3 kg loss [1.1, 1.4] and 1.6% loss [1.4, 1.8]; Table 1).
324 Although there was some indication for a higher severity of reported symptoms in CON, the incidence of GI
325 symptoms was not found to be statistically different between conditions (Table 2). Supplementation did not affect
326 sRPE following each 20TT (Table 1). Participants were successfully blinded to the intervention with a calculated
327 James's Blind Index of .67 [.48, .85] (James, Bloch, Lee, Kraemer, & Fuller, 1996).
328
329 **3.1 Exercise**
330 There was no statistical evidence to suggest that glutamine supplementation affected 20TT completion time, with
331 a mean difference [95% CI] of 11 s [-23, 44] between conditions. No statistical differences in mean 20TT power,
332 cadence and speed between the two conditions were observed (Table 3). Statistical analysis revealed only a *time*
333 effect for HR, T_{re}, T_{sk}, RPE, thermal sensation and thermal comfort over the 20TT (Table 3).
334
335 **3.2 Neuromuscular function and EMG**
336 The 20TT reduced VA impairment, from pre-exercise values for both CON (-5.6% [-1.9, -9.5]; d = -3.0) and
337 GLUT (-5.2% [-1.5, -8.8]; d = -2.8), and this was supported by a high probability that $d <$ -SESOI (-0.51) for both
338 conditions (CON = .99; GLUT = .98). No between-condition difference was found for VA. A *time* effect was
339 identified for MVC torque, although a large reduction was only observed pre- to post-exercise for CON (MD
340 [95% CI] = -24 N·m [-40, -7]; d [95% CI] = -2.8 [-4.8, 0.9]), with a smaller decrease for GLUT (MD [95% CI] =
341 -11 N·m [-28, 5]; d [95% CI] = -1.4 [-3.4, 0.6]; Figure 2). The probability that $d \prec$ -SESOI for these within-
342 condition comparisons was .94 and .48, respectively. Evidence of a *time* effect was found for the remaining muscle

contractile properties of Pt, TPt, ½ RT, RR and CD, which were reduced from initial values (Table 4), with no *condition* effect. There was no indication of *time*, *condition* or *time* x *condition* effects for muscle activation (relative %EMG of VL and VM) or RTD (Table 4).

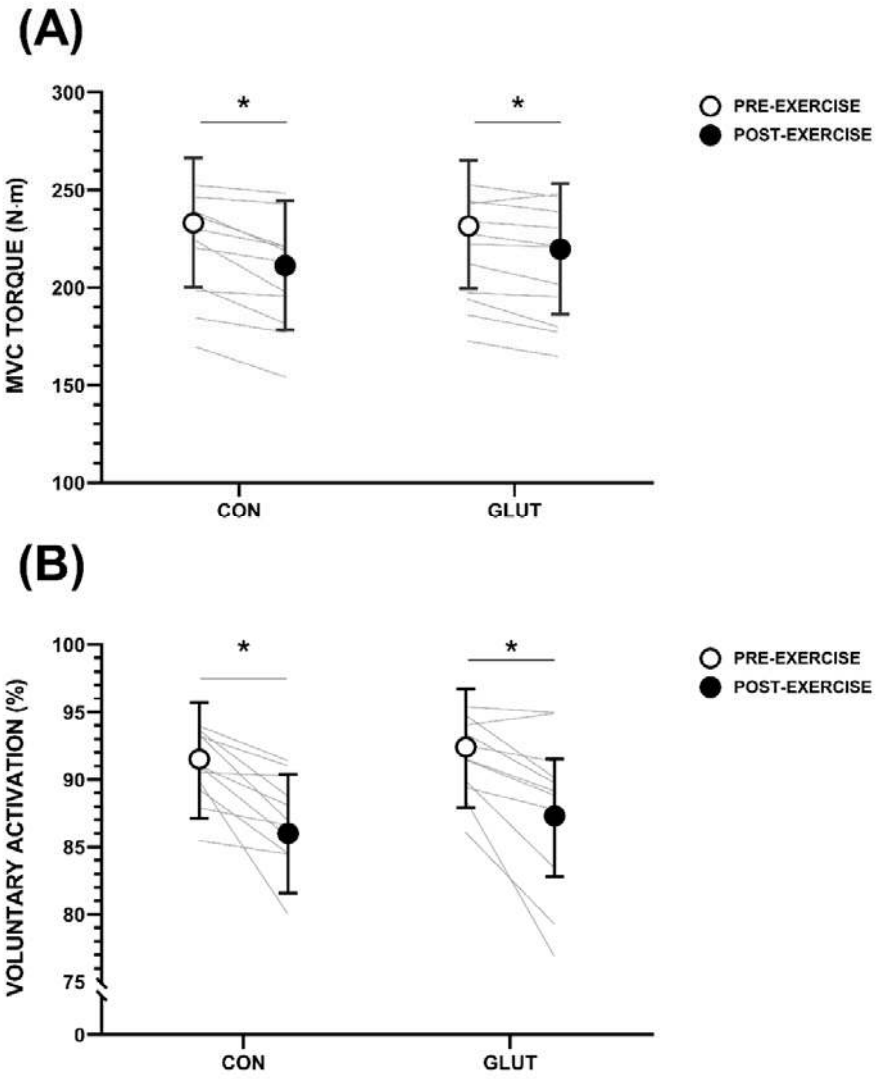
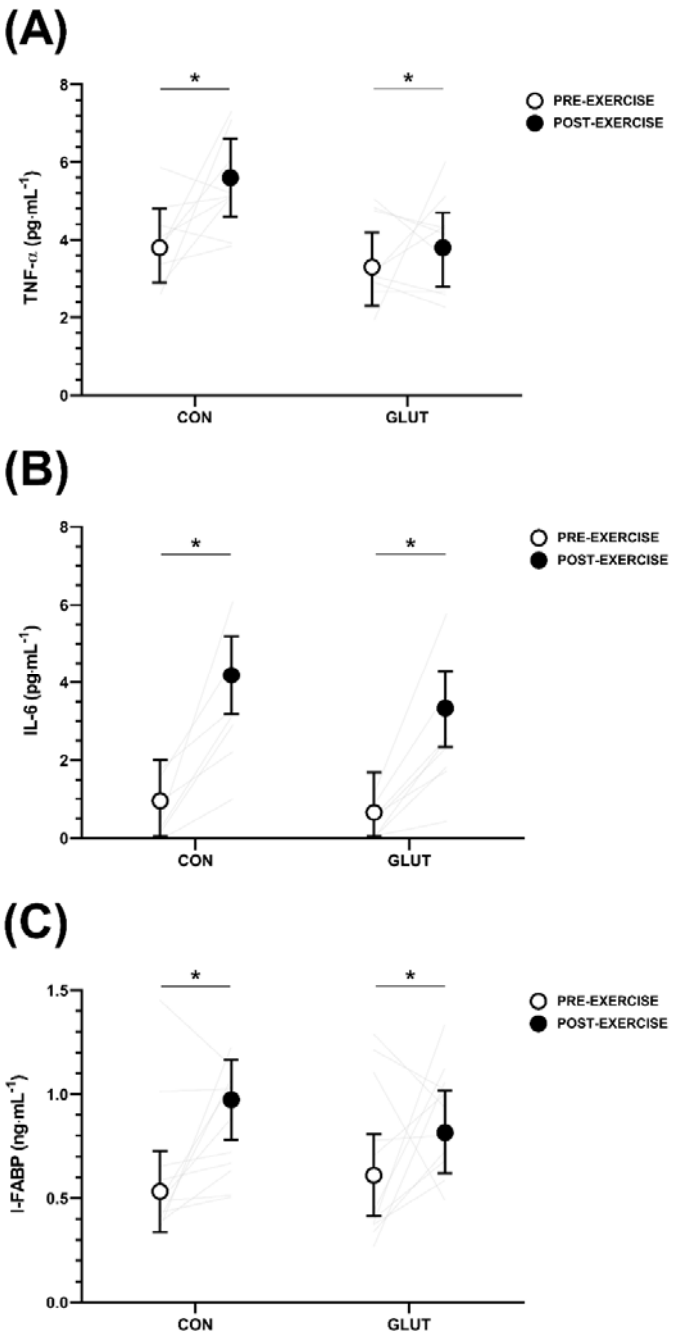


Figure 2. Neuromuscular function of the knee extensors pre and post-20TT following ingestion of glutamine (GLUT) or placebo (CON). Data displayed as posterior predicted mean ± 95% credible interval and overlayed with raw individual responses. * indicates a *time* effect.

353 **3.3 Blood markers**

354 Cycling in the heat statistically increased concentrations of IL-6 compared to pre-exercise levels for both GLUT
355 (MD [95% CI] = 2.7 pg·mL^{-1} [1.4, 4.0]; *d* [95% CI] = 4.1 [2.1, 6.1]) and CON (MD [95% CI] = 3.2 pg·mL^{-1} [1.9,
356 4.6]; *d* [95% CI] = 4.7 [2.7, 6.7]), though no *condition* or *time* x *condition* effects were observed (Figure 3; Table
357 5). A *time* effect was detected for plasma TNF-α levels, with an increase from pre-exercise values for CON (MD
358 [95% CI] = 1.8 pg·mL^{-1} [0.5, 3.1]; *d* [95% CI] = 2.7 [0.7, 4.7]), but there was little evidence for GLUT (MD [95%
359 CI] = 0.5 pg·mL^{-1} [-0.8, 1.9]; *d* [95% CI] = 0.7 [-1.2, 2.8]). While no *condition* or *interaction* effects were observed
360 for TNF-α, the probability that the within-condition *d* > SESOI was .99 for CON and .60 for GLUT, providing
361 some evidence towards a possible condition difference. A similar finding was observed for I-FABP, with evidence
362 of a clear *time* effect for CON (MD [95% CI] = 0.441 ng·mL^{-1} [0.235, 0.656]; *d* [95% CI] = 4.1 [2.2, 6.1]), but
363 less so for GLUT (MD [95% CI] = 0.206 ng·mL^{-1} [-0.025, 0.418]; *d* [95% CI] = 1.9 [0.0, 3.9]). The probability
364 that the within-condition *d* > SESOI was .99 for CON and .32 for GLUT. No *condition*, *time*, or *time* x *condition*
365 effects were observed for endotoxin.



366

367 **Figure 3. Plasma concentration of: (a) TNF-α; (b) IL-6; and (c) I-FABP at pre- and post-20TT time points**.

368 Data displayed as posterior predicted mean ± 95% credible interval and overlayed with raw individual responses.

369 * indicates a *time* effect.

370

4. DISCUSSION

A primary finding from this study was that 30-40 min of self-paced exercise in the heat resulted in an apparent increase in markers of GI damage and inflammation, indicating that athletes competing in short-duration events experience sufficient thermal and cardiovascular stress to injure the intestinal barrier (Figure 3). These data align with the increased GI damage and permeability previously reported in research utilising both fixed paced protocols of between 20-60 mins (Lambert et al., 2008; Marchbank et al., 2011; Morrison, Cheung, & Cotter, 2014; Pugh, Impey, et al., 2017) and long-duration endurance events of 3-8 hours (Lambert et al., 1999; Øktedalen et al., 1992).

In contrast to the study hypothesis, no change in endotoxin levels were detected after a 20TT in the heat (Table 5). This outcome was unexpected, given that exertional-endotoxemia has been previously observed following strenuous exercise in the heat, in both prolonged competitive events (Camus et al., 1997; Gill et al., 2015; Jeukendrup et al., 2000) and shorter-duration, fixed-intensity studies (Lim et al., 2009; Selkirk et al., 2008; Yeh, Law, & Lim, 2013). One possible explanation may be that the relative brevity of the current exercise task (i.e., ~33 min) only resulted in limited translocation of endotoxins, which could be quickly inactivated and neutralised by anti-LPS antibodies and low-density lipoproteins (Lim & Mackinnon, 2006). In contrast, the longer-duration exercise task used in previous research appears to overload the limited capacity of these clearance mechanisms, resulting in detectable endotoxemia (Bosenberg, Brock-Utne, Gaffin, Wells, & Blake, 1988; Brock-Utne et al., 1988; Camus et al., 1998).

The current study also provided evidence that acute glutamine supplementation does not alter completion time, mean power, speed or cadence during a 20 km self-paced cycling trial in the heat (Table 3). This outcome was reflected in the comparable values of physiological and perceptual strain for CON and GLUT over the 20TT. Furthermore, glutamine supplementation was not found to preserve VA more than the placebo, with a similar statistical reduction in VA observed in both conditions following the 20TT (Table 4). These data suggest athletes competing in shorter-duration events (i.e., criterium racing) in the heat are unlikely to obtain a performance benefit from glutamine supplementation. No *condition* or *time* x *condition* effects were observed for MVC torque, I-FABP and TNF-α, although this may have been due to the sample size, as within-condition effect sizes appeared to diverge between GLUT and CON (Table 4 and 5). Speculatively, the low probability that effect sizes for GLUT exceeded each respective SESOI could be considered preliminary evidence that glutamine may potentially preserve knee extensor torque (d = -1.4; Pr d < -SESOI (-1.3) = .48) and protect against GI damage (d = 1.9; Pr d

401 > SESOI (2.7) = .32). As endotoxin translocation was not observed during the current study, possibly due to the
402 short duration, the previously-hypothesised effect of glutamine supplementation on endotoxin translocation
403 remains to be elucidated (Lambert, 2009). Future research should continue to explore the possible relationship
404 between glutamine and endotoxin levels during longer-duration self-paced tasks.
405
406 Cycling in a hot environment for 20 km statistically reduced MVC torque compared to pre-exercise values (Figure
407 2). Périard and colleagues (2011) observed similar impairments in voluntary torque following a 40 km cycling
408 time trial in the heat, with the authors implicating the prolonged dynamic muscular contractile activity of the
409 exercise task. In particular, high muscle temperatures have been shown to result in more responsive contractile
410 properties, namely a faster TPt and ½RT (Todd, Butler, Taylor, & Gandevia, 2005). A similar occurrence was
411 seen in the current study, with a faster TPT and ½RT was observed for both conditions, and these data support the
412 similar post-exercise MVC torque between conditions. Conversely, there was some evidence for a greater loss of
413 torque in CON (d = -2.8*;* Pr d < -SESOI (-1.3) = .94) than in GLUT (d = -1.4*;* Pr d < -SESOI (-1.3) = .48) post-
414 exercise. However, as the similar decrease in VA and Pt for both conditions implies a comparable development
415 of central and peripheral fatigue, and the impairment in torque for both conditions could be considered marginal
416 (CON: 10% reduction; GLUT: 5% reduction), we suggest there is limited evidence that glutamine preserves MVC
417 torque to a clinically relevant degree.
418
419 To our knowledge, this is the first study to investigate the effect of glutamine supplementation on self-paced
420 exercise performance in the heat. Previous literature has focused primarily on glutamine supplementation during
421 fixed-intensity exercise tasks in the heat (Lambert et al., 2001; Pugh, Sage, et al., 2017; Zuhl et al., 2015; Zuhl et
422 al., 2014). Collectively, this research suggests that increased GI permeability and damage, stemming from
423 strenuous exercise in the heat, can be ameliorated by glutamine, with similar findings in animal models (Singleton
424 & Wischmeyer, 2006). Although the current study did not specifically assess GI permeability, glutamine
425 supplementation was not observed to attenuate the rise in markers of GI damage, I-FABP, compared to a placebo,
426 which is in contrast to the dose-response findings of Pugh, Sage, et al. (2017). However, the considerable
427 difference of within-condition effect sizes for CON (d = 4.1; Pr d > SESOI (2.7) = .99) and GLUT (d = 1.9; Pr d
428 > SESOI (2.7) = .32) provides some evidence for a possible separation due to condition. Further research using
429 larger sample sizes and correction for changes in plasma volume should be undertaken into glutamine

supplementation and its possible role in providing a stabilising and protective effect to the gut mucosa during self-paced exercise in the heat.

This study only provided limited evidence that glutamine supplementation may modulate the release of the pro-inflammatory cytokine, TNF-α, ($d = 0.7$; Pr $d >$ SESOI (0.44) = .60). This finding is dissimilar to previous research using in-vitro human PBMC models (Wischmeyer et al., 2003) as well as fixed-intensity protocols (Zuhl et al., 2015), and suggests that glutamine does not appear to protect against inflammatory cytokine release (i.e., IL-6 or TNF-α) during short-duration self-paced exercise in the heat. The increased levels of IL-6 in both conditions may have occurred due to the multi-origin nature of the molecule, which is considered both a cytokine and a myokine due to its expression during skeletal muscle contractions (Pedersen et al., 2003). The non-statistical difference in 20TT completion time between conditions would suggest a comparable level of muscle contractile activity, and thus a similar level of IL-6 released. Further, as IL-6 has been implicated in contributing to the feeling of fatigue (Vargas & Marino, 2014), it could be argued that the comparable post-exercise levels of this molecule may explain the similar cycling performance, session RPE and post-exercise VA that were observed between conditions (Tables 1, 3 and 4).

A primary limitation of this study was the small sample size. We attempted to address this limitation by utilising hierarchical models and Bayesian methods (Kruschke & Liddell, 2018; Mengersen et al., 2016). By fitting models in the Bayesian framework we were able to calculate the probability that an effect size exceeded a threshold of interest (i.e., SESOI) by drawing directly from the posterior distribution (Mengersen et al., 2016). Plasma volume change was not calculated for this study, and therefore blood marker data were not corrected for concentration changes arising from dehydration and shifts in plasma volume. While there was no statistical difference in body mass loss between conditions (Table 1), suggesting a comparable level of dehydration, it is conceivable that the observed increase in circulating markers may have been an artefact arising from plasma volume changes.

In summary, this study found that acute glutamine supplementation did not improve 20 km self-paced cycling performance in the heat, preserve neuromuscular function, or attenuate the release of inflammatory cytokines when compared to a placebo. Despite strenuous exercise (mean HR: 88% HR_{max}) and elevated core temperatures (final temperature: ~39.0 °C), the exercise task did not induce exertional endotoxemia in either condition. There was some evidence that glutamine supplementation may potentially provide a protective effect for gut mucosa

against ischaemic injury, and future studies should continue to investigate the application of glutamine in reducing inflammation and GI damage in prolonged duration exercise or multi-day sporting competitions.

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692 **Table 1.**

693 Baseline, pre- and post-exercise variables posterior predicted mean [95% credible interval]

Variable	Control	Glutamine
POMS	7 [1, 14]	8 [2, 13]
Resting T_{re} (°C)	37.0 [36.8, 37.2]	37.1 [36.9, 37.3]
Resting T_{sk} (°C)	33.1 [32.9, 33.4]	33.2 [33.0, 33.5]
USG	1.009 [1.000, 1.048]	1.016 [1.000, 1.055]
Urine colour	3 [2, 4]	3 [2, 4]
Baseline body mass (kg)	76.6 [71.0, 81.8]	77.1 [71.5, 82.3]
Pre-post body mass loss (kg)	1.3 [1.1, 1.4]	1.3 [1.0, 1.3]
Pre-post body mass loss (%)	1.6 [1.4, 1.8]	1.5 [1.3, 1.7]
Session RPE (AU)	9 [8, 9]	9 [8, 9]

694 POMS: profile of mood states; T_{re} : rectal temperature; T_{sk}: skin temperature; USG: urine specific gravity; RPE: rating of perceived exertion; AU = arbitrary
695 units.

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697

698

Table 2.

Gastrointestinal distress incidence and severity post-exercise questionnaire.

GI Distress Symptom	Control	Glutamine
Cramp	n = 0; 0 (0 – 0)	n = 1; 50 (0 – 50)
Nausea	n = 4; 19 (0 – 25)	n = 4; 13 (0 – 12.5)
Urge to defecate	n = 0; 0 (0 – 0)	n = 0; 0 (0 – 0)
Urge to vomit	n = 4; 27 (0 – 50)	n = 3; 12 (0 – 25)
Stitch/pain in gut	n = 3; 33 (0 – 75)	n = 3; 16 (0 – 30)
Flatulence	n = 0; 0 (0 – 0)	n = 0; 0 (0 – 0)

n = response rate/12; mean of responders (range).

Table 3.

Posterior predicted mean [95% credible interval] for time trial variables (mean values for each 20 km time trial).

Variable	Control	Glutamine
Completion time (min:sec)	33:22 [31:52, 34:54]	33:33 [32:02, 35:06]
Power (W)	256 [226, 285]	251 [221, 280]
Cadence (revolutions·min^{-1})	97 [91, 103]	98 [92, 104]
Speed (km·h^{-1})	36.3 [34.7, 37.8]	36.1 [34.5, 37.6]
Heart rate (beats·min^{-1})	167 [166, 173] *	166 [165, 172] *
T_{re} (°C)	38.0 [37.9, 38.2] *	38.0 [38.0, 38.2] *
T_{sk} (°C)	36.0 [36.0, 36.3] *	36.1 [36.0, 36.3] *
RPE (AU)	16 [15, 17] *	16 [16, 17] *
Thermal sensation (AU)	6 [6, 7] *	6 [6, 7] *
Thermal comfort (AU)	4 [3, 4] *	4 [3, 4] *

* indicates *time* effect. T_{re} : rectal temperature; T_{sk}: skin temperature; RPE: rating of perceived exertion; AU = arbitrary units.

Table 4.

Posterior predicted mean [95% credible interval] for pre- and post-20 km time trial neuromuscular variables.

Variable	Pre-Exercise		Post Exercise	
	Control	Glutamine	Control	Glutamine
MVC torque (N·m)	231 [194, 268]	228 [191, 266]	208 [170, 244]* *d* = -2.8 [-4.8, -0.9] Pr *d* < -SESOI (-1.33) = .94	217 [180, 254]* *d* = -1.4 [-3.4, 0.6] Pr *d* < -SESOI (-1.33) = .48
VA (%)	92.0 [87.6, 96.4]	92.7 [88.5, 97.2]	86.3 [81.9, 90.8]* *d* = -3.0 [-5.0, -1.0] Pr *d* < -SESOI (-0.51) = .99	87.6 [83.2, 91.9]* *d* = -2.8 [-4.8, -0.8] Pr *d* < -SESOI (-0.51) = .98
EMG VL (%)	-	-	76.9 [64.3, 88.8]	81.5 [69.0, 93.8]
EMG VM (%)	-	-	71.4 [60.9, 81.3]	78.4 [67.8, 88.4]
Pt (N·m)	64 [51, 78]	60 [47, 73]	55 [41, 68]* *d* = -3.9 [-5.9, -2.0]	52 [39, 66]* *d* = -3.1 [-5.0, -1.0]
TPt (ms)	76 [71, 81]	80 [75, 85]	68 [62, 73]* *d* = -2.8 [-4.8, -0.8]	70 [64, 75]* *d* = -3.6 [-5.6, -1.6]
1/2 RT (ms)	64 [52, 75]	62 [50, 74]	41 [21, 53]* *d* = -4.7 [-6.6, -2.7]	45 [34, 57]* *d* = -3.5 [-5.5, -1.5]
CD (ms)	139 [125, 153]	143 [129, 157]	109 [95, 123]* *d* = -5.4 [-7.4, -3.4]	115 [101, 129]* *d* = -5.0 [-7.0, -3.0]
RTD ($N \cdot m \cdot s^{-1}$)	850 [659, 1036]	769 [579, 951]	810 [626, 997] *d* = -0.92 [-2.8, 1.1]	761 [572, 944] *d* = -0.19 [-2.1, 1.8]
RR ($N \cdot m \cdot s^{-1}$)	590 [443, 733]	556 [408, 702]	631 [481, 774]* *d* = 2.6 [0.5, 4.5]	597 [450, 745]* *d* = 1.8 [0.21, 3.7]

* indicates *time* effect. Cohen's *d* effect size [95% credible interval] and probability (Pr) that this effect size exceeds the smallest effect size of interest (SESOI) is presented for relevant time parameter comparisons.

MVC: maximum voluntary contraction; VA: voluntary activation; EMG: electromyography; VL: vastus lateralis; VM: vastus medialis; Pt; peak twitch torque; TPt; time to peak torque; ½ RT: half relaxation time; CD: contraction duration; RTD: rate of torque development; RR: rate of relaxation.

Table 5.

Posterior predicted mean [95% credible interval] for blood marker variables.

Variable	Pre-Exercise		Post Exercise	
	Control	Glutamine	Control	Glutamine
Endotoxin (EU·mL^{-1})	0.22 [0.00, 1.19]	0.04 [0.00, 0.82]	0.87 [0.04, 1.72] *d*: 1.1 [-0.84, 3.1]	0.08 [0, 0.85] *d*: 0.25 [-1.8, 2.2]
IL-6 (pg·mL^{-1})	1.0 [0.0, 2.0]	0.7 [0.0, 1.7]	4.2 [3.2, 5.2] * *d*: 4.7 [2.7, 6.7]	3.3 [2.3, 4.3] * *d*: 4.1 [2.1, 6.1]
TNF-α (pg·mL^{-1})	3.8 [2.9, 4.8]	3.3 [2.3, 4.2]	5.6 [4.6, 6.6] * *d*: 2.7 [0.7, 4.7] Pr *d* > SESOI (0.44): .99	3.8 [2.8, 4.7] * *d*: 0.7 [-1.2, 2.8] Pr *d* > SESOI (0.44): .60
I-FABP (ng·mL^{-1})	0.533 [0.337, 0.728]	0.610 [0.413, 0.808]	0.973 [0.780, 1.163] * *d*: 4.1 [2.2, 6.1] Pr *d* > SESOI (2.66): .99	0.815 [0.621, 1.017] * *d*: 1.9 [0.0, 3.9] Pr *d* > SESOI (2.66): .32

* indicates a *time* effect. Cohen's *d* effect size [95% credible interval] and probability (Pr) that this effect size exceeds the smallest effect size of interest (SESOI) is presented for relevant time parameter comparisons.

EU: endotoxin units; IL-6: interleukin-6; TNF-α: tumour necrosis factor alpha; I-FABP: intestinal fatty acid binding protein