European Journal of Applied Physiology (2019) 119:2567–2578
https://doi.org/10.1007/s00421-019-04234-2

ORIGINAL ARTICLE

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

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Received: 27 June 2019 / Accepted: 18 September 2019 / Published online: 30 September 2019


## 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 $kg^{-1}$ 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

## 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 |

Communicated by George Havenith.

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| | |
|---|---|
| $T_{re}$ | Core (rectal) temperature |
| TNF-α | Tumour necrosis factor alpha |
| TPt | Time to peak torque |
| $T_{sk}$ | Mean skin temperature |
| USG | Urine specific gravity |
| VA | Voluntary activation |
| VL | vastus lateralis |
| VM | Vastus medialis |
| $VO_{2\,max}$ | maximal aerobic capacity |

## Introduction

Hot environmental conditions hinder endurance exercise performance (Galloway and Maughan 1997; Tatterson et al. 2000) and multiple causative factors may contribute to the premature development of fatigue (Nybo et al. 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 and 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 down-regulation of neural drive to the skeletal muscle, impairing exercise performance in the heat (Vargas and 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 et al. 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 et al. 2019; Pugh et al. 2017a; Selkirk et al. 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 and Vincent 2018; Lambert et al. 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 and Greene 2005), and reduce intestinal damage and permeability during exertional heat-stress (Zuhl et al. 2014, 2015). 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 and 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 et al. 2017b; Zuhl et al. 2014, 2015). 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 and Marino 2014, 2017), 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.

## Methods

### Participants

A convenience sample of twelve male cyclists (mean ± standard deviation); age: 32 ± 6 years, 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).

## 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.

## Experimental trials

Each experimental trial was matched for the time of day (±2 h), and upon arrival, participants ingested a GLUT or CON solution from an opaque drink bottle. The GLUT solution compromised of powdered glutamine (0.9 g $kg^{-1}$ of fat-free mass; L-Glutamine; Bulk Nutrients, Grove, Australia) mixed with 450 mL of room-temperature water and 50 mL of sugar-free lemon cordial (Diet Rite, Tru Blu Beverages, Bundamba, Australia). This dosage has been previously shown to attenuate GI permeability when compared to a placebo (Pugh et al. 2017b; Zuhl et al. 2015). The CON beverage contained matched fluid volume (i.e., water and cordial) but was void of any supplement. Body mass, fat mass and fat-free mass were measured during the familiarisation trial to calculate the supplement dose using multi-frequency bioelectrical impedance analysis (MC-780MA; Tanita Corp., Tokyo, Japan). All solutions were independently mixed immediately before ingestion, to limit glutamine degradation in the aqueous solution.

Participants provided urine and blood samples and consumed the supplement drink before undertaking a pre-exercise neuromuscular test. This was followed by nude body mass weigh, instrumentation and completion of pre-trial forms (i.e., 24-h dietary recall, 48-h physical activity recall, profile of mood states questionnaire (POMS)). All participants wore cycling apparel (i.e., bib, socks and cycling shoes). Participants were seated for 5 min in a climate-controlled laboratory (24.2 ± 1.0 °C, 60 ± 4% relative humidity) and baseline physiological (i.e., resting $T_c$, $T_{skin}$, HR) and perceptual (i.e., thermal sensation, thermal

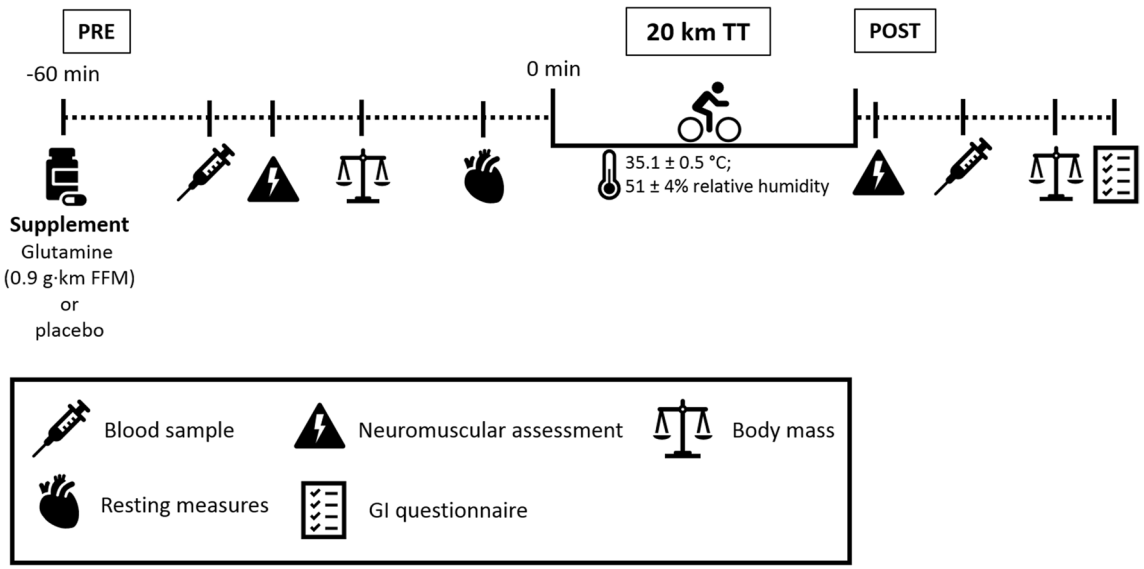


**Fig. 1** Experimental trial schematic. Participants completed both conditions in a double-blind, randomised, crossover manner, each separated by ≥ 7 days

comfort) data collected. Participants then entered a climate-controlled chamber (35.1 ± 0.5 °C, 51 ± 4% relative humidity) and completed a 20TT. All 20TTs were completed on a Velotron Pro cycle ergometer (RacerMate Inc., Washington, USA), commencing from a seated, stationary position. Velotron 3D software (Version NB04.1.0.2101, RacerMate Inc., Washington, USA) provided elapsed distance feedback over a computer-simulated flat cycling course. A constant background scene was shown, and no simulated wind resistance or computer opponents were displayed. Participants were instructed to complete the 20 km distance as quickly as possible and could freely alter gearing using a hood-mounted toggle-switch. Performance feedback was withheld during both 20TTs, and provided only after study completion. Highly reliable 20TT performance results (intra-class correlation; ICC = .93; coefficient of variation; CV = 0.9%) are reported using familiarised, trained cyclists in our laboratory (Borg et al. 2018). The time delay between completion of the supplement consumption and commencement of the 20TT was 61 ± 7 min. This lead-in time was chosen to align with peak plasma glutamine concentrations as reported in previous research (Ziegler et al. 1990). All fluid was withheld during 20TT and until after the collection of post-exercise measures.

## Thermophysiological measures

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

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

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

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

## Neuromuscular function

The neuromuscular function of the right knee extensor muscles was assessed pre- and immediately post-exercise using a Biodex System 3 dynamometer (Biodex Medical Systems, Shirley, New York, USA). Participants were seated in an upright position with the back of the chair adjusted to 95° from horizontal and the lever fulcrum 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 et al. 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).

## 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 26 T 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.

## 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 $mL^{-1}$ stock solution. Serial dilutions of this stock standard (5, 0.5, 0.05, 0.005 EU $mL^{-1}$) were prepared in duplicate, with each solution vortexed for at least 2 min between dilutions. Plasma samples were heated for 45 min at 75 °C to inactivate endotoxin-neutralising agents and diluted at 1:10 with magnesium chloride (MgCl) to overcome chelation from EDTA. Plates were incubated for 20 min at 37 °C in a SpectroStar Nano (BMG Labtech, Offenburg, Germany) before the addition of 100 µL of reconstituted Kinetic-QCL™ Reagent to each well. Plates were then read at 405 nm, and this was repeated every 61 s for 118 cycles. A log/log (time for the sample to increase 0.2 absorbance units/concentration) linear correlation of each standard was calculated ($r = -0.999$) to determine the sample concentration. Sterile tips (Biopur epTIPS; Eppendorf AG, Germany) were used for all pipetting to reduce endotoxin contamination.

## Data analysis

Data were analysed using linear mixed models in a Bayesian framework (Mengersen et al. 2016). Exploratory plots were inspected for normality before Markov chain Monte Carlo (MCMC, 50 k iterations, 1 k burn-in, thinned by a factor of 10) procedures generated posterior predicted values using the 'rjags' and 'R2jags' packages in R (Mengersen et al. 2016). Models utilised vague prior distributions for each regression coefficient (mean 0, precision 0.001) and each variance parameter (shape 0.01, scale 0.01). Models included time, condition and time × condition as fixed factors. When there was no evidence of a *time* x *condition* intervention, the term was removed from the final model.

Posterior estimates of interest were: mean and 95% credible interval [CI]; mean difference (MD, and associated 95% CI) between conditions or time points of interest; and Cohen's *d*, where $\sqrt{\text{Var}(k - l)}$ was the denominator for *d* when comparing conditions or time points *k* and *l* (Cohen 1988). Where the 95% CI (of a regression coefficient or MD) did not include zero, there was sufficient evidence of a

statistical effect or difference. Cohens *d* values were interpreted as small (0.20 – 0.49), moderate (0.50 – 0.79) and large (≥0.80) (Cohen 1988). Primary outcomes of interest in this study were considered: MVC torque, VA, I-FABP and TNF-α. For these variables, we also calculated the probability that the within condition difference exceeded the smallest effect size of interest (SESOI), denoted as $d <$ -SESOI or $d >$ SESOI, depending on the direction of the difference. The SESOI for MVC and VA was: − 1.33 and − 0.51 (Périard et al. 2011, 2014); for I-FABP: 2.66 (Pugh et al. 2017a, b; van Wijck et al. 2011); and for TNF-α: 0.44 (Lim et al, 2009; Zuhl et al. 2015). The convergence of MCMC to the posterior was visually assessed via trace plots, and posterior predictive checks were performed for all models.

## Results

No statistical differences in mood (i.e., POMS) upon arrival or baseline hydration measures (USG and urine colour) were observed between conditions (Table 1). Pre-exercise body mass, resting $T_{re}$ and $T_{sk}$ were also similar between conditions (Table 1). The absence of any statistical difference in baseline measures indicates that participants commenced each 20TT in a similar psychological and physiological state.

Post-exercise fluid loss, both absolute and relative, were not statistically different between conditions (GLUT = 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). Although there was some indication for a higher severity of reported symptoms in CON, the incidence of GI symptoms was not found to be statistically different between conditions (Table 2). Supplementation did not affect sRPE following each 20TT (Table 1). Participants were successfully blinded to the intervention with a calculated James's Blind Index of .67 [.48, .85] (James et al. 1996).

**Table 1** 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] |

*POMS* profile of mood states, $T_{re}$ rectal temperature, $T_{sk}$ skin temperature, *USG* urine specific gravity, *RPE* rating of perceived exertion, *AU* arbitrary units

**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)

### Exercise

There was no statistical evidence to suggest that glutamine supplementation affected 20TT completion time, with a mean difference [95% CI] of 11 s [− 23, 44] between conditions. No statistical differences in mean 20TT power, cadence and speed between the two conditions were observed (Table 3). Statistical analysis revealed only a *time* effect for HR, $T_{re}$, $T_{sk}$, RPE, thermal sensation and thermal comfort over the 20TT (Table 3).

### Neuromuscular function and EMG

The 20TT reduced VA impairment, from pre-exercise values for both CON (− 5.6% [− 1.9, − 9.5]; $d = -3.0$) and 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 conditions (CON = .99; GLUT = .98). No between-condition

**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:s) | 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][a] | 166 [165, 172][a] |
| $T_{re}$ (°C) | 38.0 [37.9, 38.2][a] | 38.0 [38.0, 38.2][a] |
| $T_{sk}$ (°C) | 36.0 [36.0, 36.3][a] | 36.1 [36.0, 36.3][a] |
| RPE (AU) | 16 [15, 17][a] | 16 [16, 17][a] |
| Thermal sensation (AU) | 6 [6, 7][a] | 6 [6, 7][a] |
| Thermal comfort (AU) | 4 [3, 4][a] | 4 [3, 4][a] |

$T_{re}$ rectal temperature, $T_{sk}$ skin temperature, *RPE* rating of perceived exertion, *AU* arbitrary units

[a]Indicates time effect

difference was found for VA. A *time* effect was identified for MVC torque, although a large reduction was only observed pre- to post-exercise for CON (MD [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] = − 11 N m [− 28, 5]; *d* [95% CI] = − 1.4 [− 3.4, 0.6]; Fig. 2). The probability that *d* < -SESOI for these within-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 × condition effects for muscle activation (relative %EMG of VL and VM) or RTD (Table 4).

### Blood markers

Cycling in the heat statistically increased concentrations of IL-6 compared to pre-exercise levels for both GLUT (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, 4.6]; *d* [95% CI] = 4.7 [2.7, 6.7]), though no condition or time × condition effects were observed (Fig. 3; Table 5). A *time* effect was detected for plasma TNF-α levels, with an increase from pre-exercise values for CON (MD [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% 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 for TNF-α, the probability that the within-condition *d* > SESOI was .99 for CON and .60 for GLUT, providing some evidence towards a possible condition difference. A similar finding was observed for I-FABP, with evidence 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 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 that the within-condition *d* > SESOI was .99 for CON and .32 for GLUT. No condition, time, or time × condition effects were observed for endotoxin.

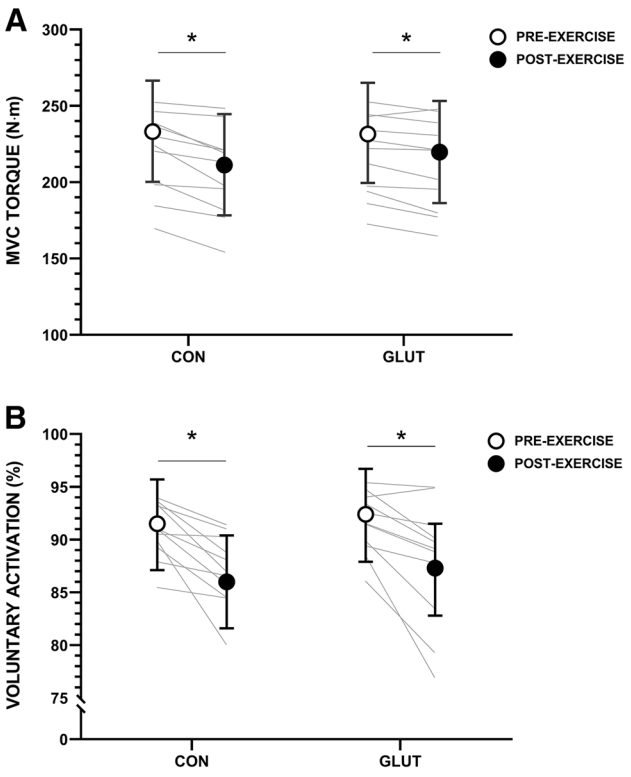


**Fig. 2** Neuromuscular function of the knee extensors pre and post-20TT following ingestion of glutamine (GLUT) or placebo (CON). Data displayed as posterior predicted mean ± 95% credible interval and overlayed with raw individual responses. *indicates a time effect

## 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 (Fig. 3). These data align with the increased GI damage and permeability previously reported in research utilising both fixed paced protocols of between 20–60 min (Lambert et al. 2008; Marchbank et al. 2011; Morrison et al. 2014; Pugh et al. 2017a) and long-duration endurance events of 3–8 h (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 et al. 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 and 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 et al. 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

**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][a]<br>$d = -2.8$ [− 4.8, − 0.9]<br>Pr $d <$ − SESOI (− 1.33) = .94 | 217 [180, 254][a]<br>$d = -1.4$ [− 3.4, 0.6]<br>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][a]<br>$d = -3.0$ [− 5.0, − 1.0]<br>Pr $d <$ − SESOI (− 0.51) = .99 | 87.6 [83.2, 91.9][a]<br>$d = -2.8$ [− 4.8, − 0.8]<br>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][a]<br>$d = -3.9$ [− 5.9, -2.0] | 52 [39, 66][a]<br>$d = -3.1$ [− 5.0, − 1.0] |
| TPt (ms) | 76 [71, 81] | 80 [75, 85] | 68 [62, 73][a]<br>$d = -2.8$ [− 4.8, − 0.8] | 70 [64, 75][a]<br>$d = -3.6$ [− 5.6, − 1.6] |
| 1/2 RT (ms) | 64 [52, 75] | 62 [50, 74] | 41 [21, 53][a]<br>$d = -4.7$ [− 6.6, − 2.7] | 45 [34, 57][a]<br>$d = -3.5$ [− 5.5, − 1.5] |
| CD (ms) | 139 [125, 153] | 143 [129, 157] | 109 [95, 123][a]<br>$d = -5.4$ [− 7.4, − 3.4] | 115 [101, 129][a]<br>$d = -5.0$ [− 7.0, − 3.0] |
| RTD (N m $s^{-1}$) | 850 [659, 1036] | 769 [579, 951] | 810 [626, 997]<br>$d = -0.92$ [− 2.8, 1.1] | 761 [572, 944]<br>$d = -0.19$ [− 2.1, 1.8] |
| RR (N m $s^{-1}$) | 590 [443, 733] | 556 [408, 702] | 631 [481, 774][a]<br>$d = 2.6$ [0.5, 4.5] | 597 [450, 745][a]<br>$d = 1.8$ [0.21, 3.7] |

*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

[a]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

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 × 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 (Tables 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 $d >$ SESOI (2.7) = .32). As endotoxin translocation was not observed during the current study, possibly due to the short duration, the previously-hypothesised effect of glutamine supplementation on endotoxin translocation remains to be elucidated (Lambert 2009). Future research should continue to explore the possible relationship between glutamine and endotoxin levels during longer-duration self-paced tasks.

Cycling in a hot environment for 20 km statistically reduced MVC torque compared to pre-exercise values (Fig. 2). Périard et al. (2011) observed similar impairments in voluntary torque following a 40 km cycling time trial in the heat, with the authors implicating the prolonged dynamic muscular contractile activity of the exercise task. In particular, high muscle temperatures have been shown to result in more responsive contractile properties, namely a faster TPt and ½RT (Todd et al. 2005). A similar occurrence was seen in the current study, with a faster TPT and ½RT was observed for both conditions, and these data support the similar post-exercise MVC torque between conditions. Conversely, there was some evidence for a greater loss of torque in CON ($d = -2.8$; Pr $d <$ − SESOI (− 1.3) = .94) than in GLUT ($d = -1.4$; Pr $d <$ − ESOI (− 1.3) = .48) post-exercise. However, as the similar decrease in VA and Pt for both conditions implies a comparable development of central and peripheral fatigue, and the impairment in torque for both conditions could be considered marginal (CON: 10% reduction; GLUT: 5% reduction), we suggest there is limited evidence that glutamine preserves MVC torque to a clinically relevant degree.

To our knowledge, this is the first study to investigate the effect of glutamine supplementation on self-paced exercise performance in the heat. Previous literature has focused

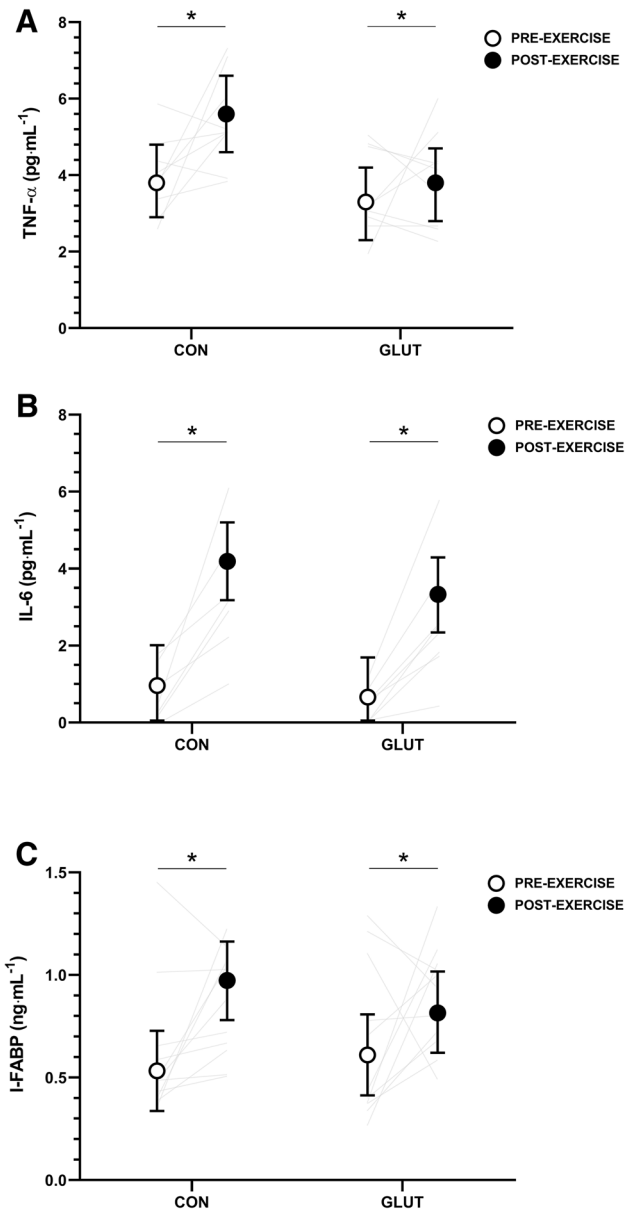


**Fig. 3** Plasma concentration of: **a** TNF-α; **b** IL-6; and **c** I-FABP at pre- and post-20TT time points. Data displayed as posterior predicted mean ± 95% credible interval and overlayed with raw individual responses. * indicates a time effect

primarily on glutamine supplementation during fixed-intensity exercise tasks in the heat (Lambert et al. 2001; Pugh et al. 2017b; Zuhl et al. 2014, 2015). Collectively, this research suggests that increased GI permeability and damage, stemming from strenuous exercise in the heat, can be ameliorated by glutamine, with similar findings in animal models (Singleton and Wischmeyer 2006). Although the current study did not specifically assess GI permeability, glutamine supplementation was not observed to attenuate the rise in markers of GI damage, I-FABP, compared to a placebo, which is in contrast to the dose–response findings of Pugh et al. (2017b). However, the considerable difference of within-condition effect sizes for CON ($d = 4.1$; Pr $d >$ SESOI (2.7) = .99) and GLUT ($d = 1.9$; Pr $d >$ SESOI (2.7) = .32) provides some evidence for a possible separation due to condition. Further research using 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 and 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 and 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:

**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]<br>*d*: 1.1 [− 0.84, 3.1] | 0.08 [0, 0.85]<br>*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][a]<br>*d*: 4.7 [2.7, 6.7] | 3.3 [2.3, 4.3][a]<br>*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][a]<br>*d*: 2.7 [0.7, 4.7]<br>Pr *d* > SESOI (0.44): .99 | 3.8 [2.8, 4.7][a]<br>*d*: 0.7 [− 1.2, 2.8]<br>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][a]<br>*d*: 4.1 [2.2, 6.1]<br>Pr *d* > SESOI (2.66): .99 | 0.815 [0.621, 1.017][a]<br>*d*: 1.9 [0.0, 3.9]<br>Pr *d* > SESOI (2.66): .32 |

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

[a]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

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.

**Acknowledgements** The authors sincerely thank Mr Logan Trim (Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, Australia) for his technical assistance with the immunoassay analysis.

**Author contributions** Study conception and design: JOO, IBS, GMM. Data collection: JOO. Data analysis: JOO, IBS, DNB, GMM. Contributed reagents/materials/analysis tools: IBS, KWB, GMM. Manuscript development: JOO, IBS, KWB, DNB, GMM.

**Funding** None declared.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interests.

## References

Allen GM, Gandevia SC, Mckenzie DK (1995) Reliability of measurements of muscle strength and voluntary activation using twitch interpolation. Muscle Nerve 18(6):593–600

Armstrong LE, Pumerantz AC, Fiala KA, Roti MW, Kavouras SA, Casa DJ, Maresh CM (2010) Human hydration indices: acute and longitudinal reference values. Int J Sport Nutr Exerc Metab 20(2):145–153

Borg G (1998) Borg's perceived exertion and pain scales. Human Kinetics, Champaign

Borg DN, Osborne JO, Stewart IB, Costello JT, Sims JNL, Minett GM (2018) The reproducibility of 10 and 20 km time trial cycling performance in recreational cyclists, runners and team sport athletes. J Sci Med Sport 21(8):858–863

Bosenberg AT, Brock-Utne JG, Gaffin SL, Wells MTB, Blake GTW (1988) Strenuous exercise causes systemic endotoxemia. J Appl Physiol 65(1):106–108

Brock-Utne JG, Gaffin SL, Wells MT, Gathiram P, Sohar E, James MF, Norman RJ (1988) Endotoxaemia in exhausted runners after a long-distance race. S Afr Med J 73(9):533–536

Camus G, Poortmans J, Nys M, Deby-Dupont G, Duchateau J, Deby C, Lamy M (1997) Mild endotoxaemia and the inflammatory response induced by a marathon race. Clin Sci 92(4):415–422

Camus G, Nys M, Poortmans JR, Venneman I, Monfils T, Deby-Dupont G, Duchateau J (1998) Endotoxaemia, production of tumour necrosis factor α and polymorphonuclear neutrophil activation following strenuous exercise in humans. Eur J Appl Physiol 79(1):62–68

Castell LM (2003) Glutamine supplementation in vitro and in vivo, in exercise and in immunodepression. Sports Med 33(5):323–345

Cohen J (1988) Statistical power analysis for the behavioral sciences, 2nd edn. L. Erlbaum Associates, Hillsdale

Dantzer R (2004) Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. Eur J Pharmacol 500(1):399–411

De Pauw K, Roelands B, Cheung SS, De Geus B, Rietjens G, Meeusen R (2013) Guidelines to classify subject groups in sport-science research. Int J Sports Physiol Performance 8(2):111–122

De-Souza DA, Greene LJ (2005) Intestinal permeability and systemic infections in critically ill patients: Effect of glutamine. Crit Care Med 33(5):1125–1135

Foster C, Florhaug JA, Franklin J, Gottschall L, Hrovatin LA, Parker S, Dodge C (2001) A new approach to monitoring exercise training. J Strength Cond Re 15(1):109–115

Gagge AP, Stolwijk JA, Hardy JD (1967) Comfort and thermal sensations and associated physiological responses at various ambient temperatures. Environ Res 1(1):1–20

Galloway SDR, Maughan RJ (1997) Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. Med Sci Sports Exerc 29(9):1240–1249

Gill SK, Teixeira A, Rama L, Prestes J, Rosado F, Hankey J, Costa RJ (2015) Circulatory endotoxin concentration and cytokine

profile in response to exertional-heat stress during a multi-stage ultra-marathon competition. Exercise Immunol Rev 21:114–128
Guy HJ, Vincent EG (2018) Nutrition and supplementation considerations to limit endotoxemia when exercising in the heat. Sports 6(1):12
ISO 9886 (2004) Evaluation of thermal strain by physiological measurements. International Organization for Standardization, Geneva
James KE, Bloch DA, Lee KK, Kraemer HC, Fuller RK (1996) An index for assessing blindness in a multi-centre clinical trial: Disulfiram for alcohol cessation—a VA cooperative study. Stat Med 15(13):1421–1434
Jeukendrup AE, Vet-Joop K, Sturk A, Stegen JHJC, Senden J, Saris WHM, Wagenmakers AJM (2000) Relationship between gastrointestinal complaints and endotoxaemia, cytokine release and the acute-phase reaction during and after a long-distance triathlon in highly trained men. Clin Sci 98(1):47–55
Kruschke JK, Liddell TM (2018) Bayesian data analysis for newcomers. Psychon Bull Rev 25(1):155–177
Lambert GP (2008) Intestinal barrier dysfunction, endotoxemia, and gastrointestinal symptoms: the 'canary in the coal mine' during exercise-heat stress? Med Sport Sci 53:61–73
Lambert GP (2009) Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. J Anim Sci 87(14 Suppl.):E101–E108
Lambert GP, Murray R, Eddy D, Scott W, Laird R, Gisolfi CV (1999) Intestinal permeability following the 1998 Ironman Triathlon. Med Sci Sports Exerc 31(Supplement):S318
Lambert GP, Broussard LJ, Mason BL, Mauermann WJ, Gisolfi CV (2001) Gastrointestinal permeability during exercise: effects of aspirin and energy-containing beverages. J Appl Physiol 90(6):2075–2080
Lambert GP, Lang J, Bull A, Pfeifer PC, Eckerson J, Moore G, O'brien J (2008) Fluid restriction during running increases GI permeability. Int J Sports Med 29(03):194–198
Lim CL, Mackinnon LT (2006) The roles of exercise-induced immune system disturbances in the pathology of heat stroke : the dual pathway model of heat stroke. Sports Med 36(1):39–64
Lim CL, Pyne D, Horn P, Kalz A, Saunders P, Peake J, Mackinnon LT (2009) The effects of increased endurance training load on biomarkers of heat intolerance during intense exercise in the heat. Appl Physiol Nutr Metab 34(4):616–624
Marchbank T, Davison G, Oakes JR, Ghatei MA, Patterson M, Moyer MP, Playford RJ (2011) The nutriceutical bovine colostrum truncates the increase in gut permeability caused by heavy exercise in athletes. Am J Physiol Gastrointest Liver Physiol 300(3):G477–G484. https://doi.org/10.1152/ajpgi.00281.2010
Mengersen KL, Drovandi CC, Robert CP, Pyne DB, Gore CJ (2016) Bayesian estimation of small effects in exercise and sports science. PLoS One 11(4):e0147311
Morrison SA, Cheung SS, Cotter JD (2014) Bovine colostrum, training status, and gastrointestinal permeability during exercise in the heat: a placebo-controlled double-blind study. Appl Physiol Nutr Metab 39(9):1070–1082
Nybo L, Rasmussen P, Sawka MN (2014) Performance in the heat-physiological factors of importance for hyperthermia-induced fatigue. Compr Physiol 4(2):657–689
Øktedalen O, Lunde OC, Opstad PK, Aabakken L, Kvernebo K (1992) Changes in the gastrointestinal mucosa after long-distance running. Scand J Gastroenterol 27(4):270–274
Osborne JO, Stewart IB, Beagley KW, Minett GM (2019) The effect of cycling in the heat on gastrointestinal-induced damage and neuromuscular fatigue. Eur J Appl Physiol 119(8):1829–1840
Pedersen BK, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, Saltin B (2003) Searching for the exercise factor: is IL-6 a candidate? J Muscle Res Cell Motil 24(2):113
Périard JD, Cramer MN, Chapman PG, Caillaud C, Thompson MW (2011) Neuromuscular function following prolonged intense self-paced exercise in hot climatic conditions. Eur J Appl Physiol 111(8):1561–1569
Périard JD, Christian RJ, Knez WL, Racinais S (2014) Voluntary muscle and motor cortical activation during progressive exercise and passively induced hyperthermia. Exp Physiol 99(1):136–148
Pfeiffer B, Cotterill A, Grathwohl D, Stellingwerff T, Jeukendrup AE (2009) The effect of carbohydrate gels on gastrointestinal tolerance during a 16-km run. Int J Sport Nutr Exerc Metab 19(5):485–503
Pugh JN, Impey SG, Doran DA, Fleming SC, Morton JP, Close GL (2017a) Acute high-intensity interval running increases markers of gastrointestinal damage and permeability but not gastrointestinal symptoms. Appl Physiol Nutr Metab 42(9):941–947
Pugh JN, Sage S, Hutson M, Doran DA, Fleming SC, Highton J, Close GL (2017b) Glutamine supplementation reduces markers of intestinal permeability during running in the heat in a dose-dependent manner. Eur J Appl Physiol 117(12):2569–2577
Rao R, Samak G (2012) Role of glutamine in protection of intestinal epithelial tight junctions. J Epithel Biol Pharmacol, 5(Suppl. 1-M7), 47–54
Selkirk GA, Mclellan TM, Wright HE, Rhind SG (2008) Mild endotoxemia, NF-kappa B translocation, and cytokine increase during exertional heat stress in trained and untrained individuals. Am J Physiol Regul Integr Comp Physiol 295(2):R611–R623
Singleton KD, Wischmeyer PE (2006) Oral glutamine enhances heat shock protein expression and improves survival following hyperthermia. Shock 25(3):295–299
Tatterson AJ, Hahn AG, Martini DT, Febbraio MA (2000) Effects of heat stress on physiological responses and exercise performance in elite cyclists. J Sci Med Sport 3(2):186–193
Todd G, Butler JE, Taylor JL, Gandevia SC (2005) Hyperthermia: a failure of the motor cortex and the muscle. J Physiol 563(2):621–631
Tucker R (2008) Thermoregulation, fatigue and exercise modality. Med Sport Sci 53:26–38
Van Wijck K, Lenaerts K, Van Loon LJ, Peters WH, Buurman WA, Dejong CH (2011) Exercise-induced splanchnic hypoperfusion results in gut dysfunction in healthy men. PLoS ONE 6(7):e22366
Vargas NT, Marino F (2014) A neuroinflammatory model for acute fatigue during exercise. Sports Med 44(11):1479–1487
Vargas N, Marino F (2017) Neuroinflammation, cortical activity, and fatiguing behaviour during self-paced exercise. Pflugers Arch Eur J Physiol 470(2):413–426
Wischmeyer PE, Riehm J, Singleton KD, Ren H, Musch MW, Kahana M, Chang EB (2003) Glutamine attenuates tumor necrosis factor-α release and enhances heat shock protein 72 in human peripheral blood mononuclear cells. Nutrition 19(1):1–6
Yeh YJ, Law LYL, Lim CL (2013) Gastrointestinal response and endotoxemia during intense exercise in hot and cool environments. Eur J Appl Physiol 113(6):1575–1583
Young AJ, Sawka MN, Epstein Y, Decristofano B, Pandolf KB (1987) Cooling different body surfaces during upper and lower body exercise. J Appl Physiol 63(3):1218–1223
Ziegler TR, Benfell K, Smith RJ, Young LS, Brown E, Ferrari-Baliviera E, Wilmore DW (1990) Safety and metabolic effects of L-glutamine administration in humans. J Parenter Enteral Nutr 14(4_suppl):137S–146S
Zuhl MN, Lanphere KR, Kravitz L, Mermier CM, Schneider S, Dokladny K, Moseley PL (2014) Effects of oral glutamine supplementation on exercise-induced gastrointestinal permeability and tight junction protein expression. J Appl Physiol 116(2):183–191

Zuhl M, Dokladny K, Mermier C, Schneider S, Salgado R, Moseley P (2015) The effects of acute oral glutamine supplementation on exercise-induced gastrointestinal permeability and heat shock protein expression in peripheral blood mononuclear cells. Cell Stress Chaperones 20(1):85–93