One week of magnesium supplementation lowers IL-6, muscle soreness and increases post exercise blood glucose in response to downhill running.

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ABSTRACT

Purpose: Magnesium supplementation modulates glucose metabolism and inflammation, which could influence exercise performance and recovery. This study investigated magnesium intake on physiological responses and performance during eccentric exercise and recovery.

Methods: Nine male recreational runners completed a counterbalanced, double-blind, placebo-controlled, cross-over study, registered at ClinicalTrial.gov. Participants consumed low magnesium diets and were supplemented with 500 mg/day of magnesium (SUP) or placebo (CON) for 7-days prior to a 10 km downhill (-10%) running time-trial (TT), separated by a 2-week washout period. At baseline and 24 hrs post TT maximal muscle force was measured. Interleukin-6 (IL-6), soluble interleukin-6 receptor (sIL-6R) and creatine kinase (CK) were measured at rest, 0 hr, 1 hr and 24 hrs post TT. Muscle soreness was measured at the previous times plus 48 hrs and 72 hrs post. Glucose and lactate were measured during the TT.

Results: Main effect of condition were detected for IL-6 (SUP: 1.36 ± 0.66 vs CON: 2.06 ± 1.14 pg/ml) ($P < 0.05$, $\eta^2 = 0.54$), sIL-6R (SUP: 27615 ± 8446 vs CON: 24368 ± 7806 pg/ml) ($P < 0.05$, $\eta^2 = 0.41$) and muscle soreness ($P < 0.01$, $\eta^2 = 0.67$). Recovery of blood glucose and muscle soreness were enhanced in SUP post TT. There were no differences in glucose and lactate during the TT, or post measures of CK and maximal muscle force.

Conclusion: Magnesium supplementation reduced the IL-6 response, enhanced recovery of blood glucose, and muscle soreness after strenuous exercise, but did not improve performance or functional measures of recovery.

Key words: Magnesium; Interleukin-6; Exercise; Recovery; Glucose; muscle soreness

Abbreviations:

ANOVA	Analysis of variance
CI	Confidence interval
CK	Creatine kinase
ES	Effect size
IL-6	Interleukin-6
sIL-6R	Soluble interleukin-6 receptor

SD Standard deviation

TT Time trial

INTRODUCTION

Magnesium is a highly abundant intracellular cation, which is involved in over 300 enzymatic reactions and plays an important role in the process of energy production and muscle function (Lukaski, 2000). The current recommended daily allowance for adult males is 400-420 mg/day (Institute of Medicine U.S., 1997). However, individuals meeting these requirements may still have an inadequate intake due to the low bioavailability of magnesium which ranges from approximately 10-75% in humans (Schuchardt & Hahn, 2017). A recent systematic review suggested that the recommended daily intake of magnesium is regularly not met and that some groups of athletes with particularly low intakes of magnesium rich foods may be up to 60% deficient (Heffernan, Horner, De Vito, & Conway, 2019). Further than this, it has been proposed that individuals who consistently take part in exercise may require a 10-20% higher intake of magnesium, in contrast to their sedentary counterparts (Nielsen & Lukaski 2006), and as such, athletes are susceptible to frequent short-term marginal disruptions in magnesium homeostasis, in part due to an increased loss of magnesium in sweat (Shirreffs & Maughan 1997), increased excretion in the urine (Bohl & Volpe 2002), and low dietary magnesium intake (Heffernan et al. 2019).

The efficacy of magnesium supplementation is currently unclear and difficult to consolidate as a result of differences in dietary magnesium intake, supplementation durations, and the nature of the exercise. Early studies of magnesium supplementation focused on chronic supplementation (Terblanche et al. 1992; Finstad et al. 2001), however these studies used relatively low doses (200-300 mg/day) and used subjects with normal dietary magnesium intakes. In contrast, higher doses of magnesium (300-500 mg/day) over relatively shorter periods (1-4 weeks) in participants with a low dietary magnesium intake have been shown to improve strength and fatigue resistance (Heffernan et al. 2019). In particular, a recent study suggested that 1-week of magnesium supplementation may be more advantageous than 4-weeks (Kass & Poeira, 2015).

There is growing evidence from animal studies that acute ingestion of high doses of magnesium can have rapid and important acute effects within a range of tissues. Several detailed studies have provided robust evidence that acute magnesium supplementation can increase glucose

availability within the blood, brain and muscle (peaking 60-80 min post ingestion), enhance lactate clearance within the brain and exercising muscle, and subsequently improved exercise performance (Chen et al. 2009; Cheng et al. 2010; Chen et al. 2014). To date no studies have examined the effects of short-term magnesium supplementation on glucose and lactate response to exercise in humans and these are important findings to investigate in humans. This is even more interesting when considering the recent evidence that high dose (500 mg/day) magnesium supplementation can reduce the post-exercise increase in the circulating concentration of the inflammatory cytokine IL-6 (Dmitrašinović et al. 2016), as IL-6 is thought to be involved in a wide number of processes that can impact exercise performance and recovery including. IL-6 is also thought to play a role in exercise induced fatigue (Vargas & Marino 2014), post exercise recovery and muscle soreness (Robson-Ansley et al. 2010) and has an established role in the modulation of glucose metabolism during exercise (Febbraio et al. 2003; Glund et al. 2007). Taken together it is conceivable that magnesium supplementation may increase glucose availability, thereby acting to reduce the IL-6 response to exercise, both of which may contribute to enhanced exercise performance or recovery. Unfortunately, the only study to investigate magnesium and IL-6 responses to exercise, did not measure functional aspects of exercise recovery such as force production, muscle damage or soreness (Dmitrašinović et al. 2016).

When assessing the downstream effects of IL-6, it is also important to consider potential changes in its receptors, which are present in membrane bound and soluble forms. Importantly, the sIL-6R is thought to be involved in both glucose metabolism (Gray et al. 2009) and sensations of fatigue (Cullen et al. 2017; Robson-Ansley et al. 2010). Yet no studies have investigated whether sIL-6R is influenced by magnesium supplementation. As such, more comprehensive studies are required to fully elucidate the role of magnesium supplementation in the context of glucose metabolism and whether this may affect the modulation of IL-6 and it's soluble receptor. Therefore, the aim of the current study was to investigate the effect of acute magnesium supplementation on exercise performance and functional recovery in recreational endurance athletes in conjunction with measures of blood glucose, lactate, IL-6 and sIL-6R.

Methods

Participants

Nine healthy male recreational endurance runners (age: 27 ± 4 years, body mass: 80 ± 11 kg

and height 180 ± 8 cm) participated in this repeated measure, counterbalanced, double blind crossover, placebo study. In the last year, participants on average ran 3 ± 1 times a week, a distance of 8 ± 3 km and had a 10 km personal best of 41 ± 4 min. Participants volunteered to participate in the study, completing informed consent and health screening forms. One participant withdrew from the study due to personal commitments. If the nutritional guidelines were not followed or participants had any form of injury / illness prior to testing, the participant was removed from the study. Ethical approval was agreed by the University Health Sciences Research Ethics Committee (SH17180029-R).

Preliminary procedures

Participants completed a baseline assessment of maximal force production of the knee extensor and flexor muscles, measured on an isokinetic dynamometer (Humac Norm Isokinectic dynamometer, CSMi Boston). Prior to all maximal leg contractions, participants were securely seated with their hip flexed at 90° and the knee joint in line with the dynamometer rotational axis. The dynamometer was set an angular velocity of 60°/s, with the range of motion of 0 - 120° for the knee joint. In preparation for maximal effort, participants completed one set of concentric and eccentric leg extensions and flexions to practice the movement and a second set at 50% of maximal effort. After which, participants completed 3 sets of 5 maximal repetitions in both eccentric and concentric actions, with 30-seconds rest between sets, and a further 1-minute rest between concentric and eccentric contractions. The dominant leg was tested in all participants and standardised verbal encouragement was provided to encourage maximal effort (Gandevia 2001). Peak torque was recorded as the highest torque output for an individual repetition across the 3 maximal sets. Participants were then thoroughly familiarised with the experimental protocol which is to be describe below.

Dietary preparations were completed prior to commencing the first supplementation period. All capsules were identical in appearance, weight, and separated into coded bags for each participant. Before the first test session, participants were provided with a list of foods and beverages rich in magnesium. Participants were instructed in detail how to find and record the quantity of an item to complete the food diary. Portion sizes were provided through the weight or referenced estimation of an item. If the participants were unsure how to record a given item, participants were instructed to send a photo of the item and packaging to the lead researcher.

Study design

Participants completed a counterbalanced, double-blind, placebo controlled, cross-over design. This study was post-hoc registered at ClinicalTrial.gov. Prior to the testing phase, participants completed baseline measurements of maximal force production of the knee extensor and flexor muscles on an isokinetic dynamometer (Humac Norm Isokinectic dynamometer, CSMi Boston). Participants were then randomly assigned to a supplementation (SUP) or placebo condition (CON) for a period of 7 consecutive days. On the 7th day of supplementation participants completed the experimental protocol consisting of a maximal effort 10 km downhill (-10% gradient) TT on a treadmill (h/p/cosmos mercury 4.0 h/p/cosmos sports & Medical GmbH, Nussdor-Traunstein, Germany), followed by assessments of muscular, perceptual and biochemical measures of recovery. This protocol has previously been shown to induce significant muscle and impair muscle function (Pokora et al. 2014), allowing for the assessment of performance and recovery. Following a two-week washout period, participants completed the experiment with the opposing treatment. A two-week washout period was deemed suitable as previous more intense acute magnesium depletion investigations observed that humans returned to baseline levels within 2-weeks (Lukaski & Nielsen, 2002), while more recent studies have used a 7-day washout period following 4-weeks of magnesium supplementation (Kass & Poeira, 2015). Both participants and investigators were blinded to the treatment until statistical analysis was completed.

Experimental protocol

In the week prior to each downhill 10 km TT, participants were instructed not to exceed 260 mg/day of magnesium and required to consume either magnesium or placebo capsules (as described below). Following a 12 hr overnight fast, participants attended the laboratory and provided an initial venous blood sample from the median cubital vein. All laboratory visits were completed at the same time of day to control for differences in circadian rhythm. Following a short rest, participants subsequently completed a self-paced 10 km downhill TT whereupon participants had been instructed to complete the distance in the shortest possible time. The speed of the treadmill was regulated via verbal command from the participant through the researcher. Participants were provided with information regarding distance at every 0.5 km. Strong verbal encouragement was provided throughout with the aim of facilitating maximal effort in each trial. At every 2 km, capillary blood samples were obtained from the fingertip and used for the assessment of blood glucose and lactate conducted on an automated benchtop analyser (Biosen C-Line Clinic, EKF-diagnostic GmbH, Barleben, Germany). These

values were then averaged to provide an overall assessment of glucose and lactate responses during the exercise (later described as 'Dur').

Further venous blood samples were obtained immediately following completion of the exercise and after 1 hr of recovery. Participants then returned to the laboratory 24 hrs later, after a 12 hr overnight fast, to provide further blood samples and to complete an assessment of maximal isometric force production of the knee extensor and flexor muscles (as described above). This provided a measure of the recovery of mechanical force production following the 10 km downhill TT (Eston et al. 1996). Immediately post the downhill 10 km TT, 24 hrs prior to maximal force testing, participants were reminded to avoid the use of recovery strategies, exercise and have sufficient sleep. Perceived muscle soreness was assessed using an ordinal visual analogue scale 0 (no pain) to 10 (unbearable pain) (Pincus et al. 2008; Robson-Ansley et al. 2010), at rest, immediately following completion of the 10 km downhill TT, 1 hr, 24 hrs, 48 hrs and 72 hrs into recovery. The timeline of experiment is displayed in Fig.1.

XXX Insert Figure 1 Here XXX

Nutritional guidelines

Participants were provided with a list of foods and beverages rich in magnesium and were instructed how to find and record the quantity of an item to complete the food diary. Examples of types of foods and beverages participants were recommended to avoid included almonds, spinach, cashews, soy milk, black beans, edamame and peanut butter. In this study, a low magnesium dietary intake was achieved by implementing a magnesium restricted diet of <260 mg/day, which is considered low for male athletic populations and if continued could lead to magnesium deficiency in the long term (Nielsen & Lukaski 2006). Food diaries were analysed using Nutritics (Nutritics LTD., Dublin, Ireland), to estimate the amount of dietary magnesium consumed and to confirm adherence to the dietary instructions. Over the 7-day supplementation period, prior to the experimental trial, participants consumed 3 capsules per day (8am, 2pm and 8pm). In the SUP condition this equated to a daily dose of 500 mg/day of magnesium (magnesium oxide, magnesium stearate, microcrystalline cellulose) (MyVitamins^{TM}), while the CON condition consumed capsules containing cornflour.

Magnesium oxide has a relatively low solubility compared to other forms of magnesium (Blancquaert, Vervaet, & Derave, 2019), however, magnesium oxide supplementation has been shown to improve exercise performance in lower doses than the current study (Setaro et al., 2014; Veronese et al., 2014). In an attempt to increase the bioavailability of magnesium, this

study implemented a supplementation regimen of low doses, at 6 hr intervals across the day (~80% of magnesium absorption), with a high overall total daily dose (500 mg). Each of the previously mentioned have been shown to enhance magnesium solubility (Fine, Santa Ana, Porter, & Fordtran, 1991; Hardwick, Jones, Brautbar, & Lee, 1990; Quamme, 2008), and the latter to increase absolute absorption (Schuchardt & Hahn, 2017).

Capsules were double blinded from the researchers and participants, with the magnesium and placebo capsules being identical in appearance and weight. On the day of testing, the capsule was consumed after the final blood sample. Throughout the study participants avoided consumption of multivitamin supplements and anti-inflammatory medications. Participants were instructed to replicate their diet in the 24 hr period in-between the downhill 10 km TT and maximal force tests (including a 12 hr overnight fast).

Blood sampling and analysis

Whole blood samples (8 ml per time point) were collected into K3EDTA vacutainers (Greiner Bio-one; Frickenhausen, Germany). CK was measured immediately using an automated analyser (Reflotron plus, Roche Diagnostics GmbH, Rotkreuz, Switzerland). The remaining whole blood sample was separated by centrifugation at 3,000 x G for a 10-minute. The resultant plasma was then stored at -80°C until subsequent analysis. Plasma IL-6 concentrations were analysed using a high sensitivity enzyme linked immunosorbent assay (ELISA) (Quantikine HS; R&D Systems Ltd., Abingdon, UK). sIL-6R was measured using a commercially available DuoSet ELISA (R&D Systems Ltd.) that has previously been validated for use with plasma samples (Cullen et al. 2016). All additional reagents were purchased from R&D Systems Ltd. Prior to analysis of sIL-6R, plasma was diluted 1:100 in a commercially available diluent (DY997, R&D Systems Ltd) to produce concentrations that were within the dynamic range of the assay. In order to minimise variation, all samples from an individual participant were analysed in the same assay and the manufacturer's instructions were carried out at all times. IL-6 and sIL-6R concentrations were corrected for changes in plasma volume, which were calculated using established methods (Dill & Costill 1974). The IL-6 assay has a detection limit of 0.031 pg/ml and had an intra-assay coefficient of variation of 3.9 ± 0.2 % across a range of 0.15 - 10 pg/ml. The sIL-6R assay has an intra-assay coefficient of variation of 4.8 ± 1.6 % across a range of 1.56 - 100 ng/ml. IL-6 and sIL-6R concentrations were identified in correspondence to a four-parameter standard curve.

Statistical analysis

Data normality were confirmed through the use of the Shapiro-Wilk test. In order to assess a potential carryover effect a Fisher's exact test was used and subsequently confirmed no carryover effect. A paired samples t-test was utilised to assess the effect of magnesium supplementation on downhill 10 km TT performance. In order to assist with the interpretation of the practical significance of this result, effect size (ES) was measured (Cohen, 1988). A one-way repeated measures ANOVA was utilised to assess the difference in peak torque between Baseline, CON and SUP conditions. A two-way repeated measures ANOVA (treatment [placebo vs magnesium] × time) was used to assess the effect of supplementation on blood glucose, IL-6 and sIL-6R responses to exercise. Corresponding effect sizes for main effects were calculated as partial eta squared (η^2). When main effects were identified, post-hoc analysis was performed using simple pairwise comparisons with Bonferroni adjustment. A post-hoc power analysis was carried out on the primary and secondary variables (IL-6 & glucose) using G*power 3.1. A Friedman test was utilised to measure differences between conditions for muscle soreness. On the occurrence of a significant result, a Wilcoxon test was then utilised to identify the differences at specific time points between conditions. Pearson correlations were used to investigate relationships between measures of performance and recovery, and biochemical variables. All data was expressed as mean ± standard deviation (SD), with statistical significance was set at $P < 0.05$. Statistical analyses were undertaken using GraphPad Prism and SPSS (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.).

Results

Dietary mineral, trace elements and macronutrients

Analysis of food diaries demonstrated that participants adhered to the magnesium restricted diet of <260 mg/day. There was no difference in reported dietary magnesium consumption between conditions (SUP = 197 ± 61 mg/day vs CON = 215 ± 52 mg/day, $P > 0.05$). A significant difference was apparent between conditions with the inclusion of 500 mg/day of magnesium for the SUP condition (SUP = 697 ± 61 mg/day vs CON = 215 ± 52 mg/day, $P < 0.001$). Significant differences were also observed between conditions for sodium and chloride ($P < 0.05$).

XXX Insert Table 1 Here XXX

10 km TT performance

There was no effect of supplementation on 10 km downhill running TT performance (SUP = 39:49 ± 4 min vs CON = 41:01 ± 3 min, P = 0.2, ES= 0.46). Performance was faster in 7 out of 9 participants in SUP than CON, which was equivalent to an average 72 seconds (4%) faster 10 km run time.

XXX Insert Figure 2 Here XXX

IL-6, sIL-6R, glucose and lactate responses to exercise

Exercise induced changes in the plasma concentration of IL-6 and sIL-6R are reported in Fig. 3. Main effects of condition ($F = 9.329$, $P = 0.016$, $\eta^2 = 0.538$) and time ($F = 18.739$, $P < 0.001$ $\eta^2 = 0.701$) were observed for IL-6. IL-6 was significantly lower during SUP (1.36 ± 0.66 pg/ml) than CON (2.06 ± 1.14 pg/ml) ($P = 0.016$, mean difference= 0.7 pg/ml, 95%CI: 0.17 - 1.23 pg/ml). Post-hoc power analysis revealed an adequate power of $p\beta > 0.96$ % for IL-6 (Cohen 1988). From pre-exercise, plasma IL-6 concentrations increased immediately post ($P = 0.004$, mean difference= 2.1 pg/ml, 95%CI: 0.72 - 3.50 pg/ml) and 1 hr post ($P = 0.012$, mean difference=1.85 pg/ml, 95%CI: 0.41 - 3.29 pg/ml) downhill 10 km TT. At 24 hrs post, plasma IL-6 was not significantly different to rest ($P = 0.78$), appearing to return to baseline levels. For sIL-6R the ANOVA revealed a main effect of condition ($F = 5.660$, $P = 0.045$, $\eta^2 = 0.41$) with sIL-6R higher in the SUP (27615 ± 8446 pg/ml) than CON (24368 ± 7806 pg/ml) ($P = 0.045$, mean difference= 3064 pg/ml, 95%CI: 94 - 6035 to pg/ml). Correlation analysis did not reveal any relationships between IL-6 and sIL-6R and measures of performance or recovery.

XXX Insert Figure 3 Here XXX

Blood glucose and lactate responses are reported in Fig. 4. There was no significant effect of condition on blood glucose, but there was a time effect ($F = 20.828$, $P < 0.001$, $\eta^2 = 0.722$). Post-hoc power analysis revealed an adequate power of $p\beta > 0.80$ % for glucose. Blood glucose concentrations were increased immediately post 10 km downhill TT ($P = 0.002$, mean difference= 1.1 mmol/L, 95%CI: 0.453 - 1.792 mmol/L), thereafter returning to resting levels in the SUP condition and below resting in the CON condition. There was a significant time x condition interaction effect, with glucose being significantly higher in SUP than CON at 1 hr

post (4.46 ± 0.15 mmol/L vs 3.72 ± 0.22 mmol/L, P = 0.005, mean difference= 0.7 mmol/L, 95%CI: 0.29 - 1.17 to mmol/L) and 24 hrs post exercise (4.40 ± 0.11 mmol/L vs 3.89 ± 0.15 mmol/L, P = 0.04, mean difference= -0.51 mmol/L, 95%CI: -0.98 to -0.03 mmol/L).

There was a main effect of time for blood lactate (F = 36.656, P < 0.001, η^2 = 0.821), increasing during exercise (P = 0.008, mean difference= 2.2 mmol/L, 95%CI: 0.59 - 3.82 mmol/L), and peaking immediately post the 10 km downhill TT (P = 0.002, mean difference= 4.4 mmol/L, 95%CI: 1.87 - 7.08 mmol/L), thereafter returning to resting levels. There were no effects of condition (F = 3.2, P = 0.11, η^2 = 0.28) or interaction effects (F = 0.67, P = 0.61, η^2 = 0.08).

XXX Insert Figure 4 Here XXX

Measures of recovery

Main effects of condition were detected for peak torque of concentric extensors (F = 10.269, P = 0.003, η^2 = 0.562), concentric knee flexors (F = 9.641, P = 0.004, η^2 = 0.547), eccentric knee flexors (F = 6.212, P = 0.013, η^2 = 0.437). Peak concentric knee extensor force was significantly decreased from baseline in SUP (P = 0.021, mean difference= -36 Nm/kg, 95%CI: -5.69 to -66.30 Nm/kg) and CON (P = 0.004, mean difference= -30 Nm/kg, 95%CI: -11.14 to -49.75 Nm/kg), demonstrating a significant impairment in muscle force production 24 hrs post 10 km downhill TT. No significant differences were detected between SUP and CON, demonstrating no effect of the intervention (Fig. 5a). Peak concentric knee flexors torque was significantly decreased from Baseline to SUP (P = 0.029, mean difference= -32 Nm/kg, 95%CI: -3.386 to -60.170 Nm/kg) and CON (P = 0.028, mean difference= -31 Nm/kg, 95%CI: -3.66 to -61.0 Nm/kg), with no difference between SUP and CON (Fig. 5c). Peak eccentric knee flexor torque was significantly decreased from Baseline to SUP (P = 0.04, mean difference= -20 Nm/kg, 95%CI: -0.68 to -40.65 Nm/kg) and CON (P = 0.004, mean difference= -22 Nm/kg, 95%CI: -3.6 to -40.85 Nm/kg), but with no difference between SUP and CON (Fig. 5d).

XXX Insert Figure 5 Here XXX

Circulating CK showed a main effect of time (F = 6.231, P = 0.029, η^2 = 0.438), however post-hoc testing revealed no consistent pattern to the data. There was no significant effect of condition (F = 0.5, P = 0.5, η^2 = 0.059) (Fig. 6a).

Muscle soreness showed a main effect of condition (F = 16.112, P = 0.004, η^2 = 0.668) and time F = 28.928, P < 0.001 η^2 = 0.783), while there was also an interaction effect (F = 2.7, P =

354 0.03, η^2 = 0.26). Muscle soreness increased immediately post (P < 0.001, mean difference= 6.8,
355 95%CI: 4.19 - 9.58), 1 hr post (P = 0.003, mean difference= 2.4, 95%CI: 0.86 - 3.91), 24 hrs
356 post (P = 0.008, mean difference= 3.2, 95%CI: 0.87 - 5.69), 48 hrs post (P = 0.009, mean
357 difference= 3.0, 95%CI: 0.77 - 5.35), and 72 hrs post downhill 10 km TT (P = 0.016, mean
358 difference= 1.6, 95%CI: 0.29 - 3.041) (Fig. 6b). CON was significantly higher than SUP at 24
359 hrs (P = 0.038, mean difference= 1.44, 95%CI: 0.11 - 2.78), 48 hrs (P = 0.021, mean
360 difference= 2.33, 95%CI: 0.45 - 4.22), and 72 hrs post (P = 0.049, mean difference= 1.55,
361 95%CI: 0.13 - 3.09). This corresponded to 32 ± 11 %, 50 ± 14 % and 53 ± 12 % lower muscle
362 soreness in SUP than CON at 24 hrs, 48 hrs and 72 hrs respectively.
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364 **XXX Insert Figure 6 Here XXX**
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366 # Discussion
367
368 The primary results of this study are that 7-days of 500 mg/day magnesium supplementation in
369 comparison to 7 days of low magnesium intake (<260 mg/day) causes a significant decrease in
370 the circulating concentration of IL-6, while increasing sIL-6R, but did not result in significant
371 improvements in performance, nor recovery of strength and muscle damage in the 24 hrs
372 following a downhill 10 km running time-trial. Magnesium supplementation did not increase
373 blood glucose concentration during exercise nor reduce blood lactate, but increased blood
374 glucose 1-24 hrs post exercise, and reduced muscle soreness 24-72 hrs after the exercise. Taken
375 together these findings provide further evidence of the potential positive physiological effects
376 of magnesium supplementation, but do not provide evidence for it as an ergogenic aid in this
377 context during acute exercise.
378 These novel findings extend our understanding of the physiological effects of magnesium
379 supplementation by demonstrating a reduction in IL-6 and increase in the sIL-6R. The
380 interaction of IL-6 and sIL-6R is highly complex and likely context dependent, however, the
381 decrease in sIL-6R observed in the CON condition, might be explained through an increased
382 formation of IL-6/sIL-6R complexes, due to the elevated IL-6 production (Baran et al. 2018).
383 Post exercise inflammation, in the form of transient increases of muscle derived IL-6, has an
384 anti-inflammatory effect protecting against insulin resistance, stimulating lipolysis and
385 increasing fat oxidation (Petersen & Pedersen, 2005). The anti-inflammatory response is
386 essential for post exercise adaptations and preventing it can hinder recovery (Mackey et al.,

2007; Mikkelsen et al., 2009; Wedell-Neergaard et al., 2019). However, inflammation also causes fibrosis (Abdelmagid et al., 2012) and induces pain (Stauber, 2004). Therefore, in situations of repeated muscle damage, when muscle soreness is prolonged in nature, attenuating inflammation and muscle soreness may enhance ones perceived 'readiness to train' or indeed performance. This could be particularly important for athletes during intensified training blocks, periods of fixture congestion in team sports, repeated competition over series of days such as major tennis tournaments, and strenuous multiple day athletic events.

Many previous studies have discussed the potential role of IL-6 during exercise, with increased circulating IL-6 concentrations often being associated with impaired subsequent exercise performance (Robson-Ansley et al. 2004; Walshe et al. 2010). Yet, we observed no respective improvement in performance despite lower concentrations of IL-6 and higher concentrations of sIL-6R. It is possible that in circumstances of intensified training or overreaching, when IL-6 may be chronically elevated (Robson-Ansley et al. 2007), that magnesium supplementation may have a beneficial effect. As such, future studies should investigate the efficacy of magnesium supplementation in periods of intensified training or repeated competition.

Previous studies are inconsistent regarding the efficacy of magnesium supplementation for improving performance and recovery from exercise. Our findings are in agreement with previous studies that magnesium supplementation does not enhance endurance performance or recovery in the context of a single bout of acute exercise in young athletic cohorts (Terblanche et al. 1992; Finstad et al. 2001). However, contradictory findings have been observed in elderly populations, using chronic magnesium supplementation in a longitudinal setting (Veronese et al. 2014); yet it is unclear whether magnesium supplementation is particularly beneficial in elderly populations or whether magnesium supplementation is simply more effective in the context of repeated exposure to exercise. It is also feasible that the effects observed in the current study were too small, or too inconsistent, to have a statistically significant effect on exercise performance. Indeed, performance was on average 71 seconds (4%) faster in SUP than CON, which equated to a moderate effect size (0.46), and while not statistically significant this may represent an important effect for practitioners seeking to improve performance by small margins in individual athletes.

In contrast to the effects observed in murine models (Cheng et al. 2010; Chen et al. 2014), we observed no effect of magnesium supplementation on blood glucose or lactate concentration during exercise. The aforementioned studies by Chen and colleagues observed large increases in blood glucose (up to 175%) following infusion of a very high dose of magnesium (equivalent to approximately 10 times the daily dose used in the current study). As such, it appears that

even the high dose used in the current study is not sufficient to induce the beneficial effects observed by Chen and colleagues. In humans, higher doses (>500 mg·day^{-1}) should be investigated with caution given the well-established laxative and gastrointestinal side effects (Portalatin & Winstead 2012). In contrast, blood glucose concentration was higher post exercise, which is in accordance with previous studies (Chen et al. 2009). This is thought to be connected to both magnesium and the Mg-ATP complex being critical for the availability of glucose via glucose metabolism, as a cofactor in glycolysis for phosphofructokinase, hexokinase, phosphoglycerate kinase, pyruvate kinase, and aldolase (Garfinkel & Garfinkel 1985). In addition to magnesium status regulating the expression and translocation of glucose transporter type 4 (GLUT4) (Romani et al. 2000; Kamran et al. 2018; Solaimani et al. 2014). This may have assisted in upholding glucose homeostasis during exercise, in turn leading to glycogen stores being less depleted in the SUP condition. This could also explain the observed lower IL-6 concentrations in the SUP condition post exercise. As GLUT4 is considered critical for the replenishment of glycogen stores post exercise (McCoy, Proietto, & Hargreaves, 1996), future studies should investigate the potential for magnesium supplementation to increase muscle glycogen repletion, as this may have important consequences in circumstances of repeated training and competition.

There was no effect of magnesium supplementation on muscle damage, as measured by CK concentration, nor maximal muscle force, but it did reduce perceived muscle soreness 48-72 hrs post exercise. Given that IL-6 has been implicated in the perception of pain (De Jongh et al. 2003) and exercise induced muscle soreness via trans-signalling through sIL-6R (Robson-Ansley et al. 2010), we also investigated the relationship between IL-6, sIL-6R and muscle soreness. Despite a reduction in IL-6 and decrease in muscle soreness at 24-72 hrs post exercise, there were no relationships between changes in IL-6, sIL-6R and perceived muscle soreness. Therefore, the observed positive effects on post exercise muscle soreness are likely due to another mechanism.

Limitations

It is important to acknowledge that the current study is not without limitation. We did not directly assess any potential changes in cellular magnesium concentration. Given that the observed responses in terms of IL-6, blood glucose and muscle soreness are likely due to molecular signalling events happening within the muscle, it would have been particularly interesting to assess any potential effects of supplementation on acute magnesium fluctuations within the muscle. Ultimately these measurements were beyond the scope of the current study.

Finally, a 10 km TT at baseline and the assessment of blood biomarkers during exercise, 48 and 72 hrs post exercise, could have further improved our understanding of the measured responses.

Conclusions

In summary, the results of our study indicate that short-term magnesium supplementation decreases plasma IL-6 concentration and has small positive effects on blood glucose and muscle soreness in the days following strenuous exercise. However, there was no beneficial effect on exercise performance, recovery of muscle force or muscle damage. Future studies should investigate the effects of magnesium supplementation in situations of repeated muscle damage and inflammation where the potential negative effects accumulate over several days.

Author Contribution Statement

CJS and TC designed the study. CJS, TC, MDC and GK conducted laboratory experiments. CJS, TC, ZY and YL analysed data. CJS and TC drafted the manuscript. All authors read and approved the manuscript.

Acknowledgements

The authors declare no conflicts of interest.

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Table and Figure Captions

Table 1 Minerals, trace elements and macronutrients during the 1-week controlled magnesium dietary intake in the magnesium supplemented (SUP) and low magnesium diet (CON) conditions. All values are mean ± standard deviation.

Fig. 1 Chronological schematic representing the experimental protocol, including supplementation periods, 10 km time trials, isokinetic dynamometer testing and venous blood sample time points.

Fig. 2 10 km downhill running time trial performance in the magnesium supplemented (SUP) and low magnesium diet (CON) conditions. Individual data points represent performance times of each participants, while lines and whiskers represent mean and SD respectively.

Fig. 3 Circulating IL-6 (A), sIL-6R (B) responses to 10 km downhill time trial in the magnesium supplemented (SUP) and low magnesium diet (CON) conditions.
a = main effect of condition
b = main effect of time
c = significantly different to Pre and 24 hrs

Fig. 4 Blood glucose (A) and lactate (B) responses to 10 km downhill time trial in the magnesium supplemented (SUP) and low magnesium diet (CON) conditions. 'Dur' represents the mean measurement taken throughout exercise the exercise.
a = main effect of condition
b = significantly different to all other time points
c = significant difference between SUP and CON

Fig. 5 Peak torque of the concentric extensors (a), eccentric extensors (b), concentric flexors (c) and eccentric flexors (d) at baseline and 24 hrs post 10 km downhill time trial in the magnesium supplemented (SUP) and low magnesium diet (CON) conditions.
a = main effect of time

b = significantly different to Baseline

Fig. 6 Creatine kinase (A) and muscle soreness (B) in responses to 10 km downhill time trial in the magnesium supplemented (SUP) and low magnesium diet (CON) conditions.
a = main effect of condition
b = main effect of time
c = significantly different to Pre and Post
d = significantly different to all other time points
e = significant difference between SUP and CON