No effect of glutamine supplementation and hyperoxia on oxidative metabolism and performance during high intensity exercise

Glutamine supplementation, hyperoxia and exercise performance

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Abstract

Glutamine enhances the exercise-induced expansion of the tricarboxylic acid intermediate pool. The aim of the present study was to determine whether oral glutamine, alone or in combination with hyperoxia, influenced oxidative metabolism and cycle time-trial performance. 8 **participants** consumed either placebo or 0.125g.kg body mass^{-1} of glutamine in 5 ml.kg body mass^{-1} placebo 1 h prior to exercise in normoxic (**control** & **glutamine** respectively) or hyperoxic (FiO$_2$=50%; **hyperoxia** & **hyperoxia and glutamine** respectively) conditions. **Participants** then cycled for 6 min at 70% $\dot{V}o_2$ max immediately before completing a brief high intensity time-trial (~ 4min) where a pre-determined volume of work was completed as fast as possible. The increment in pulmonary oxygen uptake during the performance test ($\Delta\dot{V}o_2$, p=0.02) and exercise performance {243 ± 7 (**control**) vs 242 ± 3 (**glutamine**) vs 231 ± 3 (**hyperoxia**) vs 228 ± 5 (**hyperoxia and glutamine**) s, p<0.01} were significantly improved in hyperoxic conditions. There was some evidence that glutamine ingestion increased $\Delta\dot{V}o_2$ in normoxia, but not hyperoxia (interaction drink/FiO$_2$, p=0.04) however there was no main effect or impact on performance. Overall the present data show no effect of glutamine ingestion either alone or in combination with hyperoxia, and thus no limiting effect of the **tricarboxylic acid intermediate** pool size, on oxidative metabolism and performance during maximal exercise.

Introduction

At the onset of exercise there is an increase in the tricarboxylic acid (TCA) intermediate pool size which has been suggested to facilitate increased oxidative ATP resynthesis (Sahlin, Katz, & Broberg, 1990; Wagenmakers, 1998). Conversely dissociation between the **TCA intermediate** pool size and oxidative energy provision during exercise has been consistently demonstrated during sub-maximal exercise (Bruce et al., 2001; Gibala, Gonzalez-Alonso & Saltin, 2002a; Gibala, Peirce, Constantin-Teodosiu & Greenhaff, 2002b; Dawson, Howarth, Tarnopolsky, Wong & Gibala, 2003; Howarth, LeBlanc, Heigenhauser & Gibala, 2004; Timmons, Constantin-Teodosiu, Poucher & Greenhaff, 2004; Dawson, Baker, Greenhaff & Gibala, 2005; Bangsbo, Gibala, Howart & Krustrup, 2006). However, Gibala, MacLean, Graham & Saltin, *(*1998) demonstrate that as exercise intensity **(and hence TCA cycle flux)** increases there is a concomitant **(though non-linear)** increase in the **TCA intermediate** pool size. Therefore it seems likely that only during maximal intensity exercise, once maximal **TCA intermediate** concentration has been reached, will the **TCA intermediate** pool size be limiting to TCA cycle flux and thus oxidative metabolism, (i.e. unchanging **TCA intermediate** pool size during requirement for increased TCA cycle flux).

Dichloroacetate infusion results in near-maximal activation of the pyruvate dehydrogenase complex and stockpiling of acetyl-units. During the transition to subsequent moderate intensity exercise, **dichloroacetate** infusion has consistently been shown to enhance the rate of increase of oxidative metabolism as indicated by a

reduction in phosphocreatine degradation and lactate accumulation (Timmons *et al.* 1996; Timmons, Poucher, Constantin-Teodosiu, Macdonald & Greenhaff, 1997; Timmons *et al.* 1998a Timmons, Gustafsson, Sundberg, Jansson & Greenhaff, 1998b; Howlett, Heigenhauser, Hultman, Hollidge-Horvat & Spriet, 1999a; Parolin *et al.* 2000; Roberts, Loxham, Poucher, Constantin-Teodosiu & Greenhaff, 2002). However during the transition to high intensity exercise **dichloroacetate** infusion appears to have no effect on oxidative metabolism (Savasi, Evans, Heigenhauser & Spriet, 2002; Howlett, Heigenhauser & Spriet, 1999b). Furthermore, whilst **dichloroacetate** infusion reduced **TCA intermediate** content at rest (Constantin-Teodosiu et al, 1999) the majority of studies show no effect of prior **dichloroacetate** infusion on the **TCA intermediate** pool during exercise (Gibala *et al.* 2002b; Timmons *et al.* 2004). The study by Bansgbo *et al.* (2006) is the only study to demonstrate manipulation (reduction) of the **TCA intermediate** pool during exercise following **dichloroacetate** infusion; after 15 s of high intensity exercise the **TCA intermediate** pool was reduced following **dichloroacetate** infusion, compared to saline, with no effect on oxidative metabolism. Importantly however, this effect on the **TCA intermediate** was not maintained after 3 min of exercise. Taken together**,** studies utilising **dichloroacetate** infusion prior to high intensity exercise therefore show a limitation downstream of the **pyruvate dehydrogenase complex**, hence implicating the **TCA intermediate** and / or oxygen delivery as limiting factors during high intensity exercise. Surprisingly there is a paucity of data relating to the possible role of the **TCA intermediate** in limiting oxidative metabolism during high intensity exercise. However, in contrast to the study by Bangsbo *et al.*

(2006), the rate of oxidative ATP production and power output were enhanced during severe intensity finger flexion exercise when 6 g.day^{-1} of a mixture containing 55g.l^{-1} of malate (the **TCA intermediate** making the largest contribution to the increase in **TCA intermediate** during exercise (Gibala, Tarnopolsky, & Graham, 1997) was consumed for 15 days (Bendahan *et al.* 2002).

Glutamine ingestion enhances the exercise-induced increase in the **TCA intermediate** pool following 10 min of cycle exercise at 70% $\dot{V}o_2$ max (Bruce et al. 2001), though this had no effect on substrate level phosphorylation after 10 min of exercise. However no previous study has investigated the effects of glutamine ingestion during high intensity, whole body exercise. Therefore the first aim of the present study was to determine whether glutamine ingestion (via an enhanced **TCA intermediate** pool expansion) improves oxidative metabolism and performance during maximal aerobic exercise.

Relieving a limitation at the level of the TCA cycle may only expose other limitations (e.g. oxygen delivery, enzyme activation), which prevent enhancement of oxidative metabolism (Tschakovsky & Hughson, 1999; Gurd et al. 2006). We therefore hypothesised that any glutamine-mediated enhancement of TCA cycle flux, and hence NADH & FADH production, would exacerbate the oxygen delivery limitation found during high intensity exercise (Linnarsson, Karlsson, Fagraeus & Saltin, 1974; Adams & Welch, 1980; Knight *et al.* 1993; Hogan, Richardson & Haseler, 1999; Richardson *et al.* 1999a; Richardson, Leigh, Wagner & Noyszewski, 1999b; Linossier *et al.* 2000). Therefore a second aim of this experiment was to

compare the separate and combined effects of glutamine supplementation and hyperoxia on performance and oxidative metabolism during maximal aerobic exercise.

Method

Participants

Eight trained cyclists (age: 36.0 ± 4 yrs; $\dot{V}o_2$ max: 4.6 ± 0.2 l.min^{-1}; WorkMax: 381 ± 46 W; mass: 80.4 ± 4 kg) completed the four-trial study, which was approved by the local university ethical committee.

Preliminary testing

The pre-experimental procedures were employed to replicate the protocol of Bruce *et al.* (2001) in which oral glutamine was demonstrated to enhance the exercise-induced expansion of the **TCA intermediate** pool. Full details of preliminary testing and pre-experimental procedures can be found elsewhere (Marwood & Bowtell, 2007). In brief, a submaximal test and a maximal ramp test to determine $\dot{V}o_2$ max, were completed. An incremental maximum workload (WorkMax) test was also completed to individually determine the volume of work for the time trial (Jeukendrup, Saris, Brouns, & Kester, 1996). Prior to the main trials, a familiarisation trial was performed to reduce any possible learning effect across the four trials. A separate unpublished study utilising the present exercise protocol examined performance across three identical trials; there was no significant improvement in performance across trials. In addition, the 95% limits of agreement

between adjacent trials reduced from 15.6 s / 27.9 W (mean performance: 222 s / 377 W) between trials 1 & 2 to 9.25 s / 17.0 W (mean performance: 222 s / 378 W) between trials 2 & 3, suggesting a reduction in the 95% **limits of agreement** between adjacent trials to ~ 4 % of overall performance after a single familiarisation trial. Therefore we were confident that performance during the experimental trials was reliable and without a learning effect.

Pre-experimental procedures

Participants were instructed to consume their habitual diet and refrain from strenuous physical activity in the 48 h prior to each experiment. On the evening before the experiment (day 1), **participants** completed 30 min cycling at 70% $\dot{V}o_2$ max. The workload was then doubled and 3 * 50 s bursts of exercise were completed, separated by 2 min of rest. **Participants** then cycled for a further 45 min at 70% $\dot{V}o_2$ max. This also ensured that blood lactate concentration was low at the end of exercise to minimise glycogen resynthesis from lactate during recovery. The glycogen depleting protocol was designed to deplete both type I and type II muscle fibres of glycogen as validated by Vollestad et al. (1992). **Participants** were free to drink water throughout exercise. Following the glycogen depleting exercise, **participants** consumed a prescribed diet (identical for each trial), based on their habitual diet, but which provided only 30% carbohydrate. This ensured that only limited muscle glycogen resynthesis occurred before the 2nd day of the experiment, and that the magnitude of resynthesis, and hence pyruvate availability, were similar for each trial. This design was employed to replicate the protocol of Bruce et al.

(2001) in which oral glutamine was demonstrated to enhance the exercise-induced expansion of the **TCA intermediate** pool.

Experimental Design

Participants completed four main trials: placebo & normoxia (CON), glutamine & normoxia (GLN), placebo and hyperoxia (HYP) and hyperoxia and glutamine (HPG). Trials were allocated in a systematic rotation fashion with **participants** blind to the condition. Each trial took place over the course of two days and trials were separated by 10 – 14 days.

Experimental protocol

Each experimental trial was completed across 2 days. On the evening of day 1, **participants** undertook a glycogen depletion protocol (see above). On the morning of day 2, **participants** arrived at the laboratory following an overnight fast and rested in the supine position. A cannula was inserted into an antecubital vein and a resting blood sample was obtained. **Participants** then consumed a drink containing either 5ml.kg body wt $^{-1}$ of placebo or 0.125g.kg body wt $^{-1}$ glutamine in 5ml.kg body wt $^{-1}$ of placebo and rested for 1 h before exercise commenced. Ten min before exercise, **participants** positioned themselves on the ergometer and inspired either normoxic (room) air ($FiO_2 = 21\%$) or a hyperoxic gas mixture containing 50% oxygen and balanced nitrogen ($FiO_2 = 50\%$). Exercise commenced from rest and consisted of cycling for 6 min at $70\% \dot{V}o_2$max immediately before completing a fixed-work time-trial (equal to 4 min at 95%Wmax). **Participants** received verbal

encouragement to perform maximally and were provided with standardised information relating to their progress in the time trial. **Participants** were informed of the completion of each 10 kJ until 20 kJ remaining where they were informed of each kJ completed until the finish. Following 30 min of stationary recovery from exercise, **participants** cycled for a further 5 min at 100 W, after which a tourniquet was immediately inflated (around the thigh interrogated by **near infrared spectroscopy**, see below) to 260mmHg for 1 minute and then quickly deflated.

*Pulmonary gas exchange **& near infrared spectroscopy***

Breath by breath pulmonary oxygen uptake ($\dot{V}o_2$) was measured throughout exercise; **participants** wore a nose-clip and breathed through a mouthpiece connected to a low inertia turbine volume transducer (Interface associates, USA) and mass spectrometer system (CaSE QP9000, Morgan Medical, UK) tuned to measure oxygen, carbon dioxide, nitrogen and argon. Prior to each exercise test, the turbine was calibrated using a precision 3-litre calibration syringe (Hans Rudolph Inc., USA). The system was calibrated prior to each trial using gases of known concentration spanning the range of anticipated fractional gas concentrations during each trial (normoxia: 15% O_2,; hyperoxia: 40% O_2,). The inlet port of the mouthpiece was connected to a 250L Douglas bag which was continuously filled with a gas mixture containing 50% oxygen and balanced nitrogen from a pressurised cylinder. In the normoxic trials the Douglas bag was by-passed and **participants** breathed room air. Measurement of $\dot{V}o_2$ in hyperoxia is technically difficult due to potential problems with contamination with ambient air. However, open circuit

systems such as that used in the present study appear to circumvent such problems and have been shown to be valid measures of $\dot{V}o_2$ in hyperoxia (Prieur *et al.* 1998). The present device utilises standard algorithms (Beaver, Lamarra & Wasserman, 1981) which have been utilised in previous studies measuring $\dot{V}o_2$ in hyperoxia (Bell, Paterson, Kowalchuk & Cunningham, 1999; Wilkerson, Berger & Jones, 2006). Hence we were confident that our measurement of $\dot{V}o_2$ in hyperoxia was valid.

Continuous, non-invasive measurements of muscle deoxygenation via the deoxyhaemoglobin signal (Hb) were made via **near infrared spectroscopy** (NIRO 500, Hamamatsu, Japan) throughout rest and exercise periods (see Marwood & Bowtell, 2007 for full details of these procedures). **Muscle deoxyhaemoglobin** data were normalised to the physiological range which inflation and release of a cuff (see above) around the interrogated thigh produced (%Hb), (Marwood & Bowtell, 2007).

Blood analysis

Venous blood samples (12 ml) were taken at rest (prior to placebo / glutamine ingestion), 30 min post ingestion, at the onset of submaximal exercise, at the onset and after 2 min and at termination of the time trial and after 3, 6, 10, 15, 20 min of recovery from the time trial. Samples were immediately centrifuged at 13,000 rpm for 2 min, the supernatant collected, snap frozen in liquid nitrogen and stored at -80°C and later analysed for lactate and glucose by an automated colorimetric assay

(Cobas Mira, ABX Diagnostics) and for glutamine using standard colorimetric assays (Bergmeyer, 1974).

Data analysis

$\dot{V}o_2$ and %Hb data are expressed as the increment from the mean value during the final minute of submaximal exercise ($\Delta\dot{V}o_2$ & Δ%Hb). $\Delta\dot{V}o_2$ & Δ%Hb data are also normalised against the increment in power output relative to submaximal exercise ($\Delta\dot{V}o_2$/ΔW and Δ%Hb/ΔW).

Statistics

Values are presented as mean ± standard error of the mean (SE) with an n of 8 for **near infrared spectroscopy** data and performance, 7 for pulmonary variables and 6 for blood variables (due to technical problems with pulmonary gas and blood sampling). Data from the first 6 min of exercise have been presented elsewhere (Marwood & Bowtell, 2007).

Time course dependent data were analysed by a 3 way ANOVA for repeated measures {drink (glutamine vs placebo) by inspired oxygen fraction (FiO_2: hyperoxia vs normoxia) by time}. All other data were analysed by a 2 way ANOVA for repeated measures {drink (glutamine vs placebo) by FiO_2 (hyperoxia vs normoxia)}. The specific location of any effect was identified using a pairwise analysis (t-test) corrected by a Sidak-based Holm step-down procedure for multiple comparisons.

Results

Time trial

The average volume of work to be completed during the performance test was 86.8 ± 10.4 kJ. Average time-trial power output was higher (5.7 ± 2.1%) during hyperoxic conditions (average of two hyperoxic trials versus two normoxic trials) but glutamine supplementation had no effect, {358 ± 20 (**control**) vs 357 ± 17 (**glutamine**) vs 374 ± 18 (**hyperoxia**) vs 380 ± 20 (**hyperoxia and glutamine**) W, main effect FiO_2, p=0.002, figure 1}. Time to complete the time-trial was 243 ± 7 (**control**) vs 242 ± 3 (**glutamine**) vs 231 ± 3 (**hyperoxia**) vs 228 ± 5 (**hyperoxia and glutamine**) s (main effect FiO_2, p=0.002).

Pulmonary gas exchange

Average $\dot{V}o_2$ in the final minute of steady state exercise was 3.48 ± 0.17 (**control**), 3.68 ± 0.14 (**glutamine**), 3.65 ± 0.10 (**hyperoxia**) 3.43 ± 0.19 (**hyperoxia and glutamine**) $l.min^{-1}$.

$\Delta\dot{V}o_2$ was higher in hyperoxia compared to normoxia (main effect FiO_2, p=0.02; figure 2). There was also a significant drink / FiO_2 interaction (p=0.04) showing that the effect of glutamine was different in hyperoxia compared to normoxia. Subsequent pairwise analysis revealed that $\Delta\dot{V}o_2$ was higher in the hyperoxia trial

versus control, but that following glutamine ingestion $\Delta \dot{V}o_2$ was no higher in the hyperoxic condition compared to normoxia. These effects remained when $\Delta \dot{V}o_2$ was normalised for the increment in power output (figure 3).

Near infrared spectroscopy

$\Delta\%$Hb & $\Delta\%$Hb /ΔW increased throughout exercise with no effect of glutamine ingestion or hyperoxia (main effect time, $p<0.001$; figures 4, 5).

Plasma Glutamine

At rest, plasma glutamine concentration was not different between trials {537 $\pm$ 43 (**control**) vs 651 $\pm$ 64 (**glutamine**) vs 570 $\pm$ 35 (**hyperoxia**) vs 565 $\pm$ 48 (**hyperoxia and glutamine**) μmol.l^{-1}}. Glutamine ingestion resulted in a 94% and 96% increase in plasma glutamine concentration 30 min post-consumption compared to pre-ingestion but was unchanged in placebo trials {537 $\pm$ 46 (**control**) vs 1261 $\pm$ 91 (**glutamine**) vs 633 $\pm$ 44 (**hyperoxia**) vs 1108 $\pm$ 64 (**hyperoxia and glutamine**) μmol.l^{-1}, main effect drink, $p<0.001$, figure 6}. Immediately prior to exercise, plasma glutamine concentration remained slightly elevated above pre-ingestion in the glutamine trials but was not different to the placebo trials. In the placebo trials plasma glutamine concentration was not different from rest at any time and there was no difference in plasma glutamine concentration between the two placebo-trials (control vs hyperoxia) or between the two glutamine-trials (glutamine vs hyperoxia and glutamine) at any time.

Plasma lactate

Plasma lactate concentration was not significantly different between trials and this remained the case when the data were re-calculated as the change from rest or normalised against power output (figure 7). There was no difference in peak plasma lactate concentration {12.2 ± 1.8 (**control**) vs 10.6 ± 1.0 vs (**glutamine**) vs 10.2 ± 1.4 (**hyperoxia**) vs 10.6 ± 1.1 (**hyperoxia and glutamine**) mmol.l^{-1}, figure 7} but the time to the peak measured lactate concentration following the termination of exercise was significantly longer following glutamine supplementation {1.5 ± 0.7 (**control**) vs 4.0 ± 1.0 vs (**glutamine**) vs 2.6 ± 0.5 (**hyperoxia**) vs 5.0 ± 1.0 (**hyperoxia and glutamine**) min, main effect drink, p<0.001; figure 7}.

Discussion

This is the first study to assess oxidative metabolism and performance during high intensity exercise following augmentation of the normal exercise-induced expansion of the **TCA intermediate pool.** Exercise was also conducted in hyperoxic conditions in order to blunt the anticipated oxygen delivery limitation found during high intensity exercise and thus maximise any potential effect of glutamine ingestion on oxidative metabolism. The main finding of the present study was that time-trial performance was unaffected by glutamine ingestion, whether in normoxia or hyperoxia. In contrast performance was enhanced by ~ 6% in hyperoxia, with a concomitant significant increase in $\Delta \dot{V}o_2$. Hyperoxia has previously been shown to result in enhanced performance during high intensity exercise (Linnarsson, *et al.* 1974; Adams & Welch, 1980; Knight *et al.* 1993; Hogan *et al.* 1999; Richardson *et*

al. 1999a,b; Linossier *et al.* 2000). The mechanism by which hyperoxia exerts this effect appears to be via facilitation of oxygen consumption compared to normoxic conditions, resulting in a delay before the accumulation of metabolic by-products (H^+, AMP, IMP, Pi) to fatiguing levels (Linnarsson, *et al.* 1974; Adams & Welch, 1980; Knight *et al.* 1993; Hogan *et al.* 1999; Linossier *et al.* 2000; Prieur *et al.* 2006)

Glutamine ingestion conferred no main effect on any measured parameter. However, there was a drink/FiO₂ interaction effect for both $\Delta\dot{V}o_2$ and $\Delta\dot{V}o_2/\Delta W$, whereby in the placebo trials $\Delta\dot{V}o_2$ and $\Delta\dot{V}o_2/\Delta W$ were higher in hyperoxia than normoxia, but hyperoxia had no further effect in glutamine trials. Therefore the augmentation of $\Delta\dot{V}o_2$ and $\Delta\dot{V}o_2/\Delta W$ by hyperoxia was not apparent in the glutamine-supplemented trials, suggesting a beneficial effect of glutamine ingestion in normoxic conditions. However, there was no main effect of glutamine ingestion on these parameters, nor muscle deoxygenation or plasma lactate concentration. Therefore the weight of the evidence suggests that there was no effect of glutamine ingestion on oxidative metabolism or exercise performance.

Glutamine ingestion was utilised as it has previously been demonstrated to result in an enhancement of the exercise-induced increase in the **TCA intermediate** pool size at the onset of exercise (Bruce *et al.* 2001). The present experimental protocol was designed to replicate as closely as possible that of Bruce *et al.* (2001) whilst inserting a period of high intensity exercise. The study by Bruce *et al.* (2001) demonstrated a glutamine-induced enhanced expansion of the **TCA intermediate**

pool after 10 min of exercise at 70% $\dot{V}o_2$ max. The present study, in addition to employing identical pre-exercise procedures, utilised the same exercise duration but divided into 6 min of exercise at 70% $\dot{V}o_2$ max and a ~4 min time trial. **TCA intermediate** pool size is increased 4-fold after only 5 min of cycle exercise at 70% $\dot{V}o_2$ max (Gibala *et al.* 1997) but decreases between 10 and 15 min exercise (Gibala *et al.* 2002a). Hence the present protocol was designed to maximise **TCA intermediate** pool size throughout the period of the time-trial. However a limitation of the present study is a lack of muscle **TCA intermediate** data, and no previous study has determined whether the glutamine-induced expansion of **TCA intermediate** pool is evident during higher intensity exercise. Examination of figure 6 shows a marked (94-96%) increase in plasma glutamine concentration 30 min post-ingestion, which persisted up to the onset of exercise (19% higher than pre-ingestion). This suggests that as shown previously (Hankard *et al.* 1995; Bruce *et al.* 2001), a substantial amount of the ingested glutamine escaped utilisation by the splanchnic bed. Elevated plasma glutamine concentration stimulates muscle glutamine uptake via the human muscle glutamine transporter, system N^m (Ahmed, Maxwell, Taylor, & Rennie, 1993). Therefore the decrease in plasma glutamine concentration from 30 min post-ingestion to the onset of exercise suggests that a significant amount of glutamine entered the muscle (Varnier, Leese, Thompson, & Rennie, 1995). Hence it is likely that in the present study glutamine ingestion resulted in an enhancement of the exercise-induced expansion of the **TCA intermediate** pool as in the Bruce *et al.* (2001) study. Therefore the present results

suggest that the size of the **TCA intermediate** pool does not limit oxidative metabolism or performance during maximal aerobic performance.

The lack of any effect of hyperoxia on plasma lactate concentration during maximal exercise was perhaps not surprising since a number of previous studies have found similar results (Linnarsson *et al.* 1974; Adams & Welch, 1980; Knight *et al.* 1993; Hogan *et al.* 1999; Linossier *et al.* 2000; Savasi *et al.* 2002) suggesting that whilst exercise performance is enhanced in hyperoxia, fatigue occurs at the same intracellular concentrations of potential fatiguing metabolites (H^+, AMP, IMP, Pi). Conversely, in the present study when plasma lactate concentration was normalised for power output, there still remained no differences in plasma lactate concentration between trials. However, plasma lactate concentration reflects the balance between efflux of lactate from the muscle and removal of lactate from the circulation by non-exercising muscle and the liver. Therefore, conclusions with regard to oxidative metabolism based on alterations in plasma lactate concentrations should be treated with caution. Indeed, the later peak plasma lactate concentration following glutamine ingestion in the present study demonstrates the many factors that may influence plasma lactate concentration (indeed it is possible that glutamine interferes with either the passive or active export of lactate from the muscle, via monocarboxylate transporter 1; Fei *et al.* 2000). Furthermore, the reduction in lactate concentration in hyperoxia observed in previous studies during submaximal exercise is likely due to a reduction in muscle glycogenolysis (Stellingwerff *et al.* 2005; Stellingwerff, LeBlanc, Hollidge, Heigenhauser & Spriet, 2006). However

the glycogen depletion protocol employed in the present study may have reduced muscle glycogenolysis during exercise, thus blunting this effect (Hargreaves, McConell & Proietto, 1995).

Summary

The main finding of the present study was that glutamine ingestion, previously shown to result in an enhancement of the exercise-induced increase in the **TCA intermediate** pool size (Bruce *et al.* 2001), has no effect on performance during high intensity exercise. In addition, despite some evidence to the contrary (i.e. hyperoxia did not increase $\Delta \dot{V}o_2$ and $\Delta \dot{V}o_2/\Delta W$ following glutamine ingestion, figures 2 and 3) on balance glutamine ingestion did not enhance pulmonary oxygen or muscle deoxygenation kinetics during the time trial. **In accordance with previous studies demonstrating no effect of the TCA intermediate pool size on oxidative metabolism** during submaximal exercise (Bruce *et al.* 2001; Gibala *et al.* 2002a,b; Dawson *et al.* 2003; Howarth *et al.* 2004; Timmons *et al.* 2004; Dawson *et al.* 2005) and intense (Bangsbo *et al.* 2006) exercise, the present results show no effect of glutamine ingestion, and thus no limiting effect of the **TCA intermediate** pool size, on oxidative metabolism and performance during maximal aerobic exercise.

Figure 1. Power output during the time-trial. Values are mean ± SE. There was a main effect of time ($p<0.001$) and of FiO_2 ($p=0.002$). + different to 1st minute of the time-trial (all trials, main effect time: pairwise analysis, $p<0.05$); # different to previous time point (all trials, main effect time: pairwise analysis, $p<0.05$). $ hyperoxia vs normoxia (main effect FiO_2, pairwise analysis, $p<0.05$).

Figure 2. $\Delta \dot{V}o_2$ during the time-trial. Values are mean ± SE. There was a main effect of time ($p<0.001$) and of FiO_2 ($p=0.02$). There was also a significant drink / FiO_2 interaction ($p=0.04$). + different to 1st minute of the time-trial (all trials, main effect time: pairwise analysis, $p<0.05$); # different to previous time point (all trials, main effect time: pairwise analysis, $p<0.05$). $ HYP vs CON (interaction drink / FiO_2: pairwise analysis, $p<0.05$).

Figure 3. $\Delta \dot{V}o_2/\Delta W$ during the time-trial. Values are mean ± SE. There was a main effect of time ($p<0.01$) and of FiO_2 ($p<0.05$). There was also a significant drink / FiO_2 interaction ($p=0.02$). + different to 1st minute of the time-trial (main effect time: pairwise analysis, $p<0.05$); $ HYP vs CON (interaction drink / FiO_2: pairwise analysis, $p<0.05$).

Figure 4. $\Delta\%Hb$ during the time-trial. Values are mean ± SE. There was a main effect of time for all trials ($p<0.001$). # different to previous time point (main effect time: pairwise analysis, $p<0.05$).

Figure 5. Δ%Hb/ΔW during the time-trial. Values are mean ± SE. There was a main effect of time for all trials (p<0.001). # different to previous time point (main effect time: pairwise analysis, p<0.05).

Figure 6. Plasma glutamine concentration at rest and during exercise. Plasma glutamine concentration was higher following glutamine supplementation compared to placebo (main effect drink, p<0.001). There was also a significant drink/time interaction (p<0.001). *glutamine vs placebo supplementation (interaction drink/time: pairwise analysis, p<0.05); # higher than resting concentrations (glutamine supplemented trials only; interaction drink/time: pairwise analysis, p<0.05); $ lower than at end of exercise (glutamine trials only; interaction drink / time: pairwise analysis, p<0.05).

Figure 7. Plasma lactate concentration during and following the time-trial. Values are mean ± SE. There was a main effect of time (p<0.001). + different to submaximal exercise (main effect time: pairwise analysis, p<0.05); # different to previous time point (main effect time: pairwise analysis, p<0.05); $ time to peak lactate concentration longer following glutamine supplementation (main effect drink, P<0.001).

References

Adams, R. P. & Welch, H. G. (1980) Oxygen uptake, acid-base status, and performance with varied inspired oxygen fractions. *Journal of Applied Physiology, 49* (5), 863-868

Ahmed, A., Maxwell, D. L., Taylor, P. M. & Rennie, M. J. (1993) Glutamine transport in human skeletal muscle. *American Journal of Physiology, 264* (6 Pt 1), E993-E1000

Bangsbo, J., Gibala, M. J., Howarth, K. R. & Krustrup, P. (2006). Tricarboxylic acid cycle intermediates accumulate at the onset of intense exercise in man but are not essential for the increase in muscle oxygen uptake. *Pflugers Archives, 452* (6), 737-743

Beaver, W. L., Lamarra, N. & Wasserman, K. (1981). Breath-by-breath measurement of true alveolar gas exchange. *Journal of Applied Physiology, 51 (6)*, 1662-75.

Bell, C., Paterson, D. H., Kowalchuk, J. .M. & Cunningham, D. A. (1999). Oxygen uptake kinetics of older humans are slowed with age but are unaffected by hyperoxia. *Experimental Physiology, 84 (4)*, 747-59.

Bendahan, D., Mattei, J. P., Ghattas, B., Confort-Gouny, S., Le Guern, M. E. & Cozzone, P. J. (2002) Citrulline/malate promotes aerobic energy production in human exercising muscle. *British Journal of Sports Medicine, 36* (4), 282-289

Bergmeyer, H. (1974) *Methods of Enzymatic Analysis* New York, Weinheim / Academic Press.

Bruce, M., Constantin-Teodosiu, D., Greenhaff, P. L., Boobis, L. H., Williams, C. & Bowtell, J. L. (2001) Glutamine supplementation promotes anaplerosis but not oxidative energy delivery in human skeletal muscle. *American Journal of Physiology, 280* (4), E669-E675

Constantin-Teodosiu, D., Simpson, E. J. & Greenhaff,P.L. (1999) The importance of pyruvate availability to PDC activation and anaplerosis in human skeletal muscle *American Journal of Physiology, 276* (2 Pt 1), E472-E478

Dawson, K. D., Howarth, K. R., Tarnopolsky, M. A., Wong, N. D. & Gibala, M. J. (2003) Short-term training attenuates muscle TCA cycle expansion during exercise in women. *Journal of Applied Physiology, 95* (3), 999-1004

Dawson, K. D., Baker, D. J., Greenhaff, P. L. & Gibala, M. J. (2005) An acute decrease in TCA cycle intermediates does not affect aerobic energy delivery in contracting rat skeletal muscle. *Journal of Physiology, 565* (Pt 2), 637-643

Fei, Y. J., Sugawara, M., Nakanishi, T., Huang, W., Wang, H., Prasad, P. D., Leibach, F. H. & Ganapathy, V. (2000). Primary structure, genomic organization, and functional and electrogenic characteristics of human system N 1, a Na+- and H+-coupled glutamine transporter. *Journal of Biological Chemistry, 275* (31), 23707-23717

Gibala, M. J., Tarnopolsky, M. A. & Graham, T. E. (1997) Tricarboxylic acid cycle intermediates in human muscle at rest and during prolonged cycling. *American Journal of Physiology, 272* (2 Pt 1), E239-E244

Gibala, M. J., MacLean, D. A., Graham, T. E. & Saltin, B. (1998). Tricarboxylic acid cycle intermediate pool size and estimated cycle flux in human muscle during exercise. *American Journal of Physiology, 275* (2 Pt 1), E235-E242

Gibala, M. J., Gonzalez-Alonso, J. & Saltin, B. (2002a) Dissociation between muscle tricarboxylic acid cycle pool size and aerobic energy provision during prolonged exercise in humans. *Journal of Physiology, 545* (Pt 2), 705-713

Gibala, M. J., Peirce, N., Constantin-Teodosiu, D. & Greenhaff, P. L. (2002b). Exercise with low muscle glycogen augments TCA cycle anaplerosis but impairs oxidative energy provision in humans. *Journal of Physiology, 540* (Pt 3), 1079-1086

Gurd, B. J., Peters, S. J., Heigenhauser, G. J., LeBlanc, P. J., Doherty, T. J., Paterson, D. H. & Kowalchuk, J. M. (2006) Prior heavy exercise elevates pyruvate dehydrogenase activity and speeds O2 uptake kinetics during subsequent moderate-intensity exercise in healthy young adults. *Journal of Physiology, 577* (Pt 3), 985-996

Hankard, R. G., Darmaun, D., Sager, B. K., D'Amore, D., Parsons, W. R. & Haymond, M. (1995) Response of glutamine metabolism to exogenous glutamine in humans. *American Journal of Physiology, 269* (4 Pt 1), E663-E670

Hargreaves, M., McConell, G. & Proietto, J. (1995). Influence of muscle glycogen on glycogenolysis and glucose uptake during exercise in humans. *Journal of Applied Physiology, 78* (1), 288-292

Hogan, M. C., Richardson, R. S. & Haseler, L. J. (1999). Human muscle performance and PCr hydrolysis with varied inspired oxygen fractions: a 31P-MRS study. *Journal of Applied Physiology, 86* (4), 1367-1373

Howarth, K. R., LeBlanc, P. J., Heigenhauser, G. J. & Gibala, M. J. (2004) Effect of endurance training on muscle TCA cycle metabolism during exercise in humans. *Journal of Applied Physiology, 97* (2), 579-584

Howlett, R. A., Heigenhauser, G. J., Hultman, E., Hollidge-Horvat, M. G. & Spriet, L. L. (1999a). Effects of dichloroacetate infusion on human skeletal muscle metabolism at the onset of exercise. *American Journal of Physiology, 277* (1 Pt 1), E18-E25

Howlett, R. A., Heigenhauser, G. J. & Spriet, L. L. (1999b). Skeletal muscle metabolism during high-intensity sprint exercise is unaffected by dichloroacetate or acetate infusion. *Journal of Applied Physiology, 87* (5), 1747-1751

Jeukendrup, A., Saris, W. H., Brouns, F. & Kester, A. D. (1996) A new validated endurance performance test. *Medicine and Science Sports Exercise, 28* (2), 266-270

Knight, D. R., Schaffartzik, W., Poole, D. C., Hogan, M. C., Bebout, D. E. & Wagner, P. D. (1993) Effects of hyperoxia on maximal leg O2 supply and utilization in men. *Journal of Applied Physiology, 75* (6), 2586-2594

Linnarsson, D., Karlsson, J., Fagraeus, L. & Saltin, B. (1974). Muscle metabolites and oxygen deficit with exercise in hypoxia and hyperoxia. *Journal of Applied Physiology, 36* (4), 399-402

Linossier, M. T., Dormois, D., Arsac, L., Denis, C., Gay, J. P., Geyssant, A. & Lacour, J. R. (2000) Effect of hyperoxia on aerobic and anaerobic performances and muscle metabolism during maximal cycling exercise. *Acta Physiologica Scandinavica, 168* (3), 403-411

Marwood, S. & Bowtell, J. L. (2007) Effects of glutamine and hyperoxia on pulmonary oxygen uptake and muscle deoxygenation kinetics. *European Journal of Applied Physiology, 99* (2), 149-161

Parolin, M. L., Spriet, L. L., Hultman, E., Matsos, M. P., Hollidge-Horvat, M. G., Jones, N. L. & Heigenhauser, G. J. (2000). Effects of PDH activation by dichloroacetate in human skeletal muscle during exercise in hypoxia. *American Journal of Physiology, 279* (4), E752-E761

Prieur, F., Busso, T., Castells, J., Bonnefoy, R., Benoit, H., Geyssant, A. & Denis, C. (1998). Validity of oxygen uptake measurements during exercise under moderate hyperoxia. *Medicine and Science in Sports and Exercise*, *30 (6)*, 958-62.

Prieur, F., Benoit, H., Busso, T., Castells, J., Geyssant, A. & Denis, C. (2002). Effects of moderate hyperoxia on oxygen consumption during submaximal and maximal exercise. *European Journal of Applied Physiology, 88* (3), 235-242

Richardson, R. S., Grassi, B., Gavin, T. P., Haseler, L. J., Tagore, K., Roca, J. & Wagner, P. D. (1999a). Evidence of O2 supply-dependent VO2 max in the exercise-trained human quadriceps. *Journal of Applied Physiology, 86* (3), 1048-1053

Richardson, R. S., Leigh, J. S., Wagner, P. D. & Noyszewski, E. A. (1999b). Cellular PO2 as a determinant of maximal mitochondrial O(2) consumption in trained human skeletal muscle. *Journal of Applied Physiology, 87* (1), 325-331

Roberts, P. A., Loxham, S. J., Poucher, S. M., Constantin-Teodosiu, D. & Greenhaff, P. L. (2002). The acetyl group deficit at the onset of contraction in ischaemic canine skeletal muscle. *Journal of Applied Physiology, 544* (Pt 2), 591-602

Sahlin, K., Katz, A. & Broberg, S. (1990) Tricarboxylic acid cycle intermediates in human muscle during prolonged exercise. *American Journal of Physiology, 259* (5 Pt 1), C834-C841

Savasi, I., Evans, M. K., Heigenhauser, G. J. F. & Spriet, L. L. (2002). Skeletal muscle metabolism is unaffected by DCA infusion and hyperoxia after onset of intense aerobic exercise. *American Journal of Physiology, 283* (1), E108-E115

Stellingwerff, T., Glazier, L., Watt, M. J., LeBlanc, P. J., Heigenhauser, G. J. & Spriet, L. L. (2005). Effects of hyperoxia on skeletal muscle carbohydrate metabolism during transient and steady-state exercise. *Journal of Applied Physiology, 98* (1), 250-256

Stellingwerff, T., Leblanc, P. J., Hollidge, M. G., Heigenhauser, G. J. & Spriet, L. L. (2006). Hyperoxia decreases muscle glycogenolysis, lactate production, and lactate efflux during steady-state exercise. *American Journal of Physiology, 290* (6), E1180-E1190

Timmons, J. A., Poucher, S. M., Constantin-Teodosiu, D., Worrall, V., Macdonald, I. A. & Greenhaff, P. L. (1996). Increased acetyl group availability enhances contractile function of canine skeletal muscle during ischemia. *Journal of Clinical Investigation, 97* (3), 879-883

Timmons, J. A., Poucher, S. M., Constantin-Teodosiu, D., Macdonald, I. A. & Greenhaff, P. L. (1997). Metabolic responses from rest to steady state determine contractile function in ischemic skeletal muscle. *American Journal of Physiology, 273* (2 Pt 1), E233-E238

Timmons, J. A., Gustafsson, T., Sundberg, C. J., Jansson, E., Hultman, E., Kaijser, L., Chwalbinska-Moneta, J., Constantin-Teodosiu, D., Macdonald, I. A. & Greenhaff, P. L. (1998a). Substrate availability limits human skeletal muscle oxidative ATP regeneration at the onset of ischemic exercise. *Journal of Clinical Investigation, 101* (1), 79-85

Timmons, J. A., Gustafsson, T., Sundberg, C. J., Jansson, E. & Greenhaff, P. L. (1998b). Muscle acetyl group availability is a major determinant of oxygen deficit in humans during submaximal exercise. *American Journal of Physiology, 274* (2 Pt 1), E377-E380

Timmons, J. A., Constantin-Teodosiu, D., Poucher, S. M. & Greenhaff, P. L. (2004). Acetyl group availability influences phosphocreatine degradation even during intense muscle contraction. *Journal of Physiology, 561* (Pt 3), 851-859

Tschakovsky, M. E. & Hughson, R. L. (1999) Interaction of factors determining oxygen uptake at the onset of exercise. *Journal of Applied Physiology, 86* (4), 1101-1113

Varnier, M., Leese, G. P., Thompson, J. & Rennie, M. J. (1995) Stimulatory effect of glutamine on glycogen accumulation in human skeletal muscle. *American Journal of Physiology, 269* (2 Pt 1), E309-E315

Wagenmakers, A. J. (1998) Muscle amino acid metabolism at rest and during exercise: role in human physiology and metabolism. *Exercise and Sport Science Reviews, 26,* 287-314

Wilkerson, D. P., Berger, N. J. & Jones, A. M. (2006). Influence of hyperoxia on pulmonary O2 uptake kinetics following the onset of exercise in humans. *Respiratory Physiology and Neurobiology, 153* (1), 92-106
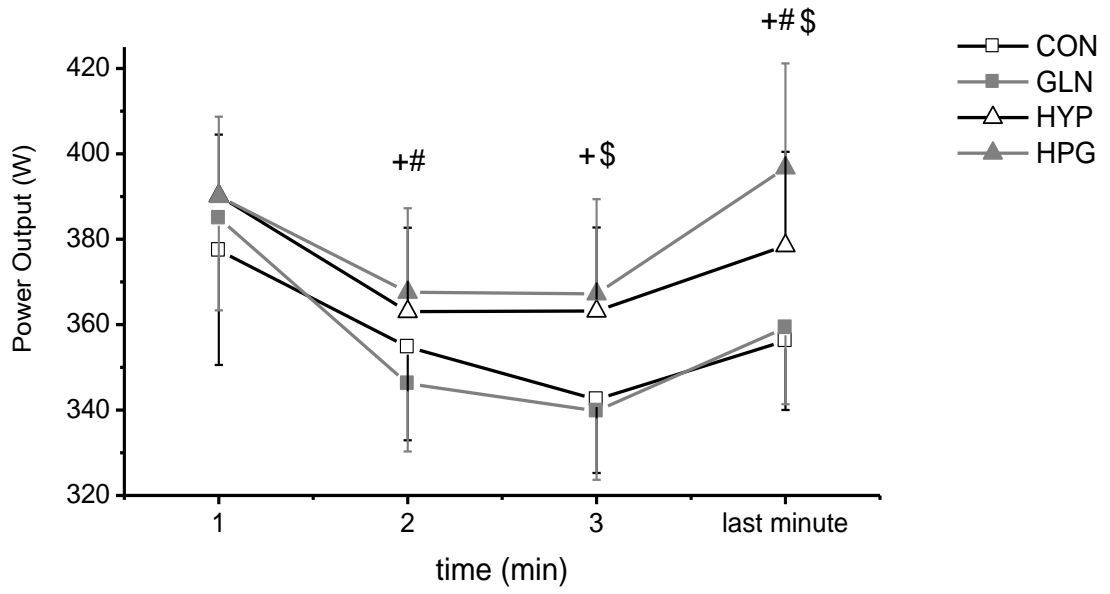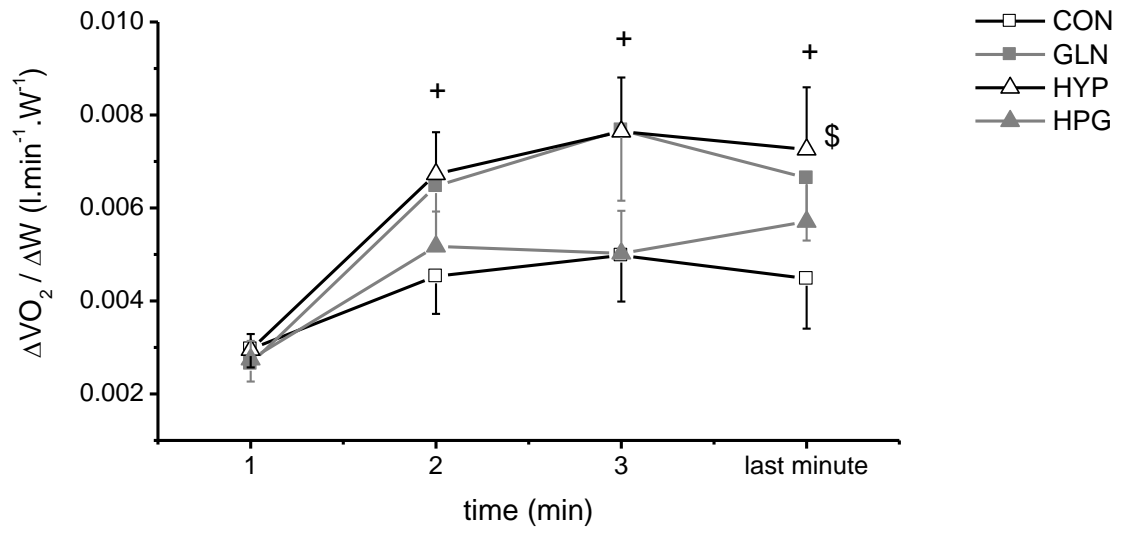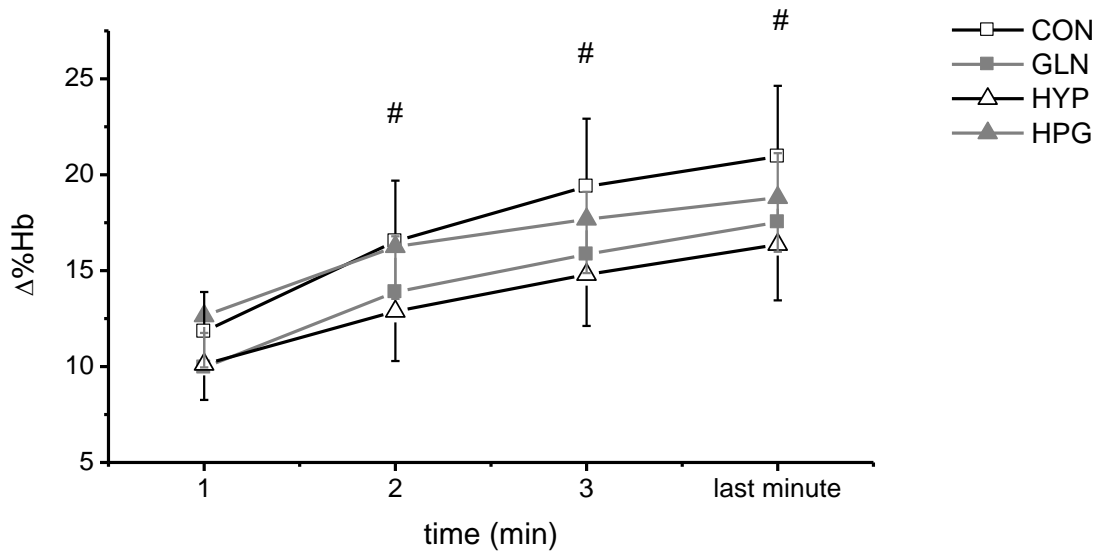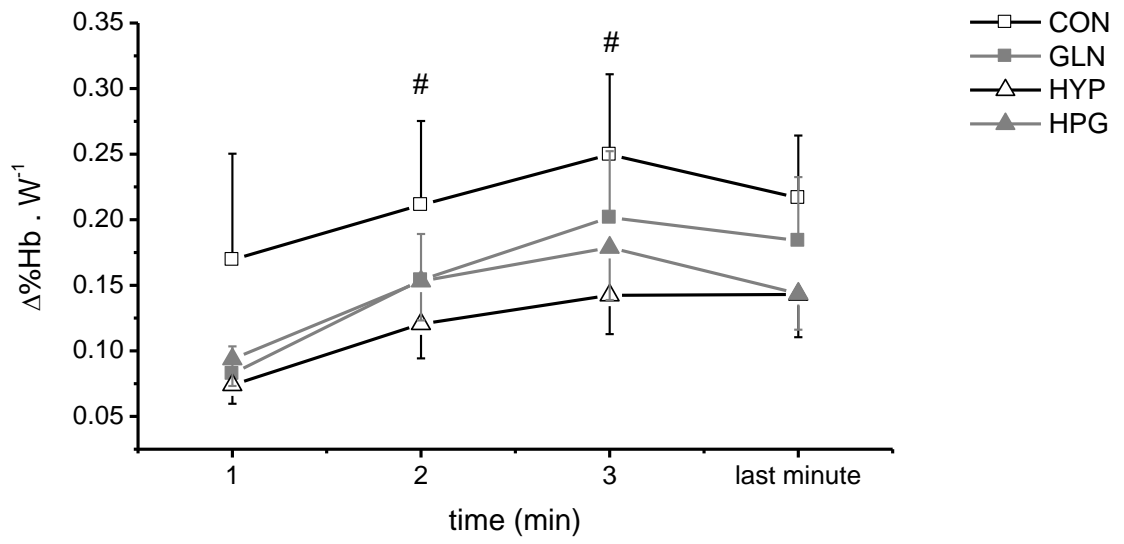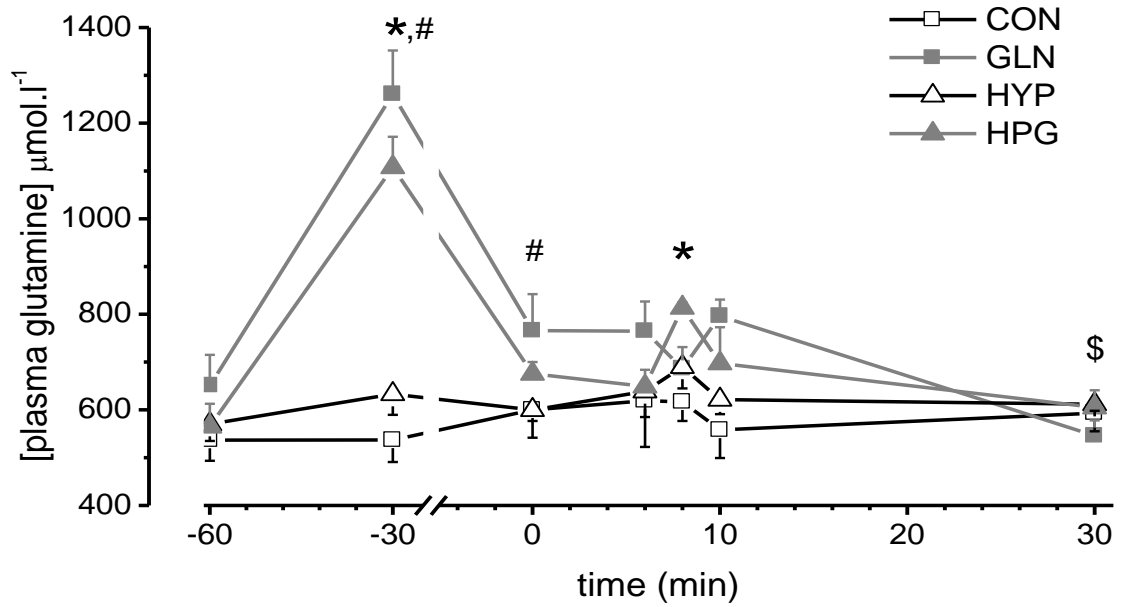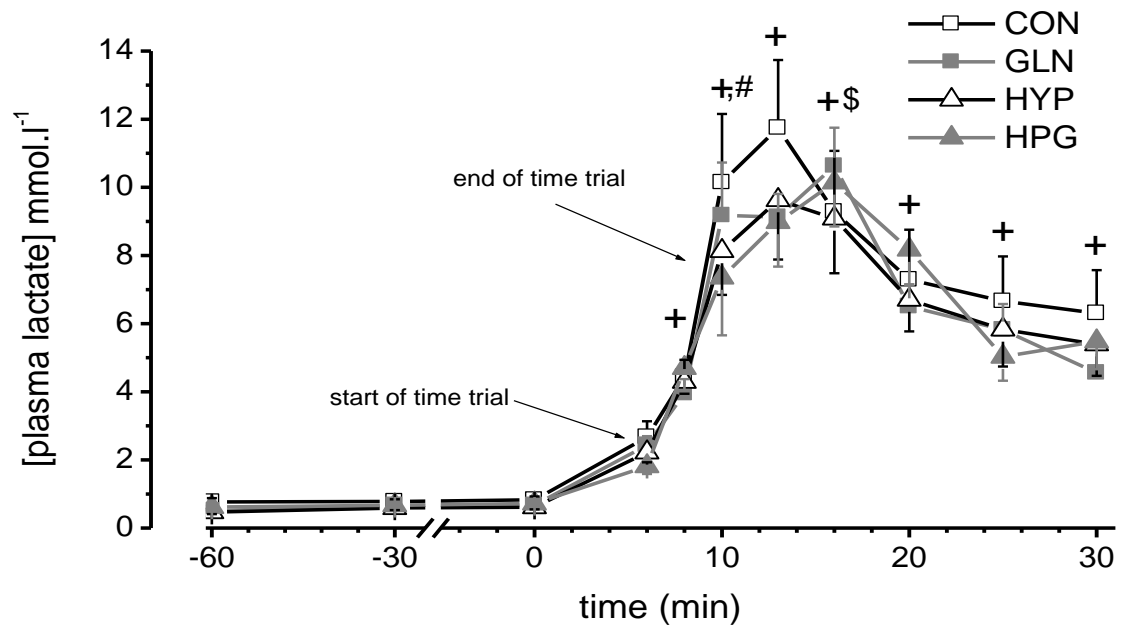
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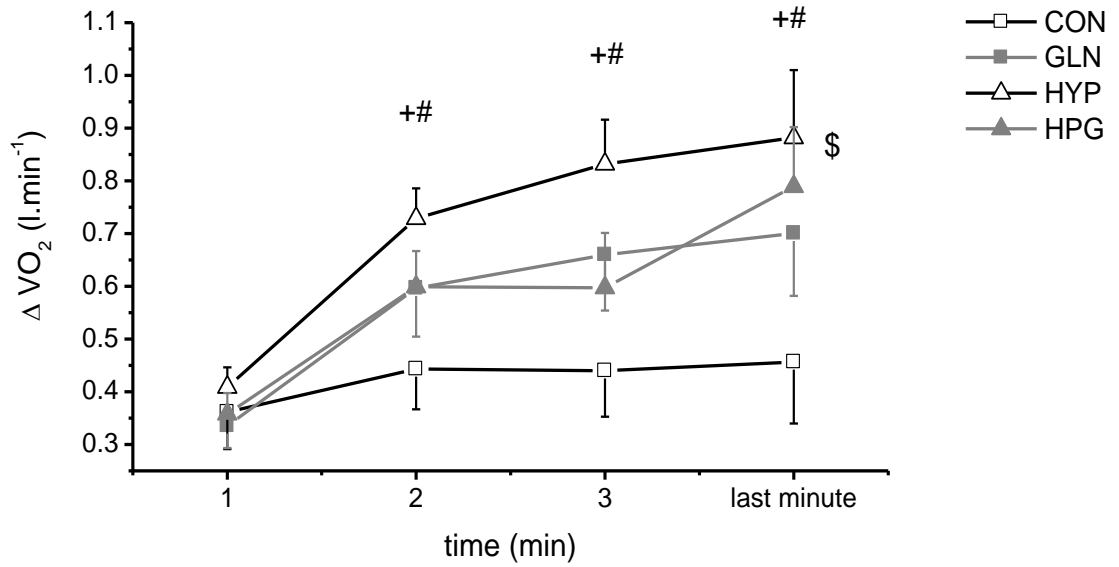
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