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Similar performance after intake of carbohydrate plus whey protein and carbohydrate only in the early phase after non-exhaustive cycling

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Running title: Protein intake after endurance exercise

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Abstract

The aim of the present study was to compare performance five hours after a 90-min endurance training session when either carbohydrate only or carbohydrate with added whey hydrolysate or whey isolate was ingested during the first two hours of the recovery period.

Methods: Thirteen highly trained competitive male cyclists completed three exercise and diet interventions (double-blinded, randomized, crossover design) separated by one week. The 90-min morning session (EX1) included a 60 min time-trial (TT_{60}). Immediately and one hour after exercise, participants ingested either 1) 1.2 g carbohydrate·kg^{-1}·h^{-1} (CHO), 2) 0.8 g carbohydrate·kg^{-1}·h^{-1} + 0.4 g isolate whey protein·kg^{-1}·h^{-1} (ISO) or 3) 0.8 g carbohydrate·kg^{-1}·h^{-1} + 0.4 g hydrolysate whey protein·kg^{-1}·h^{-1} (HYD). Additional intakes were identical between interventions. After five hours of recovery, participants completed a time-trial performance (TT_P) during which a specific amount of work was performed. Blood and urine were collected throughout the day.

Results: TT_P did not differ significantly between dietary interventions (CHO: 43:54±1:36, ISO: 46:55±2:32, HYD: 44:31±2:01 min). Nitrogen balance during CHO was lower than ISO ($p<0.0001$) and HYD ($p<0.0001$), with no difference between ISO and HYD ($p=0.317$). In recovery, the area under the curve for blood glucose was higher in CHO compared to ISO and HYD. HR, VO_2, RER, glucose, and lactate during EX2 were similar between interventions.

Conclusion: Performance did not differ after five hours of recovery whether carbohydrate only or isocaloric carbohydrate plus protein was ingested during the first two hours. Correspondingly, participants were not in negative nitrogen balance in any dietary intervention.

Keywords: Endurance exercise, Recovery, Protein supplement, Nitrogen balance

Introduction

Oxidation of carbohydrate and fat are the principal energy substrates during prolonged exercise [1-5]. In fact, most investigations of substrate selection during endurance exercise do not include protein in the calculations [1,3]. The reason that protein is excluded in the calculations is that amino acids contribute only 1-6 % of the energy during exercise [6,7]. Still, oxidation of leucine and other branched chain amino acids increase during exercise [6,8], and more as muscle glycogen content declines [9,10]. Moreover, skeletal muscle metabolism of branched-chain amino acids (BCAA) during exercise is necessary for high endurance capacity, and deletion of mitochondrial BCAA aminotransaminase dramatically reduces endurance capacity [11].

It is well-documented that the protein requirement for endurance athletes is high, and the current data suggest a daily requirement in periods of increased training load or insufficient energy intake of 1.6-2.0 g protein per kilogram bodyweight to achieve nitrogen balance [12,13]. It has also been documented that 1.8-2.0 g protein per kilogram bodyweight is required on days with extensive intensive exercise to obtain neutral or positive nitrogen balance [4,5,14]. In accordance, many cyclists ingest protein supplements after prolonged endurance training sessions to stimulate the recovery processes. Several mechanisms for the beneficial effects of protein intake after exercise were proposed: a greater increase in $mTOR^{ser2448}$ phosphorylation and myofibrillar muscle protein synthesis, a greater mitochondrial muscle protein synthesis or better adaptations to exercise, on a whole-body scale [15,16].

Intake of protein and carbohydrate also has a beneficial effect on performance in recovery after recovery periods of 4-24 hours [4,5,17]. Although many studies have shown better subsequent performance with intake of carbohydrate plus protein compared to carbohydrate only early in recovery [4,5,14,17-20], other studies report no effect of protein intake [21-23]. The reason for this discrepancy needs to be clarified. Possibilities include the type and intensity of exercise, duration of the recovery period, training status of the participants, amount of protein, as well as the type of protein consumed.

The types of protein supplements ingested after training are from many sources and include milk, soya, oat, insects and potatoes [24-27]. The rate of uptake of proteins from different sources vary, and appearance of BCAA in blood for example is much more pronounced after intake of whey compared to casein [28]. In our laboratory studies, whey isolate was supplemented [4,5,17]; whereas, other investigators used whey hydrolysate and conducted their studies in the field

rather than the laboratory [29,30]. Hydrolyzation of various protein fractions is made to increase uptake rates: theoretically, the intake of whey hydrolysate as compared to whey isolate should increase the level of plasma amino acids faster. A fast appearance of amino acids in plasma following protein consumption is associated with a larger insulin response [28], which may stimulate the synthesis of glycogen as well as suppress the degradation of protein, and thereby improving recovery of performance.

Athletes aim to optimize recovery processes particularly in connection with competition, but also during intensive training camps. Protein intake after training sessions has long been used by elite athletes [30,31], but not much attention has been given to the type of protein. The increase in plasma amino acids is relatively fast after 0.4 $g \cdot kg^{-1} \cdot h^{-1}$ of whey isolate [4,5], and nitrogen balance was positive only after intake of protein in the referred studies [4,5,17]. We have not measured protein synthesis in our studies, but a positive nitrogen balance after intake of protein is inevitably coupled to amino acid accumulation.

High-level athletes often train two times per day and the present study was designed with a five hours recovery period between the two exercises, close to the ecological conditions observed in high-level athletes. Performance was, thereby, tested after a five hours recovery from a non-exhaustive, high-intensity training session. The main aim of the present study was to test the hypothesis that co-ingestion of carbohydrate plus whey hydrolysate in the first two hours of the five hours recovery period would lead to better performance than co-ingestion of carbohydrate plus whey isolate. We also hypothesized that performance in both of these interventions would be better than with the ingestion of carbohydrates only during the two first hours of recovery.

Materials and Methods

Participants

Thirteen highly trained competitive male endurance cyclists with experience in cycling at the national and international level (mean ± SEM; age: 27.7 ± 1.2 yr.; height: 184 ± 1 cm; body mass: 77.2 ± 1.1 kg; VO_{2max} 5.3 ± 0.1 $L \cdot min^{-1}$, 68.8 ± 1.4 $ml \cdot kg^{-1} \cdot min^{-1}$) were recruited for the study. Subjects trained on average 11.5 ± 1.2 h weekly, of which 8.8 ± 1.4 h consisted of cycling. The participants were informed about the study before providing their written informed consent. The Regional Ethic Committee (REK) of Norway approved the study (2013/1528/REF Sør-Øst A) and the experimental protocol conformed to the standards set by the latest revision of the Declaration of Helsinki.

Study design

Overview. The study was completed in a double-blinded, randomized, balanced, crossover experimental design (Fig. 1). Prior to the three experimental test days with dietary interventions, physiological capacity was tested, and procedures to familiarize the participants with the main tests performed. The experimental test days consisted of two exercise sessions separated by a five-hour recovery period. Both exercise sessions consisted of high intensity endurance exercise and a 10 s sprint test was conducted before and after both exercise sessions. The first hour of the recovery period consisted of the dietary intervention and participants were provided isocaloric liquid supplements of either 1) carbohydrate (CHO), 2), carbohydrate + isolate whey protein (ISO) or 3) carbohydrate + hydrolysate whey protein (HYD). The diet during the last three hours of the recovery period was similar for all dietary interventions. The experimental test days were separated by a minimum of six days.

Physiological tests before the dietary interventions.

On the first visit, the ergometer bike (Lode Excalibur Sport, Groningen, The Netherlands) was adjusted to the preferred specification of each participant. Participants used the same setup throughout the study. Participants performed a 10-min easy self-paced warm up, before an incremental test to determine the relationship between workload (W) and oxygen uptake ($\dot{V}O_2$) by cycling 5 min at increasing workloads. The first workload was individually set between 200-250 W, and the workload increased 25 W every five min. Between 2.5-4 min of each workload, $\dot{V}O_2$ was measured (Oxycon Pro, Jager Instr; Hoechberg, Germany) and capillary blood samples were collected and analyzed for blood lactate (1500 Sport, YSI Inc.,

Yellow Springs Instr; Ohio, USA). The test was terminated when blood lactate concentration reached 3 mM. The individual cadence during this test (90-105 RPM) was used subsequently for each participant in all familiarization and experimental trials. After 10 min of rest, subjects performed a test to determine maximal work capacity (W_{max}) and maximal oxygen uptake ($\dot{V}O_{2max}$). Initial workload was the same as the second to last step of the first incremental test and workload increased 25 W per minute until voluntary exhaustion. $\dot{V}O_2$ was measured in 30 s periods using a mixing chamber and the mean of the two highest consecutive measurements was used as $\dot{V}O_{2max}$ as described [5]. W_{max} was determined as: Workload of the last stage completed + [(25 W/60s) x seconds completed in final stage]. Linear regression analysis was used to determine the workload corresponding to 75 % of $\dot{V}O_{2max}$ ($W_{75\%}$). Heart rate (HR) was measured with a Polar RS 800-CX (Kempele, Finland) with 5 s averages throughout the study.

On the second visit to the laboratory, within a week after the first test day and approximately one week before the first experimental test day, a familiarization session was performed. This included all elements of the second exercise session (EX2) of the experimental trials (see *"Second exercise session (EX2)"*), but without blood and urine sampling. If necessary, workload was adjusted during the first 30 min to achieve the load corresponding to 75 % of VO_{2max} ($W_{75\%}$). The adjusted workload was used as $W_{75\%}$ throughout the study.

Experimental trials period

Subjects were instructed to do very light or no exercise the last 24 h before the experimental test days with dietary interventions. Subjects recorded their dietary intake and exercise training for 24 h prior to the start of the first experimental intervention and were requested to follow the same diet and exercise prior to the second and third experimental intervention.

First exercise session (EX1). The athletes reported to the laboratory at 7.30 AM in a fasted state for weigh-in and venous blood sampling. EX1 was initiated with a warm-up consisting of steps of 5, 4, 4 and 2 min (tot. 15 min) at workloads eliciting 50, 55, 60 and 75 % of $\dot{V}O_{2max}$, respectively. After a 4-min recovery, subjects performed a 10-s sprint followed by a 5-min recovery period. Throughout the study, participants chose either to rest or cycle at 100 W for the 4-min recovery before the sprints. All sprints during the study were all-out efforts performed using Lode Ergometry Manager 9.4.7.0 software with a torque factor of 1.0 $Nm \cdot kg^{-1}$. Following the first sprint, the athletes performed 30 min at $W_{75\%}$. Here, $\dot{V}O_2$ and

HR were measured from 8-10, 18-20 and 28-30 min. Lactate, glucose (HemoCue Glucose 201^{+}, Ängholm, Sweden) and rating of perceived exhaustion (RPE) were measured at 10, 20 and 30 min. Lactate and glucose were both sampled from capillary blood from the fingertip during cycling. After a 5 min rest, a 60-min all-out time trial (TT_{60}) was performed. During the TT_{60}, $\dot{V}O_2$ and HR were measured between 5-7, 13-15, 28-30, 43-45 and 58-60 min. RPE and lactate and glucose blood samples were measured at 7, 15, 30, 45 and 60 min. A 4-min rest after TT_{60} was given before a new 10-s sprint was performed.

Recovery and intervention supplements ingestion period (0-2 h). After EX1, a Teflon catheter (18GA, BD Venflon Pro, New Jersey, USA) was inserted into the antecubital vein for serial blood sampling. Venous blood samples were drawn at 0, 15, 30, 60, 90, and 120 min of the recovery period.

Supplement drinks were ingested immediately after the first blood sample, and after the 60 min blood sample. Isovolumetric (7.06 $ml \cdot kg^{-1} \cdot h^{-1}$) and isocaloric drinks were provided with either 1.2 g carbohydrate $\cdot kg^{-1} \cdot h^{-1}$ (CHO), 0.8 carbohydrate$\cdot kg^{-1} \cdot h^{-1}$ + 0.4 g isolate whey protein$\cdot kg^{-1} \cdot h^{-1}$ (ISO) or 0.8 carbohydrate$\cdot kg^{-1} \cdot h^{-1}$ + 0.4 g hydrolysate whey protein$\cdot kg^{-1} \cdot h^{-1}$ (HYD). The carbohydrate portion of the drinks consisted of equal parts of maltodextrin (Maltodextrin White Pure; AppliChem, Darmstadt, Germany) and glucose (Glucose, AnalaR; VWR Prolabo, Leuven, Belgium). Whey isolate was from Lac Prodan, SP-9225 Instant (Arla Foods, Aarhus, Denmark) and whey hydrolysate was from Lac Prodan, Hydro.365 (Arla Foods, Aarhus, Denmark). All drinks were flavored by 100 $g \cdot L^{-1}$ flavoring drink (Fun light, Stabburet, Norway). A questionnaire regarding identity of supplement drinks was administered to the subjects and the results indicated the subjects were unable to distinguish any differences among the drinks.

Recovery nutrition from 2-5 hours. After the intervention supplements (0-120 min after EX1) participants were provided a meal (pasta and minced meat in tomato sauce) containing 1.87 $g \cdot kg^{-1}$ carbohydrate, 0.65 $g \cdot kg^{-1}$ protein and 0.31 $g \cdot kg^{-1}$ fat, and a carbohydrate drink containing 1.2 $g \cdot kg^{-1}$ carbohydrate 240 min after EX1. Nutrient intake in this period was similar for all interventions. All meals and supplements were made by laboratory staff and consumed in the laboratory. Water was ingested *ad libitum* and additional food, or drinks were not allowed.

Second exercise session (EX2). EX2 was initiated with the same warm-up as EX1 followed by a 4 min recovery before completing a 10-s sprint. After a 5 min recovery, subjects performed 30 min of cycling at $W_{75\%}$ and VO_2, RER, HR, RPE, glucose and lactate were measured at the same times as in EX1. After a 5 min rest, subjects completed a TT (TT_P). The work to be completed during TT_P was equivalent to 30 min (1800 s) at a workload corresponding to 100 % of $\dot{V}O_{2max}$ and was to be completed as fast as possible: Work output (kJ) = (Power at VO_{2max} (W) * 1800 s).

The athletes started TT_P at 100 W, but immediately were able to adjust their workload on a panel mounted on the bike. Time and power were blinded, but accumulated work was shown on a screen in front of the athletes, and they were informed about their progress at 10 % increments in the TT_P.
The coefficients of variations of the performance during TT_P were 7% between the first intervention to the second intervention and 8% between the second intervention to the third intervention. There was no order effect across the experimental conditions ($p = 0.133$).

HR and RPE were measured every 10 % of finished TT_P, while $\dot{V}O_2$, lactate and glucose were measured at 20, 50, 80 and 100% of finished TT_P. A blood sample from a *v antecubital* was collected at 50 and 100% of TT_P and 10 min after the last sprint of EX2. A 4 min rest after TT_P was given before a new 10-second sprint was performed.

Blood samples and analytical procedures
Venous blood samples were collected from a Venflon catheter previously inserted into a *v antecubital* except for the fasting morning sample, which was drawn using a retractable safety winged steel needle (BD Vacutainer, New Jersey, USA). Venous blood samples were then transferred into 6.5 ml tubes containing K_2EDTA, placed on ice and centrifuged for 10 min at 4 °C (2500 g). Plasma was then pipetted to Eppendorf tubes and stored at -80 °C until further analysis.

Insulin. Plasma insulin concentrations were measured with an enzyme-linked immunosorbent assay human insulin kit, K6219 (Dako, Glostrup, Denmark) as previously described [5].

Urine. Urine was collected in plastic containers from arrival to the end of EX2. Subjects were instructed to void their bladder before the start of EX1. Volume for the period was calculated from weight and density and a 12 ml sample was frozen at -20 °C for later analysis.

Nitrogen balance calculation. Nitrogen balance was calculated based on protein intake and urinary nitrogen excretion, and assuming non-urinary nitrogen losses. Nitrogen concentration in the urine was determined with the Kjeldahl method [32]. Nitrogen intake was calculated assuming the nitrogen to amino acid constant of 6.25. Total nitrogen excretion was calculated assuming 76.8 % and 77.1 % of total nitrogen loss excretion in urine when fed with normal diet or high protein diet, respectively [14].

Calculation of carbohydrate and fat oxidation

Carbohydrate and fat oxidation were calculated from the respiratory exchange ratio (RER) and oxygen uptake together with the *Table of Nonprotein Respiratory Quotient* as described by Peronnet and Massicotte [33] assuming non-protein metabolism during steady state exercise.

Statistics

All values are expressed as mean ± SEM. Two-way repeated measures analysis of variance (ANOVA) was used to determine treatment differences, with treatment and time used as factors. When differences were detected, Tukey's honestly significant difference post hoc test was performed. To investigate the differences in substrate oxidation between EX1 and EX2, a student t-test with paired measurements was used. To determine if the nitrogen balance was significantly positive/negative, we used a repeated measurement one-way ANOVA against a group of zeros. Differences were considered significant when $p < 0.05$. Statistical analyses were performed using SigmaPlot 12.5 software.

Results

During the morning session (EX1), the participants started with 30 min of cycling at $W_{75\%}$, which corresponded to 278 ± 9 W. The mean power during TT_{60} was 284 ± 14 W (1024 ± 49 kJ), 283 ± 14 W (1018 ± 50 kJ) and 282 ± 12 W (1016 ± 45 kJ) before CHO, ISO and HYD, respectively (Fig. 2A & C) with no difference among interventions (p = 0.722). For this exercise session, no differences were found among interventions for $\dot{V}O_2$, RER, HR, lactate, glucose or RPE (Fig. 3A-E).

During the TT_{60}, power, $\dot{V}O_2$, HR and RPE increased gradually (Fig. 2C and Fig. 3A-F). RPE reached ~18 of the Borg scale at the end of TT_{60} [34]. RER remained stable during the first 75% of the TT_{60} and increased at the end (Fig. 3B). Blood lactate increased gradually to ~ 4 mM (Fig. 3D). Blood glucose decreased to ~ 4 mM during the first 15 min, but increased gradually to ~ 4.5 mM at the end of EX1 (Fig. 3E).

During the initial 2 h recovery period blood glucose and insulin increased rapidly after all three dietary interventions (Fig. 4A-B). Plasma glucose was higher in CHO compared with both ISO and HYD (p for time points; 10 min: p = 0.023; 15, 30 and 60 min: p < 0.01). Plasma insulin differed between the interventions (p = 0.015). Insulin decreased from ~ 100 pM before exercise to ~ 35 pM after EX1 (Fig. 4B). Resting VO_2 was ~ 400 ml·min^{-1} 4.5 hours after EX1 and there were no differences among dietary interventions (p = 0.324, Fig. 4D).

Glucose, insulin and lactate measured immediately before EX 2 were similar after all three dietary interventions (Fig. 3D-E & Fig. 4B). Interestingly, insulin dropped rapidly to very low levels after 15 min of cycling at $W_{75\%}$ during EX2 for all dietary interventions (Figure 4C).

The metabolic responses to the 30 min cycling at $W_{75\%}$ during EX2 were not different among dietary interventions (Fig. 3A-E). However, RER was higher in all groups during EX2 compared to EX1 ($p \leq 0.001$, Fig. 3B), while glucose ($p < 0.001$) and lactate ($p < 0.001$) were lower in all groups during $W_{75\%}$ at EX2 compared to EX1 (Fig. 3D-E). There was no difference in substrate oxidation among interventions (p = 0.573). However, oxidation of carbohydrate was higher during $W_{75\%}$ at EX2 compared to $W_{75\%}$ at EX1 (EX1, 3.61 ± 0.06 g·min^{-1}; EX2, 3.91 ± 0.08 g·min^{-1}; $p < 0.001$), and also during EX2 as a whole compared to

EX1 (EX1, 3.64 ± 0.08 g·min^{-1}; EX2, 3.87 ± 0.09 g·min^{-1}; p = 0.014). Correspondingly, fat oxidation was lower during $W_{75\%}$ at EX2 and EX2 as a whole compared to EX1 (p < 0.001). $\dot{V}O_2$ and HR were similar among EX1 and EX2 during $W_{75\%}$, but RPE was slightly higher at EX2 compared to EX1 during $W_{75\%}$ (p < 0.001) (Fig. 3F).

Performance at TT_P did not differ among dietary interventions (CHO 43:54 ± 1:36, ISO 46:55 ± 2:32, HYD 44:31 ± 2:01 min, p = 0.082), and therefore, power was also similar among interventions (CHO 270 ± 12 W, ISO 258 ± 17 W, HYD 269 ± 15 W, p = 0.205) (Fig. 2A & 2D). Mean power at TT_P (EX2) was lower than at TT_{60} (EX1) despite TT_{60} lasting 13-16 min longer (p = 0.004). Lactate did not differ between dietary treatments, but the lactate concentration was lower during TT_P compared to TT_{60} (p < 0.001). Plasma glucose increased ~1 mM (from ~4 to ~5 mM) during the first 15 min after TT_P (Fig. 3E).

Mean power at the 10-s sprints were 997 - 1076 W and did not differ among interventions (p = 0.357, Fig. 2B), but there was a time effect from the first to last sprint of the day (p = 0.002).

Nitrogen intake during the protocol was 8.0 ± 0.2 g during CHO, and 18.0 ± 0.5 g during ISO and HYD. Total nitrogen excretion was not significantly different among interventions (CHO 8.2 ± 0.5 g, ISO 9.6 ± 0.7 g, HYD 8.8 ± 0.4 g, p = 0.244). Nitrogen balance during CHO was lower than ISO (p < 0.0001) and HYD (p < 0.0001), with no difference between ISO and HYD (p = 0.317) (Fig. 5). Nitrogen balance for CHO was not different from zero (-0.2 ± 0.7 g; -4.1 ± 8.9 mg·kg^{-1}; p = 0.782), but was positive for ISO (8.5 ± 0.6 g; 109.5 ± 7.6 mg·kg^{-1}; p < 0.0001) and HYD (9.3 ± 0.7 g; 118.6 ± 6.4 mg·kg^{-1}; p < 0.0001).

Discussion

The main purpose of the present study was to test the hypothesis that co-ingestion of whey hydrolysate plus carbohydrate during the first hour of a five hours recovery resulted in better recovery of performance relative to ingestion of carbohydrate or ingestion of whey isolate plus carbohydrate. Both TTp and sprint performances were similar whether whey hydrolysate or whey isolate was added to the carbohydrate drink or whether carbohydrate only was ingested. Therefore, the hypothesis was not supported.

The effect on the performance of protein intake together with carbohydrate after endurance exercise, compared to carbohydrate intake only, has yet to be concluded: some studies have reported a better performance after the recovery period [35], some do not [36,37]. In the present study, there was no difference in the performance tests completed after five hours of recovery from 90 min of high-intensity endurance exercise whether supplemented with whey isolate or whey hydrolysate co-ingested with carbohydrate, or when provided an isocaloric amount of carbohydrate only. There are several possible reasons why various contradicting effects of protein intake after exercise on subsequent exercise performance tests have been reported. In the present study, we have identified the following potential reasons: 1) amount of protein or type of protein intake, 2) amount and intensity of the exercise conducted before the dietary intervention 3) length of recovery period, and 4) type of performance tests. We will discuss these reasons in comparison with the data already published.

Importantly, we have previously found that the recovery of performance was improved after co-ingestion of whey isolate and carbohydrate compared to carbohydrate only during the early recovery period after exhaustive exercise in three studies [4,5,17]. Therefore, it was surprising that performance was similar after co-ingestion of whey and carbohydrate compared to carbohydrate only. Similarly, to our previous study, nutritional intake was 0.4 $g \cdot kg^{-1} \cdot h^{-1}$ of protein and 0.8 $g \cdot kg^{-1} \cdot h^{-1}$ of carbohydrate, or 1.2 $g \cdot kg^{-1} \cdot h^{-1}$ of carbohydrate. A minor change was that drinks were provided immediately and after 1 hour after exercise in the present study, whereas half the amount of energy was provided every 30 min in our previous studies [4,5,17]. However, we have no reason to believe that the larger supplements were the reason that co-ingestion of protein and carbohydrate did not stimulate recovery better, because other studies have seen effect of protein intake with similar quanta per drink [18,19].

In our previous studies, exhaustive exercise was conducted prior to the dietary intervention studies [4,5,17]. Saunders et al. also used time to exhaustion prior to the dietary intervention and found a positive effect of protein intake on performance [19]. However, several studies have also observed positive effects of protein intake after time-trials prior to the dietary interventions [14,18,20,38].

The present study was not designed to compare the effect of protein intake after exhaustive and non-exhaustive exercise. Instead, the exercise protocol was designed to simulate a day of training composed of two hard training sessions. Interestingly, during EX1 the athletes reported RPE as high in the present study as in our previous studies when participants cycled until exhaustion [4,5]. They also completed a similar amount of work as previous studies [4,5], but the athletes did not develop hypoglycemia during EX1 or reach voluntary exhaustion as before. It is likely that the reason for not reaching exhaustion during EX1 in the present study was that the athletes choose their workloads during TT_{60} unlike in our previous studies [4,5]. It will be important in future studies to test whether protein benefits performance more after exhaustive exercise compared to non-exhaustive exercise despite completing similar amounts of work.

The recovery period between the hard exercise session in the morning and the performance test was five hours, which reflects approximately the recovery time between two-per-day training sessions for athletes. Co-ingestion of protein and carbohydrate has resulted in better performance than carbohydrate only when the recovery period is 18 hours or longer [4,5,14,19], but better also after 4-6 hours of recovery [17,20,38]. It seems difficult to make any conclusions relative to recovery time, but glycogen stores are not fully replenished in this time window [17,39]. However, the athletes did not recover completely during the five hours of recovery as mean power during the performance test (TT_P) was lower than during the morning time trial (TT_{60}) even though the TT_{60} lasted 13-16 min longer than TT_P. Furthermore, the pacing strategy differed. At TT_{60}, mean power increased gradually throughout the test, whereas mean power gradually declined during the first 80-90% of TT_P before mean power increased during the last 10%.

Interestingly, Rauch et al. showed that glycogen depletion was similar after time trials with high and low glycogen content prior to exercise, suggesting that athletes economize their energy utilization according to energy status [40]. The metabolic responses also differed

between the morning (EX1) and afternoon session (EX2) suggesting that recovery was incomplete for all dietary interventions. Supporting this belief, was the observation that during the 30 min cycling at 75% of $\dot{V}O_{2max}$ ($W_{75\%}$) for EX1 and EX2 the metabolic responses did not differ among dietary interventions, but blood glucose and lactate were lower during EX2. Therefore, it is possible that the dietary interventions had little effect on recovery and might explain why there was no difference found among the various dietary interventions.

The performance test used could also have played a role in reducing the effectiveness of our nutritional interventions. However, we have previously demonstrated a positive effect of protein plus carbohydrate intake on performance compared to carbohydrate only when using the current time-trial protocol [5]. Time trials have been reported to have better reliability compared with time to exhaustion often showing larger differences in performance [41], and since we have previously detected a positive effect of protein on time-trial performance, during a similar time-trial, we cannot use this test protocol as an explanation for our failure to see an improvement in performance after protein plus carbohydrate supplementation.

In the present study, sprint performance after the recovery period was similar across treatments. The 10-s Wingate protocol was identical to our previous study [5] where we observed that performance was better 18 hours after exhaustive exercise when protein plus carbohydrate was co-ingested, suggesting a sensitive protocol. Although mean power at the 10-s modified Wingate test did not differ significantly after the three interventions, the mean power for these tests tended to be lower at EX2 compared to EX1. Since the capacity to develop high power depends on maximal strength and neural activation [42,43], our data suggest that these properties were not completely recovered after five hours of recovery under any dietary intervention. Therefore, neither time-trial performance (TT_P) nor sprint performance were completely recovered after five hours, indicating that EX1 was a demanding training session similarly to exhaustive exercise [5]. However, the performance was equally reduced whether the recovery drink contained protein or just carbohydrate.

Uptake of amino acids from protein hydrolysate seems faster than from intact protein [44], and it was our hypothesis that whey hydrolysate would stimulate recovery better than whey isolate. Interestingly, the insulin response (AUC) was larger during ISO compared to HYD and CHO, despite that the glucose response was higher during CHO. Amino acids are known to stimulate insulin secretion, but the reason for the different insulin response between HYD and

ISO is not obvious. Importantly, Reitelseder et al. showed that the insulin response was higher after intake of whey compared to casein, and the increased insulin response was associated with a higher concentration of BCAA [28]. An elevated insulin response will normally promote synthesis of both glycogen and protein [45,46]. However, the difference in insulin response was rather small and performance was not affected. Importantly, the amount of protein in the recovery drinks (0.8 g protein per kilogram bodyweight) was higher than required to stimulation a maximal rate of protein synthesis [47,48], and other metabolic effects cannot be excluded.

We found that for all dietary interventions, participants were not in a negative nitrogen balance prior to post recovery exercise : participants were in a neutral nitrogen balance in CHO, and in a positive and similar nitrogen balance in HYD and ISO. It has previously been argued that differences in nitrogen balance after co-ingestion of protein compared with carbohydrate contributed to the better recovery of performance [4,5,14,17]. However, in our previous studies, we observed a negative nitrogen balance after intake of carbohydrate only immediately after exercise rather than a neutral nitrogen balance [4,5,17]. In the present study, participants consumed a dinner containing 0.65 g protein per kilogram bodyweight two hours after EX1, which appeared to be sufficient to maintain a neutral nitrogen balance. We therefore speculate that protein intake exceeding the amount required to maintain a neutral nitrogen balance has no positive effect on performance. However, it is possible that recovery is adversely affected if supplementation does not prevent a negative nitrogen balance from occurring.

Strengths and limitations.

It is a strength of the present study that 13 well-trained cyclists, who have great experience with demanding training routines volunteered as participants. Furthermore, the study was double-blinded, and participants standardized their exercise and diet the day before the tests. It is also a strength that the participants completed a familiarization test before the three interventions. It could be argued that it is a weakness that plasma amino acids or muscle glycogen were not measured. However, the present study was performed to test the effect of co-ingestion of protein and carbohydrate after a typical training session with a typical recovery period between sessions on performance, with performance as our primary outcome. Therefore, we preferred not to take muscle biopsies under these conditions as recruitment of elite athletes as participants becomes much more difficult with muscle biopsies included, and

taking muscle biopsies could have possibly altered our primary outcome, performance. Another limitation is that our exercise was cycling, which is non-weight bearing, and therefore the results cannot be transferred directly to running and other types of exercise where muscle damage may be more pronounced. Conversely, a strength of the study is that the design ensured sufficient protein during the CHO treatment to elicit a neutral nitrogen balance, which gives the study high ecological validity. Further research is required to determine if the benefit of protein supplementation post exercise improves recovery by preventing the occurrence of a negative nitrogen balance.

In conclusion, performance after a five hours recovery was similar whether whey hydrolysate or isolate was co-ingested with carbohydrate or carbohydrate only was ingested after a hard endurance training session, consisting of 30 min cycling at 75% $\dot{V}O_{2max}$ and a 60 min time-trial. In disagreement to previous findings from our laboratory, whey protein consumption did not benefit recovery and the reason for this discrepancy needs additional investigation. Future studies should determine if exhaustive exercise causes muscle damage, which requires high protein intake immediately after exercise to optimize recovery, or if protein supplementation improves recovery by preventing the occurrence of a negative nitrogen balance.

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Conflict of interest

The authors declare that they have no conflicts of interest regarding the publication of this paper. There are no financial conflicts of interest to disclose. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

References

1. Romijn JA, Coyle EF, Sidossis LS, et al. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am J Physiol.* 1993;265(3 Pt 1):E380-E391.
2. Coyle EF, Coggan AR, Hemmert MK, Ivy JL. Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J Appl Physiol.* 1986;61:165-172.
3. van Loon LJ, Greenhaff PL, Constantin-Teodosiu D, Saris WH, Wagenmakers AJ. The effects of increasing exercise intensity on muscle fuel utilisation in humans. *J Physiol.* 2001;536(Pt 1):295-304.
4. Rustad PI, Sailer M, Cumming KT, et al. Intake of Protein Plus Carbohydrate during the First Two Hours after Exhaustive Cycling Improves Performance the following Day. *PLoS One.* 2016;11(4):e0153229.
5. Sollie O, Jeppesen PB, Tangen DS, et al. Protein intake in the early recovery period after exhaustive exercise improves performance the following day. *J Appl Physiol (1985).* 2018;125:1731-1742.
6. Phillips SM, Atkinson SA, Tarnopolsky MA, MacDougall JD. Gender differences in leucine kinetics and nitrogen balance in endurance athletes. *J Appl Physiol (1985).* 1993;75(5):2134-2141.
7. Rowlands DS, Hopkins WG. Effect of high-fat, high-carbohydrate, and high-protein meals on metabolism and performance during endurance cycling. *Int J Sport Nutr Exerc Metab.* 2002;12(3):318-335.
8. Wolfe RR, Goodenough RD, Wolfe MH, Royle GT, Nadel ER. Isotopic analysis of leucine and urea metabolism in exercising humans. *J Appl Physiol Respir Environ Exerc Physiol.* 1982;52(2):458-466.
9. Lemon PWR, Mullin JP. Effect of initial muscle glycogen levels on protein catabolism during exercise. *Exerc Physiol.* 1980;48:624-629.
10. Howarth KR, Phillips SM, MacDonald MJ, Richards D, Moreau NA, Gibala MJ. Effect of glycogen availability on human skeletal muscle protein turnover during exercise and recovery. *J Appl Physiol.* 2010;109(2):431-438.
11. She P, Zhou Y, Zhang Z, Griffin K, Gowda K, Lynch CJ. Disruption of BCAA metabolism in mice impairs exercise metabolism and endurance. *J Appl Physiol (1985).* 2010;108(4):941-949.
12. Tarnopolsky MA, MacDougall JD, Atkinson SA. Influence of protein intake and training status on nitrogen balance and lean body mass. *J Appl Physiol (1985).* 1988;64(1):187-193.
13. Kato H, Suzuki K, Bannai M, Moore DR. Protein Requirements Are Elevated in Endurance Athletes after Exercise as Determined by the Indicator Amino Acid Oxidation Method. *PLoS One.* 2016;11(6):e0157406.
14. Rowlands DS, Rossler K, Thorp RM, et al. Effect of dietary protein content during recovery from high-intensity cycling on subsequent performance and markers of stress, inflammation, and muscle damage in well-trained men. *Appl Physiol Nutr Metab.* 2008;33(1):39-51.
15. Ferguson-Stegall L, McCleave E, Ding Z, et al. Aerobic exercise training adaptations are increased by postexercise carbohydrate-protein supplementation. *J Nutr Metab.* 2011;2011:623182.
16. Howarth KR, Moreau NA, Phillips SM, Gibala MJ. Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans. *J Appl Physiol.* 2009;106(4):1394-1402.

17. Dahl MA, Areta JL, Jeppesen PB, et al. Coingestion of protein and carbohydrate in the early recovery phase, compared with carbohydrate only, improves endurance performance despite similar glycogen degradation and AMPK phosphorylation. *J Appl Physiol (1985).* 2020;129(2):297-310.
18. Williams M, Raven PB, Fogt DL, Ivy JL. Effects of recovery beverages on glycogen restoration and endurance exercise performance. *J Strength Cond Res.* 2003;17(1):12-19.
19. Saunders MJ, Kane MD, Todd MK. Effects of a carbohydrate-protein beverage on cycling endurance and muscle damage. *Med Sci Sports Exerc.* 2004;36(7):1233-1238.
20. Berardi JM, Noreen EE, Lemon PW. Recovery from a cycling time trial is enhanced with carbohydrate-protein supplementation vs. isoenergetic carbohydrate supplementation. *J Int Soc Sports Nutr.* 2008;5:24.
21. Berardi JM, Price TB, Noreen EE, Lemon PW. Postexercise muscle glycogen recovery enhanced with a carbohydrate-protein supplement. *Med Sci Sports Exerc.* 2006;38(6):1106-1113.
22. Nelson AR, Phillips SM, Stellingwerff T, et al. A protein-leucine supplement increases branched-chain amino acid and nitrogen turnover but not performance. *Med Sci Sports Exerc.* 2012;44(1):57-68.
23. Goh Q, Boop CA, Luden ND, Smith AG, Womack CJ, Saunders MJ. Recovery from cycling exercise: effects of carbohydrate and protein beverages. *Nutrients.* 2012;4(7):568-584.
24. Ghosh AK, Rahaman AA, Singh R. Combination of sago and soy-protein supplementation during endurance cycling exercise and subsequent high-intensity endurance capacity. *Int J Sport Nutr Exerc Metab.* 2010;20(3):216-223.
25. Oikawa SY, Bahniwal R, Holloway TM, et al. Potato Protein Isolate Stimulates Muscle Protein Synthesis at Rest and with Resistance Exercise in Young Women. *Nutrients.* 2020;12(5).
26. Pettersson S, Edin F, Hjelte C, et al. Six Weeks of Aerobic Exercise in Untrained Men With Overweight/Obesity Improved Training Adaptations, Performance and Body Composition Independent of Oat/Potato or Milk Based Protein-Carbohydrate Drink Supplementation. *Front Nutr.* 2021;8:617344.
27. Vangsoe MT, Joergensen MS, Heckmann LL, Hansen M. Effects of Insect Protein Supplementation during Resistance Training on Changes in Muscle Mass and Strength in Young Men. *Nutrients.* 2018;10(3).
28. Reitelseder S, Agergaard J, Doessing S, et al. Whey and casein labeled with L-[1-13C]leucine and muscle protein synthesis: effect of resistance exercise and protein ingestion. *Am J Physiol Endocrinol Metab.* 2011;300(1):E231-E242.
29. Hansen M, Bangsbo J, Jensen J, Bibby BM, Madsen K. Effect of whey protein hydrolysate on performance and recovery of top-class orienteering runners. *Int J Sport Nutr Exerc Metab.* 2015;25(2):97-109.
30. Hansen M, Bangsbo J, Jensen J, et al. Protein intake during training sessions has no effect on performance and recovery during a strenuous training camp for elite cyclists. *J Int Soc Sports Nutr.* 2016;13:9.
31. Sanchez-Munoz C, Zabala M, Muros JJ. Nutritional intake and anthropometric changes of professional road cyclists during a 4-day competition. *Scand J Med Sci Sports.* 2016;26(7):802-808.
32. Kjeldahl J. Neue Methode zur Bestimmung des Stickstoffs in organischen Körper. *Analytische Chemie.* 1883;22(1):366-382.
33. Peronnet F, Massicotte D. Table of nonprotein respiratory quotient: an update. *Can J Sport Sci.* 1991;16(1):23-29.

34. Borg GA. Perceived exertion. *Exerc Sport Sci Rev.* 1974;2:131-153.
35. Alghannam AF, Gonzalez JT, Betts JA. Restoration of Muscle Glycogen and Functional Capacity: Role of Post-Exercise Carbohydrate and Protein Co-Ingestion. *Nutrients.* 2018;10(2).
36. Richardson KL, Coburn JW, Beam WC, Brown LE. Effects of isocaloric carbohydrate vs. carbohydrate-protein supplements on cycling time to exhaustion. *J Strength Cond Res.* 2012;26(5):1361-1365.
37. Kloby Nielsen LL, Tandrup Lambert MN, Jeppesen PB. The Effect of Ingesting Carbohydrate and Proteins on Athletic Performance: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrients.* 2020;12(5).
38. Ferguson-Stegall L, McCleave EL, Ding Z, et al. Postexercise carbohydrate-protein supplementation improves subsequent exercise performance and intracellular signaling for protein synthesis. *J Strength Cond Res.* 2011;25(5):1210-1224.
39. Burke LM, van Loon LJC, Hawley JA. Postexercise muscle glycogen resynthesis in humans. *J Appl Physiol (1985).* 2017;122(5):1055-1067.
40. Rauch HG, St Clair Gibson A, Lambert EV, Noakes TD. A signalling role for muscle glycogen in the regulation of pace during prolonged exercise. *Br J Sports Med.* 2005;39(1):34-38.
41. Currell K, Jeukendrup AE. Validity, reliability and sensitivity of measures of sporting performance. *Sports Med.* 2008;38(4):297-316.
42. Aagaard P, Simonsen EB, Andersen JL, Magnusson P, Dyhre-Poulsen P. Increased rate of force development and neural drive of human skeletal muscle following resistance training. *J Appl Physiol (1985).* 2002;93(4):1318-1326.
43. Moss BM, Refsnes PE, Abildgaard A, Nicolaysen K, Jensen J. Effects of maximal effort strength training with different loads on dynamic strength, cross-sectional area, load-power and load-velocity relationships. *Eur J Appl Physiol Occup Physiol.* 1997;75(3):193-199.
44. Koopman R, Crombach N, Gijsen AP, et al. Ingestion of a protein hydrolysate is accompanied by an accelerated in vivo digestion and absorption rate when compared with its intact protein. *Am J Clin Nutr.* 2009;90(1):106-115.
45. Jensen J, Rustad PI, Kolnes AJ, Lai YC. The role of skeletal muscle glycogen breakdown for regulation of insulin sensitivity by exercise. *Front Physiol.* 2011;2:112.
46. Miranda L, Horman S, De P, I, Hue L, Jensen J, Rider MH. Effects of contraction and insulin on protein synthesis, AMP-activated protein kinase and phosphorylation state of translation factors in rat skeletal muscle. *Pflugers Arch.* 2008;455(6):1129-1140.
47. Witard OC, Jackman SR, Breen L, Smith K, Selby A, Tipton KD. Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *Am J Clin Nutr.* 2014;99(1):86-95.
48. Churchward-Venne TA, Pinckaers PJM, Smeets JSJ, et al. Dose-response effects of dietary protein on muscle protein synthesis during recovery from endurance exercise in young men: a double-blind randomized trial. *Am J Clin Nutr.* 2020;112(2):303-317.

Legends

Figure 1: Design of interventions. The protocol was completed three times in a double-blinded, randomized, balanced, crossover design. The participants reported to the laboratory fasted at 08:00 AM and performed the morning cycling session (EX1) that consisted of a 15-min warm-up, a 10-s sprint, 30 min at 75% of $\dot{V}O_{2max}$ ($W_{75\%}$), a 60 min time trial (TT_{60}), and finished with a second 10-s sprint. After EX1, a five hours standardized isoenergetic recovery period started in which the participants ingested either a carbohydrate only (CHO), a carbohydrate and whey hydrolysate (HYD) or a carbohydrate and whey isolate (ISO) drink after EX1 and one hour after. For the rest of the recovery period the diet was similar across interventions. Resting metabolism was measured before the afternoon session (EX2) that consisted of a 15-min warm-up, a 10-s sprint, 30 min at 75% of $\dot{V}O_{2max}$ ($W_{75\%}$), a time trial performance test (TT_P) and a 10-s sprint.

Figure 2: Performance data. (A) Mean power during the 1-h performance test (TT_{60}) in the morning exercise session (EX1) and the preloaded time trial (TT_P) in the second exercise session (EX2) after a five hours recovery in which CHO, ISO or HYD supplements were provided. (B) Mean power during the sprints before and after EX1 and EX2. (C) Power curve during TT_{60} and (D) power curve during TT_P. Values are mean ± SEM. No difference between diet interventions ($p > 0.05$).

Figure 3: Metabolic data during the exercise sessions in the morning (EX1) and the second session (EX2) after a five hours recovery supplemented with either CHO, ISO or HYD. EX1 consisted of 30 min of cycling at a load corresponding to 75 % of VO_{2max} ($W_{75\%}$), a 5 min rest before a 60 min all out time trial (TT_{60}). EX2 consisted of 30 min of cycling at $W_{75\%}$, 5 min rest before completing a time trial in which the work corresponded to 30 min of cycling at 100 % of VO_{2max} (TT_P). Figures illustrate (A) oxygen uptake (VO_2), (B) RER, (C) heart rate, (D) lactate, (E) blood glucose, and (F) RPE during EX1 and EX2. No difference between diet interventions ($p > 0.05$) in any of the metabolic data. Values are mean ± SEM.

Figure 4: Effect of the dietary interventions on blood glucose and insulin during exercise and recovery. (A) Blood glucose and (B) plasma insulin after intake of either 1.2 g carbohydrate $\cdot kg^{-1} \cdot h^{-1}$ (CHO), 0.8 carbohydrate$\cdot kg^{-1} \cdot h^{-1}$ + 0.4 g isolate whey protein$\cdot kg^{-1} \cdot h^{-1}$ (ISO) or 0.8 carbohydrate$\cdot kg^{-1} \cdot h^{-1}$ + 0.4 g hydrolysate whey protein$\cdot kg^{-1} \cdot h^{-1}$ (HYD). (C) Plasma insulin before and during EX2. (D) Resting VO_2 30 min before EX2 (4.5 hours after EX1). Data is the average from 5 min measurements. No difference between diet interventions ($p > 0.05$). C=different from CHO, I=different from ISO, E=time effect during exercise. *= time effect from 0 during recovery. Values are mean ± SEM.

Figure 5: Nitrogen balance during the protocol. Nitrogen balance was calculated from protein intake and urine nitrogen. C=different from CHO. Values are mean ± SEM. ($p < 0.05$).

Perspectives:

Previously, we have shown that athletes performed better five and 18 hours after exhaustive endurance exercise when protein and carbohydrate were co-ingested in the early recovery phase compared to intake of carbohydrate only. Thereby, protein is shown to be an important food component for athletes during exhaustive exercise, despite that carbohydrate and fat oxidation provide most of the energy. In the present study, cyclists performed a “normal” strenuous, non-exhaustive endurance exercise session in the morning. The performance at the afternoon session after five hours recovery was similar whether carbohydrate only or carbohydrate and protein was ingested in the early recovery phase. Importantly, a protein rich meal was supplemented two hours after the morning session under all dietary conditions. Consequently, for the three dietary interventions, participants were in a neutral of positive nitrogen balance. Therefore, protein intake immediately after hard endurance exercise may not be necessary as long as an adequate amount of protein is ingested to prevent a negative nitrogen balance prior to subsequent exercise.

Figure 1

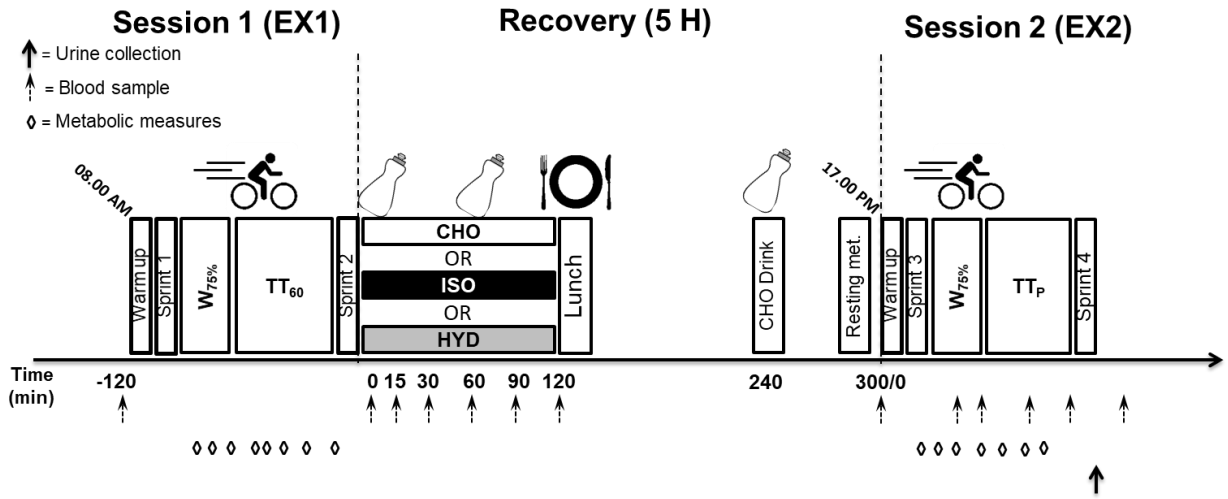

Session 1 (EX1)
Recovery (5 H)
Session 2 (EX2)
= Urine collection
= Blood sample
◊ = Metabolic measures
08.00 AM
17.00 PM
Warm up
Sprint 1
W75%
TT60
Sprint 2
CHO
OR
ISO
OR
HYD
Lunch
CHO Drink
Resting met.
Warm up
Sprint 3
W75%
TTP
Sprint 4
Time (min)
-120
0 15 30 60 90 120
240
300/0

Figure 2

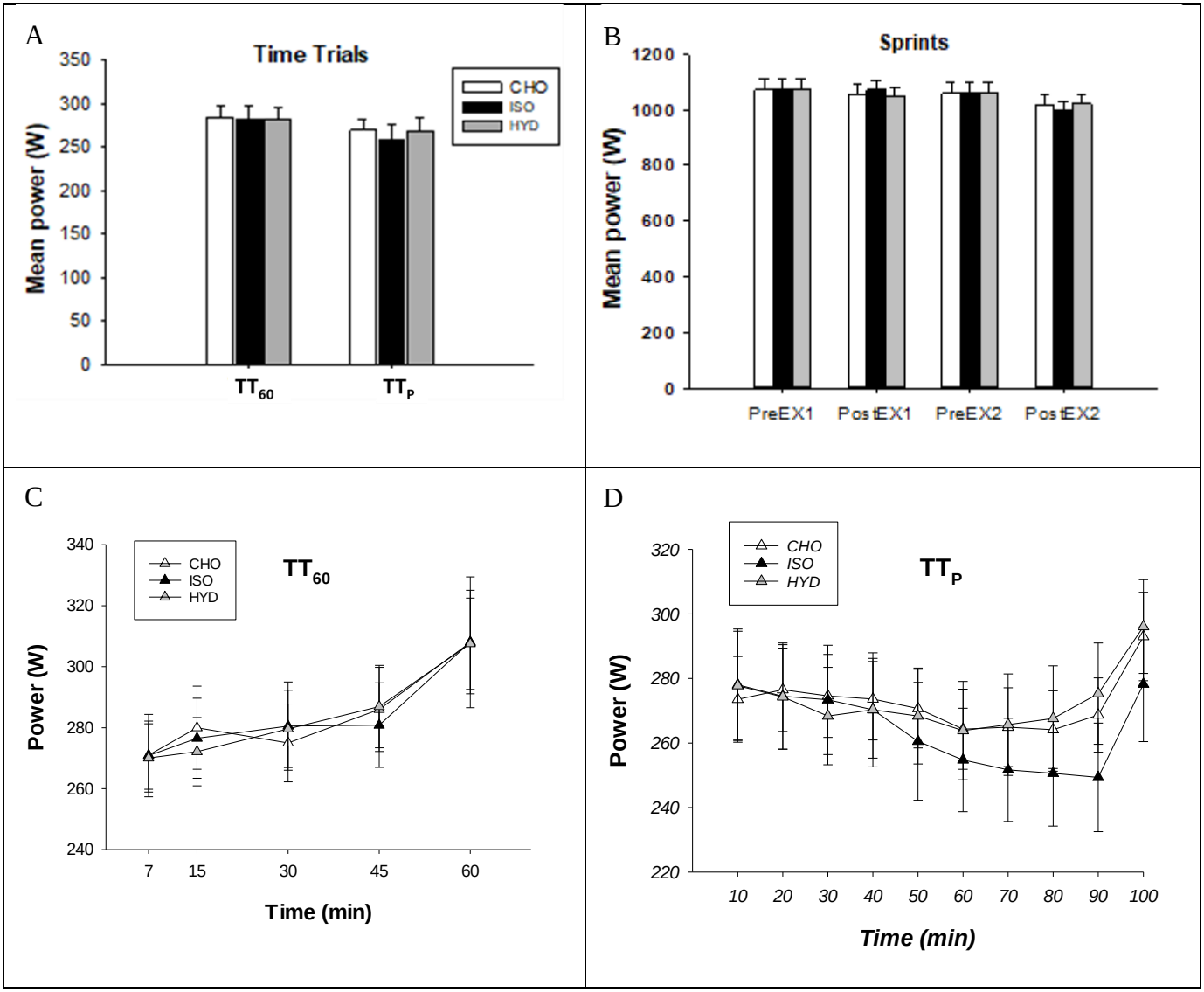
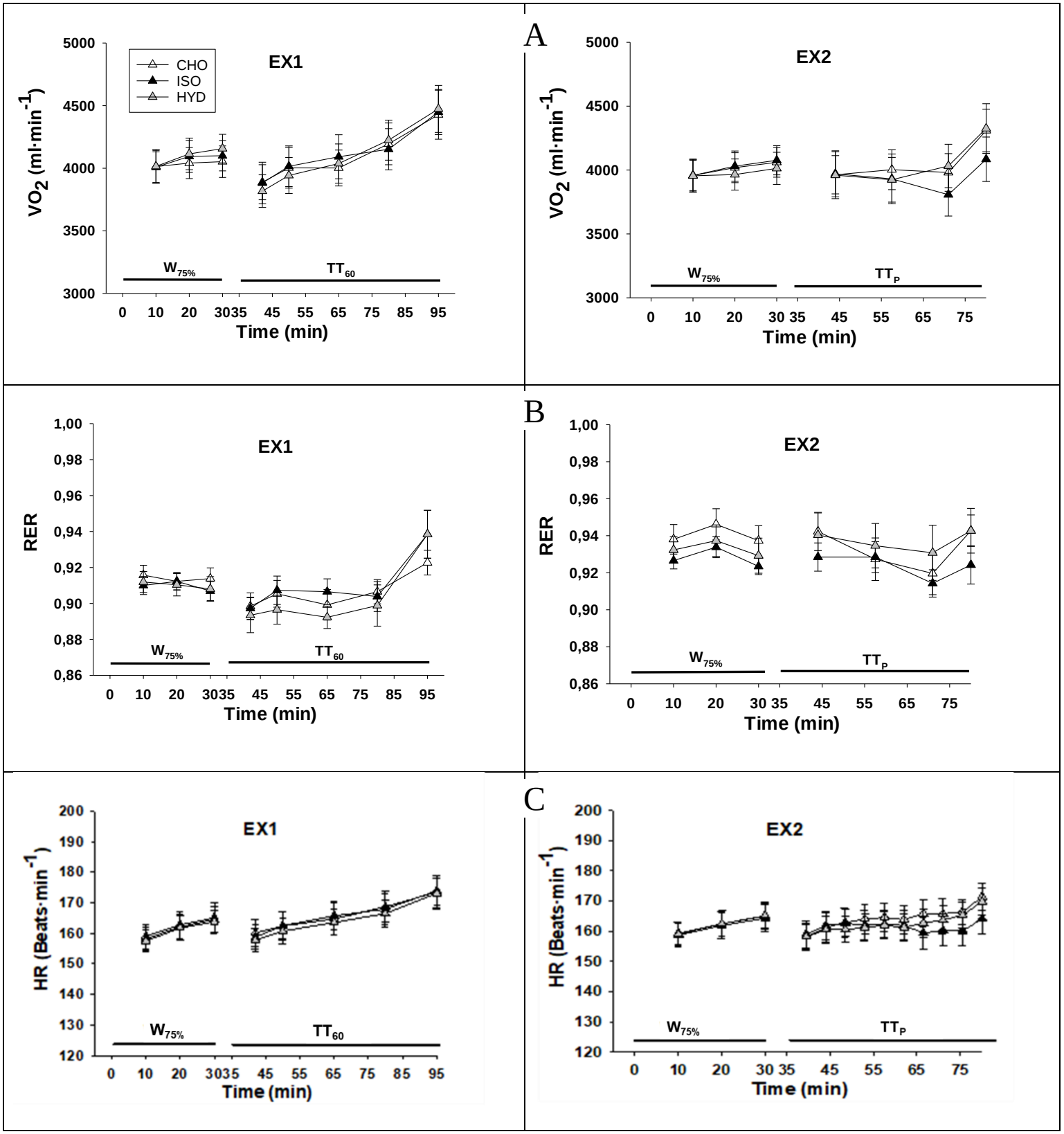
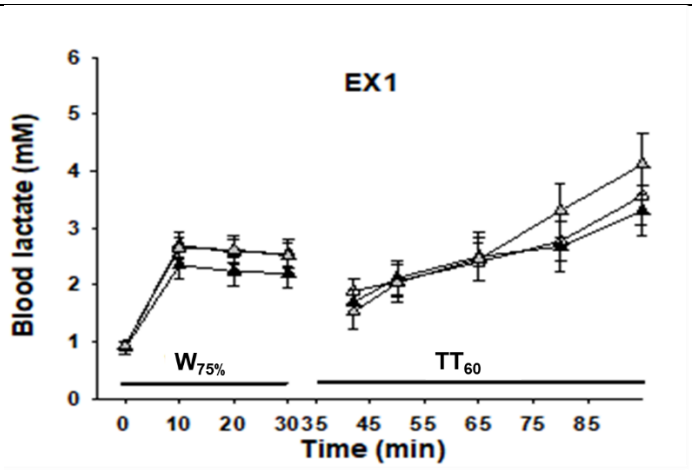
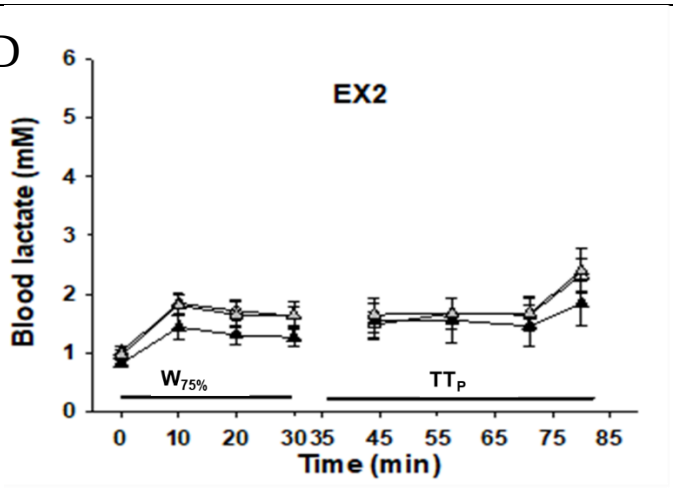

A
Time Trials
Mean power (W)
350
300
250
200
150
100
50
0
CHO
ISO
HYD
TT60
TTP
B
Sprints
Mean power (W)
1200
1000
800
600
400
200
0
PreEX1
PostEX1
PreEX2
PostEX2
C
TT60
CHO
ISO
HYD
Power (W)
340
320
300
280
260
240
7
15
30
45
60
Time (min)
D
TTP
CHO
ISO
HYD
Power (W)
320
300
280
260
240
220
10
20
30
40
50
60
70
80
90
100
Time (min)

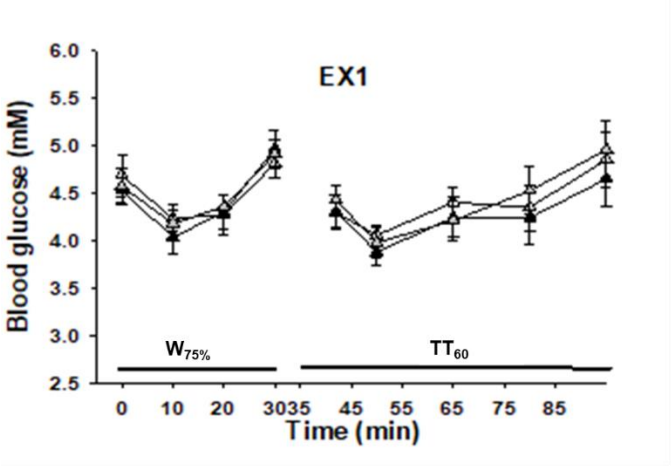
Figure 3


A
B
C
EX1
EX2
CHO
ISO
HYD
VO2 (ml·min-1)
RER
HR (Beats·min-1)
Time (min)
W75%
TT60
TTP

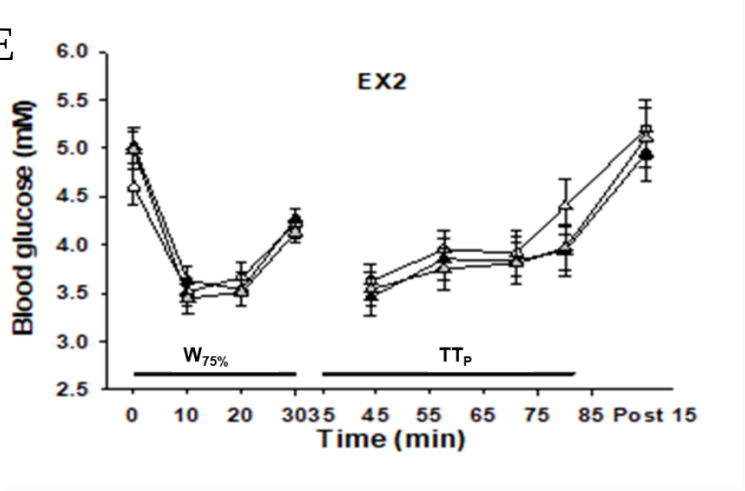

EX1
Blood lactate (mM)
W75%
TT60
Time (min)

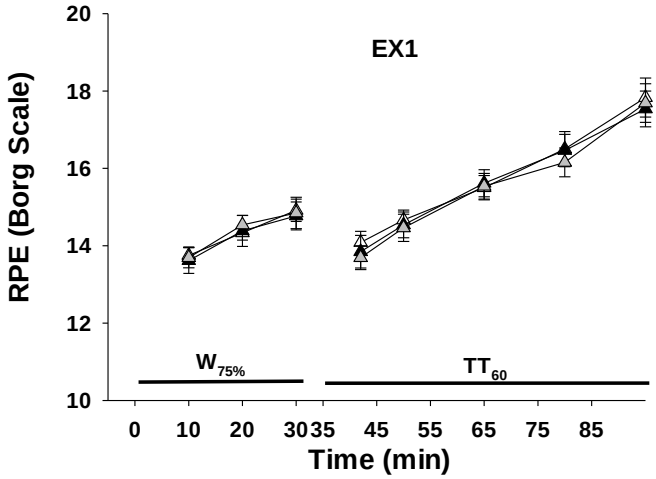
D


EX2
Blood lactate (mM)
W75%
TTP
Time (min)


EX1
Blood glucose (mM)
W75%
TT60
Time (min)

E


EX2
Blood glucose (mM)
W75%
TTP
Time (min)


EX1
RPE (Borg Scale)
W75%
TT60
Time (min)

F

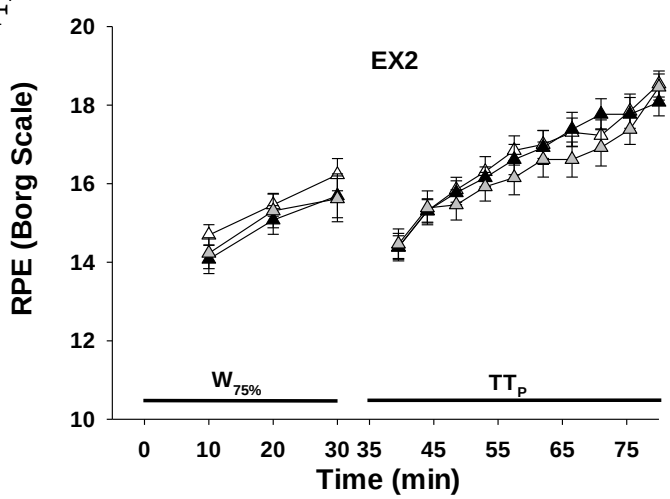

EX2
RPE (Borg Scale)
W75%
TTP
Time (min)

Figure 4

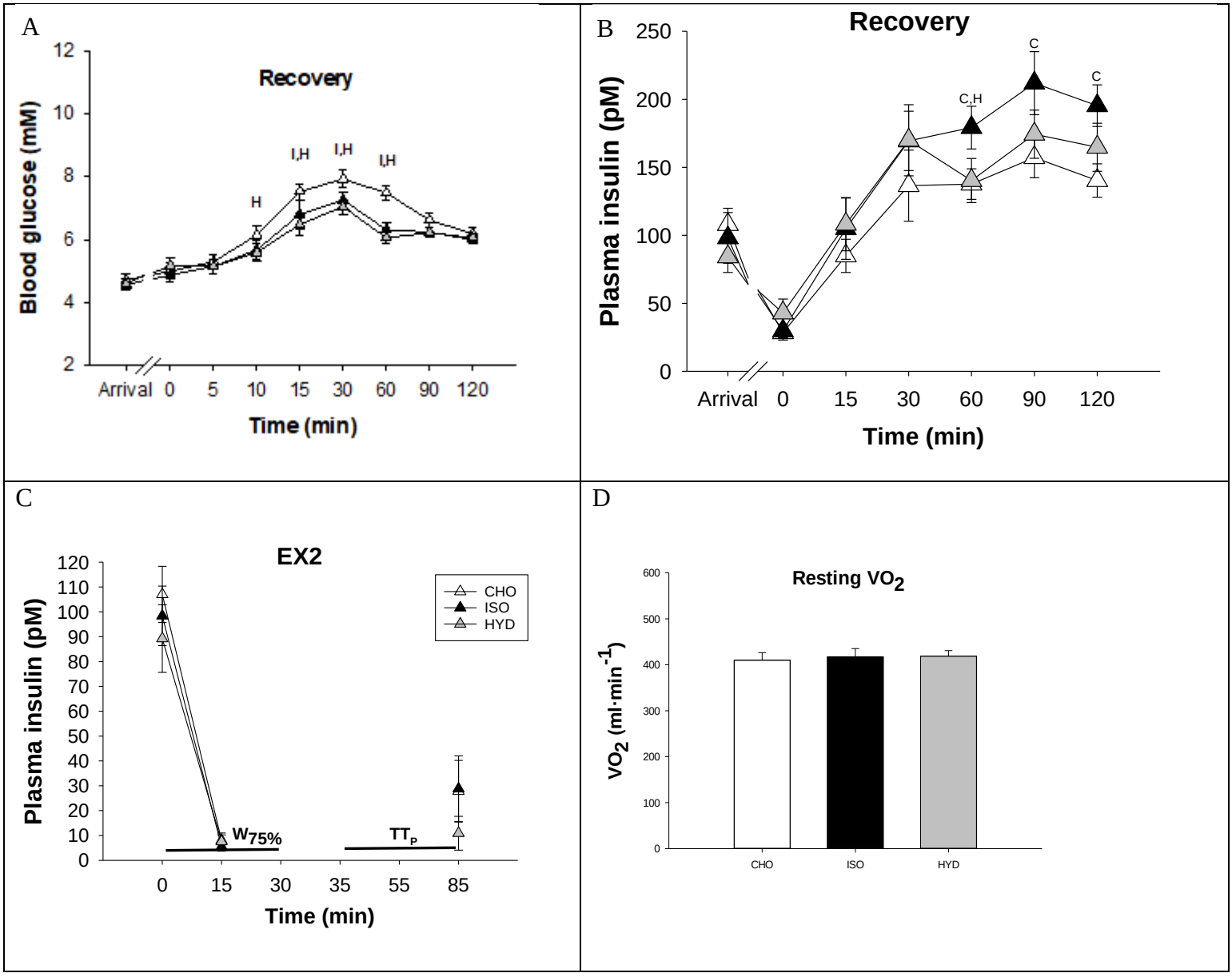

A
Recovery
Blood glucose (mM)
12
10
8
6
4
2
H
I,H
I,H
I,H
Arrival 0 5 10 15 30 60 90 120
Time (min)
B
Recovery
Plasma insulin (pM)
250
200
150
100
50
0
C
C
C,H
Arrival 0 15 30 60 90 120
Time (min)
C
EX2
Plasma insulin (pM)
120
110
100
90
80
70
60
50
40
30
20
10
0
CHO
ISO
HYD
W75%
TTP
0 15 30 35 55 85
Time (min)
D
Resting VO2
VO2 (ml·min-1)
600
500
400
300
200
100
0
CHO
ISO
HYD

Figure 5