Effect of Whey Protein Hydrolysate on Performance and Recovery of Top-Class Orienteering Runners

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This trial aimed to examine the effect of whey protein hydrolysate intake before and after exercise sessions on endurance performance and recovery in elite orienteers during a training camp. Eighteen elite orienteers participated in a randomized controlled intervention trial during a 1-week training camp (13 exercise sessions). Half of the runners (PRO-CHO) ingested a protein drink before (0.3 g kg^{-1}) and a protein-carbohydrate drink after (0.3 g protein kg^{-1} and 1 g carbohydrate kg^{-1}) each exercise session. The others ingested energy and time-matched carbohydrate drinks (CHO). A 4-km run-test with 20 control points was performed before and on the last day of the intervention. Blood and saliva were obtained in the mornings, before and after run-tests, and after the last training session. During the intervention, questionnaires were fulfilled regarding psychological sense of performance capacity and motivation. PRO-CHO and not CHO improved performance in the 4-km run-test (interaction $p < .05$). An increase in serum creatine kinase was observed during the week, which was greater in CHO than PRO-CHO (interaction $p < .01$). Lactate dehydrogenase ($p < .001$) and cortisol ($p = .057$) increased during the week, but the change did not differ between groups. Reduction in sense of performance capacity during the intervention was greater in CHO ($p < .05$) than PRO-CHO. In conclusion, ingestion of whey protein hydrolysate before and after each exercise session improves performance and reduces markers of muscle damage during a strenuous 1-week training camp. The results indicate that protein supplementation in conjunction with each exercise session facilitates the recovery from strenuous training in elite orienteers.

Keywords: sports nutrition, orienteering, muscle damage, IgA, cytokines, creatine kinase

Elite endurance training is characterized by very large training loads, which often includes training on consecutive days and several times a day with reduced glycogen stores and possible structural sarcolemma disruption and degradation of other muscle proteins (Phillips, 2012; Tarnopolsky, 2004). Therefore, optimization of acute and long-term recovery is of special importance for training adaptations and performance in elite sport. On the other hand, high training load with insufficient recovery can lead to a state of burnout or overtraining and decreases in immune function associated with increased risk of infections (Meeusen et al., 2013; Walsh et al., 2011).Nutrition plays an important role in the recovery process (American Dietetic et al., 2009; Hawley, 2013). A fast restoration of muscle and liver glycogen stores is essential for the energy status to have an effective next training pass a few hours later, and it is

well documented that intake of 1 to 1.5 g carbohydrate/kg bodyweight immediately after training accelerates the rate of glycogen replenishment (American Dietetic Association et al., 2009; Hawley, 2013). Apart from the high-energy demand in endurance training, nutritional aspects should also cover the need of specific nutrients to optimize adaptations to training stimuli. Endurance training results in a remodeling of the skeletal muscle structure with synthesis of new mitochondria, and increased level of different oxidative enzymes and transport proteins (Egan & Zierath, 2013). Furthermore, endurance running is characterized by eccentric muscle actions, especially during downhill running, increasing the risk of muscle damage during intensified training. These factors point toward a need for protein intake during the recovery process, but the knowledge is limited when it comes to the effect of timing of protein intake in endurance sport.

A few studies have tested the effects of protein supplementation on endurance performance and recovery in trained athletes beyond the subsequent exercise session (Hill et al., 2013;Luden et al., 2007; Nelson et al., 2012; Skillen et al, 2008; Thomson et al., 2011; Witard et al., 2011). No effects on time to exhaustion (TTE) (Hill et al. , 2013), running performance (Luden et al., 2007) and cycling sprint performance (Nelson et al., 2012) at the end of a training period have been reported, whereas others have reported an attenuation of the reduction in TTE

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(Skillen et al., 2008) or performance in time trials (Witard et al., 2011) toward the end of intensive endurance training periods (3 to 14 days) compared with isocaloric carbohydrate supplementation. Only one of the prolonged studies with protein supplementation included runners (Luden et al, 2007). In a crossover trial by Luden et al (2007), runners were provided with either a protein-CHO-antioxidant supplement or CHO supplement matched for carbohydrate content after each exercise session for 6 days. No effect on 5 km (women) and 8 km (men) race time was detected between the two intervention periods, but a reduction in the enhanced creatine kinase (CK) level was observed in the protein group. Nevertheless, in higher mileage runners a tendency toward a beneficial effect on race performance was observed after protein supplementation. However, since the later trial included antioxidant supplementation, it is difficult to relate the observations to the protein content alone. Another problem is that some of the prior studies have examined the effects of added protein rather than isocaloric treatment comparisons (Luden et al., 2007), which limits the ability to attribute treatment differences to protein. So, results from previous research do not give a clear picture regarding timing of protein intake for endurance runners and a possible beneficial effect on the recovery process.

The effect of protein intake in connection to a single bout of training is very difficult to study due to the expected small effect on the recovery process. Long-term training studies are also quite difficult, mainly due to the needed strict control of performed training and food and drink intake. One model to investigate the importance of protein intake in connection to training would be an intense, one-week training camp for endurance athletes where the training load is extremely high and tightly controlled, and it is possible to supply and record the exact amount, content and timing of the nutritional intake.

In the present trial, we aimed to examine the effect of intake of whey protein hydrolysate plus carbohydrate compared with intake of isocaloric CHO beverages before and after each running session during a training camp for elite orienteering runners with a very high training load. The athletes in the current study were provided with an energy-balanced diet containing sufficient amounts for CHO to support optimal resynthesis of the glycogen stores (American Dietetic Association et al., 2009).

We hypothesized that the beverages containing whey protein hydrolysate provided before and after each training session would enhance recovery and attenuate muscle damage, and subsequently improve exercise performance on the last day of the training camp compared with isocaloric carbohydrate beverages.

Materials and Methods

Design

The project was designed as a controlled intervention trial during a 1-week training camp. The subjects were block-randomized to obtain a similar number of women and men in each group. Elite orienteers participated and performed 13 exercise sessions during the camp. Half of the subjects (PRO-CHO) ingested a whey protein hydrolysate drink before and a whey protein hydrolysate-carbohydrate drink after each exercise session. The other half of the runners (CHO) ingested an energy-matched carbohydrate drink before and after each exercise session. A 4-km run-test on a 400 m center track with control orienteering points every 200 m, was performed before and on the last day of the training camp. Blood and saliva samples were obtained at Day 1, 3, 5, 6, 7 in the morning, as well as after the 4-km run-test, and after the final afternoon training bout on day seven. The diet was controlled for energy and macronutrients.

The trial complied with the Declaration of Helsinki and was approved by the local ethics committees in the Central Denmark Region (1–10–72–562–12). All subjects gave their informed consent to participate before the experiments.

Subjects

The best 18 elite orienteers in Denmark above 18 years of age who were at a national or international level were invited to the training camp. All participants were part of the Danish National team in orienteering. Subject characteristics are shown in Table 1. The two groups were similar with regard to numbers of men and women, age, height, weight, body fat percentage as measured by the sum of four skin fold-measurements (Durnin & Womersley, 1974), VO_{2max}, fitness level (Table 1) and performance in the baseline 4-km run-test before start of the intervention at the training camp (Figure 1).

Sports Drinks

PRO-CHO ingested 0.3 g protein kg^{-1} (Whey protein hydrolysate Lacprodan® HYDRO.365, Arla Food Ingridiens Group P/S) before each exercise session, whereas CHO received a similar amount of energy from carbohydrate (Maxim Energy Drink, Maxim International, Ishoej, Denmark). The preexercise drink was masked in taste by noncaloric ice tea (Iced tea lime, Nestea, Spain). The preexercise drink was served within the last 10 min before each exercise session. After each exercise session, PRO-CHO had 0.3 g protein kg^{-1} in combination with 1 g carbohydrate kg^{-1}, whereas CHO received a similar amount of energy from carbohydrate (1.3 g carbohydrate kg^{-1}). The postexercise drink was ingested within the first 15 min after each exercise session and nothing else was ingested until two hours after each training session except water. Energy-matched beverages were chosen to exclude any effect of the addition of protein in PRO-CHO, due to an extra amount of energy compared with CHO. The amount of carbohydrate in the postexercise beverages corresponded to the recommended amount (1 to 1.2 g carbohydrate kg^{-1}) (American Dietetic Association et al., 2009), with the aim of ensuring that any effect of the addition of protein to the sports beverages compared

Table 1   Subject Characteristics

Characteristics	CHO		PRO-CHO		*p**
	Women (*n* = 5)	Men (*n* = 4)	Women (*n* = 5)	Men (*n* = 4)	
Age (yrs)	20.0± 2.1	21.2 ± 1.7	21.2 ± 3.1	23.8 ± 2.8	.16
Height (m)	1.66 ± 0.07	1.88 ± 0.09	1.69 ± 0.03	1.80 ± 0.09	.76
Weight (kg)	56.0 ± 4.5	75.5 ± 4.8	57.5 ± 2.5	68.1 ± 4.1	.57
Body Fat (%)	22 ± 2	10 ± 3	24 ± 4	11 ± 4	.55
Fitness (O_2 ml min^{-1} kg^{-1})	55.8 ± 4.4	65.8 ± 3.0	58.9 ± 2.8	66.1 ± 1.9	.48
VO_2max (O_2 L min^{-1})	3.13 ± 0.46	4.97 ± 0.44	3.38 ± 0.17	4.50 ± 0.16	.86

Note. Values are mean ± SD. VO_2max = maximal oxygen uptake. **p*-values for test for differences between groups.
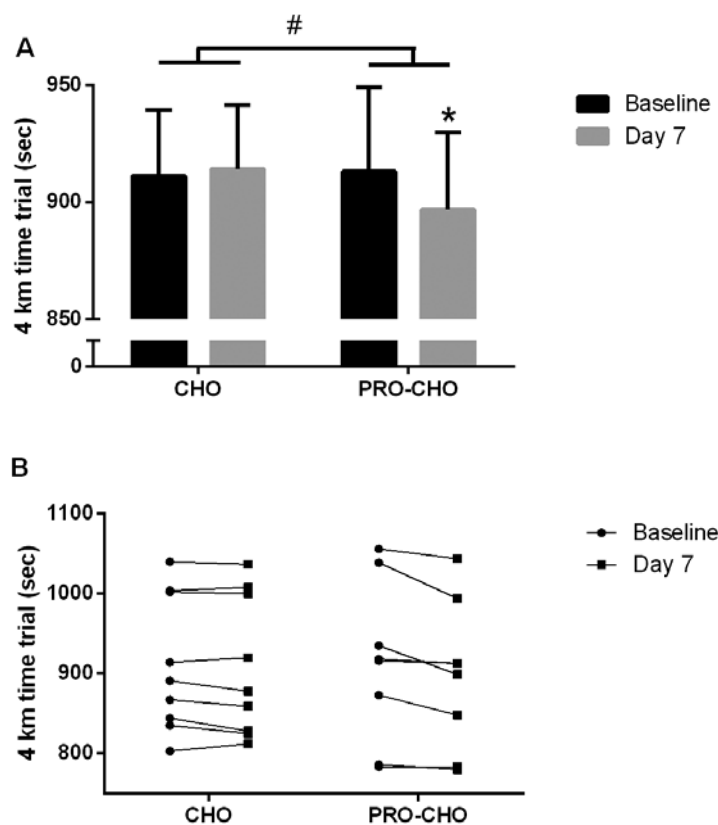

Figure 1 — Performance in 4-km run-test with 20 control orienteering points at baseline and at Day 7. Data in A) are shown as mean ± SE and individual data are shown in B). **p* < .01: Improvement in performance in PRO-CHO at Day 7 compared with baseline. #*p* < .05: Interaction between time and treatment.

with CHO was not related to an insufficient carbohydrate intake in either of groups.

Subjects were blinded to which beverage was consumed during the training camp. Beverages were prepared by laboratory technicians, who did not participate in the analysis of the data, and the beverages were intended to have a similar taste and color. Subjects were not informed regarding the specific contents of the pre- and postexercise beverages or their potential effects on performance and recovery. They were informed that the study would test different sports drinks with different compositions during the training camp. Nevertheless; due to the substantial macronutrient differences between the beverages, it is likely that subjects detected the different tastes/textures between the beverages.

Diet Control

In the present trial, we took particular care to control extraneous dietary bias. Apart from the sports drinks pro-

vided, the diet was similar in the two groups in relation to macronutrient composition (PRO/CHO/FAT 15/63/22% of energy intake, respectively). Diet composition was analyzed using the software program MADLOGVITA (MADLOG Aps, Denmark). The protein content in the basic diet provided for both groups was 1.8 g protein kg^{-1} day^{-1} and sufficient to meet recommended intake for elite endurance athletes (1.7 g kg^{-1} day^{-1}) (American Dietetic Association et al., 2009). PRO-CHO had a total protein intake of 3.0 g kg^{-1} day^{-1} when two preexercise drinks and two postexercise drinks were provided during the two daily exercise sessions. The total carbohydrate intake for PRO-CHO was ~8.3 to 9.3 g kg^{-1} day^{-1} and 8.8 to 10.8 g kg^{-1} day^{-1} in CHO, depending on the training load. The diet provided adequate carbohydrate (>8 g CHO kg^{-1} day^{-1}) in both groups to ensure sufficient glycogen resynthesis when exercising 1 to 3 hours per day with a moderate to high intensity (American Dietetic Association et al., 2009). The daily energy intake was calculated to balance the estimated daily energy expenditure: Basal metabolic rate × Physical Activity Level (PAL) + planned running distance × 1 kcal km^{-1} kg^{-1} excluding exercise session (NNR 2004). PAL was set to 1.5 corresponding to a sedentary lifestyle (NNR 2004). The athletes were weighed each morning before breakfast using a body composition analyzer (TBF-310GS Body Composition Analyzer, Tanita Corporation of America, INC, Illinois, USA). If the morning weight changed by more than ±0.5 kg that could not be explained by fluid changes measured by bioimpedance, the energy content was adjusted to ±125 kcal day^{-1} to reestablish energy balance. No significant weight change was detected from the first to last day of the training camp (PRO-CHO -0.1 ± 0.2 kg, CHO -0.3 ± 0.2 kg). The athletes were not allowed to ingest any dietary supplements or sports products except the administrated sports drinks. The food was served as a buffet and the athletes had to weigh all the food items in accordance with their individual diet plan.

A researcher was available during all meals to support the athletes and monitor food intake making adjustments when necessary.

Training Schedule for the Training Camp

The subjects performed 13 exercise sessions during the week. The training was planned for each individual by the Danish national orienteering coach based on training and injury history. The runners had their heart rate (HR) registered by a HR monitor (RS800 or RS800CX, Polar Electro Denmark ApS) during the exercise sessions. Furthermore, the runners registered whether they were running or had to perform alternative training to reduce the risk of overuse injuries (mountain-bike or cross-trainer). Alternative training sessions were performed with the same intensity (in terms of heart rate) and duration as the superseded running session. Training loads within the two groups are reported in Table 2.

Test Protocol (VO$_2$max and 4-km Run-Test)

Protocol for the VO$_2$max Test: After warm-up, the subjects were running for 2 min before the slope of the treadmill was raised 2%. Thereafter, the slope of the treadmill was raised 2% every 90 s until voluntary exhaustion. Respiratory variables (averaged for each 15-s period) were measured continuously through a mouthpiece connected to an automated metabolic cart (AMIS 2001, Innovision, Odense, Denmark). The mean of the three highest 15-second values was recorded as maximal oxygen uptake (VO$_2$max). HR was measured continuously by a wireless HR monitor (Polar Sport Tester, Polar Electro OY, Kempele, Finland). To ensure that a true VO$_2$max was attained, at least two of the following three criteria had to be fulfilled: (1) a VO$_2$max plateau was reached, (2) a HR was within ±5 beats min^{-1}

Table 2   Training Load

	CHO (*n* = 9)	PRO-CHO (*n* = 8)[a]	*p*
Total training load[b]	13:30 ± 01:46	13:14 ± 02:30	.82
Total running time[b]	09:06 ± 04:14	10:21 ± 04:49	.63
Alternative training[b]	04:23 ± 03:08	02:34 ± 02:47	.29
Number of running sessions	8.8 ± 3.1	10.0 ± 2.8	.41
% of maximal heart rate			
> 90%	9 ± 5	10 ± 5	.90
>85%	19 ± 8	20 ± 8	.81
>80%	34 ± 11	35 ± 11	.83
>70%	68 ± 14	67 ± 13	.79
<70%	32 ± 14	33 ± 13	.79

[a]Data from one subject in PRO-CHO was lost.

[b]Time is hr:min.

[c]% of total time

of maximal HR, and (3) VCO_2 (L min^{-1})/VO_2 (L min^{-1}) > 1.1.

Protocol for the 4-km Run-Tests. the tests were performed at classic athletic stadiums on a 400 m running track after a standardized 15 min warm-up. The subjects were familiarized with the test in previous training sessions. Control orienteering points were set up every 200 m. The first test was performed in Denmark three days before start of the intervention (~3°C). The last test was performed at the last day of the training camp (Day 7) in Portugal (~12°C). Lactate was measured 1 min after finish of the test together with blood sampling. The orienteers were placed in heats of four, who started at two different starting points. The two orienteers that started at the same spot were as different in performance as possible to avoid pacing. The test at Day 7 was performed in the same way, at the same time of the day as the first test, and with a standardized breakfast before each test. No sports drinks were served before the tests, since the aim was to test the accumulated effect of the sports drinks served during the camp, and not the acute effect of a drink served right before the last test.

The orienteers were asked to rate their perceived exertion in a questionnaire, designed by the authors, each morning and after the last exercise session each day. The measure categories were *sense of performance capacity* and *motivation for training* before and during exercise. The orienteers completed the questionnaires in the morning before breakfast and in the late afternoon after the last daily training session, rating their perceptions of motivation and sense of performance capacity on a scale from 0 to 100. The subjects were instructed to treat the scale as continuous, i.e., to rate anywhere on the scale in accordance with the verbal anchors noted on the scale (horrible 0 to 10, very bad 15 to 25, poor 30 to 40, medium 45 to 55, good 60 to 70, very good 75 to 85, very very good 90 to 95, perfect 100).

Analytical Methods

All blood samples were drawn from a cubital vein into sealed vials except the analysis for lactate, hemoglobin, and glucose, which were obtained by finger sticks and analyzed immediately. After separation by centrifugation the remaining plasma samples from Li-Heparin vials were stored at –80 degrees until analysis.

CK, myoglobin, and lactate dehydrogenase (LDH) were analyzed in plasma samples at Aarhus University Hospital, Denmark. Variation coefficient (CV) for CK determination was 2.8 to 3.9%, whereas CV% for LDH was 3.0 to 4.0% and for myoglobin 6.8 to 9.0%. Plasma samples taken after the 4-km run-tests and 0 and 1 hour after the last training session were adjusted for possible changes in fluid compartmentalization in the following way: CK/LDH/myoglobin concentration × hemoglobin concentration in morning sample day 7/hemoglobin concentration in the test sample.

The cytokine levels were determined by multiplex bead array. The levels of granulocyte macrophage–colony-stimulating factor (GM-CSF), interferon-gamma (IFN-c), interleukin-1beta (IL-1b), IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, and tumor necrosis factor α (TNFα) were measured in plasma using the ultrasensitive multiplex kit LHC 6004 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The analyses were performed using BIO-RAD Luminex 100 and the Bioplex Manager version 6.0 software (BIO-RAD, Hercules, CA, US). Values below the detection limit were given half the value of the detection limit.

Salivary morning samples were analyzed for IgA by Salivary Secretory IgA Enzyme Immunoassay kit (Salimetrics Europe, Ltd., Item No. 1 to 1602, Suffolk, UK) using a BioRad Model 550 Microplate Reader and Microplate Manager version 5.2.1. The samples were obtained over 3 min and the analytic results were corrected for differences in salivary flow rate by multiplying the results by the flow rate (ml min^{-1}). CV% for IgA was 3.6%

Statistics

All data were log-transformed where appropriate based on an inspection of the standardized residuals (CK, LDH, cortisol, sense of performance capacity, and motivation for training). Performance data, CK, LDH, cortisol, IgA, lactate, sense of performance capacity and motivation for training were analyzed using multivariate repeated measurements ANOVA with treatment (PRO-CHO and CHO) and time interactions. The unequal standard deviations and correlations in the two groups were taken into account in all the analyses by letting the standard deviations and correlations vary between groups. The data were analyzed using Stata version 12.1. Data are presented as mean ± standard error of the mean (SE), if nothing else is stated. Furthermore, group differences identified by statistical analysis are presented as 95% c.i.

Results

Performance and Training

The performance in the 4-km run-test did not differ significantly between the groups at baseline. On Day 7 a significant interaction between time and treatment was observed ($p < .05$). PRO-CHO improved performance by 17 ± 6 second (95% c.i. 7 to 26 second, $p < .01$), whereas no change was observed in CHO (4 ± 3 second, 95% c.i. -12 to 4 second), $p = .40$) (Figure 1). No difference in lactate (mmol/L) was observed between test and between groups (baseline CHO: 10.2 ± 0.5, PRO-CHO 10.5 ± 0.9; Day 7 CHO: 10.0 ± 0.8, PRO-CHO 10.6 ± 0.9, all $p > .05$).

Total exercise time, time spent on running or alternative exercise, time spent in different intensity zones, and the relative proportion of the exercise time spent in different intensity zones was similar in both groups (Table 2).

Plasma Proteins

A significant interaction between time and treatment was detected in plasma CK ($p < .01$; Figure 2). Plasma CK
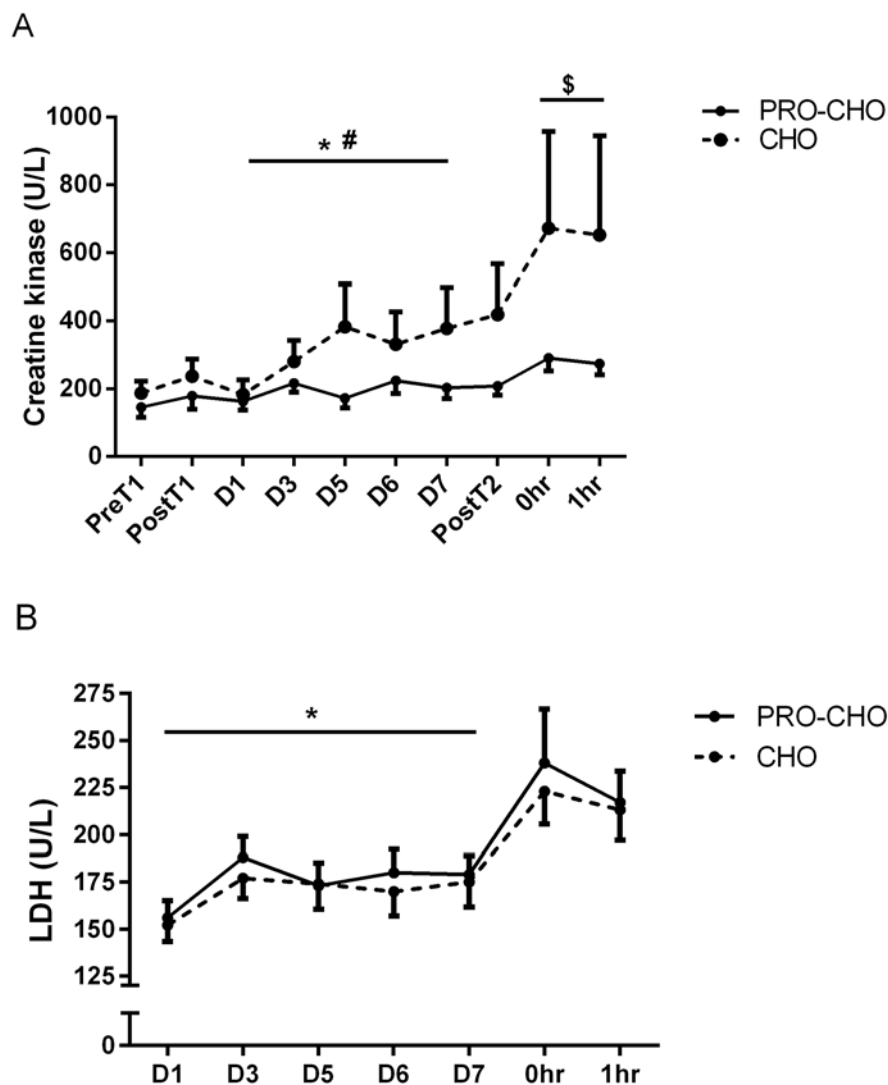
A

B

Figure 2 — Plasma creatine kinase (CK) (A) and plasma lactate dehydrogenase (LDH) (B), as markers for muscle damage. Illustrated are data for morning samples at Day 1 to 7 and data obtained after the last running session (0 and 1hr after running) . CK data from test1 (T1) performed before the intervention period and at day 7 (T2) are included in (A) . CK and LDH increased in the morning samples during the week (*$p < .01$) and increased further after the last running session ($^$$p < .01$). Significant interaction between time and treatment was observed for CK ($^#p < .01$), but not LDH ($p = .31$).

was higher in CHO in the morning of Day 3, 5, 6 and 7 compared with Day 1 (all $p < .01$), whereas no significant change compared with baseline was detected in PRO-CHO during the week.

No difference in CK between groups was detected before the baseline 4-km run-test. The change in CK during the 4-km run-test was not significantly different between groups before the intervention ($p = .97$) and Day 7 ($p = .88$). During the last training at Day 7, CK increased by 294 U L^{-1} (95% c.i. -110 to 699 U L^{-1}) in CHO and 87 U L^{-1} (95% c.i. 66 to 108 U L^{-1}) in PRO-CHO ($p < .01$), respectively, and remained elevated one hour after termination of the exercise ($p < .01$) with no difference in response between groups (interaction, $p = .13$) (Figure 2).

Plasma LDH increased in the morning samples during the week ($p < .01$), but no treatment effect was observed (interaction, $p = .15$). After the last training session, LDH was higher than in the morning ($p < .01$) with no difference between groups (Figure 2).

Plasma myoglobin was below detection level (<53 µg L^{-1}) in all morning samples with the exception of two subjects in CHO and one subject in PRO-CHO at Day 7. Immediately after and one hour after the last exercise session, myoglobin was above the detection level in six of nine subjects in CHO (218 µg L^{-1}, 95% c.i 2 to 434 µg L^{-1}, n = 6) and in eight of nine subjects in PRO-CHO (147 µg L^{-1}, 95% c.i. 15 to 278 µg L^{-1}, n = 8).

Plasma Cortisol

Plasma cortisol in the morning was significantly higher compared with baseline at Day 5, 6 and 7 than at Day 1 ($p < .01$). Furthermore, cortisol at Day 7 was significantly higher than at Day 3 ($p < .05$). No difference between the groups was observed. Immediately after and one hour after the last training session, plasma cortisol was lower compared with the level at the morning of Day 7 (effect of time, $p < .01$) with no difference between the groups (results for cortisol are given in supplemental data Table 3).

Table 3 Cortisol

Cortisol (nmol*L^{-1})	CHO	PRO-CHO
Day 1	798 ± 88	762 ± 77
Day 3	817 ± 98	802 ± 92
Day 5	847 ± 95	848 ± 106
Day 6	831 ± 91	848 ± 105
Day 7	848 ± 93	888 ± 103
Day 7 (0 hr) [a]	710 ± 65	850 ± 89
Day 7 (1 hr) [a]	590 ± 76	792 ± 103

[a]Adjusted for changes in hemoglobin compared with mornings sample.

Cytokines

Cytokines levels were overall very low. No significant interaction between time and treatment was observed in any of the ten cytokines between samples obtained at Day 1 and Day 7. The level of cytokines was the same before and after the 4-km run-test and was not influenced by the treatment (data for IL-6 and TNF-α are given in a supplemental figure).

Salivary IgA

Salivary IgA obtained in the morning did not change significantly during the week (effect of time $p = .43$, interaction $p = .11$) (see supplemental data Table 6). Furthermore, salivary IgA at the end and 1 hour after the last training session was not different from the level at the morning of Day 7. Results for salivary IgA are given in supplemental data) (see supplemental data Table 6).

Sense of Performance Capacity and Motivation for Training

The reported sense of performance capacity level before the first exercise session each day was reduced during the week (time, $p = .05$). At Day 7, the reported sense of performance capacity was significantly lower compared with Day 1, 2, 3, 4 and 5 (all, $p < .05$) (Figure 3). The reduction was significantly greater in CHO compared with PRO-CHO (interaction, $p < .05$). Similarly, the reduction in the sense of performance capacity reported

after the daily training was greater in CHO compared with PRO-CHO (interaction $p < .05$).

Motivation for training reported before the first exercise session each morning diminished during the week ($p = .05$) (Figure 4). A tendency toward a greater reduction in motivation was observed in CHO than PRO-CHO (interaction, $p = .08$). The change in motivation reported after the daily training sessions during the week was different between groups (interaction $p < .05$).

Discussion

The main finding in the present trial was an improved performance in elite orienteers at the end of a strenuous 1-week training camp, when whey protein hydrolysate was ingested before and after each exercise session together with carbohydrate (PRO-CHO). This was not the case when subjects ingested energy-matched carbohydrate (CHO) beverages. Furthermore, PRO-CHO compared with CHO reduced the increase in plasma CK and attenuated the reduction in sense of performance capacity during the 1-week training camp. It is important to emphasize that the weather conditions in Portugal during the final test was better than the baseline test in Denmark (temperature ~10°C and no wind vs. temperature ~0°C and windy), which may explain the improvement in time in PRO-CHO in spite of the increase in muscle damage markers and changes in perceptions of performance at the final test day. Therefore, the important perspective is to focus on the differences between PRO-CHO and CHO.

Various recovery regimes affect training outcomes and performance. Intake of nutrients plays a pivotal role and is one of the key components during recovery after endurance exercise. Ingestion of protein-carbohydrate compared with carbohydrate during and/or after endurance exercise has been shown to be more appropriate for short-term recovery according to muscle functionality (Skillen et al, 2008), muscle damage and soreness (Hall et al, 2013; Luden et al, 2007; Nelson et al, 2012; Rowlands et al, 2007; Saunders et al, 2004; Skillen et al, 2008; Thomson et al, 2011; Valentine, 2007), endurance capacity (Betts et al, 2007; Saunders et al, 2004) or performance in subsequent tests (Berardi et al, 2008; Ferguson-Stegall et al, 2011; Hall et al, 2013; Rowlands et al, 2008), but no difference between groups in these parameters have also been reported (Breen et al, 2010; Goh et al, 2012; Millard-Stafford et al, 2005; Romano-Ely et al, 2006). An important aspect, at least from the elite athletes' point of view, is performance trials where the test circumstances are as close to real life training and competition conditions as possible. In many of the previous trials, the athletes have initiated the exercise after overnight fasting (Betts et al, 2007; Thomas et al, 2009), or after suboptimal energy- and carbohydrate intake (Ivy et al, 2003; Saunders et al, 2004; Saunders et al, 2007), which limits the generalizability and application of the results to real world practice. Therefore, we provided both groups with a carbohydrate-rich energy-balanced
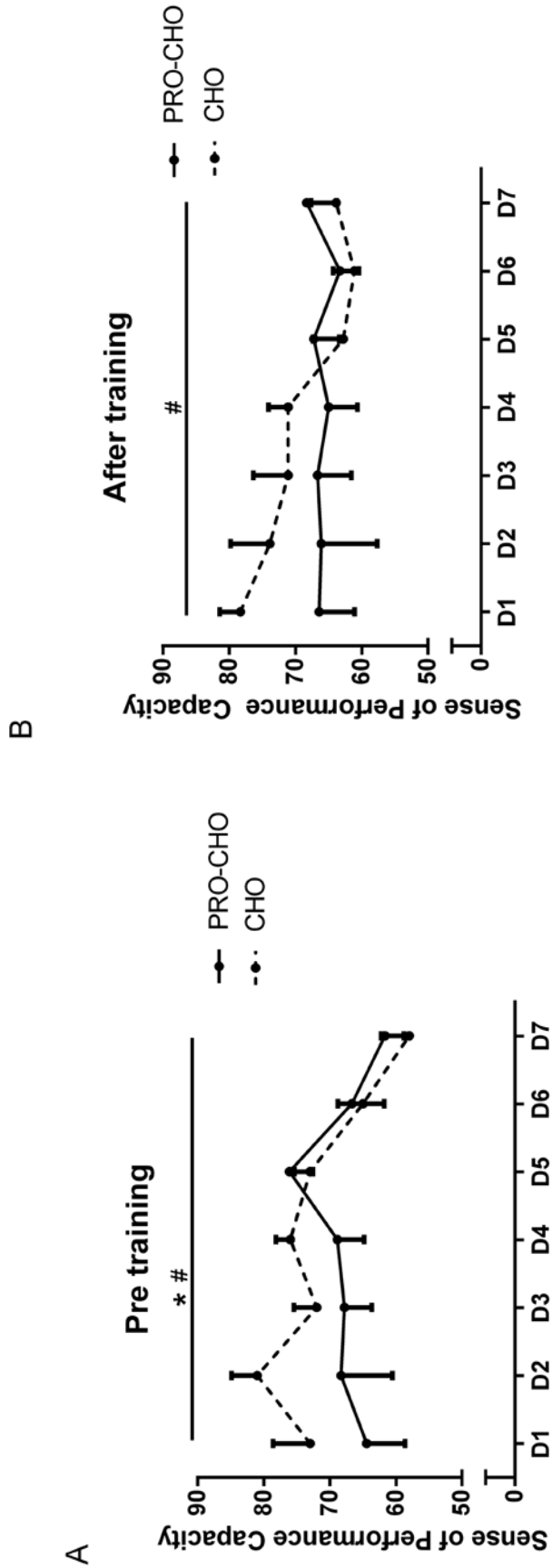
Figure 3 — Sense of Performance Capacity measured in the morning (A) and measured after the last training session each day (B) was reduced during the week (*$p < .05$), more in CHO compared with PRO-CHO (#$p < .05$)
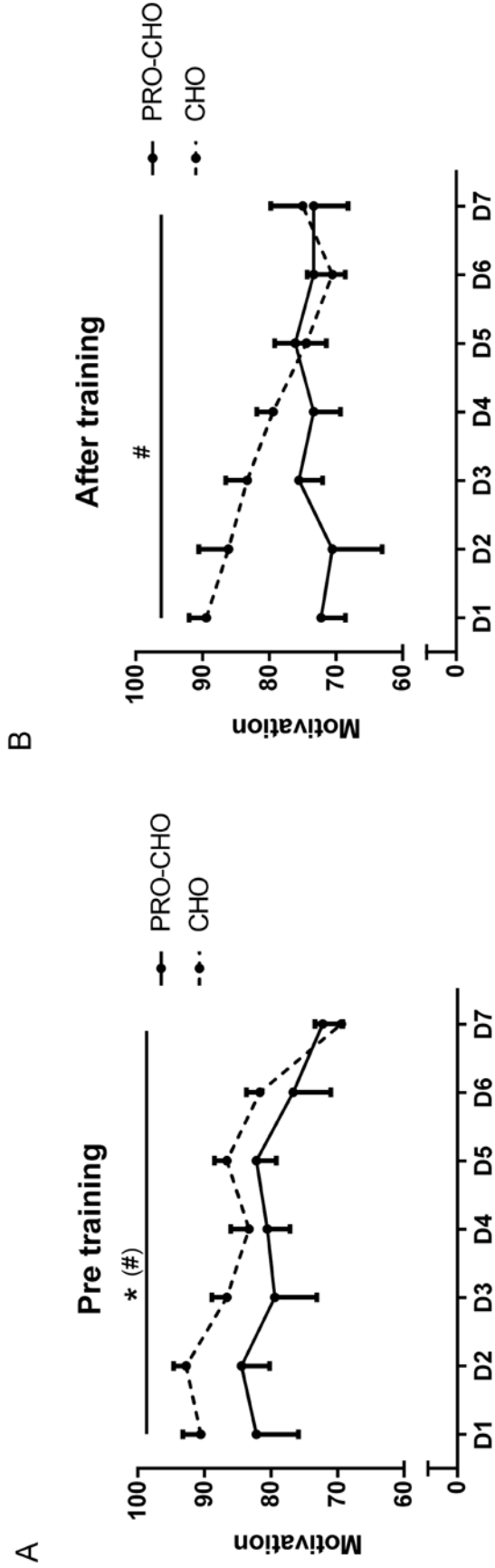

Figure 4 — Motivation for training measured in the morning (A) was reduced during the week (*$p = .05$) and tended to be more reduced in CHO than PRO-CHO ([#], $p = .08$). Motivation for training measured after the last training session each day (B) was overall not reduced during the week ($p = .30$), but a significant interaction between treatment and time was observed (#$p < .05$).

Table 4   Immunoglobulin A

IgA (mg * L^{-1})	CHO	PRO-CHO
Day 1	196 ± 33	203 ± 47
Day 5	295 ± 77	209 ± 44
Day 6	232 ± 57	223 ± 42
Day 7	227 ± 60	198 ± 37
Day 7 (0 hr)	226 ± 56	168 ± 32
Day 7 (1 hr)	219 ± 48	134 ± 22

Note. The analytic results were corrected for differences in salivary flow rate by multiplying the results by the flow rate (ml min^{-1}).

diet containing sufficient energy to support glycogen resynthesis and a daily protein intake in accordance with recommendations for elite endurance athletes (>1.7 g protein kg^{-1} day^{-1}) (American Dietetic Association et al., 2009). Furthermore, the athletes had a standardized carbohydrate-rich breakfast two hours before the run-test. By saturating muscle glycogen restoration, we assumed that the design would reveal the effect of adding proteins to the beverages provided before and after each exercise session.

We aimed to test the accumulated effect of the ingested beverages on performance. Therefore, no intervention beverage was served before the initiation of the 4-km run-tests, to exclude the observed effects being due to an acute effect of the last beverages as in many of the previous studies (Berardi et al, 2008; Ferguson-Stegall et al, 2011; Hall et al, 2013; Rowlands et al, 2008). A limited number of trials have studied the more long-termed effect of PRO-CHO recovery supplementation. The results are mixed. Ergogenic effects on performance (Thomson et al, 2011; Witard et al, 2011) or endurance capacity (Skillen et al, 2008) after PRO-CHO compared with CHO supplementation has been reported, but no significant difference has also been observed (Hill et al, 2013; Luden et al, 2007; Nelson et al, 2012). Witard et al (2011) observed a reduction of the decrement in performance in a time trial after one week of intense training, when trained cyclist received protein supplements to increase dietary protein intake. Similarly, Thomson et al (2011), after three days interval training and 39 hour recovery observed an ergogenic effect on mean sprint power (+2.5%) and an attenuation of increase in CK (-19%), when cyclists ingested PRO-CHO in the first 1.5 hour recovery period of each exercise session compared with isocaloric CHO. The nonsignificant observations reported in the literature (Hill et al, 2013; Luden et al, 2007; Nelson et al, 2012) may be explained by a limited number of subjects (Hill et al, 2013), small amount of protein in the control beverages (Hill et al, 2013) and a positive nitrogen balance in the control situation, which indicates no need for additional protein (Nelson et al, 2012). In addition, an ergogenic effect of PRO-CHO compared with CHO may only be present if the training period is strenuous (Luden et al, 2007). Furthermore, it can be hypothesized that the effect of PRO-CHO may

be more marked in runners than cyclists due to the large volume of eccentric work during downhill running in particular.

In the present trial, the orienteers had either the recommended amount of protein (1.8 g kg^{-1} day^{-1}) or a protein-rich diet (3.0 g kg^{-1} day^{-1}), which however still was within normal range of the recommendations (19% of the energy) (NNR 2004). The orienteers in PRO-CHO consumed the extra protein immediately before and after each running session. Therefore, our observations indicate that ingestion of protein close to each the training session is important during a period of intensified training. Nevertheless, the observation may also be explained by the higher total daily protein intake in the protein-supplemented group. Unfortunately the current study was not designed to investigate the impact of protein timing, and future studies are needed within this field to clarify this aspect.

The smaller blood CK increase in PRO-CHO compared with CHO is consistent with other reports for trained cyclists after PRO-CHO ingestion during and/or postexercise compared with controls matched in CHO content (Luden et al, 2007; Saunders et al, 2004; Saunders et al, 2007; Valentine et al, 2008) or total calories (Nelson et al, 2012; Romano-Ely et al, 2006; Rowlands et al, 2008; Rowlands et al, 2007; Skillen et al, 2008; Thomson et al, 2011; Valentine et al, 2008), but no effect has also been observed (Breen et al, 2010; Ferguson-Stegall et al, 2011; Goh et al, 2012; Green et al, 2008; Millard-Stafford et al, 2005). An attenuation of the increase in CK may indicate a lower relative disruption of cellular structural integrity and an improved recovery, which suggest that the athletes may tolerate a greater training load after PRO-CHO ingestion.

The finding of a significant attenuation of CK and no clear effect on LDH after PRO-CHO compared with CHO in the current study, has been observed by others (Thomson et al, 2011), whereas some have observed attenuation of both parameters (Luden et al, 2007; Romano-Ely et al, 2006). In the latter studies, antioxidants were provided making comparison between studies difficult (Luden et al, 2007; Romano-Ely et al, 2006). LDH is present in the kidney, red blood cells and stomach as well as in the skeletal muscles, hence LDH may be a less specific marker for sarcolemma disruption than CK. An increase in CK may also reflect damage in the cardiac muscle, but we consider that the latter is unlikely in the elite endurance athletes.

A limitation of the present trial is the absence of direct markers of muscle damage. The validity of some markers of muscle damages (such as CK levels) has been questioned, as they may not be strongly correlated with muscle protein breakdown, muscle function or histochemical staining for muscle damage (Beaton et al, 2002; Crameri et al, 2007; Hansen et al, 2009; Warren et al, 1999). Because of the highly applied nature of the present trial with elite orienteers, it was not possible to obtain biopsies for direct measurements of muscle damage.
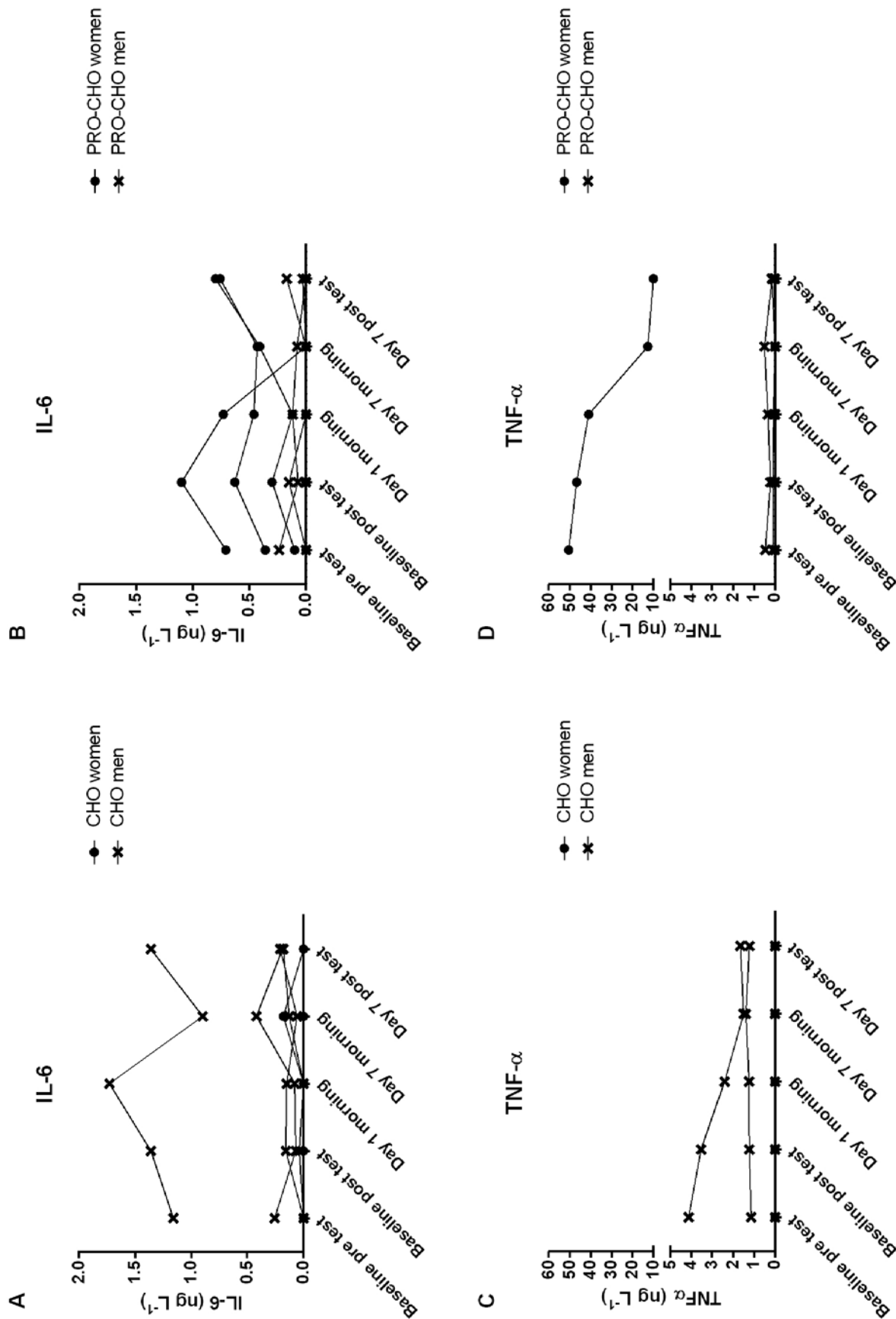
Supplemental Figure — Plasma IL-6 for CHO (A) and PRO-CHO (B); Plasma TNF-α for CHO (C) and PRO-CHO (D).

The potential mechanisms explaining how PRO-CHO may influence recovery are not clearly elucidated. Postexercise protein ingestion stimulates synthesis of myofibrillar protein and related muscle proteins, which is important for tissue remodeling (Breen et al, 2011; van Loon, 2013). In the present trial, 0.3 g protein kg^{-1} beverage^{-1} was provided both pre- and postexercise to maximize muscle protein synthesis rate (van Loon, 2013). The PRO-CHO beverage served before the exercise sessions may have reduced skeletal muscle breakdown by supplying the intercellular free amino acids pool with amino acids. The hypothesis behind this is that a decrease in the free amino acid pool in the muscle tissue may act as a signal to promote muscle protein breakdown (Tipton & Wolfe, 1998). Replenishing the pool by ingestion of high quality whey protein hydrolysate before and after each exercise session may have suppressed the signal for muscle breakdown in PRO-CHO. The above-mentioned factors may individually or cooperatively explain the positive effects of PRO-CHO observed in the current study. Whether the positive effect on performance and muscle damage is a result of the pre- and/or the postexercise protein supplements alone or in synergy cannot be elucidated based on the present design, but points to the need for further investigations.

Studies have shown that intake of carbohydrate during exercise may attenuate exercised induced immunosuppression associated with intensive training (Scharhag et al, 2006), while the importance of protein have not been adequately investigated (Walsh et al, 2011). We did not detect treatment differences in any of the pro- or anti-inflammatory cytokines or in salivary IgA either in the morning resting samples or in response to the last training session. The last training session was probably not strenuous enough to induce a marked effect (a standardized warm-up and 25-min exercise at high intensity above 85% of maximal heart rate).

A reduction in motivation for training and sense of performance capacity during the week was observed in the present trial together with the increase in cortisol, which may be indicators of overreaching (Meeusen et al, 2013). An interesting observation was that a significant attenuation of the reduction in the sense of performance capacity and a tendency toward a reduction in the decrease in motivation was observed after PRO-CHO compared with CHO. We have to emphasize that our questionnaire was not validated against internationally accepted questionnaires such as POMS (Morgan et al, 1987). Furthermore, it is problematic for the comparison between groups that the motivation was higher the first day of the training camp in the CHO group. Nevertheless, future investigation may elucidate the potential link between PRO-CHO ingestion and the observed improved mental recovery during the training camp.

In conclusion, ingestion of whey protein hydrolysate before and whey protein hydrolysate plus carbohydrate after each exercise session compared with isocaloric carbohydrate ingestion had an ergogenic effect on performance in elite orienteers at the end of a strenuous 1-week training camp. The protein supplement was provided in a dose, which we anticipated would maximize muscle protein synthesis, accelerate myocellular repair and help to restore cellular integrity and homeostasis. In line with this, an attenuation of the increase in CK as a marker for sarcolemmal disruption was observed in the protein-supplemented group. Our results indicate that whey protein hydrolysate supplementation in elite orienteers before and after each exercise session improve recovery and their ability to cope with a strenuous training load.

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