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**Article Title:** Pre-Sleep Protein Supplementation Does Not Improve Recovery During Consecutive Days of Intense Endurance Training: A Randomized Controlled Trial

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Pre-sleep protein supplementation does not improve recovery during consecutive days of intense endurance training: A randomized controlled trial.

Effect of pre-sleep protein ingestion on recovery from endurance exercise

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Abstract

Recent studies demonstrate that protein ingestion immediately before sleep improves muscle recovery during the night following resistance exercise. Whether this feeding strategy benefits recovery from endurance training has yet to be established. The aim of this study was to investigate the effects of whey protein isolate ingested every night before sleep on subsequent performance and circulatory markers of muscular recovery during a week of intensified endurance training mimicking a training camp. In a parallel design, thirty-two trained runners underwent a one-week intervention with a rigorously controlled diet (g kg\(^{-1}\) d\(^{-1}\): 7.2 carbohydrate, 1.8 protein, 1.0 fat) and exercise program (11 sessions) while receiving either a protein (0.5 g kg\(^{-1}\) d\(^{-1}\)) or carbohydrate (0.5 g kg\(^{-1}\) d\(^{-1}\)) beverage every night before sleep. Blood samples were obtained on the morning of day 1, 4, 7 and 8 and analyzed for markers of muscle damage (CK: creatine kinase, LDH: lactate dehydrogenase, Mb: myoglobin). Post-intervention 5-km time-trial-performance was significantly impaired in both groups (+11 ± 24 sec, \(p < 0.01\)). Plasma CK (+227 ± 221 \%, \(p < 0.01\)), LDH (+18 ± 22 \%, \(p < 0.01\)) and Mb (+72 ± 62 \%, \(p < 0.01\)) increased gradually throughout the week with no difference between groups (\(p > 0.05\)). In conclusion, pre-sleep protein ingestion did not reduce the decline in performance or ameliorate the rise of circulatory markers of muscle damage during a week of intensified training when compared to isocaloric carbohydrate ingestion. The study was registered at www.clinicaltrials.gov (identifier: ID: NCT03147833).

Keywords: muscle damage, endurance performance, dietary protein.
Introduction

Periodically, most elite endurance athletes undertake high intensity exercise e.g. during training camps or meets. Uncustomary intense and frequent exercise places the musculoskeletal system under great strain. To maintain training intensity and/or performance and reduce the risk of overuse injuries, efficient muscle recovery is of special importance during such periods. While the cellular mechanisms driving the acute regenerative processes are not well elucidated a growing number of studies have unveiled benefits of protein feeding strategies in regards to optimizing recovery from endurance exercise (Moore et al., 2014) including attenuated circulatory markers of sarcolemma disruption (Hansen et al., 2015; Saunders et al., 2004; Saunders et al., 2007; Valentine et al., 2008), improvements in protein balance (Howarth et al., 2009; Koopman et al., 2004), glycogen resynthesis (Levenhagen et al., 2001; van Loon et al., 2000) and markers of immune function (Witard et al., 2014). Furthermore, studies show accelerated regain of strength and function following a single bout of muscle damaging resistance exercise when supplementing with whey protein (Buckley et al., 2010; Cooke et al., 2010). Traditionally, protein feeding strategies have focused on the time immediately surrounding or during the exercise session. However, emerging evidence indicates that ingestion of dietary protein prior to overnight sleep may potentiate recovery. During the last decade a series of studies have shown that protein provision immediately prior to or during overnight sleep is digested and released into the circulation, hence stimulating muscle protein synthesis (Groen et al., 2012; Kouw et al., 2017; Trommelen et al., 2017). This approach adds to the conventional way of administering protein in the hours surrounding exercise sessions. Following resistance exercise, pre-sleep protein feeding promotes overnight recovery by enhancing muscle protein synthesis (Res et al., 2012). Furthermore, a
12-week resistance training study demonstrated additional gains in muscle mass and strength when protein rather than non-caloric placebo was ingested before sleep (Snijders et al., 2015). Thus, the beneficial effects of pre-sleep protein supplementation on muscle protein synthesis rates seems well substantiated. However, the potential impact of pre-sleep protein feeding may reach beyond improved gains in muscle mass and strength. It is well established that protein ingestion increases both myofibrillar and mixed muscle protein synthesis after endurance exercise (Moore et al., 2014). This response is clearly an expression of increased muscle protein turnover inducing tissue repair, tissue remodeling and adaptation to endurance training, more so than promoting muscle protein accretion. Previous findings from our lab suggest that ingestion of 0.3 g protein x kg⁻¹ body weight immediately before and after endurance training sessions improves performance and reduces markers of muscle damage in elite runners during a strenuous one-week training camp (Hansen et al., 2015). The benefits of the protein feeding strategy in Hansen et al. (2015) were observed in spite of the control group meeting the current recommendations of daily protein intake for endurance athletes (~1.7 g kg⁻¹ d⁻¹) (Jager et al., 2017).

The present trial aimed to study if protein feeding before sleep will aid athletes in maintaining performance capacity during a period of intensified training. We hypothesized that ingestion of protein supplements every night prior to sleep would reduce muscle damage and the decline in performance after one week of intensified training compared to controls receiving an isocaloric carbohydrate supplement. To our knowledge, this is the first study to assess the effect of pre-sleep protein supplements on recovery from endurance training.
Methods

Experimental protocol.

The study was designed as a controlled, double-blinded randomized paired design (figure 1). Following preliminary testing, the subjects were divided into two groups ingesting either a whey protein isolate drink (PRO, n = 17) or an isocaloric carbohydrate drink (CHO, n = 17) immediately before sleep every night of the one-week training-intervention. A 5-km time trial (TT) was performed on day one and day eight.

On the morning of day 1, 4, 7 and 8 subjects reported to the lab for blood sampling, body weight measurements and questionnaires. The blood samples were analysed for markers of muscle damage (CK: creatine kinase, LDH: lactate dehydrogenase and Mb: myoglobin) and cortisol.

All meals during the study were provided by the research team and controlled for energy and macronutrient content.

Prior to giving their written consent to participate, all subjects were informed of the possible risks of all procedures. The study was registered at www.clinicaltrials.gov (identifier: ID: NCT03147833), approved by the ethics committee of the Central Region of Denmark (1-10-72-292-16), and adhered to the Helsinki Declaration.

Subjects & pairing.

Thirty-four trained male runners were included in the study. The subjects were paired based on their fitness level (VO2peak), 5-km Time Trial (TT)-performance, physical characteristics (age and weight) and training history.

To get an accurate estimate of the participants training status they were asked to upload their last two months of training history from their sport watches to an internet-based
training management software (Sportlyzer, Tartu, Estonia). Additionally, all participants filled out a questionnaire regarding their training habits.

Within each pair, subjects were randomly assigned to either PRO or CHO and completed the exact same training program. The subjects were unaware of the pairing throughout the study period. Subject characteristics are shown in table 1.

**Exercise testing**

Prior to inclusion, volunteers underwent a screening protocol consisting of a 5-km TT and assessment of peak oxygen consumption.

*5-km TT.* A 5-km TT was performed on three occasions. 1) prior to inclusion of the subjects. 2) At day 1 of the intervention (baseline test, PRE) and 3) at the end of the intervention (day 8, POST). The participants were asked to perform the run as fast as possible on all three occasions. The first TT was used to determine if the volunteer was eligible for inclusion in the study and as a familiarization trial. Only subjects able to complete the run in less than 21 minutes underwent further assessments. To avoid anyone pacing their run the subjects were unaware of this inclusion criteria. To minimize the influence of changing weather conditions on the ground surface, the 5-km TT was performed on a 2.5 km paved path (out and back) instead of synthetic track surfacing. All runners wore a heart rate (HR)-monitor. To avoid pacing, HR-monitors were covered with black tape and start times were staggered by 1 min.

*\( \text{VO}_2\text{peak} \).* \( \text{VO}_2\text{peak} \) was determined using a progressive running test on a treadmill, during which gas exchange measurements were carried out every 10s using a computerized mixing bag system (O2CPX with Innocore software, Innovision Aps, Glamsbjerg, Denmark). \( \text{VO}_2\text{peak} \) was defined as the highest oxygen uptake averaged during any 30s of test. Before
commencing the test, a ten-minute self-chosen warm up was allowed. The test was initiated at a speed of 15 km hour\(^{-1}\). After two minutes, speed was increased by 1 km hour\(^{-1}\) which was repeated every minute until volitional exhaustion.

**Assessment of body weight and composition.**

Body composition was determined using dual-energy x-ray absorptiometry (DXA) (GE Lunar DXA scan, GE Healthcare, United States). Body weight was monitored at all laboratory visits using a bioelectrical impedance analyzer (TBF-310GS Body Composition Analyzer, Tanita Corporation of America, INC, Illinois, USA).

**Diet control**

During the study all meals, snacks and drinks, excluding water, were provided by the research team. Diet compositions were analysed using a software program (Vitakost Aps, Kolding, Denmark). Total daily energy expenditure was estimated as basal metabolic rate (Cunningham, 1980) \(\times\) daily physical activity level factor beside training (set to 1.5) + estimated energy expenditure during training (Keytel et al., 2005). All suppers were consumed at the university cafeteria. An evening snack was to be consumed before 8 p.m. Only the water and the experimental drink were allowed after 8 p.m.

The energy content of the basic diet balanced the estimated individual energy expenditure. Energy provisions were evaluated and adjusted in case of changes in body weight exceeding \(\pm\) 0.5 kg from baseline and based on the individual subject’s perception of satiety. Macronutrient composition of the basic diet is presented in table 1. The food intake 24h prior to the two performance tests were exactly matched. Water was allowed *ad libitum* at all times. No subject reported any waste of food.
**Pre-sleep drinks**

Each subject consumed a pre-sleep drink immediately before bedtime every day during the study period. The subjects were blinded to the content of the pre-sleep beverages. To mask the content, each drink (protein/carbohydrate) came in two flavours (Protein: apple and grapefruit; Carbohydrate: berry and pomegranate). Each subject received the same flavour drink every night by random allocation. The drinks contained either 0.5 g x kg body weight\(^{-1}\) of whey protein isolate (Lacprodan ALPHA-20, Arla Foods Ingredients Group P/S) or maltodextrin. The drinks were matched for caloric content (kJ x kg body weight\(^{-1}\)). The subjects added water to their liking but were advised to use ~250mL.

**Training**

All runners were set to complete 11 training sessions excluding the two time-trials. No additional training was allowed. The training was planned for each individual pair of runners to challenge their training status without increasing the risk of overreaching and injuries. Two daily running sessions (morning and afternoon) were performed except on day 4 when only one session was completed.

The morning run was planned by the research team and coaches but undertaken by the subjects individually. The afternoon sessions were planned and supervised by experienced coaches and performed collectively (specified training program is provided as Suppl. 1B). All training sessions were monitored using sport watches. The training data was uploaded every evening to Sportlyzer. To reduce the risk of overuse injuries three subjects complaining of musculoskeletal pain in the lower extremities performed some training sessions as cycling at intensities and durations similar to the superseded running sessions.
Blood samples

On the morning of day 1, 4, 7 and 8, blood samples were drawn from a cubital vein into Li-Heparin blood collection tubes. After centrifugation (10 min, 5°C, 1200 rpm), plasma was stored at -80°C until analysis. Levels of cortisol, CK, LDH and Mb were analysed at Aarhus University Hospital, Denmark. The coefficient of variation (CV%) for the analyses was as follows; CK: 5.6 – 7.8 %; LDH: 6.0 – 8.2 % and Mb: 11.0 – 12.7 %; cortisol: 15.8 – 18.0 %.

Questionnaires

At each lab visit the subjects were asked to rate their sense of performance and their level of motivation for training on a scale from 1 – 100 (1 being the lowest score), designed by the authors.

Statistics

The data was analyzed using Stata version 14.2 (StataCorp Tx, USA). Normality of all data was checked by QQ-plots, and plots of residuals vs. the fitted values. Data were log-transformed where appropriate (CK, LDH, Mb, cortisol and training history outcomes). Data collected repeatedly through the study period was analyzed for time x treatment interaction by a repeated-measures mixed-effects-model. Unequal standard deviations and correlations within and between subjects were included in the analyses by letting the standard deviations and correlations vary between and within subjects.

Subject characteristics were analyzed using an unpaired t-test. Data are presented as mean ± standard deviations (SD), if nothing else is stated. A p-value < 0.05 was considered statistically significant.
Results

Thirty subjects (PRO: 14; CHO: 16) completed the study. Prior to the intervention two subjects dropped out due to time constraints. During intervention, two subjects were excluded (illness unrelated to the study; poor compliance) both from the PRO-group.

Performance and training

An overall decline in TT-performance (+11 ± 24 s, \(p = 0.01\)) was observed between day 1 (18 min 31 s ± 1 min 19 s) and day 8 (18 min 42 s ± 1 min 21 s), with no significant difference in decline between groups (\(p > 0.05\); figure 2). The difference in weather conditions between the two test days was minor (Day 1: 13°C and dry; Day 8: 8°C and damp from previous night’s rain).

At baseline, VO\(_2\)max (table 1) and training history (Suppl. 1A) did not differ between groups (\(p > 0.05\)). On average, the subjects increased their weekly running distance 3-fold during the intervention compared to the reported training history, with no differences between groups (\(p > 0.05\)). This increase refers to running exclusively. On average the participants increased their weekly training volume (all activities included; expressed as weekly training duration) form 7.6 ± 4.5 h to 9.2 ± 1.8 hours with no difference between groups (\(p = 0.9\)). Further data on training history is provided in Suppl. 1A.

Blood samples

Plasma CK (\(p < 0.001\)), LDH (\(p < 0.001\)) and Mb (\(p < 0.001\)) increased during the study period, but no significant differences were observed between groups at any time point (figure 3). Although plasma cortisol levels did not change during the intervention in either group, a significant difference was observed at baseline with lower levels in PRO vs CHO (531.1 ± 117.2 vs 616.8 ± 88.8 nmol L\(^{-1}\); \(p < 0.05\)).
Diet control and bodyweight

Body weight (figure 4) did not differ significantly at baseline between the two groups. Surprisingly, an overall time ($p < 0.001$) and time x treatment effect ($p < 0.05$) was observed, but the post hoc pairwise comparison did not reveal any differences between treatments at specific time points. Yet a small but significant decrease in body weight was observed at day 8 for both treatments (CHO: -0.7± 0.6 kg; PRO: -0.3 ± 0.5 kg; $p ≤ 0.05$).

Macronutrient content in the standardized diet (i.e. excluding the intervention beverages) was not different between the groups (all parameters $p > 0.05$, table 1).

Motivation and sense of performance capacity

Both motivation ($p < 0.05$) and sense of performance capacity ($p < 0.001$) dropped throughout the study week with no significant differences between the groups (figure 5). However, the motivation tended to be higher in CHO than in PRO at baseline ($p = 0.07$).

Discussion

This study provides novel data regarding the effect of pre-sleep protein supplementation during a prolonged endurance training intervention. In addition to the pre-sleep beverages the participants were subjected to a rigorously controlled diet and training program. Contrary to our hypothesis, similar impairments (~1%) in the 5-km TT-performance after the intervention were observed in the two groups. This finding coincided with similar rises in circulatory markers of muscle damage and decline in motivation and sense of performance capacity.

Performance. Unlike our previous study, where protein supplements were given in temporal relation to the exercise, we did not observe a beneficial effect of pre-sleep protein feeding in the present trial even though the sample size was comparable (Hansen et al., 2015).
Like us, others have reported performance benefits of increased protein intake ingested close to training sessions during consecutive days of intensified endurance training (Rowlands et al., 2008; Skillen et al., 2008; Thomson et al., 2011; Witard et al., 2011). However, no consensus has been established on this topic (Hill et al., 2013; Luden et al., 2007; Nelson et al., 2012). Likewise, consistency is lacking among more acute studies measuring performance capacity within 24 hours after a single exercise bout combined with protein supplementation, both when compared to isocaloric (Betts et al., 2007; Ferguson-Stegall et al., 2011; Hall et al., 2013; Millard-Stafford et al., 2005; Rowlands et al., 2007) or non-isocaloric placebo treatments (Betts et al., 2007; Breen et al., 2010; Goh et al., 2012; Millard-Stafford et al., 2005; Saunders et al., 2004).

**Muscle damage.** As expected, a continuous rise in circulatory markers of muscle cell disruption was observed throughout the training period as observed previously (Hansen et al., 2015). This implies that the subjects had been exposed to considerable strain throughout the week. Nevertheless, our study did not reveal any difference between groups in any of the blood markers. This finding is contrary to a number of previous longitudinal studies showing an ameliorating effect of protein/amino acid ingestion on markers of sarcolemma disruption when ingested immediately before and/or after training (Hansen et al., 2015; Luden et al., 2007; Thomson et al., 2011). The remarkable difference between the present study and previous training studies showing positive effects of protein supplementation on muscle recovery and performance, is the timing of the supplementary protein intake. While one of the studies showing beneficial effects of protein on recovery from endurance exercise provided the supplementation at night, this was still immediately after exercise (Thomson et al., 2011). Thus, although the current study was not designed to investigate the impact of
protein timing *per se*, we permit ourselves to speculate that supplementation of protein-derived amino acids beyond the hours immediately surrounding the exercise is of little importance when aiming to improve recovery from endurance training performed during the day. Supporting this notion, Levenhagen and colleagues showed that immediate protein feeding following a 60-min bike-ride at 60% of VO$_2$max, induced greater uptake of amino acids across the leg and greater leg and whole-body protein synthesis than when the protein supplement was given 3 hours later (Levenhagen et al., 2001). Furthermore, substantial evidence suggests that the protein net balance during (Rennie et al., 1981) and/or in the early hours after endurance training, in the fasted (Levenhagen et al., 2002; Sheffield-Moore et al., 2004) or carbohydrate fed state (Howarth et al., 2009; Koopman et al., 2004; Levenhagen et al., 2002) is negative. However, acute protein ingestion post exercise shifts the whole-body net protein balance to positive and increases muscle protein synthesis (Breen et al., 2011; Howarth et al., 2009; Koopman et al., 2004; Levenhagen et al., 2002; Levenhagen et al., 2001). These latter findings seem to support that protein derived amino acids ingested immediately following endurance exercise, compared to several hours later, has a greater potential in attenuating muscle damage and/or facilitate faster repair by accelerated muscle protein turnover. In line with our speculations, it is noteworthy, that in the study by Res et al. (2012) showing improvements in overnight recovery (muscle protein synthesis) following resistance exercise, the training sessions were completed at 09.00 pm and both the post-training drink (60 g carbohydrate and 20 g whey protein) as well as the pre-sleep drink (40 g casein vs. water) was consumed within 2.5 hours after completing the exercise. Thus, in the current study we may have seen a positive effect of the protein supplementation had the timing of the exercise sessions been in close connection to the protein feeding (i.e. at night). However,
undertaking intense training late at night does not mimic the typical training pattern of an elite athlete. Thus, such a design would not serve our purpose.

All subjects in the present trial ingested a standardized diet and thus achieved the recommend amount of daily protein (1.7 g/kg) (Jager et al., 2017; Kerksick et al., 2017). It could be argued that the ample amount of protein supplied in the basic diet may have obfuscated the potential benefit of the pre-sleep supplementation. Had the basic dietary provision of protein been suboptimal, a beneficial effect of the pre-sleep supplementation may have been present by counteracting a negative nitrogen balance. However, as this was designed as an applied study, meant to reflect the typical behaviour of a competitive athlete focusing on a high level of performance during training and competition, feeding the control group a suboptimal diet would not serve our purpose.

Despite our best efforts, the mean body mass of the runners dropped slightly (CHO: -0.9 %; PRO: -0.5 %) at the end of the study. However, as the decline in body mass occurred between day 7 and day 8 and not as a development throughout the study, it could be related to insufficient hydration. One may argue that this might have influenced the POST-test performance negatively (Armstrong et al., 1985). However, the effect of slight hypo-hydration on performance seems trivial at temperatures of ~8°C (day 8) (Murray, 2007). Regardless, the loss of body mass was not different between groups and the energy availability was standardized across groups.

Surprisingly, cortisol was lower in PRO vs CHO at baseline. Notably, the biological (>40 %) and diurnal variation in plasma cortisol is relatively large in the morning. In this regard, two CHO subjects showed relative high values (787 and 809 nmol L⁻¹), whereas one PRO
subject had a particularly low value (232 nmol L$^{-1}$). Thus, the difference may be related to a statistical type 1 error, due to a relatively small sample size.

**Limitations.** Given the design of the present study and recovery in general, we would have liked to monitor sleep. Unfortunately, we were unable to acquire equipment to do so accurately. Furthermore, the present study only included men. This of course complicates extrapolation of our findings to the population of female runners. The decision regarding the inclusion of men only, was based on our own (unpublished) and observations by others, suggesting that the presence of estrogen may attenuate the levels of CK in the circulation following muscle damaging exercise (Enns & Tiidus, 2010). However, the lack of female participants may be viewed as a limitation to the study.

In summary, we provided moderately trained runners a whey protein isolate or an isocaloric carbohydrate beverage every night before sleep, along with a standardized diet during a strenuous training week. Pre-sleep whey protein ingestions did not reduce impairments in performance or ameliorate the increase in markers of muscle cell disruption. Compared to existing literature, we speculate that when supplemented to a well-balanced diet the timing of protein intake is of importance in order to elicit an ergogenic effect.

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Conflict of interest statement

MSL, UH and MH are responsible for the study design and preparation of the manuscript. MSL and MH are responsible for the data collection, analysis and interpretation. DC and AAJ recruited the subjects, conducted tests and training and assisted in analysing data and preparing the manuscript. MSL and URM currently hold positions at Arla Foods Ingredients P/S as industrial PhD-student and research scientist, respectively. The views expressed in the manuscript are those of the authors and do not necessarily reflect the position or policy of Arla Foods Ingredients P/S, Denmark. The other authors have no conflict of interest.
“Pre-Sleep Protein Supplementation Does Not Improve Recovery During Consecutive Days of Intense Endurance Training: A Randomized Controlled Trial” by Larsen MS et al.

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References


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Figure 1. Study design. Overview of study design incl. 8 days intervention. ♂ Diet control. ♂ Training session. Two symbols indicate training in the morning and in the afternoon. ♂ Blood samples and body weight measurements in the morning. ♂ Protein (n=14) or carbohydrate (n=16) supplement.
**Figure 2.** Performance tests. 5-km time-trial performance (sec) at day 1 (PRE) and day 8 (POST) by group. Grey lines are individual tests, black lines are means ± SD. Overall time effect ($p = 0.01$)
Figure 3. Markers of sarcolemma disruption. Development in markers of sarcolemma disruption (CK, LHD and Mb). Values are means ± SD. Overall time effects were observed for CK, LDH and Mb (p < 0.01). Significant difference from day 1 (** p < 0.01).
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**Figure 4.** Body weight. Data are means ± SD. Overall time and time x treatment effect. * significantly different from all other days within group (p < 0.05). # significantly different from day 4 in CHO exclusively (p = 0.003).
Figure 5. Daily sense of performance capacity and motivation. Data are means ± SD. * significantly different form day 1 ($p < 0.05$).
Table 1. Subject characteristics, diet and training

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Diet</th>
<th>Training</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Age</td>
<td>Body mass</td>
<td>Body mass DXA</td>
</tr>
<tr>
<td>years</td>
<td>kg</td>
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<tr>
<td>CHO</td>
<td>26.3 ± 6.8</td>
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<tr>
<td>PRO</td>
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<tr>
<td>P</td>
<td>0.70</td>
<td>0.88</td>
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</table>

Data are Presented as means ± SD.
Supplementary tables.

**Training history.** Training 2 months prior to inclusion in the study.

<table>
<thead>
<tr>
<th></th>
<th>Running</th>
<th>Endurance</th>
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<tr>
<td></td>
<td>Cho</td>
<td>Pro</td>
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<tr>
<td><strong>Training history</strong></td>
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<tr>
<td>n</td>
<td>16</td>
<td>14</td>
<td></td>
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<tr>
<td>Duration (h × wk⁻¹)</td>
<td>4.2 ± 2.6</td>
<td>3.3 ± 1.5</td>
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<tr>
<td>Distance (km × wk⁻¹)</td>
<td>43 ± 30</td>
<td>36 ± 26</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>Intervention</strong></td>
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<td></td>
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<tr>
<td>Duration (hours)</td>
<td>9.4 ± 1.8</td>
<td>8.9 ± 1.8</td>
<td>0.52</td>
</tr>
<tr>
<td>Distance (km)</td>
<td>104 ± 23</td>
<td>98 ± 22</td>
<td>0.43</td>
</tr>
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</table>

Data is based on training logs and questionnaires. Statistical analysis regarding training history was performed on actual running sessions only, using an unpaired t-test (data on distance and duration was log-transformed). Endurance training in this case covers a range of disciplines i.e. cycling.
Training Program

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
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</thead>
<tbody>
<tr>
<td><strong>Morning sessions (individual, continuous runs)</strong></td>
<td><strong>HR-zone</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Zone 1-2</td>
<td>Zone 1-2</td>
<td>Zone 1-2</td>
<td>Zone 1-2</td>
<td>Zone 1-2</td>
<td>Zone 1-2</td>
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<tr>
<td>25 - 45 min</td>
<td>20 – 40 min</td>
<td>25 - 45 min</td>
<td>20 - 40 min</td>
<td>25 - 45 min</td>
<td></td>
</tr>
<tr>
<td><strong>Afternoon session (supervised HIIT sessions)</strong></td>
<td><strong>HR-zone</strong></td>
<td></td>
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<tr>
<td>Zone 4-5</td>
<td>Zone 3-4</td>
<td>Zone 4-5</td>
<td>Zone 3-4</td>
<td>Zone 4-5</td>
<td>Zone 4-5</td>
</tr>
<tr>
<td>Intervals</td>
<td>Tempo runs</td>
<td>Intervals</td>
<td>Tempo runs</td>
<td>Intervals</td>
<td>Tempo runs</td>
</tr>
<tr>
<td>5-7, 2min intervals with 1 min jogging breaks</td>
<td>RP (5 min)</td>
<td>7-11, 2min intervals with 1 min breaks</td>
<td>MP (5 min)</td>
<td>5-7, 3 min intervals with 1.5 min breaks</td>
<td>RP (5 min)</td>
</tr>
<tr>
<td>3 min break</td>
<td>MP (5 min)</td>
<td>2 min break</td>
<td>MP (5 min)</td>
<td>2 min break</td>
<td></td>
</tr>
<tr>
<td>3 min break</td>
<td>RP (5 min)</td>
<td>7, 30 sek intervals with 30 s breaks</td>
<td>MP (5 min)</td>
<td>7, 30 sek intervals with 30 s breaks</td>
<td></td>
</tr>
<tr>
<td>8-10, 1 min intervals with 30 s jogging breaks</td>
<td>MP (5 min)</td>
<td>7, 30 sek intervals with 30 s breaks</td>
<td>RP (5 min)</td>
<td>5-7, 3 min intervals with 1 min breaks</td>
<td></td>
</tr>
</tbody>
</table>

Training programs. The training programs were adjusted to each pair of subjects to suit their training status. The adjustments regarded the duration of the continuous run in the morning and the number of intervals performed during high intensity interval sessions (HIIT) in the afternoon. Heart rate (HR) zones (% of HR-max); 1: 50-59; 2: 60 - 69 3: 70 - 79; 4: 80 - 89; 5: 90 - 100. RP: race pace; MP: moderate pace.