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Running head: Manipulation of CHO availability

ABSTRACT

Purpose: We investigated the effect of a chronic dietary periodization strategy on endurance performance in trained athletes. **Methods:** 21 triathletes ($\dot{V}O_{2max}$: 58.7 ± 5.7 $mL \cdot min^{-1} \cdot kg^{-1}$) were divided into 2 groups: a "sleep-low" (SL, n = 11) and a control group (CON, n = 10) consumed the same daily carbohydrate (CHO) intake (6 $g \cdot kg^{-1} \cdot d^{-1}$) but with different timing over the day to manipulate CHO availability before and after training sessions. The "sleep low" strategy consisted of a 3-week training/diet intervention comprising three blocks of diet/exercise manipulations: 1) "train-high" interval training sessions (HIT) in the evening with high-CHO availability; 2) overnight CHO restriction ("sleeping-low"), and 3) "train-low" sessions with low endogenous and exogenous CHO availability. The CON group followed the same training program but with high CHO availability throughout training sessions (no CHO restriction overnight, training sessions with exogenous CHO provision). **Results:** There was a significant improvement in delta efficiency during submaximal cycling for SL versus CON (CON: +1.4 ± 9.3 %, SL: +11 ± 15 %, $P<0.05$). SL also improved supra-maximal cycling to exhaustion at 150% of peak aerobic power (CON: +1.63 ± 12.4 %, SL: +12.5 ± 19.0 %; $P = 0.06$) and 10 km running performance (CON: -0.10 ± 2.03 %, SL: -2.9 ± 2.15 %; $P < 0.05$). Fat mass was decreased in SL (CON: -2.6 ± 7.4; SL: -8.5 ± 7.4 %PRE, $P < 0.01$), but not lean mass (CON: -0.22 ± 1.0; SL: -0.16 ± 1.7 %PRE). Conclusion: Short-term periodization of dietary CHO availability around selected training sessions promoted significant improvements in submaximal cycling economy, as well as supra-maximal cycling capacity and 10 km running time in trained endurance athletes. **Key words:** Dietary manipulation, carbohydrates, triathletes, exercise-nutrient interactions

INTRODUCTION

The increasing sophistication of our knowledge of the interactions between nutrition and exercise has opened up new possibilities for the preparation of athletes for competition performance (18). A novel concept in the preparation of athletes for endurance events involves the integration of two approaches with seemingly opposing objectives: 1) promoting the nutritional environment (i.e., high carbohydrate availability) before and during prolonged/high-intensity exercise to optimize performance via optimal fuel availability (35), and 2) creating an intracellular environment in skeletal muscle in which training responses and adaptations are enhanced via the restriction of carbohydrate (CHO), so called "training low". It is now well recognized that muscle energy status exerts profound effects on resting fuel metabolism and patterns of fuel utilization during exercise as well as acute regulatory processes underlying gene expression and cell signaling. As such, these nutrient-exercise interactions have the potential to activate or inhibit many biochemical pathways (signaling proteins, gene expression, transcription rate of several genes, enzymes activity) with putative roles in training adaptation (3, 16, 18, 21, 22, 40).

Previous studies using a variety of such "train-low" strategies to allow some workouts to be undertaken with either low muscle glycogen levels and/or low exogenous CHO availability have reported robust up-regulation of selected markers of training adaptation (increased whole body fat oxidation, increased activities of oxidative enzymes) compared with training with normalized glycogen stores and high CHO availability (5, 22, 29, 32, 38, 40). However, despite evidence of changes in molecular and cellular markers, it has proved difficult to demonstrate a concomitant enhancement in sports performance. Part of the reason for this 'disconnect' between

'mechanistic' and performance outcomes, is that the dietary-training strategies that successfully augment markers of training adaptation simultaneously reduce the intensity at which athletes can train during key high intensity interval training (HIT) sessions (22, 40). As such, it is important that attempts to manipulate CHO availability and promote training adaptation be periodized such that the goals of various components of the training program are not compromised. In this regard, a recent investigation by Lane and colleagues (24) has examined a unique manipulation of nutrient-exercise interaction: withholding feeding after an intense evening training session, and subsequently sleeping with low-CHO availability. They examined the effect of this strategy on acute skeletal muscle and whole-body responses to a bout of prolonged steady-state cycling the following morning (i.e.," train-high, sleep low") and showed an upregulation of several markers of lipid metabolism, but little change in cellular pathways involved in skeletal muscle mitochondrial biogenesis. However, there was evidence for small, but significant shifts in DNA methylation that corresponded with inverse changes in transcription for metabolically adaptive genes (24). Furthermore, Bartlett and al**.** (5) reported that the exercise-induced increase in p53 phosphorylation is greater when subjects perform high-intensity interval running in conditions of reduced CHO availability, compared to when they commence the same exercise with normal or elevated CHO availability. Whether these early adaptive responses involving epigenetic modification (i.e., diet-exercise interactions) underpin chronic training-induced adaptations and/or result in enhanced athletic performance has not been investigated.

Accordingly, we determined the effects of a chronic (3 weeks) "train-high, sleep-low intervention in trained triathletes on selected metabolic and performance outcomes. The periodization strategy integrated three diet-exercise manipulations within a real-world training

program: high-intensity training with high-CHO availability aimed at maximizing adaptation and performance; overnight CHO restriction (low-CHO availability) to prolong the signaling response after exercise (17, 34); and a prolonged, submaximal training session commenced with low-CHO availability to promote lipid metabolism. We hypothesized that compared to a control condition in which all training sessions were undertaken with high CHO availability, chronic dietary periodization would enhance endurance performance.

METHODS

Subjects

Twenty-one endurance-trained male triathletes volunteered to participate in this study. All subjects had been competing for > 2 years and were training a minimum of 10 hours per week *(Table 1).* Subjects were given medical clearance for participation in the study by a cardiologist, and were not taking any prescribed medication during the study. The experimental design was approved by the local Ethics Committee (Paris IDF VI, France), and the protocol was performed in line with the Declaration of Helsinki. After comprehensive verbal and written explanations of the study and its aims, all subjects gave their written informed consent for participation.

Study Overview

The study used a parallel group design, with the subject cohort being randomly divided into two groups who undertook the same endurance training program for 3 consecutive weeks. Only the timing of dietary CHO intake differed between the groups. One group (n=11) implemented a “sleep low” strategy for manipulating CHO availability (high-intensity workout

with high-CHO availability followed by a CHO-restricted recovery plus an overnight fast; then a prolonged submaximal workout the following morning commenced with low-CHO availability) into the training schedule, while the control group (n= 10) maintained regular CHO intake over the day and undertook each training session with normal/high CHO availability. Selected whole-body measures and a variety of performance tests were undertaken before, during and in the 3 days after completion of the training program *(Figure 1).*

Training protocol

After preliminary testing sessions for the determination of physiological and anthropometric measures, all subjects commenced a 6-week supervised training program, divided into two 3-week phases (3 weeks of baseline and 3 weeks of training/diet intervention). During the first 3 weeks (Baseline), subjects completed their usual training regimen (10-15 $h \cdot wk^{-1}$: 40% running, 35% cycling, 25% swimming). The aim of this first training block was to assess subjects' compliance to the study demands and ensure they all attained similar baseline fitness measures before study commencement. During the second 3 weeks (training/diet intervention) subjects of both groups completed the same standardized training program (*Table 2*), but with two different nutritional protocols (described in detail subsequently). The training program consisted of six sessions over four consecutive days, including high intensity training (HIT) sessions in the afternoon and low intensity training (LIT) sessions the next morning. The training intensity was individually prescribed according to each individual's maximal aerobic power (MAP). LIT sessions consisted in 60 min cycling at 65% MAP (218.8 ± 20.4 W - 95% CI: 227.5 and 210.7), while HIT sessions consisted alternatively in 8 x 5 min cycling at 85% MAP (286 ± 26.7 W- 95% CI: 297.5 and 274.7) or 6x5 min running at their individual 10 km intensity with 1

min recovery between sets (37). The HIT protocol has previously been reported to utilize ~50% of starting muscle glycogen stores in well trained subjects who commence the sessions with normal glycogen levels (37). Furthermore, it has previously been shown that HIT protocols rapidly activate signaling pathways with putative roles in the regulation of mitochondrial biogenesis (4), while HIT training is an integral part of an endurance athletes training repertoire (4). One LIT session per day was prescribed for the other days of the week for a total training volume of 10-15 h. Subjects performed all training sessions using their own equipment with monitoring (activity, duration, intensity, rate of perceived exertion), heart rate was also recorded.

Nutritional protocol

Before commencing the modified training/diet program, participants were randomly assigned to either a "sleep low" group (SL, n=11) or control (CON, n=10). All subjects received standardized dietary instructions depending on the dietary group to which they were assigned and instructed to follow prescribed menus that consisted of the same total food intake, with different timing of intake to ensure a divergent periodization over each day according to the allocated dietary treatment. Total daily CHO intake was similar for both SL and CON groups (~6 $g \cdot kg\ BM^{-1} \cdot d^{-1}$), but intake was allocated differently over the day to achieve high or low CHO availability before/after the various training sessions *(Table 2)*. Specifically, over 4 consecutive days in each week in which the training-diet intervention was implemented, the "sleep low" group was deprived of CHO intake from the period immediately after the HIT to completion of the LIT session. In addition, CHO was not consumed during HIT and LIT sessions. The evening meal for SL was CHO-free and the LIT sessions the following morning were performed after an overnight fast. Once the LIT session was completed, subjects then refueled with high quantities

of CHO-rich foods and drinks until the next HIT session. For the CON group, CHO availability was maintained throughout the recovery period and during each training session; a 6% CHO-electrolyte drink (Gatorade Performance Series®, Pepsico, USA) was consumed during training sessions and each meal included CHO-rich foods. To preserve muscle protein synthesis, both groups consumed a high protein sugar-free drink (High Protein 15 g per 20 mL, UHS Bruno, France) just prior going to bed.

The subjects completed a food diary throughout the last week of the baseline and throughout training/diet intervention and were instructed to provide details of the quantity, type and preparation of foods (food weights, pictures of dishes, details of fats and oils added in cooking or as dressings). The diaries were analyzed by the same scientist using the Nutrilog 2.31 software (Nutrilog SAS, Marans, France).

Exercise Tests

Pre-trial $\dot{V}O_{2max}$

On their first visit to the lab, subjects underwent an incremental cycling test at a self-selected cadence, previously described by Hawley and Noakes (20), on an electronically braked cycle ergometer (Excalibur Sport, Lode®, Groningen, The Netherlands). Saddle and handlebar heights were set to match the usual positions used by participants, and these were standardized between sessions. The cycle ergometer was equipped with individual racing pedals and toe clips, allowing participants to wear their own shoes. Subjects warmed-up for 6 min at 100 W then power output was increased by 25 W each successive 2 min until volitional exhaustion. Participants wore a face mask covering their mouth and nose to collect breath (Hans Rudolph,

Kansas City, MO, USA). During the test, oxygen uptake ($\dot{V}O_2$), carbon dioxide uptake ($\dot{V}CO_2$), minute ventilation ($\dot{V}E$) and the respiratory exchange ratio (RER) were continuously recorded and monitored as breath by breath values (Quark, Cosmed®, Rome, Italy). The gas and flow analyzers were calibrated prior to each test using ambient air, known-concentration gas and a 3 L syringe. $\dot{V}O_{2max}$ was determined based on the highest 30-s average value. MAP (W) was calculated as MAP = W completed + 25 x (t/120) where W is the last completed workload and t is the number of seconds in the last workload (20).

Performance tests

We investigated changes in endurance performance using three different successive exercise tests (15min rest between): subjects underwent two laboratory tests and one field-based protocol in the fed state. All tests were undertaken before the baseline training period to allow familiarization by subjects. These tests were then repeated immediately before (PRE) and after (POST) the training/diet intervention. Measurements collected during each exercise test included respiratory gas exchange, HR, ratings of perception of effort (RPE) and blood lactate concentrations. Whole body rates of CHO and fat oxidation (in $g \cdot min^{-1}$) were calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ values measured during the submaximal cycling test. The last minute of the intensity of interest and nonprotein RER values were used according to the following equations (23):

CHO oxidation = $4.210\dot{V}CO_2 - 2.962\dot{V}O_2$

Fat oxidation = $1.695\dot{V}O_2 – 1.701\ \dot{V}CO_2$

Submaximal test

The first laboratory test was a cycling test at a self-selected cadence to assess cycling efficiency (submaximal test). The test started with 6 min at 100 W followed by a 6-min stage at 70% of MAP. Efficiency represents a ratio between mechanical work during cycling and the energy expenditure (EE). We calculated it using delta efficiency that represents the incremental ratio measured between two stable states (here 100W and 70% of MAP). DE is often considered as a better cycling performance indicator and a valid estimate of muscular efficiency (10).

$$DE\ (\%) = \frac{\Delta \text{ work rate (joules)}}{\Delta \text{ energy expenditure (joules)}} \times 100$$

EE (kcal·min^{-1}) was obtained from the rate of oxygen uptake, using the equations developed by Brouwer (7) and based on thermal equivalent of O_2 for non protein RER.

EE was calculated as follow:

$$\boldsymbol{EE = \frac{\text{thermal equivalent of O2 x } \dot{V}\text{O2}}{1000}}$$

Supramaximal test

The second laboratory test was a supramaximal cycling test designed to last ~60-70 seconds. This test commenced at a submaximal work rate (3 min at 2 W·kg^{-1}). During this time subjects progressively increased their pedal cadence to 120 rev·min^{-1}, then the work rate was instantaneously increased to 150% of MAP (26). Subjects were instructed to pedal as strongly and as long as possible at this intensity and the test ended when cadence fell below 70 rev·min^{-1}. Care was taken to ensure that all subjects performed the tests in similar conditions, without standing up on the pedals and with strong verbal encouragement.

Simulation of triathlon race

The third test was a field-based protocol, designed to simulate the final leg of a triathlon race. The test commenced with 40 min cycling at 70% of MAP on a bicycle ergometer (6) at a self-selected cadence, immediately followed by a 10 km simulated running race. The 40-min cycling period was divided into three parts lasted 15 min, 15 min and 10 min, respectively, at 70% of MAP and separated by 45-s of active recovery at 100 W. During these short active recovery periods, the face mask was quickly released and cleaned, while subjects were provided with a drink bottle to allow hydration as in a race setting. Immediately after the cycling exercise, the subjects moved to a 340 m indoor running track and began a 10 km time-trial (TT). During the TT, subjects wore a HR monitor whose screen was hidden by a piece of masking tape to avoid any influence in performance. The lap time was continuously recorded by a researcher presents on the track. During the run, they were allowed to drink, when they wanted, a CHO-rich drink (45 g CHO per liter, Gatorade Performance Series®-Endurance Formula) as per current sports nutrition guidelines for competition performance. No significant difference was observed for the quantity of CHO ingested between both tests (P=0.62) and between groups (respectively for SL group; PRE vs. POST: 14.7 ± 7.21 g vs. 15.3 ± 6.43 g; P=0.47 and for CON group PRE vs. POST: 18.0 ± 15.5 g vs. 15.1 ± 11.3 g; P=0.50).

Body composition

Before and after the training/diet intervention period, measurement of whole body composition was undertaken on all subjects using dual-energy X-ray absorptiometry (Lunar IDXA, General Electric, Madison, USA). All measurements were taken early in the morning and in a fasted state (30) .

Blood parameters

Blood samples (5 mL) were collected from participants following an overnight fast on five occasions (before and after training/diet intervention, and before the last training session of each week). Samples were taken from a superficial forearm vein using standard venipuncture techniques and collected into EDTA tubes (Greiner Bio-one; Frickenhausen, Germany) for immediate processing.

Blood samples were centrifuged at 3,000 rev·min^{-1} for 10 min at +4 °C to separate plasma from red blood cells. The plasma samples were then divided into 1500 µL aliquots and stored in Eppendorf tubes at -80 °C until analysis. To avoid inter-assay variations, all blood samples were analyzed in a single batch at the end of the study. Epinephrine and norepinephrine concentrations were determined in plasma by enzyme-linked immunoabsorbent assay with commercially available high sensitivity ELISA kits (Demeditec Diagnostics GmbH, Kiel, Germany). All blood samples were analyzed in duplicate at the appropriate wavelength on a spectrophotometer Dynex MRXe (Magellan Biosciences, Chelmsford, MA, USA). Calibration curves were obtained for the standards using a non-linear regression for curve fitting.

Statistical analysis

All statistical analyses were conducted using Statistica 7.1 software (StatSoft). All data are expressed as mean ± SD. Data distribution was first checked using a Shapiro-Wilk normality test. Data which were not normally distributed were log-transformed (RPE values during submaximal test). A repeated-measures analysis of variance was used to calculate the effect of the dietary strategy (SL vs CON) and the period (PRE and POST) on performance, blood

parameters and body composition. When a significant effect was found, post hoc tests were performed using Newman–Keuls procedures. Effect sizes were, calculated using partial eta squared (η_P^2) values. Values of 0.1, 0.3 and over 0.5 were respectively considered as small, medium and large effect (12). For all tests, the significance level was set at $P < 0.05$.

RESULTS

Dietary intervention

The analysis of food records over the training/diet intervention period revealed that participants adhered to the prescribed dietary protocols and achieved a similar total CHO intake over a day. However, as intended, there was a different pattern of intake according to their group allocation. The SL group reported an average intake of 5.44 ± 1.2 $g \cdot kg^{-1} \cdot d^{-1}$ CHO (breakfast: 1.8 ± 0.66 $g \cdot kg^{-1}$; lunch: 2.2 ± 0.83 $g \cdot kg^{-1}$; snack: 1.8 ± 0.80 $g \cdot kg^{-1}$; dinner: 0.0 ± 0.1 $g \cdot kg^{-1}$), 1.57 ± 0.28 $g \cdot kg^{-1} \cdot d^{-1}$ protein (PRO) and 1.05 ±0.19 $g \cdot kg^{-1} \cdot d^{-1}$ FAT while the CON group consumed 5.65 ± 0.99 $g \cdot kg^{-1} \cdot d^{-1}$ CHO, 1.61 ± 0.22 $g \cdot kg^{-1} \cdot d^{-1}$ PRO and 1.02 ± 0.16 $g \cdot kg^{-1} \cdot d^{-1}$ FAT. Total energy intake during the intervention was similar between groups and over time. The SL group reported a mean daily intake of 2530 ± 660 $kcal \cdot d^{-1}$ during the baseline and 2685 ± 500 $kcal \cdot d^{-1}$ during the training/diet intervention period, while the intake of the CON group was reported as 2715 ± 645 $kcal \cdot d^{-1}$ and 2835 ± 505 $kcal \cdot d^{-1}$ during baseline and training/diet intervention periods, respectively. There was a similar change in reported macronutrient intake between baseline (usual dietary habits) and the training/diet intervention period (prescribed menus) for each group. Mean CHO intake significantly increased in the intervention period compared with the baseline (+26.5% and +24.9%, $P < 0.01$, for SL and CON groups, respectively), this was associated with an increase in protein intake (+16.1% and +19.2%, $P < 0.01$, for SL and CON

groups respectively). In both groups, there was a reduction in reported intake of fat during the training/diet intervention period compared with baseline (-13.1% and -16.4%, $P < 0.01$, for SL and CON groups, respectively). Dietary data are presented in table format in the supplementary content [see Table, Supplemental Digital Content 1, Total energy and macronutrient intake for SL and CON groups before starting the training program (BASELINE) and during the training/diet intervention (TRAINING) (mean ± SD), http://links.lww.com/MSS/A628].

Performance tests

Simulated triathlon race: 10 km running performance. There was a significant main effect of dietary group for 10 km running performance with all SL subjects improving their running time (PRE: 40:23 ± 03:22 min:s; POST: 39:10 ± 03:02 min:s) (*Figure 2).* This equates to a mean improvement of ~3% (-2.9 ± 2.1%, $P < 0.01$, d=0.38). No change was observed for CON (PRE: 41:26 ± 02:13 min:s; POST: 41:24 ± 02:43 min:s; change = -0.10 ± 2.7 %, NS).

Supramaximal test. SL increased supramaximal cycling time to exhaustion (PRE: 52.7 ± 13.8 s; POST: 57.8 ± 6.4 s; change = +12.5 ± 19%; $P= 0.062$, d=0.41) with no difference for CON (PRE: 57.8 ± 6.4 s; POST: 58.8 ± 10.7 s; change = +1.63 ± 12.4, NS).

Perceived exertion scores

Perceived exertion scores for various testing sessions are summarized in Table 3. A decrease in RPE was observed for SL group during tests performed at a fixed intensity (submaximal and cycling test).

Submaximal test. A significant interaction effect (strategy x period, $P < 0.05$, d=0.28) was recorded for the RPE values. A significant decrease in mean RPE values was recorded only in the SL group between PRE and POST tests (SL group: -5.9 ± 6.5 %, P<0.05, d= 0.72 *vs.* CON group: + 0.7 ± 3.9 %, NS).

Simulated triathlon race: cycling test. A significant effect of interaction (strategy x period, $P < 0.01$, d=0.42) was recorded for the RPE values at the end of the cycling test. A significant decrease in mean RPE values was recorded only in the SL group between PRE and POST tests (SL group: -9.2 ± 8.7 %, P < 0.05, d= 1.0 *vs.* CON group: +2.9 ± 5.9 %, d= 0.24).

Perceived exertion during training. RPE values recorded during all LIT training sessions (performed in a fasted state for the SL group) performed during the training intervention were significantly higher in the SL group when compared with CON group (respectively for SL and CON group: 14.3 ± 0.29 *vs.* 12.7 ± 0.43, $P < 0.05$, d= 4.36).

Physiological parameters

The results of the submaximal test are presented in *Table 4*. There was a significant improvement in DE values for the SL group ($P<0.05$, d=0.48) and a decrease in HR ($P = 0.058$, d=0.45). Furthermore, in this group CHO oxidation was significantly decreased ($P < 0.001$, d=0.66). There was a significant effect of time for RER values with a decrease between PRE and POST tests ($P < 0.05$) but with no difference between groups.

Blood parameters

Plasma epinephrine concentration increased during the training/diet intervention period (blood collection performed the morning after an overnight fast, before the last prescribed training session of the week) compared to baseline values (78.5 ± 29.3 pg·mL^{-1}) for SL (respectively for W2 and W3, 94.4 ± 41.8 pg·mL^{-1}, d=0.44; and 94.7 ± 37.5 pg·mL^{-1}, d=0.48) (*Table 5*) but not CON (baseline: 56.7 ± 30.0 pg·mL^{-1}, W1: 49.6 ± 25.0 pg·mL^{-1}, W2: 41.8 ± 23.1 pg·mL^{-1}, W3: 44.3 ± 18.3 pg·mL^{-1}).

Body composition

There was a significant decrease in body mass (BM) in the SL group over the training block (PRE: 70.6 ± 5.0 kg *vs.* POST: 69.6 ± 5.0 kg, $P < 0.001$, d=0.19). This was the result of a significant loss of fat mass in the SL group over this period (PRE: 9.70 ± 4.08 kg *vs.* POST: 8.86 ± 3.8 kg $P < 0.01$, d=0.22). No changes were detected in the CON group over the course of training (BM: PRE: 72.8 ± 3.9 kg *vs.* POST: 72.4 ± 4.1kg; Fat mass: PRE: 8.9 ± 2.3 kg *vs.* POST: 8.6 ± 2.4 kg). No difference in fat free mass was observed for both groups after training period. Body composition data are presented in table format in the supplementary content [see Table, Supplemental Digital Content 2, Body composition before and after the training program for SL and CON group (mean ± SD), http://links.lww.com/MSS/A629].

DISCUSSION

We investigated the effects of a chronic dietary periodization strategy in endurance-trained triathletes who undertook a strenuous three-week training block. We "clamped" energy intake for both SL and CON groups so that self-reported total energy intakes (~2700 kcal.d^{-1})

and macronutrient composition were similar (6 $g \cdot kg^{-1} \cdot d^{-1}$ CHO, 1.6 $g \cdot kg^{-1} \cdot d^{-1}$ PRO and 1.0 $g \cdot kg^{-1} \cdot d^{-1}$ FAT), but altered the timing of nutrient intake so that CHO availability was different for selected training sessions. The main findings were that: (1) 10 km running performance was improved when athletes periodized their CHO intake and slept and performed selected training sessions with low-CHO availability; (2) submaximal cycling efficiency was enhanced and ratings of perceived exertion were lower during constant exercise after this "sleep-low" strategy, and (3) body mass and body fat mass were reduced in response to altering the timing of CHO intake during the 3-week training block.

To the best of our knowledge, this study is the first to report a robust improvement in athletic endurance performance in response to a chronic training regimen in endurance-trained athletes after integrating workouts with low CHO availability. Our periodized diet-exercise protocol used three different types of dietary CHO manipulation to specifically alter the availability of this substrate before, during and/or after targeted training sessions. The combination of high intensity training with high CHO availability, low CHO availability post-exercise (i.e., sleeping low), and low intensity training with low CHO availability was designed to integrate the potential benefits of three different training/nutrition responses on whole-body and performance outcomes. Specifically these were: nutritional support for high intensity workouts, a prolonged period of potentially elevated post-exercise signaling responses, and an enhanced metabolic stimulus to prolonged submaximal training, respectively. Each of these diet/exercise strategies has been individually identified as beneficial for competitive athletes, and their integration into a periodized training program has previously been proposed (9) and practiced by elite athletes (35, 36). However, the novel aspect of the current study is that we

successfully implemented these exercise-dietary strategies in trained athletes in association with both lab- and field-based measures of performance. Hence, a major finding was a clear and robust improvement of 10 km running performance after implementation of a "sleep-low" strategy: every subject in the SL group showed an enhanced performance in 10-km race time (average improvement: 73 ± 20 s), whereas no improvement was recorded for the CON group.

Our dietary periodization strategy also enhanced the capacity for high-intensity exercise lasting 60-70 sec in endurance-trained subjects. This improvement is similar in magnitude to that previously reported by Lindsay et al. (26) who implemented a HIT training program in well-trained cyclists (6 sessions undertaken within a 28 d training block). HIT is well known to rapidly improve endurance performance and fatigue resistance, even for well-trained athletes (14, 26). Our study provides evidence, however, that periodizing CHO availability around these training sessions is an important determinant of the performance results. Although both groups in our investigation followed the same training program, incorporating 9 HIT sessions over 3 weeks, the timing of CHO availability around the specific training sessions were associated with a difference in performance outcomes. Furthermore, in relation to the previous studies of "train low" strategies in trained individuals which have failed to find superior performance outcomes (i.e. greater than the control group), we note that their protocols exposed the HIT rather than accompanying low intensity sessions to low CHO availability (22, 29); this choice reduced the intensity of work that could be achieved in these key workouts and potentially counteracted the benefits of the session (metabolic adaptations and performance improvement) (4).

Only two studies have reported an enhanced improvement in performance tasks following "train low" strategies in comparison to a traditional training diet with sustained high CHO availability; however, issues with the test protocols or the training status of the subjects have made it difficult to extrapolate the results of these investigations to the preparation of competitive athletes. Hansen et al. (16) reported a greater increase (19.7 ± 2.4 min *vs.* 11.9 ± 1.3 min) in time to exhaustion of a unilateral isokinetic "leg kicking" protocol after a 10-week training program in the leg which undertook 50% of sessions with lowered glycogen (2-a-day training program with the second session commenced after depleting glycogen via the first session) compared to the leg which undertook the same training with opportunity for daily glycogen restoration. However, the subjects in that study were previously untrained individuals who undertook a training protocol in which exercise intensity for both legs was clamped. More recently, Cochran et al. (11) studied the chronic (2 weeks) effect of lower-CHO availability between two HIT sessions undertaken in a single day on whole-body exercise performance (cycling time-trial and repeated-sprints test). Active adults were provided with either a CHO-rich or CHO-restricted meal during the 3 h recovery between the two training sessions on each of 6 days during the study period. The training-induced improvement of the mean power output during a 250-kJ time trial performance was greater (P = 0.02) in the group that undertook the second session with reduced CHO availability (pre: 211 ± 66 W, post: 244 ± 75 W) compared with the group that refueled prior to that session (pre: 203 ± 53 W, post: 219 ± 60 W). By contrast, there was no difference between groups in the improvement in a repeated sprints test (5 x 15 s all out sprints). It should be noted that subjects in that study were not trained, and the HIT sessions were undertaken at a constant power.

To date, studies that have investigated different train-low strategies in trained populations (22, 40) have failed to detect enhancements in performance beyond those attained by training with the more traditional dietary support of high-CHO availability. Despite strong evidence of enhanced metabolic capacity at cellular (increased levels of key enzymes: β-hydroxyacyl-coA-dehydrogenase and citrate synthase) and whole body levels (increased fat oxidation at submaximal work intensities) after various "train low" strategies, it appears that these adaptive markers do not always translate into superior performance (3). This highlights that sports performance is multi-factorial and can be enhanced by a variety of training inputs and physiological/neurological/psychological adaptations. Since previous studies have observed a reduction in power outputs and work during high-intensity workouts (22, 40) normally associated with a real-world training program, it is likely that a compromise in one aspect of athletic preparation could counteract the benefits achieved in another and compromise the overall outcome (22, 40).

The current study included several novel features to address the multiple goals in the preparation of competitive athletes. We focused on the recruitment of endurance-trained triathletes for whom an enhancement of performance is incremental and challenging to achieve. Second, we included a 3-week baseline phase to harmonize the training programs and fitness levels of the athletes as well as to familiarize them with the training and performance protocols. Finally, we periodized CHO availability in the training program according to the goals of different training sessions to balance the divergent nutritional approaches to high quality training and enhanced adaptive response. We believe that this approach was a key element which underpinned the outcomes of the investigation: even though all participants had been adhering to

a high training load for many years, those who undertook the periodized CHO support were able to achieve a substantial improvement in their performance level after only 3 weeks. We include a high protein sugar-free drink as part of the 'train-high, sleep-low' regimen ingested before subjects went to bed. The ingestion of proteins following an exercise bout increases skeletal muscle protein synthesis, and has been described to support muscle repair and remodeling (1, 28). Thus the ingestion of high-quality protein during recovery may also have maximized training adaptations by promoting the synthesis of mitochondrial proteins or metabolic enzymes (31). These conditions may have all played some part in the enhanced training adaptations and improved performance.

The clear performance improvements observed in this study were accompanied by significant reductions in the physiological and perceived loads during submaximal testing sessions performed at fixed intensities. Cycling efficiency was improved and HR values decreased only in the SL group, indicating a decrease in the relative energy cost of a given exercise intensity. A decrease in the energy cost of cycling has previously been shown to improve the performance of a running protocol undertaken immediately after the cycling bout (39). These findings may, in part, explain the enhanced 10 km running performance we observed after athletes had undertaken a bout of prior cycling exercise.

Interventions that influence an individuals' perception of effort are known to affect endurance performance (27). The RPE values for morning training sessions undertaken by our subjects were higher for the SL group, who performed these sessions in a fasted state, compared to the CON group. However, perceived exertion during performance tests at a fixed intensity

with high CHO availability were lower for the SL group compared to no changes were detected in the CON group. We cannot exclude the possibility that our novel exercise-dietary protocol induced a placebo effect among subjects (15). Indeed, there is growing body of evidence of the impact of the participant's beliefs and expectations on performance outcomes (15, 27).

The observed improvements in endurance performance were accompanied by a small but significant changes in body composition after 3 weeks of the training/diet intervention. Indeed, body mass decreased (by ~1 kg) only in the SL group, with this outcome being mainly attributed to a 1.1% decrease in fat mass. The small but non-significant difference in energy intake between groups does not appear sufficient to account for these observations, although we acknowledge the limitations of self-reported dietary data. Indeed, the apparent energy intake of the group was less than might be expected for athletes undertaking the current training program and remaining in energy balance. However, the propensity to under-reporting energy intake via food diaries is well-known, especially in athletic populations (8), and there is no reason to suspect that this would occur more prominently in one group of our subjects than the other. Another explanation for the leaner body composition in the SL group could be increased lipid oxidation (13), as inferred by the reduction in RER values during the submaximal cycling test. This decrease in body mass may have contributed to the faster running performance. Indeed, running economy, an important determinant of endurance running performance, is negatively correlated with body mass (2). Our periodization of CHO availability and the inclusion of high-intensity training may be an effective method to alter body composition and thus improve endurance performance during the weeks preceding competitions. It should be noted that both elements seem important,

since the implementation of the HIT sessions alone (as undertaken by the CON group) did not result in a body composition change.

A potential limitation of our study design was that we focused on a real-world intervention and measures of athletic performance. As such, we did not have the opportunity to undertake the invasive techniques needed to explore some of the mechanisms that may have contributed to the observed performance outcomes (i.e., muscle biopsies, tracer techniques to determine substrate turnover and oxidation). Nevertheless, a companion study that utilized essentially the same exercise-nutrient strategy (24) attempted to examine some of the possible cellular and molecular aspects related to the 'train-high, sleep-low' protocol. The variations in CHO availability integrated into our dietary periodization protocol were chosen to achieve different metabolic and training goals. First, the HIT sessions were undertaken with high CHO availability to facilitate the highest attainable power outputs and running/cycling intensities and gain the associated neural, metabolic and biomechanical adaptations (4). The subsequent withholding of CHO for a prolonged period after the HIT training sessions ("sleep low") may have further contributed to the observed performance increase by extending the duration of the exercise-stimulated enhancement of transcriptional activation of specific genes involved in mitochondrial biogenesis and muscle fuel utilization that underpin the adaptation to training (33, 34). Finally, increased catecholamine activity is observed and is also known to enhance metabolic adaptation via mechanisms including an increase in the expression of the transcriptional co-activator PGC-1α(25). The final element of the dietary periodization protocol involved the completion of a steady-state bout of exercise with low CHO availability (overnight fasting, reduced muscle glycogen stores and absence of CHO intake during the session). Each of

these elements has been shown to amplify the training response with mechanisms including enhanced activation of key cell signaling kinases (e.g. AMPK, p38MAPK), transcription factors (e.g. p53, PPARδ) and transcriptional co-activators (e.g. PGC-1α), leading to a co-ordinated up-regulation of both the nuclear and mitochondrial genomes. All theses adaptations lead to an up-regulation of the lipid metabolism as observed in other studies with a higher lipid oxidation and higher resting glycogen content (5, 16, 24, 40), improving performance in long duration exercises.

In conclusion, this study is the first to report a robust improvement in a 'real world' test of endurance performance after 3 weeks of a program involving periodization of CHO availability (high-intensity training with high CHO availability, recovery with low CHO availability and low intensity training with reduced muscle glycogen and in a fasted state) in endurance-trained triathletes. The enhancement in performance observed after our dietary periodization protocol ("train high, sleep low") was not seen in a control group of athletes who undertook the same training regimen but with a more traditional approach to nutrition (i.e., high-CHO availability around training sessions). The manipulation of the timing of CHO intake in relation to exercise isolates periods of the training program according to their different priorities of performance or adaptation. These results reinforce the growing evidence that CHO availability is a potent mediator in the adaptive response to endurance training (5, 19, 32) and that periodizing it within the training program to include both sessions with high fuel-support to promote high quality/high intensity training and sessions/recovery promoting enhanced metabolic adaptation can lead to a superior training outcome. A limitation of our study design was that the involvement of endurance-trained individuals and the focus on real-world

interventions and measurement of performance reduced the opportunity to undertake the invasive techniques often needed to explore potential mechanisms for the observed outcomes. Accordingly future studies are needed to better understand the mechanisms underlying changes in performance with "train-low" protocols, as well as to determine the applicability of this approach to training in already well-trained athletes.

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Conflicts of Interest and Source of Funding

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FIGURE CAPTIONS

Figure 1- Diagram of the experimental protocol

Figure 2- 10 km running performance before (PRE) and after (POST) 3 weeks of training with periodized CHO availability (SL group) vs. training with maintained CHO availability (CON group). Individual changes (dashed lines) and group mean (continuous lines). *: $P < 0.05$ compared to PRE SL values.

SUPPLEMENTAL DIGITAL CONTENT

Supplementary Digital Content 1 – Total energy and macronutrient intake for SL and CON groups before starting the training program (BASELINE) and during the training/diet intervention (TRAINING) (mean ± SD).

Supplementary Digital Content 2 - Body composition before and after the training program for SL and CON group (mean ± SD).

Figure 1

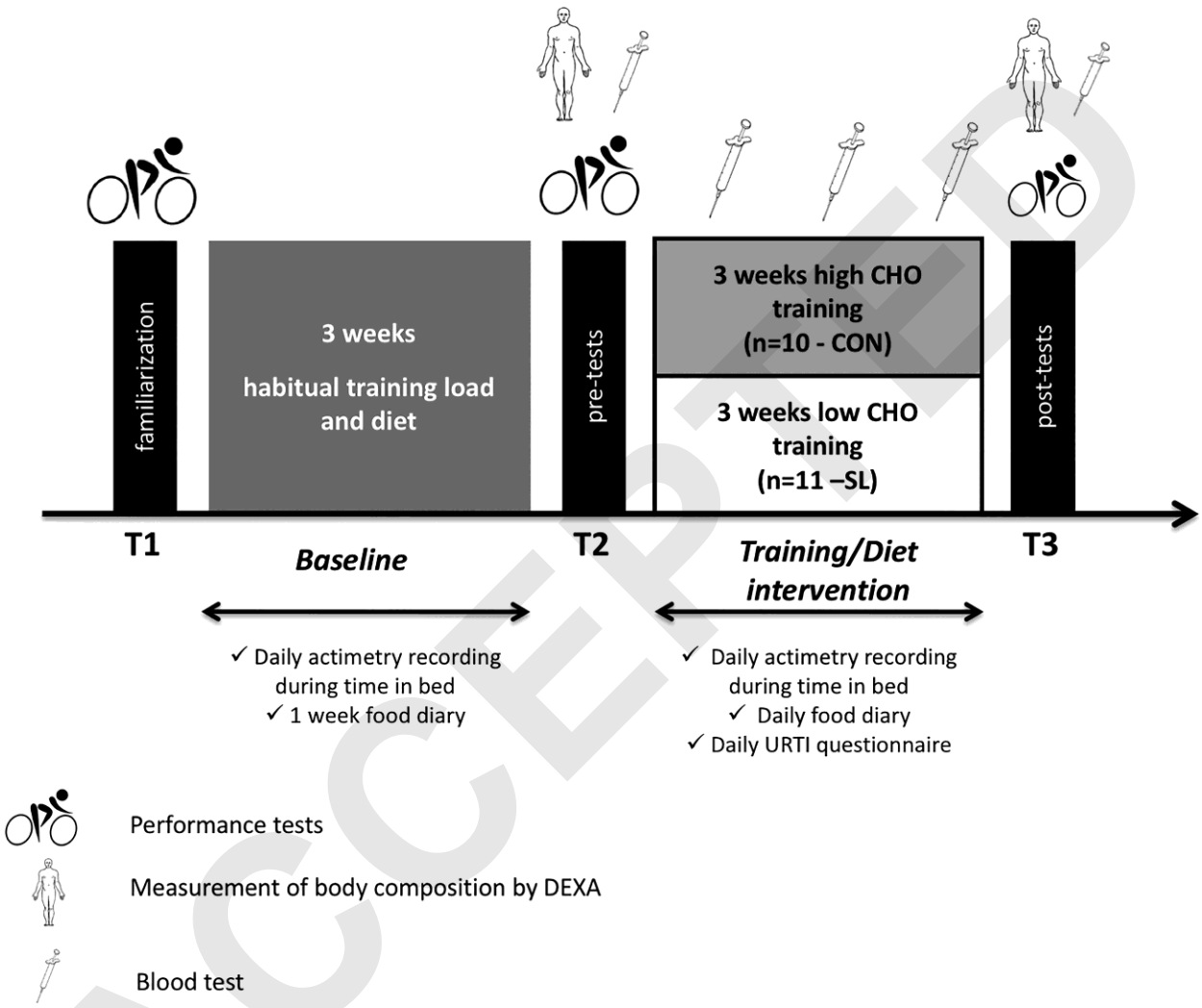

Figure 2

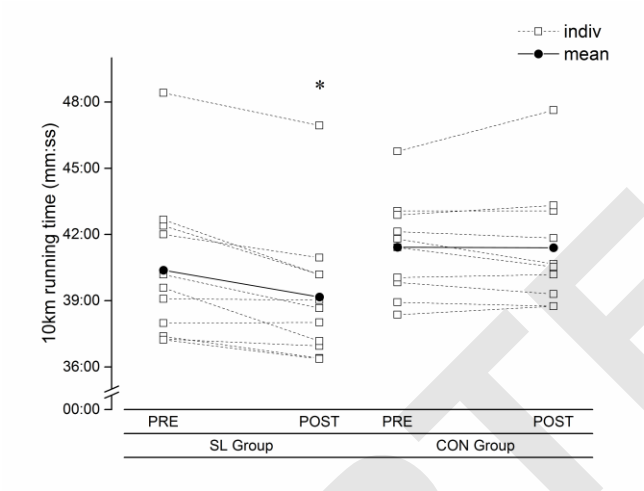

Table 1- Characteristics of subjects in the SL and CON groups (mean ± SD).

	Sleep Low group (SL) (n=11)	Control group (CON) (n=10)
Age (yr)	32.0 ± 4.2	29.3 ± 5.2
Height (m)	1.78 ± 0.04	1.81 ± 0.05
Body mass (kg)	70.6 ± 5.0	72.8 ± 3,9
$VO_{2\ max}$ ($ml.min^{-1}.kg^{-1}$)	60.1 ± 6.8	60.2 ± 4.5
MAP (W)	325 ± 33	349 ± 25
Hours of training ($h.wk^{-1}$)	12:38 ± 02:10	11:00 ± 02:07

MAP: maximal aerobic power

Table 2- Sample weekly protocol for training and CHO intake ($g \cdot kg^{-1}$) to achieve different CHO availability around training sessions

	D1			D2 and D3			D4			D5 to D7
	Diet SL	**Train**	**Diet CON**	**Diet SL**	**Train**	**Diet CON**	**Diet SL**	**Train**	**Diet CON**	
Before 10 am	Breakfast (2 g)		Breakfast (2 g)			Breakfast (2 g)			Breakfast (2 g)	
					LIT 🚲	Sports Drink (0.5 g)		LIT 🚲	Sports Drink (0.5 g)	
				Breakfast+ Sports Drink (2.5 g)			Breakfast + Sports Drink (2.5 g)			
Midday	Lunch (2 g)		Lunch (2 g)	Lunch+Sport Drink (2.5 g)		Lunch (2 g)				Free LIT : 1 session per day Water during training
	Snack (2 g)			Snack (2 g)						
After 5 pm		HIT 🚲	Sports drink (0.5 g)		HIT D2 🚲 D3 🚹	Sports drink (0.5 g)	Usual diet		Usual diet	Usual diet
			Snack (1 g)			Snack (1 g)				
	Dinner (No CHO)		Dinner (1g)	Dinner (No CHO)		Dinner (1g)				
	Prot Drink (No CHO)		ProtDrink (No CHO)	ProtDrink (No CHO)		ProtDrink (No CHO)				

Total content of meals and snacks for day are identical for the Sleep Low and the Control, but the CHO content ($g \cdot kg^{-1}$) is spread as indicated.
HIT, high intensity training sessions, 🚲 cycling: 8x 5 min @ 85% of MAP + 1 min recovery, 🚹 running: 6 x 5 min @ 10 km triathlon speed + 1 min recovery; LIT, Low intensity training session, 🚲 cycling: 1h, 65% of MAP

Table 3-RPE values (Rating Perceived Exertion) for the different performance tests before and after the training program for SL and CON group (mean ± SD)

				End of a triathlon race	
		submaximal test	supramaximal test	cycling test	10km running test
SL group	PRE	16.3 ± 1.4	17.8 ± 1.0	15.82 ± 1.3	17.4 ± 1.5
	POST	15.3 ± 1.4 *	17.7 ± 1.3	14.40 ± 1.5 *	17.6 ± 1.3
CON group	PRE	15.5 ± 1.0	17.6 ± 1.1	15.8 ± 1.2	16.8 ± 1.9
	POST	15.6 ± 1.0	17.2 ± 1.6	16.1 ± 1.4	16.6 ± 1.4

*: $P < 0.05$ as compared to PRE values

Table 4- Physiological parameters recorded during the submaximal cycling test performed before and after the training program for SL and CON groups (mean ± SD)

		PRE		POST	
		100 W	*70% MAP*	*100 W*	*70% MAP*
Heart Rate	SL	112.8 ± 10.1	157.7 ± 9.4	110.4 ± 9.4	153.7 ± 11.0
(bpm)	CON	112.9 ± 9.9	155.8 ± 8.7	110.0 ±8.5	154.2 ± 7.1
VO_2	SL	26.7 ± 2.2	47.4 ± 4.8	27.7 ± 2.7	46.7 ± 4.9
($mL.min^{-1}.kg^{-1}$)	CON	26.5 ± 1.6	47.8 ± 4.3	26.5 ± 2.8	47.9 ± 5.0
RER values	SL	0.86 ± 0.03	0.94 ± 0.03	0.84 ± 0.04	0.91 ± 0.03
	CON	0.85 ± 0.06	0.94 ± 0.03	0.85 ± 0.04	0.91 ± 0.03
	SL	1.21 ± 0.27	3.24 ± 0.62	1.07 ± 0.29	2.89 ± 0.42 *
CHO oxidation ($g.min^{-1}$)	CON	1.21 ± 0.46	3.39 ± 0.53	1.16 ± 0.30	3.13 ± 0.71
Fat oxidation	SL	0.44 ± 0.11	0.34 ± 0.18	0.53 ± 0.14	0.46 ± 0.14
($g.min^{-1}$)	CON	0.46 ± 0.19	0.35 ± 0.19	0.47 ± 0.16	0.45 ± 0.32
Blood lactate	SL	1.8 ± 0.7	4.8 ± 2.1	2.3 ± 1.4	4.2 ± 2.3
($mmol.L^{-1}$)	CON	2.2 ± 0.9	5.0 ± 1.6	2.0 ± 1.1	4.2 ± 1.0
			70% - 100W		*70% - 100W*
Delta Efficiency	SL		26.5 ± 4.5		29.6 ± 8.0 *
(DE-%)	CON		26.3 ± 2.5		26.5 ± 2.4

VO_2: oxygen uptake; RER: respiratory exchange ratio; *: $P < 0.05$ as compared to PRE values.

Supplementary Digital Content 1 – Total energy and macronutrient intake for SL and CON groups before starting the training program (BASELINE) and during the training/diet intervention (TRAINING) (mean ± SD).

		Total energy intake ($kcal.d^{-1}$)	Carbohydrate intake ($g.kg^{-1}.d^{-1}$)	Lipid intake ($g.kg^{-1}.d^{-1}$)	Protein intake ($g.kg^{-1}.d^{-1}$)
SL group	BASELINE	2530 ± 660	4.44 ± 1.3	1.33 ± 0.49	1.39 ± 0.28
	TRAINING	2685 ± 500	5.44 ± 1.2 *	1.05 ± 0.19 *	1.57 ± 0.28 *
CON group	BASELINE	2715 ± 645	4.79 ± 1.12	1.32 ± 0.43	1.44 ± 0.37
	TRAINING	2835 ± 505	5.65 ± 0.99 *	1.02 ± 0.16 *	1.61 ± 0.22 *

*: $P < 0.05$ as compared to PRE values

Supplementary Digital Content 2 - Body composition before and after the training program for SL and CON group (mean ± SD).

		Body mass (kg)	Fat Free mass (kg)	Fat mass (kg)
SL group	PRE	70.57 ±5.02	57.86 ± 29.1	9.70 ± 4.08
	POST	69.6 ± 4.97 *	57.76 ± 27.3	8.86 ± 3.78 *
CON group	PRE	72.79 ± 3.94	60.82 ± 45.3	8.86 ± 2.26
	POST	72.42 ± 4.13	60.68 ± 45.3	8.63 ± 2.36

*: $P < 0.05$ as compared to PRE values