Influence of Periodizing Dietary Carbohydrate on Iron Regulation and Immune Function in Elite Triathletes

McKay, Alannah K A; Heikura, Ida A; Burke, Louise M; Peeling, Peter; Pyne, David B; van Swelm, Rachel P L; Laarakkers, Coby M; Cox, Gregory R

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Abstract

Sleeping with low carbohydrate (CHO) availability is a dietary strategy that may enhance training adaptation. However, the impact on an athlete's health is unclear. This study quantified the effect of a short-term "sleep-low" dietary intervention on markers of iron regulation and immune function in athletes. In a randomized, repeated measures design, 11 elite triathletes completed two four-day mixed cycle-run training blocks. Key training sessions were structured such that a high-intensity training (HIT) session was performed in the field on the afternoon of days 1 and 3, and a low-intensity training (LIT) session on the following morning in the laboratory (days 2 and 4). The ingestion of CHO was either divided evenly across the day (HIGH), or restricted between the HIT and LIT sessions, so that the LIT session was performed with low CHO availability (LOW). Venous blood and saliva samples were collected prior to and following each LIT session and analyzed for interleukin-6 (IL-6), hepcidin-25 and salivary immunoglobulin-A (s-IgA). Concentrations of IL-6 increased acutely after exercise ($p<0.001$), but did not differ between dietary conditions or days. Hepcidin-25 increased 3 h post-exercise ($p<0.001$), with the greatest increase evident after the LOW trial on day 2 (2.5±0.9 fold increase±90% CL). The s-IgA secretion rate did not change in response to exercise, however, was highest during the LOW condition on day 4 ($p=0.046$). There appears to be minimal impact on markers of immune function and iron regulation when acute exposure to low CHO availability is undertaken with expert nutrition and coaching input.

Key words: sleep low; train low; hepcidin.

Introduction

Acute carbohydrate (CHO) restriction around exercise can enhance molecular adaptations to training by augmenting cell signaling, gene expression, enzyme activity and lipid oxidation (Impey et al., 2018). These outcomes inform nutritional guidelines, which advise athletes to strategically approach CHO periodization (Jeukendrup, 2017). One strategy is the "sleep low" sequence (Burke et al., 2018), which involves commencing a high-intensity training session in the evening with high CHO availability, before restricting CHO intake at the subsequent meal and overnight. This protocol supports high quality training for the first session, augmented by an enhanced period of cellular signaling related to delayed restoration of muscle glycogen. The following morning's submaximal exercise session is performed under conditions of low muscle and liver glycogen, promoting higher reliance on fat oxidation, greater metabolic stress and cellular adaptation (Bartlett et al., 2015). While careful integration of such strategies can enhance adaptation and performance (Marquet et al., 2016), the potential implications on athlete health are relatively unknown. Accordingly, concern centers on transient immunodepression associated with endurance exercise, which might otherwise be attenuated by CHO ingestion. Current research shows that low or declining levels of salivary immunoglobulin-A (s-IgA) are associated with increased risk of upper respiratory illness (URI) (Bishop & Gleeson, 2009); however, both increases (McKay et al., 2018) and decreases (Louis et al., 2016) in s-IgA levels are reported in response to a 3-week periodized CHO intervention; thus, the acute impact of this strategy is unclear.

Training with low CHO availability can also increase post-exercise concentrations of the inflammatory cytokine interleukin-6 (IL-6) (Hennigar et al., 2017), which may have downstream implications for the iron-regulatory hormone hepcidin (Badenhorst et al., 2015). Adherence to a low CHO diet (3 g/kg) for 24 h can amplify the immediate post-exercise IL-6, and 3 h post-exercise hepcidin response as compared to high (10 g/kg) CHO availability (Badenhorst et al., 2015).

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Increases in hepcidin levels occur ~3-6 h post-exercise (Peeling et al., 2009), potentially reducing dietary iron absorption and macrophage iron recycling (Nemeth et al., 2004). Despite the potential long-term implications to athlete health and performance, studies exploring these effects in elite athletes are lacking. Therefore, we quantified the effect of a sleep-low protocol in the daily training environment on markers of inflammation, iron regulation and immune function in elite triathletes. Given that exercise modality can also influence the post-exercise inflammatory response (Nieman et al., 1998), we compared responses between exercise modalities.

Methods

Participants

Four male (22.5 ± 3.0 y, 64.3 ± 4.1 kg, 39 ± 9 mm for sum of 7 skinfolds, 74.8 ± 6.2 and 76.6 ± 1.6 $ml \cdot kg^{-1} \cdot min^{-1}$ for cycle and treadmill $\dot{V}O_{2peak}$ respectively; mean±SD) and seven female (26.4 ± 2.0 y, 56.4 ± 5.4 kg, 62 ± 20 mm for sum of 7 skinfolds, 66.2 ± 5.1 and 65.3 ± 4.4 $ml \cdot kg^{-1} \cdot min^{-1}$ for cycle and treadmill $\dot{V}O_{2peak}$, respectively) triathletes with >18 months of elite training history were recruited. Skinfold measurements were undertaken by an accredited anthropometrist according to International Society for the Advancement of Kinanthropometry standards. Athletes were free from injury, illness, or iron deficiency (serum ferritin >30 $\mu g \cdot L^{-1}$); none were taking iron supplements during the testing period. Written informed consent was obtained prior to participation. Approval was granted by the Australian Institute of Sport (AIS) Ethics Committee.

This study was conducted during an AIS training camp over a 4-week period between two World Triathlon Series events. In a randomized, crossover design athletes completed two 4-day experimental trials with high CHO availability (HIGH) or adopting an alternate day sleep-low protocol (LOW) (see Figure 1). Athletes completed familiarization tests prior to intervention commencement, from which, workloads for subsequent testing sessions were derived. Tests included a 4-min maximal cycling test to determine cycling $\dot{V}O_{2peak}$ (Gore et al., 1998) and mean maximal

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power (MMP), and a graded treadmill test to determine $\dot{V}O_{2peak}$ and running velocity at $\dot{V}O_{2peak}$ ($v\dot{V}O_2$), as described elsewhere (Tanner & Gore, 2013). Additionally, a venous blood sample was collected from female athletes to measure sex hormone concentrations and determine menstual phase. Athletes were subsequently pair-matched based on performance measures and menstrual phase, before being randomized into their first dietary condition.

Nutrition Interventions

During experimental trials, a standardized daily CHO intake of 6 (females) or 8 $g \cdot kg^{-1}$ body mass (males) was consumed, with differences attributed to disparities in lean body mass and habitual CHO intake between sexes. Athletes completed a directed daily dietary record and ate in a dining hall setting where all meal options where prepared from standardized recipes, allowing free-living with a high degree of dietary control. Athletes were provided with individualized CHO targets for each meal, and were guided to self-select food and fluid to accommodate dietary preferences while meeting dietary targets. A research team member attended all meals to ensure compliance, before undertaking subsequent analysis (Food Works 8 Professional program; Xyris Software Australia Pty Ltd, Australia). Although each athlete's individual CHO intake was matched across experimental trials, CHO intake was periodized differently across days according to trial allocation. During LOW, a high intensity training (HIT) session was performed in the afternoon to deplete muscle glycogen stores on days 1 and 3 (Table 1). CHO intake was scheduled predominantly pre HIT with post-session consumption restricted (<0.5 $g \cdot kg^{-1}$) ahead of a low intensity aerobic session (LIT) the following morning. During HIGH, the CHO intake was scheduled evenly throughout the day and following the HIT session to facilitate overnight recovery of muscle glycogen stores.

Training

Athletes completed an individualized training program, involving 3-4 daily sessions of running, swimming, cycling and resistance training (Table 1). Training was structured to include a field-based

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HIT session on the afternoons of day 1 and 3, and a laboratory-based LIT session the following morning (day 2 and 4). This design achieved 2 repetitions of the sleep-low protocol within each 4-day period. This protocol incorporated mixed-mode training practices for elite triathletes, with cycling sessions performed on days 1 and 2 and running sessions on days 3 and 4.

High Intensity Training Sessions

To ensure high external validity and account for the proximity of the upcoming international event, all HIT sessions were individually prescribed and led by an international-level coach who understood the study goals. During the HIT cycling session (day 1), athletes completed an interval-based session (~45 min) at an outdoor velodrome on a fixed-gear bike, in addition to cycling to and from the training venue (LOW: 31.2±5.1 km, 76.1±9.0 min return; HIGH 28.8±0.9 km, 70.5±1.9 min return). The HIT running session (day 3) was interval-based, prescribed from time and/or distance to equal ~10 km (LOW: 53.2±5.5 min, mean heart rate (HR) 166±6 bpm; HIGH: 53.2±9.5 min, mean HR 165±11 bpm).

Low Intensity Training Sessions

Athletes reported to the laboratory for LIT sessions at a standardized time in an overnight fasted and rested state. A 4 mL baseline venous blood sample was collected from an antecubital vein before athletes consumed a snack in accordance with their dietary allocation (1.5 v 0.0 $g \cdot kg^{-1}$ CHO for HIGH and LOW, respectively); 25 min later, they provided a saliva sample. Thirty min post-snack, athletes commenced their planned LIT task. The 60 min cycle trial (day 2) was performed in the laboratory on the athlete's own bicycle mounted to a stationary cycle ergometer. The 45 min running trial (day 4) was conducted as a hybrid laboratory-field test, with the first and final 5 min completed on a motorized treadmill, and the remainder completed on a flat, outdoor course. The first and final 5 min of each trial were completed at 65% MMP (cycle) or $vV\dot{O}_2$ (run) with the remaining distance

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completed at ~55% MMP or $vV\dot{O}_2$. During HIGH, athletes consumed 0.5 $g \cdot kg^{-1}$ CHO during exercise, while water was consumed during LOW. Mean HR and respiratory exchange ratio were determined for the final 5 min of each session, while venous blood and saliva samples were collected immediately and 15 min post-exercise, respectively. Athletes received a whey protein shake immediately following saliva collection (0.3 $g \cdot kg^{-1}$ whey protein) and a CHO-rich breakfast 1 h post-exercise (2 $g \cdot kg^{-1}$ CHO) to ensure recovery and avoid hunger. Venous blood samples were collected 1 and 3 h post-exercise.

Experimental Procedures

Blood Sampling

Venous blood samples were collected into 4 mL SST gel separator tubes and left to clot for 30 min before centrifugation (2200 G for 10 min). Serum was divided into 1 mL cryotubes and frozen at -80°C until batch analysis of iron, serum ferritin, IL-6 and hepcidin-25 concentrations. Pre-exercise serum iron and ferritin concentrations were quantified via a COBAS Integra 400 automated biochemistry analyzer (Roche Diagnostics, Switzerland). IL-6 concentrations were analyzed in duplicate pre-, post-, 1 h and 3 h post-exercise using an ELISA (Quantikine HS, R&D Systems, Minneapolis, USA; CV=5.7%). Hepcidin-25 measurements were performed on serum obtained pre- and 3 h post-exercise (www.hepcidinanalysis.com, Nijmegen, The Netherlands) using a combination of weak cation exchange chromatography and time-of-flight mass spectrometry (Kroot et al., 2010; Swinkels et al., 2008). When values were below the lower limit of detection of 0.5 nM, this value divided by the square root of 2 was used (Croghan & Egeghy, 2003).

Saliva Sampling

Prior to, and following each LIT session, an unstimulated saliva sample was obtained 10 min after a 40 ml water mouth rinse. Prior to sample collection, a >20 min window where no CHO was ingested

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was employed to minimize potential sample contamination. Samples were collected using a saliva collection aid (Salimetrics, State College, PA) over a timed 2 min period into 2 ml cryotubes and subsequently weighed. Assuming a saliva density of 1.00 $g \cdot mL^{-1}$ (Cole & Eastoe, 1988), flow rate ($mL \cdot min^{-1}$) was calculated by multiplying saliva volume by collection time. Samples were centrifuged at 1500 G for 15 min, before s-IgA concentrations were analyzed in duplicate (EIA kits, Salimetrics, State College, PA; CV=5.8%). Secretion rates ($\mu g \cdot min^{-1}$) were calculated by multiplying the salivary flow rate by s-IgA concentration.

Statistical Analysis

Data were analyzed in SPSS (version 25.0, IBM, USA) using a linear mixed model and presented as mean±standard deviation. A random intercept for subjects was included to adjust for baseline levels and inter-individual homogeneity. Initial models included all possible interactions, but non-significant interaction terms were dropped from the models for ease of interpretation.

Results

Self-recorded dietary intakes (Table 2) did not differ for energy, CHO, protein, fat and iron between treatments, however timing of CHO intake across the day was different. As intended, CHO intake before the HIT session on days 1 and 3 was markedly lower in HIGH (4.6±0.6 $g \cdot kg^{-1}$) than LOW (6.7±1.1 $g \cdot kg^{-1}$; $p<0.001$), while intakes after HIT were greater in HIGH (2.6±0.7 $g \cdot kg^{-1}$) than LOW (0.3±0.1 $g \cdot kg^{-1}$; $p<0.001$).

Measures of training performance from the morning LIT sessions showed no differences in power output ($F(1,19)=0.11$, $p=0.741$) or running speed ($F(1,18)=0.48$, $p=0.497$) between trials (Table 3). Moderately lower RER (-4.2%; $p<0.001$) and higher HR (3.8%; $p=0.053$) occurred during LOW, compared to HIGH. Moderate increases in HR (4.7%; $p=0.019$) and large increases in RER (8.4%; $p<0.001$) were evident during cycling compared to running.

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Resting serum ferritin levels were 56±21 and 52±16 $\mu g \cdot L^{-1}$ during LOW, and 50±17 and 50±15 $\mu g \cdot L^{-1}$ during HIGH (cycle and run, respectively). No differences in serum ferritin were seen between dietary interventions or exercise modalities ($p>0.05$). Concentrations of IL-6 (Figure 2) gradually increased from pre- to 3 h post-exercise during all trials ($F(3,161)=28.5$, $p<0.001$). No clear differences between exercise modalities or dietary interventions were evident ($p>0.05$). Finally, hepcidin-25 increased substantially from pre- to 3 h post-exercise ($F(1,78)=20.2$, $p<0.001$), with a greater increase seen after cycling than running ($F(1,78)=4.2$, $p=0.043$). Furthermore, the increase in hepcidin-25 was greatest during cycling when adhering to LOW than HIGH ($F(1,78)=5.2$, $p=0.026$).

Markers of mucosal immunity (Table 4) showed that s-IgA concentrations increased post-exercise (17-68%) during all trials ($F(1,81)=8.6$, $p=0.004$) but were not different between exercise modalities or dietary interventions ($p>0.05$). Salivary flow rates decreased consistently (19-39%) post-exercise ($F(1,81)=4.3$, $p=0.042$), with trivial differences between exercise modalities ($F(1,81)=0.70$, $p=0.404$) and dietary interventions ($F(1,81)=0.11$, $p=0.917$). s-IgA secretion rates were greater during the LOW run than all other trials ($F(1,83)=4.1$, $p=0.046$). No other clear differences in s-IgA secretion rates were apparent.

Discussion

This study examined the influence of a sequenced periodization of CHO availability ("sleep low") on markers of inflammation, iron metabolism and immune function in elite athletes. No differences in the IL-6 response to exercise between trials or dietary conditions were evident, however, the 3 h post-exercise hepcidin response was highest during the LOW cycling trial compared to all other trials. Exercise had an acute effect on mucosal immune markers, but differences between trials or dietary conditions were trivial. It appears that acute rescheduling of daily CHO intake to delay glycogen restoration from previous exercise sessions to create low CHO availability for a subsequent

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session of low intensity exercise causes minimal perturbations to the immune system or iron regulation in elite triathlon training settings.

We investigated the interactions between exercise modality and CHO availability, by exposing run and cycling sessions of equal metabolic stress to two different scenarios of CHO availability. However, HR and RER measures during LIT sessions were higher during cycling than running, showing our failure to accurately match the exercise intensity between modalities. We attribute this shortcoming to differences between a graded exercise test (run) and a 4 min MMP test (cycle) for prescribing exercise intensity. While both protocols should elicit a similar $\dot{V}O_{2peak}$ (Gore et al., 1998), the lack of cumulative fatigue during the shorter protocol (4 min MMP test) likely resulted in a higher power at $\dot{V}O_{2peak}$ than during the incremental test. As such, the prescribed cycling power occurred at a higher intensity than the equivalent target pace for running. Therefore, while direct comparison of modalities is not entirely appropriate, this study provides insights on the impact of exercise intensity to iron regulation and the immune system when CHO availability is manipulated. Importantly, our dietary intervention appears to have been successful, with an increased HR and decreased RER achieved during LOW compared to HIGH, despite the similarity of external workloads. Differences can be attributed to increased metabolic stress and fat utilization in tandem with reductions in endogenous and exogenous CHO availability (Yeo et al., 2008).

Increases in s-IgA concentration and decreases in salivary flow rate were evident after each LIT session, which confirms previous findings (Li & Gleeson, 2004; McKay et al., 2018), where exercise-induced dehydration and activation of the sympathetic nervous system decreased salivary flow rate to elicit a concentrating effect on s-IgA levels (Bishop et al., 2000). When these factors were accounted for, there were no differences in secretion rates between all four LIT trials. It is possible that the low intensity, short duration exercise bout (45-60 min) failed to achieve a

sufficiently large exercise stress to elicit alterations to immune function. An increase in s-IgA secretion rate was evident from day 2 to day 4 in the LOW condition only, suggestive of enhanced mucosal defense in response to low CHO availability. Indeed, we previously reported increases in resting s-IgA secretion rates while athletes completed a 3-week block of training involving periodized CHO availability (McKay et al., 2018). Conversely, 3-weeks of an alternate day "sleep low" intervention yielded a small decrease in resting s-IgA levels in trained triathletes (Louis et al., 2016), however was deemed unlikely to be clinically meaningful and did not impact illness rates. Collectively, it appears that acute 'sleep-low' approaches to training are not detrimental to the immune system when the training performed with low CHO availability is of short duration and low intensity.

Despite inducing differences in metabolism and fuel utilization, training with low CHO availability did not increase the inflammatory response to exercise. Although IL-6 levels can be increased after exercise performed with low muscle glycogen stores (Steensberg et al., 2001), our intervention may not have sufficiently depleted muscle glycogen stores to create functional differences between conditions. Specifically, the HIT sessions performed on days 1 and 3 were coach-designed to balance the study goals with up-coming international competition, and were intended to maximize performance rather than deplete glycogen stores. Conversely, it is possible that the high daily training loads of our triathlete population created intrinsic glycogen depletion patterns that overshadowed the differential effects of timing of dietary intake, particularly since total CHO intake was similar. Furthermore, since the magnitude of the IL-6 increase is exercise duration- and intensity-dependent (Fischer, 2006), the small IL-6 response to a short, LIT session reduces the potential for detection of differences.

Despite the lack of differences in IL-6 concentrations between trials, there was a greater increase in post-exercise hepcidin concentrations following the LOW-LIT cycle session. This is an unexpected outcome if an augmented IL-6 response is considered the primary mechanism for post-exercise hepcidin changes. However, we recently reported that although an increase in IL-6 is necessary to invoke this change, its contribution to the magnitude of hepcidin response in exercise settings appears small (Peeling et al., 2017; McKay et al., 2019) and other factors are likely to be involved. Exercise intensity may be a regulating factor since the augmented response was only evident in the cycling session. These outcomes strengthen the evidence for the intentional planning of LIT sessions during periods of low CHO availability, since this approach appears to minimize any potential impact on iron metabolism.

Since maintenance of good health underpins the consistency of training and optimal performance (Raysmith & Drew, 2016), it is important to understand the impact of emerging sports nutrition strategies on outcomes such as inflammation, iron metabolism and the immune system. The highly applied nature of this study created limitations to experimental control in the general prescription of the training program, as well as catering for individual athlete requirements. However, the fusion of laboratory-based testing within the daily training environment gives our results strong 'real-world' credibility, and outcomes are highly relevant for elite-level triathletes. While we have examined the acute impact of this dietary strategy, the potential long-term effects of repeated bouts of CHO restriction merit further investigation. Our findings suggest periodizing CHO availability to promote adaptation throughout the training cycle had limited impact on alterations to markers of athlete health, at least when occasional and planned exposure to low CHO availability was undertaken with expert nutrition and coaching input. Accordingly, when sessions performed with low CHO availability are low intensity and short in duration, there is likely only minimal impact on exercise-induced alterations in immune function and iron regulation.

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Table 1. Prescribed training program and carbohydrate (CHO) targets (male athlete; 8.0 g·kg^{-1}CHO) for each 4-day experimental trial when adopting a sleep-low (LOW) and sleep high approach (HIGH). HIT: High-intensity training session; LIT: Low-intensity training session; LSD: Long, slow duration session.

	DAY 1		**DAY 2**		**DAY 3**		**DAY 4**	
	HIGH	*LOW*	*HIGH*	*LOW*	*HIGH*	*LOW*	*HIGH*	*LOW*
Breakfast	2.5 g·kg^{-1}	3.5 g·kg^{-1}	1.5 g·kg^{-1}	0 g·kg^{-1}	2.5 g·kg^{-1}	3.5 g·kg^{-1}	1.5 g·kg^{-1}	0 g·kg^{-1}
Session 1	*Run* 40 min LSD		**LIT Cycle** 0.5 g·kg^{-1}CHO	0 g·kg^{-1}CHO	*Run* 20 min LSD + 4 x 45 sec builds		**LIT Run** 0.5 g·kg^{-1}	0 g·kg^{-1}
Post training Snack	-		2 g·kg^{-1}CHO	2 g·kg^{-1}CHO	-		2 g·kg^{-1}	2 g·kg^{-1}
Session 2	*Gym* *30 mins*		-		*Gym* *30 mins*		-	
Session 3	*Swim* 60 min AT main set		*Run* 40 min LSD + 6 x 30 sec builds		*Swim* 60 min AT main set		*Cycle* 90 min aerobic	
Lunch + Snacks	2.5 g·kg^{-1}	4 g·kg^{-1}	2 g·kg^{-1}	3 g·kg^{-1}	2.5 g·kg^{-1}	4 g·kg^{-1}	2 g·kg^{-1}	3 g·kg^{-1}
Session 4	**HIT Cycle**		*Swim* 60 min		**HIT Run**		*Swim* *60 min*	
	0.5 g·kg^{-1}	0.5 g·kg^{-1}	Aerobic Pull		0.5 g·kg^{-1}	0.5 g·kg^{-1}	*Aerobic Pull*	
Dinner	2.5 g·kg^{-1}	0 g·kg^{-1}	2 g·kg^{-1}	3 g·kg^{-1}	2.5 g·kg^{-1}	0 g·kg^{-1}	2 g·kg^{-1}	3 g·kg^{-1}

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Table 2. Daily total energy intake, macronutrient concentrations and daily iron content of athletes when adopting a sleep-low (LOW) and sleep high approach (HIGH); Mean (±SD).

		Day 1		Day 2		Day 3		Day 4	
		LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH
Energy ($kJ \cdot kg^{-1}$)	Mean	277	279	300	299	267	261	293	287
	SD	38	40	43	37	30	36	39	49
Carbohydrate ($g \cdot kg^{-1}$)	Mean	7.5	7.7	7.7	8.2	6.9	7.1	7.7	8.0
	SD	1.3	1.2	1.4	1.6	1.1	1.1	1.3	1.5
Fat ($g \cdot kg^{-1}$)	Mean	2.3	2.5	2.9	2.7	2.4	2.3	2.8	2.4
	SD	0.5	0.5	0.5	0.4	0.5	0.5	0.6	0.5
Protein ($g \cdot kg^{-1}$)	Mean	3.4	3.1	3.2	3.3	3.1	3.0	3.4	3.4
	SD	0.8	0.7	0.8	0.6	0.7	0.7	1.0	0.6
Iron (mg)	Mean	23.24	24.9	26.2	25.0	23.9	22.4	20.2	21.9
	SD	5.8	7.6	4.0	5.5	5.5	8.4	3.6	5.7

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Table 3. Internal and external load variables for low intensity sessions when adopting a sleep low (LOW) and sleep high approach (HIGH). Data are presented as mean (±SD). *indicates values are significantly greater than day 4. #indicates values are significantly lower than HIGH trials.

	Trial	Day 2 - Cycle	Day 4 - Run
55% Intensity	LOW	164 ± 23	5:15 ± 0:19
(W OR min/km)	HIGH	164 ± 22	5:09 ± 0:12
65% Intensity	LOW	212 ± 27	4:45 ± 0:26
(W OR min/km)	HIGH	216 ±33	4:45 ± 0:26
Mean Heart Rate	LOW	158 ± 9*	151 ± 13
(bpm)	HIGH	152 ± 9*	144 ± 9
RER	LOW	0.84 ± 0.04#*	0.76 ± 0.02#
(Respiratory exchange ratio)	HIGH	0.87 ± 0.03*	0.80 ± 0.03

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Table 4. s-IgA concentration, flow rate and secretion rate pre- and post-exercise in athletes adopting a sleep low (LOW) or sleep high (HIGH) dietary approach. Data are presented as Mean (±SD).* Indicates a significant increase from pre-exercise. # Indicates a significant decrease from pre-exercise. ^ Significantly greater than all other trials.

	Day 2 – Cycle		**Day 4 - Run**	
	Pre-exercise	*Post-exercise*	*Pre-exercise*	*Post-exercise*
s-IgA Concentration ($\mu g \cdot mL^{-1}$)				
LOW	162 ± 82	229 ± 147*	195 ± 87	229 ± 108*
HIGH	145 ± 78	243 ± 180*	134 ± 59	224 ± 136*
Salivary Flow Rate ($mL \cdot min^{-1}$**)**				
LOW	0.62 ± 0.35	0.50 ± 0.35#	0.83 ± 0.49	0.64 ± 0.36#
HIGH	0.74 ± 0.45	0.59 ± 0.55#	0.80 ± 0.59	0.49 ± 0.27#
s-IgA Secretion Rate ($\mu g \cdot min^{-1}$)				
LOW	98 ± 73	92 ± 49	165 ± 137^	144 ± 116^
HIGH	122 ± 136	126 ± 120	95 ± 59	101 ± 71

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Figure Caption List

Figure 1. Overview of the randomized crossover design for elite triathletes undertaking two four-day experimental trials, inclusive of a 48 h breakdown of the carbohydrate periodization around key high intensity (HIT) and low intensity (LIT) sessions.

Figure 2. Concentrations of interleukin-6 (A and B) and hepcidin-25 (C and D) on days 2 and 4 when adopting a sleep low (LOW) or sleep high approach (HIGH). * Indicates a significant increase from pre-exercise. # Indicates a significantly greater response compared both the HIGH condition and running trials. Data presented as Mean (±SD).

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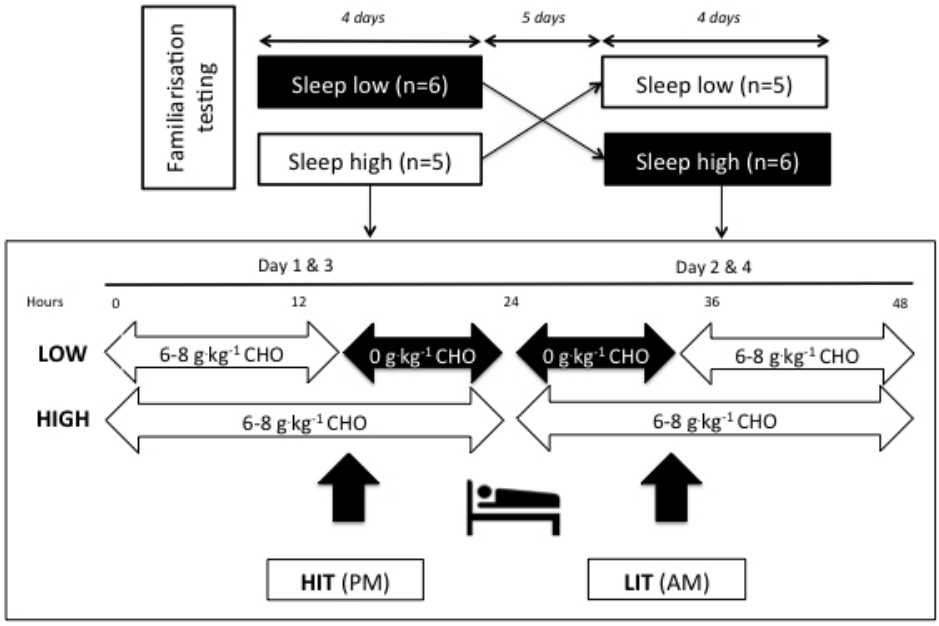

Figure 1. Overview of the randomized crossover design for elite triathletes undertaking two four-day experimental trials, inclusive of a 48 h breakdown of the carbohydrate periodization around key high intensity (HIT) and low intensity (LIT) sessions.

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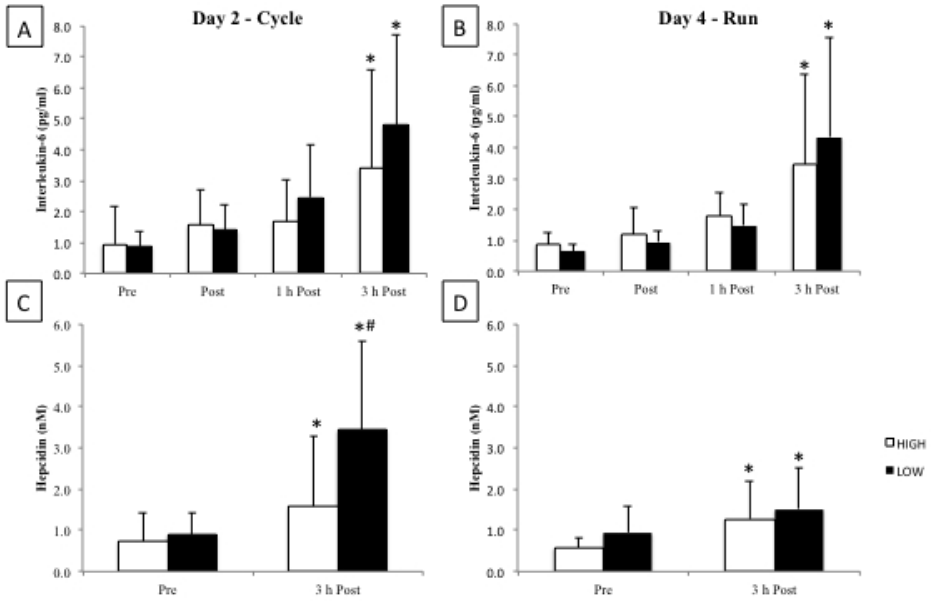

Figure 2. Concentrations of interleukin-6 (A and B) and hepcidin-25 (C and D) on days 2 and 4 when adopting a sleep low (LOW) or sleep high approach (HIGH). * Indicates a significant increase from pre-exercise. # Indicates a significantly greater response compared both the HIGH condition and running trials. Data presented as Mean (±SD).

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