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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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1 **Abstract**

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

19

20 **Key words:** sleep low; train low; hepcidin.

21 **Introduction**

22 Acute carbohydrate (CHO) restriction around exercise can enhance molecular adaptations to training
23 by augmenting cell signaling, gene expression, enzyme activity and lipid oxidation (Impey et al.,
24 2018). These outcomes inform nutritional guidelines, which advise athletes to strategically approach
25 CHO periodization (Jeukendrup, 2017). One strategy is the “sleep low” sequence (Burke et al.,
26 2018), which involves commencing a high-intensity training session in the evening with high CHO
27 availability, before restricting CHO intake at the subsequent meal and overnight. This protocol
28 supports high quality training for the first session, augmented by an enhanced period of cellular
29 signaling related to delayed restoration of muscle glycogen. The following morning’s submaximal
30 exercise session is performed under conditions of low muscle and liver glycogen, promoting higher
31 reliance on fat oxidation, greater metabolic stress and cellular adaptation (Bartlett et al., 2015).
32 While careful integration of such strategies can enhance adaptation and performance (Marquet et al.,
33 2016), the potential implications on athlete health are relatively unknown. Accordingly, concern
34 centers on transient immunodepression associated with endurance exercise, which might otherwise
35 be attenuated by CHO ingestion. Current research shows that low or declining levels of salivary
36 immunoglobulin-A (s-IgA) are associated with increased risk of upper respiratory illness (URI)
37 (Bishop & Gleeson, 2009); however, both increases (McKay et al., 2018) and decreases (Louis et al.,
38 2016) in s-IgA levels are reported in response to a 3-week periodized CHO intervention; thus, the
39 acute impact of this strategy is unclear.

40

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

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

53

54 **Methods**

55 **Participants**

56 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
57 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for cycle and treadmill $\text{VO}_{2\text{peak}}$ respectively; mean \pm SD) and seven female (26.4 ± 2.0 y,
58 56.4 ± 5.4 kg, 62 ± 20 mm for sum of 7 skinfolds, 66.2 ± 5.1 and 65.3 ± 4.4 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for cycle
59 and treadmill $\text{VO}_{2\text{peak}}$, respectively) triathletes with >18 months of elite training history were
60 recruited. Skinfold measurements were undertaken by an accredited anthropometrist according to
61 International Society for the Advancement of Kinanthropometry standards. Athletes were free from
62 injury, illness, or iron deficiency (serum ferritin >30 $\mu\text{g}\cdot\text{L}^{-1}$); none were taking iron supplements
63 during the testing period. Written informed consent was obtained prior to participation. Approval
64 was granted by the Australian Institute of Sport (AIS) Ethics Committee.

65

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

72 power (MMP), and a graded treadmill test to determine VO_{2peak} and running velocity at VO_{2peak}
73 (vVO_2), as described elsewhere (Tanner & Gore, 2013). Additionally, a venous blood sample was
74 collected from female athletes to measure sex hormone concentrations and determine menstrual
75 phase. Athletes were subsequently pair-matched based on performance measures and menstrual
76 phase, before being randomized into their first dietary condition.

77 **Nutrition Interventions**

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

93

94 **Training**

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

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

101

102 *High Intensity Training Sessions*

103 To ensure high external validity and account for the proximity of the upcoming international event,
104 all HIT sessions were individually prescribed and led by an international-level coach who understood
105 the study goals. During the HIT cycling session (day 1), athletes completed an interval-based session
106 (~45 min) at an outdoor velodrome on a fixed-gear bike, in addition to cycling to and from the
107 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).
108 The HIT running session (day 3) was interval-based, prescribed from time and/or distance to equal
109 ~10 km (LOW: 53.2±5.5 min, mean heart rate (HR) 166±6 bpm; HIGH: 53.2±9.5 min, mean HR
110 165±11 bpm).

111

112 *Low Intensity Training Sessions*

113 Athletes reported to the laboratory for LIT sessions at a standardized time in an overnight fasted and
114 rested state. A 4 mL baseline venous blood sample was collected from an antecubital vein before
115 athletes consumed a snack in accordance with their dietary allocation (1.5 v 0.0 g·kg⁻¹ CHO for
116 HIGH and LOW, respectively); 25 min later, they provided a saliva sample. Thirty min post-snack,
117 athletes commenced their planned LIT task. The 60 min cycle trial (day 2) was performed in the
118 laboratory on the athlete's own bicycle mounted to a stationary cycle ergometer. The 45 min running
119 trial (day 4) was conducted as a hybrid laboratory-field test, with the first and final 5 min completed
120 on a motorized treadmill, and the remainder completed on a flat, outdoor course. The first and final 5
121 min of each trial were completed at 65% MMP (cycle) or vVO₂ (run) with the remaining distance

122 completed at ~55% MMP or $v\dot{V}O_2$. During HIGH, athletes consumed $0.5 \text{ g}\cdot\text{kg}^{-1}$ CHO during
123 exercise, while water was consumed during LOW. Mean HR and respiratory exchange ratio were
124 determined for the final 5 min of each session, while venous blood and saliva samples were collected
125 immediately and 15 min post-exercise, respectively. Athletes received a whey protein shake
126 immediately following saliva collection ($0.3 \text{ g}\cdot\text{kg}^{-1}$ whey protein) and a CHO-rich breakfast 1 h post-
127 exercise ($2 \text{ g}\cdot\text{kg}^{-1}$ CHO) to ensure recovery and avoid hunger. Venous blood samples were collected
128 1 and 3 h post-exercise.

129

130 **Experimental Procedures**

131 *Blood Sampling*

132 Venous blood samples were collected into 4 mL SST gel separator tubes and left to clot for 30 min
133 before centrifugation (2200 G for 10 min). Serum was divided into 1 mL cryotubes and frozen at -
134 80°C until batch analysis of iron, serum ferritin, IL-6 and hepcidin-25 concentrations. Pre-exercise
135 serum iron and ferritin concentrations were quantified via a COBAS Integra 400 automated
136 biochemistry analyzer (Roche Diagnostics, Switzerland). IL-6 concentrations were analyzed in
137 duplicate pre-, post-, 1 h and 3 h post-exercise using an ELISA (Quantikine HS, R&D Systems,
138 Minneapolis, USA; CV=5.7%). Hepcidin-25 measurements were performed on serum obtained pre-
139 and 3 h post-exercise (www.hepcidinanalysis.com, Nijmegen, The Netherlands) using a combination
140 of weak cation exchange chromatography and time-of-flight mass spectrometry (Kroot et al., 2010;
141 Swinkels et al., 2008). When values were below the lower limit of detection of 0.5 nM, this value
142 divided by the square root of 2 was used (Croghan & Egeghy, 2003).

143

144 *Saliva Sampling*

145 Prior to, and following each LIT session, an unstimulated saliva sample was obtained 10 min after a
146 40 ml water mouth rinse. Prior to sample collection, a >20 min window where no CHO was ingested

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

154 *Statistical Analysis*

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

159 **Results**

160 Self-recorded dietary intakes (Table 2) did not differ for energy, CHO, protein, fat and iron between
161 treatments, however timing of CHO intake across the day was different. As intended, CHO intake
162 before the HIT session on days 1 and 3 was markedly lower in HIGH ($4.6\pm 0.6 \text{ g}\cdot\text{kg}^{-1}$) than LOW
163 ($6.7\pm 1.1 \text{ g}\cdot\text{kg}^{-1}$; $p<0.001$), while intakes after HIT were greater in HIGH ($2.6\pm 0.7 \text{ g}\cdot\text{kg}^{-1}$) than LOW
164 ($0.3\pm 0.1 \text{ g}\cdot\text{kg}^{-1}$; $p<0.001$).

165 Measures of training performance from the morning LIT sessions showed no differences in power
166 output ($F(1,19)=0.11$, $p=0.741$) or running speed ($F(1,18)=0.48$, $p=0.497$) between trials (Table 3).
167 Moderately lower RER (-4.2% ; $p<0.001$) and higher HR (3.8% ; $p=0.053$) occurred during LOW,
168 compared to HIGH. Moderate increases in HR (4.7% ; $p=0.019$) and large increases in RER (8.4% ;
169 $p<0.001$) were evident during cycling compared to running.

170 Resting serum ferritin levels were 56 ± 21 and 52 ± 16 $\mu\text{g}\cdot\text{L}^{-1}$ during LOW, and 50 ± 17 and 50 ± 15 $\mu\text{g}\cdot\text{L}^{-1}$ 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 ($F(1,78)=5.2$, $p=0.026$).

178 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.

185

186 Discussion

187 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

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

196

197 We investigated the interactions between exercise modality and CHO availability, by exposing run
198 and cycling sessions of equal metabolic stress to two different scenarios of CHO availability.
199 However, HR and RER measures during LIT sessions were higher during cycling than running,
200 showing our failure to accurately match the exercise intensity between modalities. We attribute this
201 shortcoming to differences between a graded exercise test (run) and a 4 min MMP test (cycle) for
202 prescribing exercise intensity. While both protocols should elicit a similar $\text{VO}_{2\text{peak}}$ (Gore et al.,
203 1998), the lack of cumulative fatigue during the shorter protocol (4 min MMP test) likely resulted in
204 a higher power at $\text{VO}_{2\text{peak}}$ than during the incremental test. As such, the prescribed cycling power
205 occurred at a higher intensity than the equivalent target pace for running. Therefore, while direct
206 comparison of modalities is not entirely appropriate, this study provides insights on the impact of
207 exercise intensity to iron regulation and the immune system when CHO availability is manipulated.
208 Importantly, our dietary intervention appears to have been successful, with an increased HR and
209 decreased RER achieved during LOW compared to HIGH, despite the similarity of external
210 workloads. Differences can be attributed to increased metabolic stress and fat utilization in tandem
211 with reductions in endogenous and exogenous CHO availability (Yeo et al., 2008).

212

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

219 sufficiently large exercise stress to elicit alterations to immune function. An increase in s-IgA
220 secretion rate was evident from day 2 to day 4 in the LOW condition only, suggestive of enhanced
221 mucosal defense in response to low CHO availability. Indeed, we previously reported increases in
222 resting s-IgA secretion rates while athletes completed a 3-week block of training involving
223 periodized CHO availability (McKay et al., 2018). Conversely, 3-weeks of an alternate day “sleep
224 low” intervention yielded a small decrease in resting s-IgA levels in trained triathletes (Louis et al.,
225 2016), however was deemed unlikely to be clinically meaningful and did not impact illness rates.
226 Collectively, it appears that acute ‘sleep-low’ approaches to training are not detrimental to the
227 immune system when the training performed with low CHO availability is of short duration and low
228 intensity.

229

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

242

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

253

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

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277 RvS, CML and GRC; data interpretation and manuscript preparation were undertaken by AKAM,
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Table 1. Prescribed training program and carbohydrate (CHO) targets (male athlete; 8.0 g·kg⁻¹CHO) 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
<i>Breakfast</i>	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	<i>Run</i> 40 min LSD		LIT Cycle 0.5 g·kg ⁻¹ CHO 0 g·kg ⁻¹ CHO		<i>Run</i> 20 min LSD + 4 x 45 sec builds		LIT Run 0.5 g·kg ⁻¹ 0 g·kg ⁻¹	
<i>Post training Snack</i>	-		2 g·kg ⁻¹ CHO	2 g·kg ⁻¹ CHO	-		2 g·kg ⁻¹	2 g·kg ⁻¹
Session 2	<i>Gym</i> 30 mins		-		<i>Gym</i> 30 mins		-	
Session 3	<i>Swim</i> 60 min AT main set		<i>Run</i> 40 min LSD + 6 x 30 sec builds		<i>Swim</i> 60 min AT main set		<i>Cycle</i> 90 min aerobic	
<i>Lunch + Snacks</i>	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 0.5 g·kg ⁻¹ 0.5 g·kg ⁻¹		<i>Swim</i> 60 min Aerobic Pull		HIT Run 0.5 g·kg ⁻¹ 0.5 g·kg ⁻¹		<i>Swim</i> 60 min Aerobic Pull	
<i>Dinner</i>	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

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 (\pm SD).

		Day 1		Day 2		Day 3		Day 4	
		LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH
Energy (kJ·kg⁻¹)	Mean	277	279	300	299	267	261	293	287
	SD	38	40	43	37	30	36	39	49
Carbohydrate (g·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·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·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

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

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

361 **Figure Caption List**

362

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

366

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

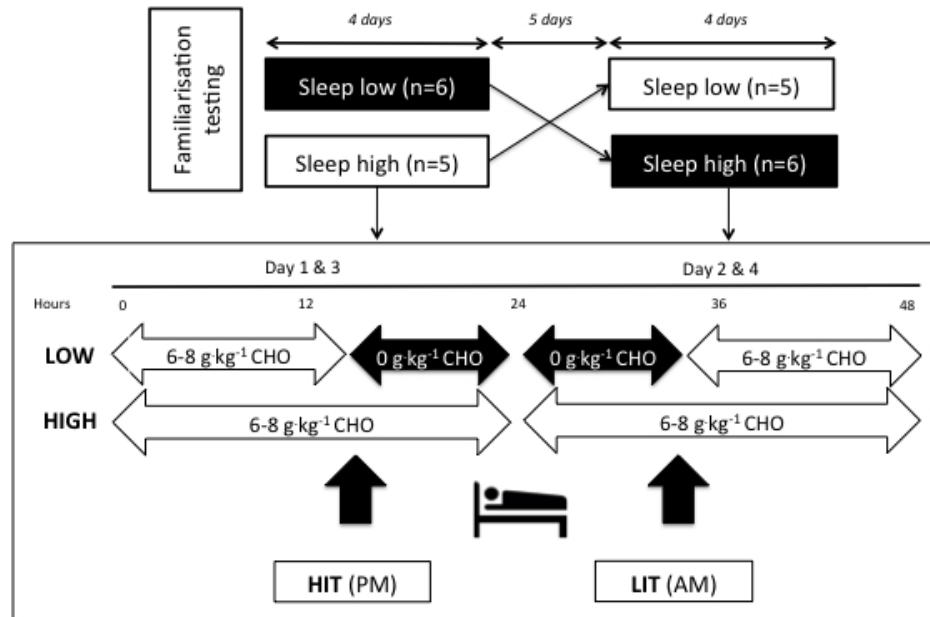


Figure 1. Overview of the randomized crossover design for elite triathletes undertaking two four-day experimental trials, inclusive of a 48 h breakdown of the carbohydrate periodization around key high intensity (HIT) and low intensity (LIT) sessions.

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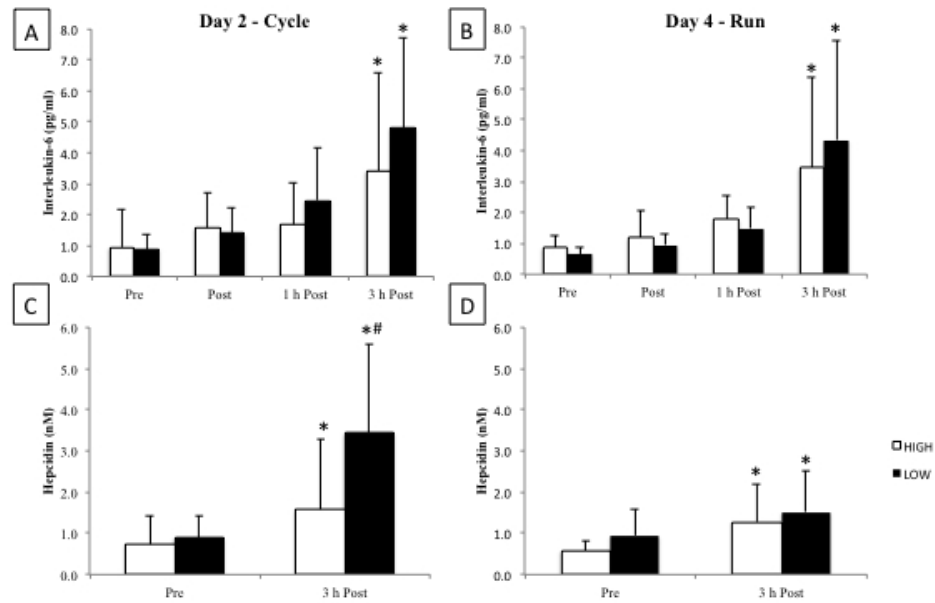


Figure 2. Concentrations of interleukin-6 (A and B) and hepcidin-25 (C and D) on days 2 and 4 when adopting a sleep low (LOW) or sleep high approach (HIGH). * Indicates a significant increase from pre-exercise. # Indicates a significantly greater response compared both the HIGH condition and running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

211x132mm (72 x 72 DPI)