Sustained Exposure to High Carbohydrate Availability Does Not Influence Iron-Regulatory Responses in Elite Endurance Athletes

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This study implemented a 2-week high carbohydrate (CHO) diet intended to maximize CHO oxidation rates and examined the iron-regulatory response to a 26-km race walking effort. Twenty international-level, male race walkers were assigned to either a novel high CHO diet (MAX = 10 g/kg body mass CHO daily) inclusive of gut-training strategies, or a moderate CHO control diet (CON = 6 g/kg body mass CHO daily) for a 2-week training period. The athletes completed a 26-km race walking test protocol before and after the dietary intervention. Venous blood samples were collected pre-, post-, and 3 hr postexercise and measured for serum ferritin, interleukin-6, and hepcidin-25 concentrations. Similar decreases in serum ferritin (17–23%) occurred post-intervention in MAX and CON. At the baseline, CON had a greater postexercise increase in interleukin-6 levels after 26 km of walking (20.1-fold, 95% CI [9.2, 35.7]) compared with MAX (10.2-fold, 95% CI [3.7, 18.7]). A similar finding was evident for hepcidin levels 3 hr postexercise (CON = 10.8-fold, 95% CI [4.8, 21.2]; MAX = 8.8-fold, 95% CI [3.9, 16.4]). Postintervention, there were no substantial differences in the interleukin-6 response (CON = 13.6-fold, 95% CI [9.2, 20.5]; MAX = 11.2-fold, 95% CI [6.5, 21.3]) or hepcidin levels (CON = 7.1-fold, 95% CI [2.1, 15.4]; MAX = 6.3-fold, 95% CI [1.8, 14.6]) between the dietary groups. Higher resting serum ferritin (p = .004) and hotter trial ambient temperatures (p = .014) were associated with greater hepcidin levels 3 hr postexercise. Very high CHO diets employed by endurance athletes to increase CHO oxidation have little impact on iron regulation in elite athletes. It appears that variations in serum ferritin concentration and ambient temperature, rather than dietary CHO, are associated with increased hepcidin concentrations 3 hr postexercise.

Keywords: ferritin, hepcidin, race walk

Iron is a key micronutrient involved in multiple biological processes relevant to athletic performance, including oxygen transport/delivery and energy production at a cellular level (Beard, 2001). Despite the importance of adequate iron stores for athletes, exercise-induced mechanisms of iron loss can result in a negative iron balance, likely contributing to the high prevalence of iron deficiency seen in athlete populations (Sim et al., 2019). One key mechanism responsible for the increased rates of iron deficiency in athlete populations is the impact of exercise on the key iron-regulatory hormone, hepcidin. This hormone regulates iron metabolism by...
internalizing ferroportin (Nemeth et al., 2004b), which impedes the absorption of iron from the gut and recycling of iron by macrophages. Hepcidin activity can be directly upregulated by the inflammatory cytokine interleukin-6 (IL-6; Nemeth et al., 2004a), with exercise-induced increases in hepcidin concentrations attributed to increases in IL-6 that occur postexercise. Here, hepcidin concentrations peak 3–6 hr postexercise (Peeling et al., 2009), creating a postexercise window in which iron metabolism may be impaired. However, it appears that the athlete’s iron status interacts with the magnitude of this response (Peeling et al., 2014).

In addition to regulating hepcidin expression, IL-6 plays an important role in glucose homeostasis during exercise (Pedersen & Fischer, 2007). When muscle glycogen stores are low, the IL-6 response to exercise is increased (Steensberg et al., 2001), signaling the liver to increase hepatic glucose output in an attempt to maintain blood glucose concentrations during exercise. Investigations of acute (Badenhorst et al., 2015) and chronic (McKay et al., 2019a) low carbohydrate (CHO) availability in the athlete’s diet have shown clear increases in the postexercise IL-6 response compared with conditions of high CHO availability. However, while exercise-induced elevations are associated with increases in hepcidin postexercise, whether exacerbated IL-6 levels in periods of low CHO availability are associated with augmented hepcidin levels postexercise is less definitive, with differences in hepcidin levels between dietary conditions in the acute study not reaching significance (Badenhorst et al., 2015) and differences in the baseline iron status between groups potentially confounding the results in the chronic investigation (McKay et al., 2019b). Therefore, the extent to which CHO manipulation can influence the postexercise hepcidin response remains unclear.

The influence of CHO manipulation on endurance performance has been extensively studied. High CHO availability prior to and during exercise enhances CHO storage and utilization, characteristics that are deemed beneficial to performance, as CHO oxidation produces more adenosine triphosphate (ATP) per unit of oxygen than fat (Leverve et al., 2007). With that in mind, the highly adaptable state of the gastrointestinal tract (Cox et al., 2010) can be trained by repetitive exposure to high doses of CHO during exercise to increase gastric emptying rates (Cunningham et al., 1991), improve intestinal CHO absorption, and minimize negative gastrointestinal symptoms (Costa et al., 2017a). Collectively, these processes may increase CHO oxidation during exercise and improve endurance performance (Jeukendrup, 2017). Importantly for iron metabolism, it may be proposed that an increase in CHO availability during exercise will better sustain blood glucose concentrations and supply the muscle with a more stable energy source, effectively blunting the signal for hepatic glucose production, minimizing IL-6 levels, and potentially attenuating the hepcidin response to exercise. Accordingly, a dietary strategy that can maximize endogenous CHO stores prior to exercise and increase exogenous CHO oxidation during exercise may be of benefit to both iron metabolism and performance. Therefore, the aim of the current study was to implement a novel 2-week CHO-rich dietary intervention and quantify the subsequent impact on inflammatory responses and hepcidin levels relevant to iron metabolism in athletes.

Methods

Participants

Twenty international-level male race walkers were invited to attend one of two 3-week training camps in either January or May of 2018. The athlete characteristics are provided in Table 1. One athlete completed in both training camps, while two athletes were removed from the sample analysis due to unusually high iron stores (serum ferritin > 300 μg/L). These exclusions resulted in a total of 19 data sets from 18 athletes for analysis. Prior to participation, the athletes provided informed consent. The study was approved by the ethics committee of the Australian Institute of Sport.

Study Design

In a parallel groups design, the athletes were assigned to one of two dietary interventions: (a) a novel, higher CHO diet (MAX), utilizing a combination of evidence-based and theoretical approaches to increase endogenous CHO stores and exogenous CHO availability, or (b) a moderate CHO control diet (CON). Dietary allocations were made based on athlete dietary preference, while matching for characteristics, such as age, body mass (BM), VO2max, and training history. The athletes completed a race walking test protocol prior to (baseline) and following the dietary intervention (postintervention) to assess any changes in iron regulation in response to exercise (see Figure 1). In the 3 days prior to each race walking test protocol, the athletes consumed a “race preparation diet,” with the intention to replicate the nutrition practices that would typically occur prior to competition. The athletes then commenced their allocated dietary intervention for 2 weeks while following a standardized training program. This program was undertaken as a group, and it included two long walks (+25 km), an interval track session, and 14-km high-intensity session performed on an incline. The remaining sessions were selected at the athlete’s discretion to accommodate current fitness levels, residual fatigue, and training goals. All training was monitored by the research team and recorded in a training diary, with the athletes on average completing approximately 13.5 hr of training per week (including resistance training) and covering approximately 138 km/week, which is representative of the typical volumes performed by elite male race walkers (Burke et al., 2017, Carr et al., 2020). After the 2-week intervention period, the 3-day “race preparation diet” was repeated, which this time was in accordance with their prescribed dietary intervention goals.

Dietary Intervention

Diets were created by a panel of accredited sports dietitians to provide a mean total daily energy intake of approximately 225 KJ/kg BM, equivalent to a daily energy availability of approximately 40 kcal (168 KJ/kg fat free mass). Dietary intake was highly controlled, with all meals served to the athletes in a communal living environment and intake subsequently quantified using dietary analysis software (Food Works 8 Professional program; Xyris Software Australia Pty Ltd., Kenmore Hills, Australia). The CON

| Table 1 Subject Characteristics of Athletes Adhering to a CON and MAX CHO Diet |
|-----------------------------|-----------------------------|-----------------------------|
| Characteristic              | CON (n = 10)                | MAX (n = 9)                 |
| Age (years)                 | 29.5 ± 4.6                  | 29.6 ± 4.3                  |
| Body mass (kg)              | 69.4 ± 8.5                  | 69.6 ± 4.2                  |
| VO2max (ml/min/kg)          | 62.1 ± 5.3                  | 63.8 ± 5.8                  |
| 10 km personal best (min:s) | 41:11 ± 1:28                | 40:39 ± 1:16                |
| 20 km personal best (min:s) | 84:42 ± 4:31                | 83:28 ± 3:44                |

Note: Values are presented as mean ± SD. CHO = carbohydrate; CON = moderate; MAX = very high; VO2max = maximal aerobic capacity.
diet was designed to meet the lower end of the range of recommended CHO intakes for endurance athletes (Thomas et al., 2016), providing 6 g/kg of BM CHO daily, with 30 g/hr of CHO consumed during training sessions exceeding 60 min in duration. The 3-day race preparation diet increased CHO ingestion to 8 g/kg of BM daily, in an attempt to maximize endogenous CHO stores prior to the race walking test protocol. The MAX intervention used a combination of strategies for increasing the capacity of the athlete to absorb and utilize CHO as a fuel source during exercise. This approach included a daily CHO intake (10 g/kg BM CHO) at the upper end of current sports nutrition guidelines (Thomas et al., 2016). During training, emphasis was placed on athletes consuming an increasing amount of CHO across the 2-week intervention (from 60 up to 90 g/hr of multiple-transportable CHO). A total of 5 mg/kg BM of sucralose was also consumed daily, provided as a 795 mg/L solution, with athletes instructed to drink one-third of the solution three times per day, away from any other food or fluid ingestion. Animal models have demonstrated that chronic (2-week) ingestion of sucralose can upregulate the intestinal absorption of CHO by increasing the activity of sodium-dependent glucose transporter (Margolskee et al., 2007). Prior to baseline testing, both the MAX and CON groups consumed the same 3-day race preparation diet. However, in the 3 days prior to postintervention testing, the MAX group’s daily CHO intake increased to 12 g/kg of BM inclusive of low-residue foods to reduce gut fiber content (Thomas et al., 2016). The dietary targets for each study phase are shown in Figure 1, with the actual dietary intakes detailed in Table 2.

**Figure 1** — Schematic representation of the study design, including CHO targets, and an overview of standardized race walking protocol. CHO = carbohydrate; CON = moderate; MAX = very high.

**Table 2** Mean Daily Energy and Nutrient Intake of Athletes Adhering to the CON and MAX CHO Diets

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Baseline race preparation diet</th>
<th>Dietary intervention</th>
<th>Postintervention race preparation diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>MAX</td>
<td>CON</td>
</tr>
<tr>
<td>Energy (kJ/kg FFM)</td>
<td>219 ± 26</td>
<td>225 ± 13</td>
<td>221 ± 8</td>
</tr>
<tr>
<td>Carbohydrate (g/kg BM)</td>
<td>7.6 ± 0.8</td>
<td>8.0 ± 0.5</td>
<td>6.6 ± 0.2* ,#</td>
</tr>
<tr>
<td>Protein (g/kg BM)</td>
<td>1.8 ± 0.3</td>
<td>2.0 ± 0.1</td>
<td>2.1 ± 0.1#</td>
</tr>
<tr>
<td>Fat (g/kg BM)</td>
<td>1.4 ± 0.3</td>
<td>1.4 ± 0.1</td>
<td>1.8 ± 0.1* ,#</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>17.6 ± 2.6</td>
<td>17.8 ± 1.5</td>
<td>19.7 ± 2.6#</td>
</tr>
</tbody>
</table>

*Note.* Values are presented as mean ± SD. CHO = carbohydrate; CON = moderate; MAX = very high; BM = body mass; FFM = fat free mass.

*Significant difference compared with MAX. **Significant difference compared with the dietary intervention. *Significant difference compared with baseline.

A 26-km race walking protocol was completed at baseline and postintervention to assess the impact of each dietary intervention on inflammatory and iron-regulatory responses to exercise. For each test, the athletes arrived at the laboratory at the same time each morning and consumed a standardized breakfast consisting of 10 g/kg BM CHO at the upper end of current sports nutrition guidelines (Thomas et al., 2016). During training, emphasis was placed on athletes consuming an increasing amount of CHO across the 2-week intervention (from 60 up to 90 g/hr of multiple-transportable CHO). A total of 5 mg/kg BM of sucralose was also consumed daily, provided as a 795 mg/L solution, with athletes instructed to drink one-third of the solution three times per day, away from any other food or fluid ingestion. Animal models have demonstrated that chronic (2-week) ingestion of sucralose can upregulate the intestinal absorption of CHO by increasing the activity of sodium-dependent glucose transporter (Margolskee et al., 2007). Prior to baseline testing, both the MAX and CON groups consumed the same 3-day race preparation diet. However, in the 3 days prior to postintervention testing, the MAX group’s daily CHO intake increased to 12 g/kg of BM inclusive of low-residue foods to reduce gut fiber content (Thomas et al., 2016). The dietary targets for each study phase are shown in Figure 1, with the actual dietary intakes detailed in Table 2.

**Race walking test protocol.** A 26-km race walking protocol was conducted at baseline and postintervention to assess the impact of each dietary intervention on inflammatory and iron-regulatory responses to exercise. For each test, the athletes arrived at the laboratory at the same time each morning and consumed a standardized breakfast consisting of 2 g/kg of BM CHO. Fifteen minutes prior to the start of exercise (105 min after breakfast), a 4-ml venous blood sample was drawn from an indwelling cannula placed into a forearm vein. The athletes then commenced a combined laboratory and field race walking protocol. Kilometers 0–1, 6–7, 12–13, 18–19, and 24–25 were performed on a motorized treadmill at either 12 or 13 km/hr. This speed equated to approximately 75% VO₂max and corresponded approximately to the athlete’s 50-km race pace. The athletes then immediately commenced the final kilometer (25–26), with a speed increment of...
1.3 km/hr (to 13.3 or 14.3 km/hr). This speed increase was used to replicate the top-end finishing speed or end spurt that typically occurs during the final stages of a race walking event (Hanley, 2013). The remaining kilometers were performed on an outdoor circuit (5-km loop) at a consistent speed, nominated by the athlete, which was maintained between trials. Prior to exercise, and following each kilometer completed on the treadmill, the athletes were provided with a CHO solution consisting of a 2:1 glucose to fructose ratio. During baseline testing, this solution was 8% CHO and provided approximately 30 g/hr. The athletes adhering to the CON diet replicated this intake during the postintervention testing; however, the athletes allocated to the MAX intervention consumed a 24% CHO solution providing approximately 90 g/hr. Immediately postexercise, a 2.5-ml blood sample was collected. At 30 min postexercise, the athletes consumed a protein drink and rested in the laboratory until 3 hr postexercise, where a final blood sample (2.5 ml) was collected.

Venous Blood Analysis

A total of three venous blood samples were collected during each race walking test protocol. Samples were collected into a 4-ml (preexercise) or 2.5-ml (post- and 3 hr postexercise) serum separator tube, which were subsequently left on the bench top at room temperature for 30 min to clot. The samples were then centrifuged at 2,200 g, 4 °C, for 10 min. Serum iron and ferritin analyses were immediately determined on preexercise samples via a COBAS Integra 400 automated biochemistry analyzer biochemistry analyzer (Roche Diagnostics, Rotkreuz, Switzerland). The remaining serum was aliquoted and frozen at −80 °C until a batch analysis was conducted. Preexercise, an additional 2 ml K3EDTA tube was collected and whole blood was immediately analyzed for hemoglobin and hematocrit by fluorescent flow cytometry on a XN-L 550 analyzer (Sysmex Corporation, Kobe, Japan).

Concentrations of IL-6 were analyzed pre- and postexercise using a commercially available ELISA (Quantikine HS, R&D Systems, Minneapolis, MN) on a FLUOstar OPTIMA plate reader (BMG Labtech, Ortenberg, Germany). The coefficient of variation for IL-6 determination was 4.9%. Hepcidin measurements were performed on pre- and 3-hr postexercise samples by a combination of weak cation exchange chromatography and time-of-flight mass spectrometry using synthetic hepcidin-24 as an internal standard for quantification (Kroot et al., 2010; Swinkels et al., 2008). Hepcidin-25 concentrations are expressed as nmol/L (nM). The lower limit of detection of this method is 0.5 nM, and data points below this value were substituted with the lower limit of detection divided by √2 (Croghan & Egeghy, 2003).

Statistical Analysis

The data were analyzed with a general linear mixed model using the R package lm4 (R Core Team, 2017) to accommodate the unbalanced design and the repeated measurements (Jennrich & Schlucht, 1986). A random intercept for both athlete and camp was included to adjust for the baseline levels and interindividual homogeneity, and to account for the partial cross-over design. All models were estimated using restricted maximum likelihood. Visual inspection of the residual plots did not reveal any obvious deviations from homoscedasticity or normality. The p values were obtained using Type II Wald F tests with Kenward–Roger degrees of freedom, as implemented in the R package car (Fox & Weisberg, 2011). To account for differences in ambient temperature during each trial, the mean ambient temperature for each individual’s trial was included as a covariate in the analysis of IL-6 and hepcidin concentrations. Furthermore, given the strong influence that iron status can have on the postexercise hepcidin response (Peeling et al., 2014), serum ferritin was used as a covariate in the analysis of hepcidin. Pre- to postexercise changes in IL-6 and hepcidin are expressed as X-fold changes. To construct a confidence interval (CI) around the fold change, a bootstrapped CI based on 10,000 replications was calculated using the R package boot (Canty & Ripley, 2017).

Results

Serum ferritin concentrations decreased from the baseline to postintervention in both groups, F(1, 17)=5.08, p = .039, with the magnitude of decrease similar between groups, F(1, 17)=0.726, p = .411; Table 3. No differences in serum ferritin were evident between groups at the baseline or postintervention, F(1, 10)=2.0, p = .189. The serum iron concentrations were similar between groups, F(1, 15)=0.53, p = .479, and trials, F(1, 16)=0.85, p = .371. The hemoglobin concentrations, F(1, 17)=6.95, p = .017, and hematocrit, F(1, 17)=4.96, p = .040, decreased from the baseline to postintervention; however, no differences between groups were evident. In the MAX group, the ambient temperature was greater during postintervention, compared with the baseline, F(1, 17)=8.715, p = .009; however, no difference between trials for CON or differences between dietary groups were evident. Significant differences in humidity were also evident between the dietary interventions, F(1, 16)=4.59, p = .048, and trials, F(1, 17)=19.47, p = .004.

The concentrations of IL-6 consistently increased from pre- to postexercise, F(1, 51)=157.32, p < .001, the magnitude of which was generally greater in the CON than the MAX group, F(1, 51)=5.11, p = .03; Figure 2a. While these differences did not reach significance between trials, F(1, 51)=1.49, p = .22, a greater postexercise increase was evident at the baseline in CON (20.1-fold increase; 95% CI [9.2, 35.7]), compared with MAX (10.2-fold increase; 95% CI [3.7, 18.7]), mean estimate = 10.8 pg/ml; 4.2 to 14.6 pg/ml). The equivalent comparison during the postintervention trial revealed no differences between the CON (13.6-fold increase; 95% CI [9.2, 20.5]) and MAX groups (11.2-fold increase; 95% CI [6.5, 21.3]), mean estimate = −1.6 pg/ml; −5.3 to 4.9 pg/ml).

An increase in hepcidin-25 concentrations was evident at 3 hr postexercise, F(1, 51)=70.24, p < .001; Figure 2b. Specifically, the increase in hepcidin-25 was greater at the baseline in CON (10.8-fold increase; 95% CI [4.8, 21.2]) than MAX (8.8-fold increase; 95% CI [3.9, 16.4]; mean estimate = 8.7 nM; 4.8 to 11.4 nM). However, postintervention, no differences in the hepcidin-25 increase were evident between the CON (7.1-fold increase; 95% CI [2.1, 15.4]) and MAX group (6.3-fold increase; 95% CI [1.8, 14.6], mean estimate = 3.6 nM; −5.4 to 0.6 nM). Serum ferritin, F(1, 27)=9.80, p = .004, and ambient temperature, F(1, 37)=6.74, p = .014 were both significant variables in the hepcidin model, indicating that higher resting serum ferritin and hotter trial temperatures were associated with higher hepcidin levels 3 hr postexercise.

Discussion

This study demonstrates that the 2-week novel MAX dietary intervention provided no additional benefits to inflammatory control or hepcidin activity following a 26-km standardized race walking protocol, compared with a more moderate CHO intake. This outcome is in agreement with some of our previous work,
indicating that chronic macronutrient manipulations may not have a strong influence on iron regulation in elite endurance athletes (McKay et al., 2019b). Instead, it appears that resting iron status (serum ferritin concentrations) and environmental conditions (ambient temperature) had a larger influence on the increase in hepcidin levels 3 hr postexercise than dietary CHO intake.

A similar decrease in iron status (determined via serum ferritin) was evident after 2 weeks in both the CON (−23%) and MAX (−17%) group. This short-term reduction in serum ferritin occurred, despite dietary iron intakes being well above the recommended daily intake of 8 mg/day for males in both groups (Trumbo et al., 2001). Our findings are in-line with previous studies showing that iron stores can decrease when individuals undertake heavy periods of physical training (Auersperger et al., 2013; Karl et al., 2010), even when dietary iron intake is adequate (McKay et al., 2019a). Although this pattern may reflect a positive adaptation to the training stimulus with iron stores used to synthesize new tissues and proteins, it appears that decreases in serum ferritin can occur in as little as 2 weeks with high volume/intensified training loads. Therefore, preparation for this type of training should occur, as is the case for specialized interventions, such as altitude exposure, to ensure that the athlete’s iron status is sufficient to support the desired adaptive response (Govus et al., 2015). This requirement is a key consideration for dietitians and sports physicians working with endurance athletes who might be at a higher risk of compromised iron stores.

Differences in IL-6 levels were evident between groups at baseline, but not postintervention, which might relate to the attenuated IL-6 response that occurred within the CON group postintervention. Training can attenuate IL-6 levels postexercise in both recreationally active individuals (Croft et al., 2009) and elite race walkers (McKay et al., 2019a). As a result of training, the muscle becomes less dependent on glucose and muscle glycogen as a substrate during submaximal exercise (Hennigar et al., 2017), subsequently minimizing the signal for muscle-derived IL-6 release. Meanwhile, the MAX group experienced a similar IL-6 response during both trials; that is, they did not experience the same attenuation in IL-6 postintervention as CON, despite demonstrating similar improvements in peak aerobic capacity following the dietary intervention to the CON group (unpublished data). A potential explanation is that the increased amount of CHO ingested by the MAX group during the postintervention trial (90 g/hr) resulted in larger perturbations to gastrointestinal integrity and function and, combined with the hotter trial temperature, potentially signaled an increased inflammatory response to exercise (Costa et al., 2017b; Starkie et al., 2005). Nevertheless, the difference in the baseline values between groups, in combination with the high variability in IL-6, makes it difficult to isolate any meaningful impact of the dietary interventions. It seems that moderate CHO intake may be sufficient in preventing large increases in postexercise IL-6 concentrations, and there is no additional benefit (to the inflammatory response) from increasing CHO ingestion further.

At 3 hr postexercise, the hepcidin response was greater in the CON than the MAX group at the baseline, with no difference between groups evident postintervention, again, likely related to an attenuated response in CON postintervention. While increases in hepcidin levels occurred in a similar manner to that of IL-6, our results demonstrated that higher serum ferritin concentrations were associated with greater hepcidin levels, implying that serum ferritin has a larger role in determining the magnitude of postexercise hepcidin increase. Indeed, the previous modeling of the iron-regulatory response to exercise indicated that serum ferritin, rather than IL-6, has the greatest influence on hepcidin concentrations 3 hr postexercise (Peeling et al., 2017). Furthermore, our earlier work has shown that dietary-induced changes in IL-6 can occur without

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### Table 3 Hematological Variables and Environmental Conditions During the 26-km Race Walking Protocol for Athletes Adhering to a CON and MAX CHO Diet During the Baseline and Postintervention Trials

<table>
<thead>
<tr>
<th></th>
<th>MAX Baseline</th>
<th>MAX Postintervention</th>
<th>CON Baseline</th>
<th>CON Postintervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ferritin (µg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean</td>
<td>51</td>
<td>43**</td>
<td>81</td>
<td>62**</td>
</tr>
<tr>
<td>SD</td>
<td>27</td>
<td>22</td>
<td>33</td>
<td>34</td>
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<tr>
<td>Serum iron (µmol/L)</td>
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<td></td>
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</tr>
<tr>
<td>Mean</td>
<td>13.7</td>
<td>14.1</td>
<td>17.8</td>
<td>15.6</td>
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<tr>
<td>SD</td>
<td>2.2</td>
<td>4.2</td>
<td>6.0</td>
<td>8.2</td>
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<tr>
<td>Hemoglobin (g/dl)</td>
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<td></td>
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<tr>
<td>Mean</td>
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<td>14.7**</td>
<td>15.5</td>
<td>14.6**</td>
</tr>
<tr>
<td>SD</td>
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<td>1.7</td>
<td>1.0</td>
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<tr>
<td>Hematocrit (%)</td>
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<tr>
<td>Mean</td>
<td>42.9</td>
<td>42.2**</td>
<td>44.4</td>
<td>42.4**</td>
</tr>
<tr>
<td>SD</td>
<td>2.0</td>
<td>0.8</td>
<td>4.6</td>
<td>2.9</td>
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<tr>
<td>Temperature (°C)</td>
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<tr>
<td>Mean</td>
<td>17.6</td>
<td>19.6**</td>
<td>16.4</td>
<td>15.0</td>
</tr>
<tr>
<td>SD</td>
<td>3.9</td>
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<td>Humidity (%)</td>
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<tr>
<td>Mean</td>
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<td>49**</td>
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<tr>
<td>SD</td>
<td>10</td>
<td>5</td>
<td>11</td>
<td>8</td>
</tr>
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</table>

*Note. Values are presented as mean ± SD. CON = moderate; MAX = very high; CHO = carbohydrate.

**Significant difference compared with MAX. **Significant difference compared with baseline.
knowledge, only one study has examined the impact of heat on
IL-6 and hepcidin activity; however, in this instance, heat was combined
with hypoxia, and the impact of temperature alone was not deter-
mined (Hayashi et al 2020). Therefore, we are unsure of the mecha-
nisms underpinning the association between heat and hepcidin
concentrations evident in our study. It is of particular interest
that this relationship appeared to occur independent of IL-6, despite
the fact that exercising in the heat can augment the IL-6 response
to exercise (Starkie et al., 2005), which potentially implicates a
noninflammatory mechanism of upregulation. However, it should
be emphasized that our findings do not infer causality; therefore,
there is the possibility that a third factor, affected by both hepcidin
levels and ambient temperature, has mediated this association.
Nevertheless, our findings provide support for further work to
be undertaken to establish the impact of an athlete’s thermal
environment on the hepcidin response to exercise and the subse-
quent impact on iron absorption.

There are inherent limitations associated with implementing
applied field-based research with elite athletes that should be
acknowledged. First, employing an outdoor exercise protocol meant
that the differences in environmental conditions may have affected
our ability to directly assess the impact of the dietary interventions.
While environmental conditions were accounted for statistically,
the impact that changing conditions had on factors, such as blood
.pressure, sweat rates, and plasma volume, was not quantified here.
Furthermore, given no research has yet examined iron-regulatory
responses to exercising in the heat, the full implications of the current
study remain unclear. In addition, there were many elements included
in the novel MAX dietary intervention, including differing CHO
intakes, gut-training strategies, low residual diets, and the periodic
ingestion of sucralose. Accordingly, the current study was unable to
isolate the direct impact each of these factors had on iron metabolism,
instead, assessing the practical implications to iron regulation from
implementing this strategy as a whole. Finally, this study exclusively
studied male athletes. With the prevalence of iron deficiency higher in
females, compared with males athletes (Rowland, 2012), assessing
the potential for CHO-based nutritional interventions to improve iron
metabolism in females is warranted.

The other variable associated with hepcidin concentrations in
the current study was the ambient temperature of each trial. With
daily and seasonal differences in weather occurring between the
two training camps, and a large proportion of each trial conducted
outdoors, we deemed it important to account for the effect of
ambient temperature on the inflammatory and hepcidin response to
exercise. Although we demonstrated that hotter trial temperatures
were associated with a greater hepcidin response, ambient temper-
ature was not associated with increased IL-6 concentrations. To our
knowledge, only one study has examined the impact of heat on

Figure 2 — (a) IL-6 concentrations pre- and postexercise and
(b) hepcidin-25 concentrations pre- and 3 hr postexercise with athletes
adhering to a CON and MAX CHO diet during the baseline and
postintervention trials. Results presented as mean ± SD. IL-6 = interleukin-
6; CON = moderate; MAX = very high; CHO = carbohydrate; CI =
confidence interval. *Significant increase from preexercise. #Differences
between dietary interventions based on CIs.

subsequent alterations to hepcidin concentrations (McKay et al.,
2019b). Taken together, it appears that serum ferritin concentrations
may be the most dominant factor in the regulation of postexercise hepcidin levels. Given the similarities between the
IL-6 and hepcidin response to exercise, it is possible that IL-6,
driven by the dietary intervention, also influenced the hepcidin
levels. However, it is more likely that the observed changes to the
hepcidin levels in the 3-hr postexercise period occurred as part of a
homeostatic response to the lower serum ferritin concentrations
(Peeling et al., 2014; Peeling et al., 2017).

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**Conclusion**

Very high CHO diets (10–12 g/kg/day), such as those practiced by
endurance athletes as part of gut-training strategies, do not have a clear
impact on iron regulation in elite athletes. Our data confirms
previous research demonstrating that serum ferritin levels have a
strong influence on postexercise hepcidin concentrations (McKay
et al., 2019b; Peeling et al., 2014; Peeling et al., 2017). Moreover,
ambient temperature may also be associated with the increase in
hepcidin concentrations 3 hr postexercise; this relationship war-
rants further investigation.

**Acknowledgments**

This study was financially supported by grants from the Australian
Catholic University Research Fund and the Alliance for Potato Research
and Education. The study was designed by A.K.A. McKay, P. Peeling,
D.B. Pyne, N. Tee, I.A. Heikura, A.P. Sharma, J. Whitfield, M.L. Ross, and
L.M. Burke; the data were collected and analyzed by A.K.A. McKay, N.
Tee, M. Welveart, I.A. Heikura, A.P. Sharma, J. Whitfield, M.L. Ross,
R.P.L. van Swelm, C.M. Laarakkers, and L.M. Burke; drafting the article
or revising it critically for important intellectual content was undertaken by
A.K.A. McKay, P. Peeling, D.B. Pyne, and L.M. Burke. All authors
approved the final version of the paper.
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