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Original research

Acute carbohydrate ingestion does not influence the post-exercise iron-regulatory response in elite keto-adapted race walkers

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ABSTRACT

Objectives: Adhering to a low carbohydrate (CHO) high fat (LCHF) diet can alter markers of iron metabolism in endurance athletes. This investigation examined the re-introduction of CHO prior to, and during exercise on the iron-regulatory response to exercise in a homogenous (in regard to serum ferritin concentration) group of athletes adapted to a LCHF diet.
Design: Parallel groups design.
Methods: Three weeks prior to the exercise trials, twenty-three elite race walkers adhered to either a CHO-rich (n = 14) or LCHF diet (n = 9). A standardised 19–25 km race walk was performed while athletes were still adhering to their allocated dietary intervention (Adapt). A second test was performed three days later, where all athletes were placed on a high CHO diet (CHO Restoration). Venous blood samples were collected pre-, post- and 3 h post-exercise and measured for interleukin-6 (IL-6) and hepcidin-25.
Results: The post-exercise IL-6 increase was greater in LCHF ($p < 0.001$) during both the Adapt (LCHF: 13.1-fold increase; 95% CI: 5.6–23.0, CHO: 8.0-fold increase; 5.1–11.1) and CHO Restoration trials (LCHF: 18.5-fold increase; 10.9–28.9, CHO: 6.3-fold increase; 3.9–9.5); outcomes were not different between trials ($p = 0.84$). Hepcidin-25 concentrations increased 3 h post-exercise ($p < 0.001$), however, they did not differ between trials ($p = 0.46$) or diets ($p = 0.84$).
Conclusions: The elevated IL-6 response in athletes adapted to a LCHF diet was not attenuated by an acute increase in exogenous CHO availability. Despite diet-induced differences in IL-6 response to exercise, post-exercise hepcidin levels were similar between diets and trials, indicating CHO availability has minimal influence on post-exercise iron metabolism.

Practical implications

- Chronic adherence to a LCHF diet increases the inflammatory response to exercise, which may have downstream effects on a number of body systems.
- The increased inflammatory response in keto-adapted athletes is not attenuated by an acute increase in CHO consumption.
- IL-6 has a small role in determining the hepcidin response to exercise, therefore a LCHF diet is unlikely to be detrimental to post-exercise iron metabolism.
- The pleiotropic nature of IL-6 implies other body systems regulated by IL-6, including bone health and immune functioning, may be influenced by adherence to a LCHF diet.

1. Introduction

Strenuous exercise causes perturbations to the body, with robust evidence of immune and inflammatory responses subse-

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quent to an exercise bout.[1] Concentration of interleukin-6 (IL-6), an important cytokine involved in the inflammatory cascade, peaks immediately post-exercise, with the magnitude of increase dependent on factors such as exercise duration, modality, intensity and muscle glycogen status.[1] Indeed, several studies have reported an amplification of the inflammatory response to exercise when undertaken with low carbohydrate (CHO) availability.[2,3] Under conditions of glycogen depletion, and/or a limited supply of exogenous carbohydrate during exercise, there is a greater increase in the muscle release of IL-6 to stimulate glucose production via hepatic glycogenolysis or gluconeogenesis, and to provide additional substrate for muscle and other energy-requiring systems.[4]

Alterations in the IL-6 response to exercise have the potential to modify a range of downstream targets. Iron status, playing an important role in oxygen transport, energy metabolism, and neurological and immunological functioning,[5] may be involved in such outcomes. Adequate iron stores are important for athletes to support optimal adaptation to training, ultimately influencing athletic performance. Despite the importance of iron, both acute and chronic exercise can result in multiple avenues of iron loss and altered iron metabolism.[6] One such process is the exercise-associated increase in the iron-regulatory hormone hepcidin.[7] In the context of exercise, it is well-established that a transient increase in hepcidin occurs ~3–6 h post-exercise in response to increases in IL-6,[7,8] potentially creating a period of reduced iron absorption, which may compromise an athlete's iron status. Furthermore, there is evidence that when exercise is undertaken with low glycogen stores, the increase in hepcidin levels 3 h post-exercise is amplified.[3] This result raises the possibility that training chronically with low CHO availability may, over time, negatively affect iron status.

Recently, this concept was investigated in a study examining the influence of a 3-week ketogenic, low CHO high fat (LCHF) diet on the iron-regulatory response to exercise in elite-level race walkers.[9] During the post-intervention testing of this study, the increase in IL-6 following a 19–25 km race walk was greater in the LCHF group (13.6-fold increase over pre-exercise), as compared to the athletes who had adhered to a CHO-rich diet and consumed CHO during exercise (7.6-fold increase). Furthermore, an attenuated post-exercise hepcidin-25 response in the CHO group was noted post-intervention, as compared to baseline testing (7.1 vs 4.8-fold increase), whereas the equivalent change after adhering to a LCHF (4.3 vs 6.3-fold increase) was unclear. However, a confounding feature of this initial study was a marked difference in resting serum ferritin (sFer) levels between groups post-intervention. Since sFer is a second regulating factor of post-exercise hepcidin levels,[10] higher levels in the LCHF group potentially contributed to a greater 3 h post-exercise hepcidin response.[9] Therefore, it is unclear whether differences in sFer or the dietary intervention was the dominant factor in explaining the higher hepcidin response. It is also unknown whether the greater iron-regulatory response evident with the LCHF dietary intervention was an acute alteration to the exercise feeding, or a chronic adaptation to the 3-week training-diet intervention. Therefore, the aim of this investigation was to quantify the effect of re-introducing CHO prior to, and during exercise, on the IL-6 response to exercise and its downstream effect on hepcidin, within a homogenous (in regard to sFer concentration) subset of elite, keto-adapted race walkers.

2. Methods

Twenty-seven internationally competitive race-walking athletes participated in this study. Four athletes were subsequently excluded from analysis due to injury (n = 1), illness (n = 1) and unusually high sFer levels (>500 μg/L; n = 2), resulting in a total of 23 athletes, consisting of 17 males and 6 females (see Table 1). All athletes met the International Association of Athletics Federation's race walking standard for a World Championship or Olympics, in either the 20 or 50 km event. Informed consent was obtained from all athletes and ethical approval was granted by the Human Ethics Committee of the Australian Institute of Sport (AIS; approval: 20161201).

In the three weeks prior to this investigation, all athletes adhered to one of three dietary interventions while completing an intensified block of training. The three dietary interventions were: a high CHO diet (HCHO; n = 8); periodised CHO availability (PCHO; n = 6) or a LCHF diet (n = 9). Throughout the dietary intervention, daily CHO intakes for the HCHO and PCHO groups were identical, with differences attributed to the timing of ingestion around key training sessions. On the basis of identical CHO intakes, the data from athletes adhering to the HCHO and PCHO diets were aggregated into one group for this study (CHO; n = 14). Further details regarding each dietary intervention employed in the lead-up to this study are reported elsewhere.[11] Following the 3-week adaptation period, athletes completed a race walking protocol on two occasions to determine the iron-regulatory response to exercise (Fig. 1). This protocol included a trial on day 1 (Adapt), performed while athletes were still adhering to (and were therefore adapted to) their allocated dietary intervention. A 3-day recovery period was subsequently employed, during which time, athletes remained on their allocated diets. The second testing session was then performed on day 5, which examined the acute effects of de-adaptation to the LCHF diet (CHO Restoration), with athletes consuming CHO for the first time 2 h prior to, and during the exercise task.

The methods used for this race-walking protocol are identical to those presented in more detail elsewhere.[9] Athletes arrived to the laboratory following an overnight fast and a baseline venous blood sample drawn from an indwelling cannula. Athletes then received a standardised breakfast providing 2 g kg^{-1} body mass (BM) CHO. The exception here occurred during the Adapt trial, where the LCHF group consumed a high fat isocaloric meal in accordance with their dietary guidelines (see Fig. 1). A second venous blood sample was then collected 15 min prior to the start of the exercise protocol (105 min post-breakfast). Athletes then commenced a standardised race walking protocol, which involved an evenly paced 19 or 25 km race-walking trial for female and male athletes, respectively. During this protocol, kilometres 0–1, 6–7, 12–13, 18–19 (all athletes) and 24–25 (males only) were completed indoors on a motorised treadmill. Athletes walked at their target speed (10 or 11 km h^{-1} for females and 12 or 13 km h^{-1} for males), established previously during a graded exercise test. This speed equated to 77.1 ± 6.1% and 76.4 ± 2.5% VO_{2max} for CHO and LCHF, respectively. The remaining distance was completed on a 5 km outdoor circuit on the grounds surrounding the AIS physiology laboratory. Heart rate and RPE were collected during the last 30 s of the exercise protocol. Athletes consumed a pre-prepared CHO solution regularly throughout the exercise protocol, equating to ~70 g/h CHO. For athletes adhering to the LCHF diet, high fat snacks and non-caloric electrolyte drinks replaced CHO beverages during the Adapt trial to match the total energy consumed during CHO-fuelled trials. Immediately post-exercise, a third venous blood sample was drawn, before athletes rested in the laboratory for a 3 h period. Given the long protocol duration, a recovery drink in accordance with the athlete's dietary allocation (either 0 or 1.5 g kg^{-1} BM CHO) was provided 30 min after exercise to limit hunger. Athletes abstained from any further food intake until a final blood sample was collected at 3 h post-exercise.

For each athlete, 4 blood samples per testing session were collected into 4 ml SST gel separator tubes. Samples were left to clot for 30 min at room temperature, then centrifuged at 2200 G, 4 °C for 10 min. Serum was immediately frozen at −80 °C until batch analysis was conducted. Concentrations of serum iron and sFer were

Table 1
Characteristics of race walking athletes adhering athletes adhering to a carbohydrate rich diet (CHO) or low carbohydrate, high fat (LCHF) diet. Training volume data calculated from subsequent 3 weeks on dietary intervention. Data presented as mean ± standard deviation.

	CHO		LCHF	
	Males (n = 9)	Females (n = 5)	Males (n = 8)	Females (n = 1)
Age (y)	24.5 ± 3.6	27.2 ± 3.4	28.7 ± 3.6	30.7
Body Mass (kg)	67.1 ± 5.8	56.4 ± 3.9	67.8 ± 6.0	51.5
VO_{2max} (ml kg min^{-1})	58.7 ± 4.0	56.5 ± 4.1	62.2 ± 4.8	53.6
10 km personal best (min:sec)	41:10 ± 1:04	44:52 ± 0:21	40:52 ± 1:16	42:43
20 km personal best (min:sec)	84:04 ± 3:45	92:08 ± 1:35	83:36 ± 2:39	90:20
Weekly training volume (km)	124 ± 22	99 ± 18	131 ± 15	116

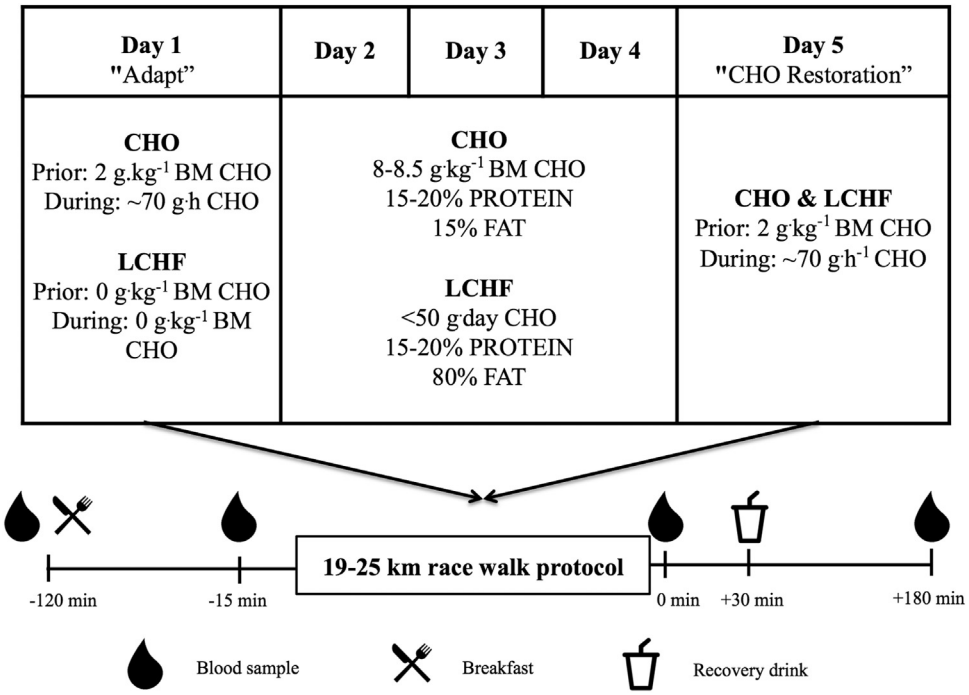

Fig. 1. Schematic representation of the study design, including the standardised laboratory and field race walking protocol and dietary intake targets.

determined on fasting samples via a COBAS Integra 400 automated biochemistry analyser (Roche Diagnostics, Switzerland). Concentrations of IL-6 immediately pre- and post-exercise were analysed using a commercially available ELISA (Quantikine HS, R&D Systems, Minneapolis, USA). The coefficient of variation for IL-6 determination was 4.1%. Hepcidin-25 concentrations were analysed with serum obtained when athletes were fasted and at 3 h post-exercise using a combination of weak cation exchange chromatography and time-of-flight mass spectrometry.[12,13] A detailed description of methods used for blood analysis has been reported previously.[9]

Data were analysed with a General Linear Mixed Model using the R package lme4[14] to accommodate the unbalanced design and repeated measurements.[15] A random intercept for subjects was included to adjust for baseline levels and inter-individual homogeneity. All models were estimated using Restricted Maximum Likelihood. Visual inspection of residual plots revealed no obvious deviations from homoscedasticity or normality. P-values were obtained using Type II Wald F tests with Kenward–Roger degrees of freedom as implemented in the R package car.[16] Initial models included all possible interactions, but non-significant interaction terms were dropped from the models for ease of interpretation. In the case of hepcidin-25, sFer was included as a covariate in the model to account for its influence on the 3 h post-exercise hepcidin response.[17] The magnitude of effects is presented as x-fold change. To construct a 95% confidence interval (CI) around the fold change, a bootstrapped confidence interval based on 10,000 replications was calculated using the R package boot.[18]

Table 2
Haematology and performance variables from the 19–25 km race walk protocol performed at Adapt and CHO Restoration for the CHO and LCHF groups. Data presented as mean ± standard deviation.

	Adapt		CHO restoration	
	CHO	LCHF	CHO	LCHF
Serum ferritin (μg/L)	58.7 ± 47.5	57.9 ± 45.6	59.7 ± 46.6	60.3 ± 54.2
Serum iron (μmol/L)	14.1 ± 4.2	11.5 ± 3.3	14.7 ± 4.2	11.6 ± 1.9
Heart rate (bpm)	151 ± 12	167 ± 12	156 ± 19	161 ± 9
RPE (6–20)	14 ± 2	17 ± 2	13 ± 2	15 ± 1
Total time (h:min)	1:50 ± 0:15	2:04 ± 0:13	1:49 ± 0:15	1.55 ± 0:07

3. Results

Fasting serum ferritin and serum iron concentrations in each of the dietary conditions are summarised in Table 2. No substantial differences in serum ferritin were evident between the Adapt and CHO restoration trials ($F(1,21) = 0.51$, $p = 0.48$) or between the CHO and LCHF groups ($F(1,22) = 0.01$, $p = 0.93$). Furthermore, serum iron concentration did not differ between trials ($F(1,22) = 0.24$, $p = 0.63$), however, it was moderately (~20–25%) higher in CHO compared to LCHF ($F(1,22) = 4.00$, $p = 0.06$).

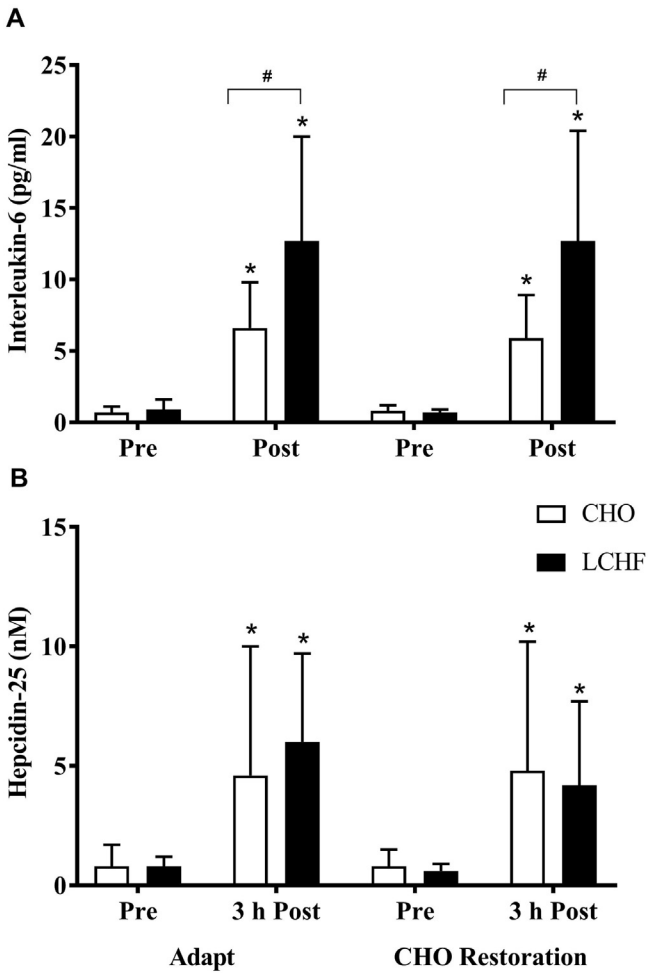

Fig. 2. (A) Interleukin-6 (IL-6) concentrations pre- and post-exercise and (B) hepcidin-25 concentrations pre- and 3 h post-exercise in the carbohydrate (CHO) and low carbohydrate, high fat (LCHF) groups during Adapt and CHO Restoration trials. Data presented as mean ± standard deviation. Pre-exercise hepcidin-25 analysis was performed on fasted samples and IL-6 analysis performed on fed samples. * significant within-group difference from pre-exercise, # significant between-group change.

A significant ~5 to 10-fold increase in IL-6 (Fig. 2) was evident post-exercise in both dietary conditions ($F(1,64) = 119.07$, $p < 0.001$), with only trivial differences evident between the Adapt and CHO Restoration trials ($F(1,68) = 0.14$, $p = 0.71$). There were substantial differences in the increase in IL-6 between diets ($F(1,66) = 18.0$, $p < 0.001$), with a greater post-exercise response evident in the LCHF group during both the Adapt (13.1-fold increase; 95% CI: 5.6–23.0) and CHO Restoration trials (18.5-fold increase; 10.9–28.9) than the CHO group (Adapt: 8.0-fold increase; 5.1–11.1, CHO Restoration: 6.3-fold increase; 3.9–9.5). Moderate to large increases in hepcidin-25 occurred 3 h post-exercise (compared to pre-exercise) during the Adapt and CHO Restoration trials ($F(1,66) = 58.75$, $p < 0.001$). In contrast, no clear differences were evident between trials ($F(1,69) = 0.56$, $p = 0.46$) and diets ($F(1,21) = 0.04$, $p = 0.84$).

4. Discussion

This study further explores previous findings[9] indicating that adherence to a ketogenic LCHF diet can augment the post-exercise inflammatory (IL-6) response and hepcidin levels 3 h post-exercise. With elevated hepcidin being a potentially unfavourable prospect for optimal iron status, our interest was in determining whether these alterations were a downstream outcome of elevated IL-6 levels, or otherwise attributable to the homeostatic influence of higher sFer levels. Our findings confirm that strenuous exercise undertaken with chronic adaptation to low endogenous and exogenous CHO availability is associated with a greater post-exercise IL-6 response than when exercise is undertaken with high availability of CHO. Furthermore, an acute increase in exogenous CHO availability in keto-adapted athletes did not attenuate the elevated IL-6 response. It appears sFer concentration is the major influence on the magnitude of post-exercise hepcidin response to strenuous exercise. We make this assertion on the basis that groups matched for baseline sFer levels show similar increases in hepcidin-25 concentration at 3 h post-exercise, despite diet-induced differences in IL-6 responses.

The results of this study confirm our previous findings that strenuous exercise undertaken after chronic adaptation to low CHO availability is associated with an increased release of the inflammatory cytokine, IL-6.[9] However, the increased IL-6 response to exercise persisted after the acute re-introduction of CHO into the diet on the day of exercise, which was implemented according to current sports nutrition guidelines for the pre-event meal and CHO intake during endurance exercise.[19] An explanation for this outcome is that muscle glycogen stores, rather than exogenous CHO availability is the main driver of IL-6 release,[4] and that the amount of CHO consumed prior to the CHO Restoration trial (2 g kg^{-1} BM) was inadequate in achieving functional replenishment of muscle glycogen stores. Support for the first part of this theory is provided by observations that acute strategies to lower muscle glycogen prior to exercise can yield a clear increase in post-exercise IL-6 concentrations.[3,4]

We were unable to perform muscle biopsies on these elite athletes to measure muscle glycogen concentration, either in the fasted stated nor after the 2 h post-meal period before exercise. However, muscle glycogen content can be reduced by 5-days of adherence to a high fat diet (19% CHO, 68% fat).[20] Moreover 24 h of high CHO intake (9.9 g kg^{-1} BM) was necessary to replenish muscle glycogen stores to similar levels as those seen in athletes consistently adhering to a HCHO diet.[20] In terms of the ketogenic LCHF diet, there is one account[21] of greatly enhanced rates of muscle glycogen synthesis in long-term keto-adapted athletes (i.e. hourly rates of ~20 mmol/kg wet weight muscle) in the 2 h after exercise, where gluconeogenic substrates were presumably in supply. However, other studies of keto-adapted athletes have reported substantially lower muscle glycogen stores at rest than in athletes consuming a high CHO, energy-matched diet.[22,23] Therefore, we speculate that muscle glycogen was lower in the LCHF group than the CHO groups at the start of exercise in the current study despite the pre-exercise meal, however, muscle biopsy studies are needed to confirm this assertion.

Previous investigations have reported an increase in post-exercise hepcidin concentrations, associated with elevated IL-6 levels, when exercise was undertaken with low CHO availability. For example, Badenhorst et al.[3] manipulated CHO status prior to two protocols of interval running, by depleting glycogen with a separate exercise session undertaken 24 h earlier, and then providing diets of either 3 g kg^{-1} or 10 g kg^{-1} BM CHO during the recovery period. The consumption of the HCHO diet attenuated the post-exercise IL-6 increase, with a trend towards lower hepcidin levels 3 h post-exercise, when compared with the trial involving a low CHO availability diet. Meanwhile, our previous investigation[9] examined the chronic adoption of strategies that reduce CHO availability of both endogenous and exogenous sources. This reduction in CHO was associated with amplified IL-6 levels immediately post-exercise and a moderate increase in hepcidin levels 3 h post-exercise compared to athletes adhering to high CHO dietary strategies before and during exercise. However, since there were

differences in the sFer stores of the athletes between groups, a factor known to influence post-exercise hepcidin activity,[10,17] we were unable to isolate the reason for our findings.

The results of the current study showed no differences in the magnitude of the post-exercise increase in hepcidin levels subsequent to a strenuous 25 km race walking protocol between scenarios of high endogenous/high exogenous CHO availability, low endogenous/low exogenous CHO availability and low endogenous/high exogenous CHO availability. Since post-exercise hepcidin-25 levels were not influenced by changes in the inflammatory response to the exercise bout associated with the availability of endogenous CHO stores, it appears the outcomes of our earlier study,[9] were potentially influenced by the underlying sFer levels of the athletes. This data may suggest that sFer concentration plays a more dominant role in regulating changes in post-exercise hepcidin levels. A positive association between iron status and hepcidin levels has previously been established, both at rest[24] and post-exercise,[10,17] with large correlations evident between hepcidin and serum iron ($r = 0.63$), and sFer ($r = 0.69$).[10] It is likely that a suppressed hepcidin response in athletes with low iron levels occurs as a homeostatic response,[25] prompting the body to enhance iron absorption/recycling and increase iron stores. In clinical investigations, hepcidin increases have previously been induced by elevations in IL-6,[26] typically from much higher circulating concentrations (~250 pg/ml) than those reported in the current study (~10 pg/ml). Exercise-associated IL-6 increases appear to be a small contributor ($r = 0.21$) to the variance seen in the post-exercise hepcidin levels of endurance athletes,[10] and therefore, its effect may not be large enough to override the strong homeostatic influence of underlying iron status in regulating changes to hepcidin concentrations. Although in the current study the 3-week LCHF diet was unlikely to be detrimental to post-exercise iron metabolism, it should be noted that a previous repeated measures design study of exercise-associated responses to acute glycogen manipulation reported greater hepcidin and inflammatory responses to exercise undertaken with a low glycogen preparation despite similar sFer levels.[3] As such, given these acute studies have demonstrated that dietary manipulation can alter hepcidin activity,[3] consideration could be given to the possibility that prolonged adherence to a low CHO diet may result in an adaptive state, whereby these acute alterations may subside as the dietary adherence is maintained over time.

Chronic exposure to a LCHF diet, either alone[21,23] or combined with acute consumption of CHO prior to and during an event[27] is practiced by some endurance and ultra-endurance athletes. As such, the metabolic response to exercise in keto-adapted athletes, even when CHO is consumed during exercise, is an area of continued interest. While it appears diet-induced alterations in IL-6 are unlikely to influence post-exercise hepcidin activity, IL-6 is a pleiotropic cytokine that regulates a host of other body systems.[28] Therefore, increases in IL-6 concentrations induced by adherence to a LCHF diet should be considered in relation to other inflammatory-mediated processes vital to maintaining good health in athletes, including bone metabolism,[29] nutrient (i.e. zinc) regulation[30] and immune function.[28] This information may be particularly important in maintaining athlete training consistency by minimising the time lost to illness and injury.

5. Conclusion

Adherence to a LCHF diet heightened the post-exercise IL-6 response, but did not influence the magnitude of hepcidin elevation in the hours after prolonged, endurance exercise. The influence of IL-6 is unlikely the primary driver of increased hepcidin activity during exercise undertaken by elite athletes, where only relatively small increases in the cytokine levels occur. Instead, it appears that baseline iron status (established via sFer) is a more dominant factor regulating the post-exercise hepcidin response. Furthermore, the acute consumption of exogenous CHO in keto-adapted athletes prior to, and during exercise was ineffective at attenuating the post-exercise IL-6 response to levels similar to athletes consuming a CHO-rich diet. Given the pleiotropic nature of this cytokine, the increased IL-6 activity may negatively influence other body processes. The long-term impact of adhering to LCHF on other health outcomes warrants further investigation.

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