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ORIGINAL ARTICLE

Macronutrient Intake and Energy Availability In Young Male Elite Cyclists: The Importance of Adequate Cho Intake

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Abstract. *Background and Aims:* Macronutrient intake before, during and after exercise may influence performance and inflammatory responses in elite athletes. In this context, we analyze the adequacy of nutritional status, daily energy and macronutrient intake as well as intake during and after acute training, energy availability (EA) and inflammatory response after acute training associated with carbohydrate intake (CHO) in ten young male elite cyclists. *Methods:* Ten Slovenian competitive cyclist, aged between 15 and 30 years participated in this study. Energy intake (EI) and macronutrient intake were assessed using 3-day dietary protocols. Venous blood samples were collected before and after acute exercise to analyse serum biochemical parameters, as well as pro-inflammatory (CRP, IL-6 and TNF-α) and anti-inflammatory (adiponectin) cytokines. *Results:* The average macronutrient composition of daily EI was 6.1 g/kg body mass (BM) of CHO, 1.7 g/kg BM of protein, and 29% EI fat, and in most cases did not meet exercise intensity recommendations. Less than 50% of all participants consumed sufficient CHO daily and during exercise (mean intake was 6.1 g/kg BM and 32g/h, respectively). Protein intake was the only category in which most participants (70%) met the recommended daily amounts (1.7 g/kg BM) and the amounts during and after exercise (0.08 g/kg BM and 0.5 g/kg BM). Fat intake during (15% EI meal) and immediately after exercise (32% EI meal) exceeded the recommended amount and less than 40% of all participants adhered. EI was below estimated requirements (45 kcal/kg BM) and most of our participants had subclinical EA (35 kcal/kg FFM). We observed a positive correlation between daily CHO and EA (p=0.024) and serum ferritin (p=0.014). Finally, adequate CHO intake during and after exercise reduces pro-inflammatory IL -6. *Conclusion:* Young male elite cyclists did not meet energy demands and specific macronutrient requirements. Inadequate carbohydrate intake, low EA, and ferritin concentrations may exacerbate inflammation after acute exercise. These findings may have both short- and long-term implications for the health and performance of young cyclists.

Key words: energy availability, carbohydrate intake, cyclists, inflammation

Introduction

Implementing a nutrition program is one of many important behaviours which can lead to successful athletic performance (1). Adequate nutrition is known to enhance an athlete's training and recovery and contribute to the maintenance of adequate body composition, as well as optimal immune, endocrine, and musculoskeletal function (2). While specific physiological requirements may vary by sport, and several studies have described 24-hour energy and macronutrient intake, relatively few studies have been conducted on a sample of young participants (3). The success of young cyclists is the result of appropriate training as well as correct nutritional intake and eating habits. Many studies conducted on athletes have reported protein and fat intake in line with high energy recommendations; however, despite the fact that carbohydrate (CHO) intake is an important fuel for training and racing performance in cyclists (6,7,8), its intake has been reported as low (4,5). Fatigue in endurance sports during aerobic and anaerobic activities is due to the depletion of muscle and liver glycogen, which makes a high CHO intake at all times before, during and after exercise an important target (7).

In accordance with these findings, sports nutritionists recommend that male cyclists undertaking high-intensity training or competition consume more than half of their daily energy intake in the form of CHO to replenish depleted glycogen stores (9). To meet the daily macronutrient requirements, athletes participating in high-intensity training and competition should consume 7-10 g/kg/day of CHO to maintain adequate blood glucose levels and replenish muscle glycogen stores (7,10). Longer training sessions of high-performance cyclists may require a higher CHO intake (12 g/kg/day) to replenish their muscle glycogen stores. In addition, the importance of post-exercise CHO intake on the recovery process in high-performance cyclists has also been reported in the literature (10). However, a combination of simple CHO with fructose resulted in increased exogenous CHO oxidation during exercise, an important response to improve exercise performance (11).

Adequate CHO intake has been shown to reduce inflammatory responses associated with increased levels of inflammatory cytokines such as interleukin-6 (IL-6), tumour necrosis factor α (TNF-α) and C-reactive protein (CRP) after exercise (12, 13). Moreover, IL-6, which is involved in a variety of pro- and anti-inflammatory processes, is commonly measured to assess the degree of inflammation (12, 13, 14). Post-exercise inflammation associated with an increase in IL-6 has been recognised as a key stimulus for increased levels of the iron-regulating hormone hepcidin (14,15), and is associated with low muscle glycogen stores (16). This observation suggests that iron status, which is tightly regulated by hepcidin (17), is dependent on CHO intake after exercise (12).

As little is known about macronutrient intake in young elite cyclists, this study aimed to investigate the daily macronutrient intake during and after acute exercise in young male elite cyclists. The aim of the study was to assess the nutritional status of young male competitive cyclists so as to better understand their dietary intake and determine whether their dietary intake is in line with the relevant recommendations. Based on previous studies, it was hypothesised that the energy intake of young male cyclists is below their energy expenditure and does not meet the recommended levels. Moreover, the study also aimed to test the hypothesis that the inflammatory state in young male elite cyclists changes in the post-exercise period in relation to their CHO intake. To this end, body composition and biochemical parameters were measured, and pro-inflammatory and anti-inflammatory circulating cytokines were determined after acute exercise.

Methods

Study design and participants

The aim of this prospective study was to examine participants' daily dietary intake and blood parameters before and immediately after a time-to-exhaustion test. The study protocol was approved by the Slovenian National Medical Ethics Committee (No. 93/07/13). All measurements were performed at the Faculty of Heath Sciences, University of Primorska, Izola, Slovenia.

Ten healthy Slovenian competitive cyclists, aged between 15 and 30 years, who had competed either at national level or in European races participated in the study. Written informed consent was obtained from all subjects prior to their participation in the study. The inclusion criteria were: a) having trained for at least 6 months and b) being aged between 15 and 30 years, c) having a body mass index (BMI) of 18.5-27.0 kg/m^2, and d) being free from acute or chronic inflammatory disease. Participants recorded their food intake in 3-day food diaries and kept a diary of their physical activity in the week before the fatigue test. On the day of the test, participants' resting metabolic rate measurements and anthropometric measurements were taken between 7:00 and 9:00 am after an overnight fast. The acute exercise test was performed on a cycle ergometer (Monark, Model Ergomedic 894E, Varberg, Sweden) following a ramp protocol until exhaustion. Blood samples were taken before the start of the exercise and immediately after exhaustion.

Anthropometric measurements

Participants' body mass (BM) (kg), body composition (total percentage of body fat (%BF), percentage of fat-free mass (% FFM), percentage of muscle mass (% MM), and height (cm) were measured in a standing position, with subjects wearing light clothing and no shoes. Body composition was assessed using the Tanita BC 418 MA multiple-frequency bioelectrical impedance analysis (BIA) scale (Tanita Corporation, Arlington Heights, IL) and data were analysed with the software provided by the same producer. The height of the participants was measured in a standing position using a stadiometer (Invicta Plastic, Oadby, England) with an accuracy of 0.1 cm. BMI was calculated as weight/height^2 (kg/m^2).

Dietary assessment and analysis

Dietary intake was assessed throughout the competitive season using 3-day food diaries and photographic records. Prior to data collection, all participants participated in a hands-on workshop conducted by the author to ensure all subjects were familiar with the study procedures related to energy intake. Participants were asked to record the following information: time of day, food description including preparation methods and brand names, amount prepared, amount remaining, volume and type of fluid consumed. They were also asked to record their intake of dietary supplements. Participants were asked to photograph everything they consumed on the days evaluated. After submitting their nutrition logs, the records were carefully reviewed, and each athlete participated in an interview with the authors to clarify any ambiguities. Nutrient composition and energy content were analysed using the Open Platform for Clinical Nutrition web tool (OPEN) http://opkp.si/.

Recommended ranges for energy and macronutrient intakes were obtained from Vitale et al. (26) and Burke (6) and are shown in Table 2. Intakes were considered inadequate if values were below or above the recommended ranges.

Energy expenditure and physical activity

Subjects' resting metabolic rate (RMR) was measured using a handheld indirect calorimeter (MedGem Microlife, Golden, CO) under standard conditions and in the fasting state. To estimate average daily energy expenditure, participants were asked to record their activity patterns over the 7-day period. The physical activity diary was completed using a 7-level intensity scale and was used to record the frequency, type and duration of daily physical activity (18). Based on the data obtained, metabolic equivalents (METs) and energy expenditure during physical activity (EEE) were calculated. METs for each activity were assigned according to a standardised protocol published by Ainsworth et al. (19). Based on the METs model, the total amount of physical activity per day was calculated by multiplying the time spent on the activities by the intensity index expressed in MET, where 1 MET equals 1 kcal/kg/min. Energy availability (EA) was calculated as energy intake minus energy expenditure during exercise (EEE) relative to FFM.

Serum analysis

Venous blood samples for biochemical and hormonal analyses were collected after overnight fasting and after exercise. Blood samples were collected between 7 and 9 am into empty 4-ml EDTA-containing vacuum tubes and centrifuged. Serum23 was immediately separated, frozen and stored at -20 ˚C until analysis.

Serum concentrations of bilirubin, glucose, triglycerides (TG), total cholesterol, low-density lipoprotein, (LDL cholesterol), high-density lipoprotein (HDL cholesterol), CRP, and ferritin were analysed using Olympus reagents and the AU 680 analyser (Beckman Coulter). Hct and Hb were analysed with the LH780 hematological analyser (Beckman Coulter, Sweden) using Beckman Coulter reagents.

Serum concentrations of adiponectin (BioVendor, Lab.Med.Inc., Brno, Czech Republic), IL-6 and TNF-α (both Thermo Fischer Scientific Inc., Rockford, USA) were determined in duplicate on a microplate reader (Tecan, Männedorf, Switzerland) using commercially available enzymatic reagents. The inter-assay and intra-assay CVs were typically < 10% (for TNF-α 5.2%, for APN 6.3%, for IL-6 7.1%). CVs for all other biochemical parameters were < 2.5%.

Statistical methods

Statistical analyses were performed using SPSS (version 20.0 IBM, Armonk, NY). Before the analysis, all data which were not normally distributed were log-transformed to approximate a normal distribution (in our experiment: data for the concentration of IL-6). Data are presented as mean values ± SD. Pearson correlations were calculated to assess the associations between different biochemical variables and diet. A one-sample t-test was used to compare the means with the lower and upper limits of dietary recommendations for carbohydrate (7-10 g/kg BM) and protein (1.4-2 g/kg BM) intake. Statistical significance was assessed at alpha 0.05. In addition, the percentage of athletes with adequate reference values was calculated (4).

Results

Table 1 shows the baseline descriptive characteristics of all subjects aged 19.6 ± 1.3 years who participated in the study. The average weight (SD) of all subjects was 68.8 (7.6) kg and their average BMI was 20.8 (1.6) kg/m^2, which was within the recommended values. The subjects had a high percentage of lean muscle mass 89.0 (4.0) %, and a low percentage of fat mass 10.7 (4.2) %, which is also reflected in their high resting metabolic rate (1750 (163) kcal/day). All biochemical and haematological parameters were within reference values.

Total energy and macronutrient intake

The mean dietary macronutrient daily intake, during and after exercise, and the reference values (considered as recommendations) are summarised in Table 2. Energy intake versus energy expenditure was 3119±691 kcal versus 3761± 327 kcal (p = 0.009) (data not shown), while EA (35 kcal/kg FFM) was close to the lower limit of the reference value. Only 30% of the athletes reached the recommended ranges of energy intake. Furthermore, the t-test results for both EA and energy intake were significantly ($p < 0.05$) below the upper limit of the recommendations (EA: 45 kcal/kg FFM; energy intake: 80 kcal/kg BM, respectively) (Table 2).

Table 1. Participants' anthropometric, metabolic, biochemical and haematological characteristics.

Variable	Mean (SD)	Minimum value (RV)	Maximum value (RV)
Height (cm)	181.8 (1.2)	176.3	182.0
Weight (kg)	68.8 (7.6)	59.8	85.5
Fat mass (%)	10.7 (4.2)	5.7	17.30
Fat free mass (%)	89.0 (4.0)	82.6	89.1
Muscle mass (%)	85.1 (3.4)	79.0	85.1
Water (%)	65.2 (2.9)	60.5	69.1
BMI (kg/m^2)	20.8 (1.6)	19.4	24.7
RMR (kcal/day)	1750 (163)	1880	2290
MET (h/day)	13.9 (3.6)	8.9	20.4
Total cholesterol(mmol/l)	4.0 (0.8)	3.1 (4.0)	5.8 (5.2)
Glucose (mmol/l)	4.6 (0.7)	2.8 (3.6)	5.2 (6.1)
TG (mmol/l)	1.1 (0.3)	0.8 (0.6)	1.9 (1.7)
HDL-C (mmol/l)	1.3 (0.3)	0.8 (1.4)	2.1 (2.8)
LDL-C (mmol/l)	2.2 (0.7)	1.4 (2.0)	3.9 (3.5)
Bilirubin (nmol/l)	17 (8)	12 (0)	39 (17)
Ferritin (μg/l)	128 (83)	36 (28)	313 (365)
Hb (g/l)	148 (8)	137 (140)	157 (180)
Hct (1)	0.44 (0.02)	0.41(0.40)	0.47 (0.54)

Values are means (SD). BW, body weight; BMI, body mass index; RMR, resting metabolic rate; MET, metabolic equivalent; TG, triacilglycerides; HDL-C, high density lipoproteins; LDL-C, low density lipoproteins; Hb, haemoglobin; Hct, Haematocrit; RV, reference value.

Table 2. Macronutrient Intakes vs. Recommendations.

Variable	Mean (SD)	RV [a,b]	% of Athletes that had Adequate Intake	p-value vs. Lower End of RV	p-value vs. Upper End of RV
Daily energy and macronutrient intake					
Energy availability kcal/kg FFM	35 (3)	30-45	60	0.083	***0.003***
Energy intake kcal/kg BM	45 (8)	50-80	30	0.116	***0.000***
Carbohydrates g/kg BM g	 6.1 (1.4) 422 (105)	 7-10 -	 40 -	 0.084 -	 ***0.000*** -
Proteins g/kg BM	1.7 (0.5)	1.4-2	70	0.062	0.102
Fats % Energy intake	29 (5)	20-30	100	**0.000**	0.658
Saturated fatty acids % Energy intake	10 (4)	≤ 10	50	**0.000**	0.982
Intake during exercise					
Carbohydrates (g/h)	32 (19)	30-70	50	0.748	***0.000***
Proteins (g/kg BM)	0.08 (0.06)	0-0.25	100	**0.003**	***0.000***
Fats (% Energy intake meal)	15 (12)	0-10+	40	**0.004**	0.854
Intake post exercise					
Carbohydrates (g/kg BM)	1.2 (0.5)	1-1.3	80	0.137	0.783
Proteins g/kg BM g	 0.5 (0.2) 35 (17)	 0.2-0.5 20	 90 90	 **0.003** **0.000**	 0.927 -
Fats % Energy intake meal g	 32 (8) 26 (10)	 0-20+	 20	 **0.000**	 **0.001**

Text in ***bold italics*** indicates where macronutrient intake is significantly ($p < .05$) less than recommended intake, text in **bold** indicates where intake is significantly ($p < .05$) greater than recommended intake at the upper or lower end of the ranges as indicated (one-tailed single-sample t-test). +avoid high fat meal; RV, recommended values; a (Vitale and Getzin. 2019); b (Burke 2001); FFM, fat free mass; BM, body mass; * $p < 0.05$

Less than 50% of all participants consumed sufficient CHO on a daily basis and during exercise. The mean total daily intake of CHO (6.1±1.4 g/kg BM/day) was below the recommended value. 23±8% of the total CHO was sugar, and a large proportion of this was fructose (23±13%, data not shown).

The mean CHO intake during exercise (32±19 g/h) was within the recommended values; however, the observed CHO intake during exercise was significantly lower than the upper limit according to the t-test (p=0.000). Half of the CHO intake during exercise consisted of sugars (51±37%), of which 12±16% was fructose (data not shown). Adequate CHOs were consumed after exercise, as 80% of all subjects met or exceeded the recommended amounts.

The only category in which most participants (70%) met the recommended daily amounts, as well as amounts during and after exercise, was protein intake. The mean daily protein intake was 1.7 g/kg BM and, the mean post exercise protein intake was 0.5 g/kg BM, which was close to the upper limit of the reference value, while its intake during exercise was low (0.08 g/kg BM).

In addition, daily fat intake (29.2±5.4% of energy intake) was at the upper limit of the recommended values. The mean intake of fat and saturated fat was significantly higher (p=0.000) than the lower limit of the recommended ranges. In addition, fat intake during exercise was significantly higher than the lower limit (p=0.004), and most participants (60%) showed inadequate fat intake. Post-exercise fat intake was significantly above the upper limit of the recommended range (p=0.001). Only 20% of the subjects complied with the recommended amounts.

Circulating pro- and anti-inflammatory cytokine levels

The values of anti-inflammatory (adiponectin) and pro-inflammatory cytokines (CRP, TNF-α and IL-6) after acute exercise training are summarised in Table 3. The results show an increase in inflammation after exercise, with a significant difference observed between pre- and post-exercise only for circulating IL-6 ($p<0.001$). On the other hand, no significant differences were observed for circulating pro-inflammatory CRP and TNF-α levels before and after acute exercise, and adiponectin also remained unchanged.

Figure 1 shows the basal and post-exercise IL-6 levels corresponding to adequate and inadequate CHO intake before, during, and after exercise, respectively. In the present study, daily, during and post-exercise CHO intakes reached the recommended values in 40 %, 50 %, and 80 % of athletes, respectively.

Basal IL-6 levels were lower when the daily CHO (> 7 g/kg BM) and post-exercise (> 1.3 g/kg BM) intakes were within recommended levels (Figure 1); however, the differences between groups were not statistically significant. The same trend was observed for IL-6 serum levels after exercise; when CHO intake was adequate during or after exercise, IL-6 levels were lower. However, even in this case, the differences between the groups were not statistically significant.

Correlation analyses were performed to investigate the possible relationship between CHO and daily fructose intake, CHO intake during and after training, EA and serum ferritin levels, and anti-inflammatory APN and pro-inflammatory IL-6 in elite cyclists after acute training (Table 4). Daily CHO intake correlated positively with EA (r=0.701, p=0.024) and serum ferritin (r=0.743, p=0.014) (Fig. 2A). Moreover, daily fructose intake correlated significantly with serum ferritin levels (r=0.901, p=0.000) (Fig. 2B).

Discussion

One of the main findings of this study was that young Slovenian elite cyclists had inadequate energy and macronutrient intakes; only 40% of young cyclists had adequate daily CHO intakes and only 30% had adequate daily energy intakes, while 50% had adequate CHO intakes during exercise. Moreover, participants with inadequate daily CHO or inadequate CHO intake during and after exercise had higher serum levels IL -6 after exercise; however, these differences were not statistically significant. Another finding of the present study was that daily CHO and fructose intake were significantly and positively related to serum ferritin levels.

Athletes participating in weight-sensitive sports, such as endurance road cyclists, seem to have a

Table 3. Mean serum concentrations of pro-inflammatory and anti-inflammatory biomarkers before and immediately after the completion of an acute exercise.

Variable	PRE	POST	95 % CI
CRP (mg/l)	0.46 (0.32)	0.57 (0.34)	(-0.227, 0.007)
APN (μg/ml)	19.64 (11.87)	19.64 (7.92)	(-5.568, 5. 554)
Log_{10}IL-6	0.52 (0.39)	1.11 (0.35)	(-0863, -0.407)*
TNF-α (pg/ml)	1.07 (0.68)	1.28(0.95)	(-0.821,-0.358)

Values are means (SD). APN, adiponectin; CRP, reactive protein C; TNF-α, tumour necrosis factor α; IL-6, interleukin 6. Serum inflammatory and anti-inflammatory markers concentrations were measured before (PRE) and immediately after completion of an acute exercise (POST). PRE and POST values were compared using paired samples t-test * $p < 0.001$

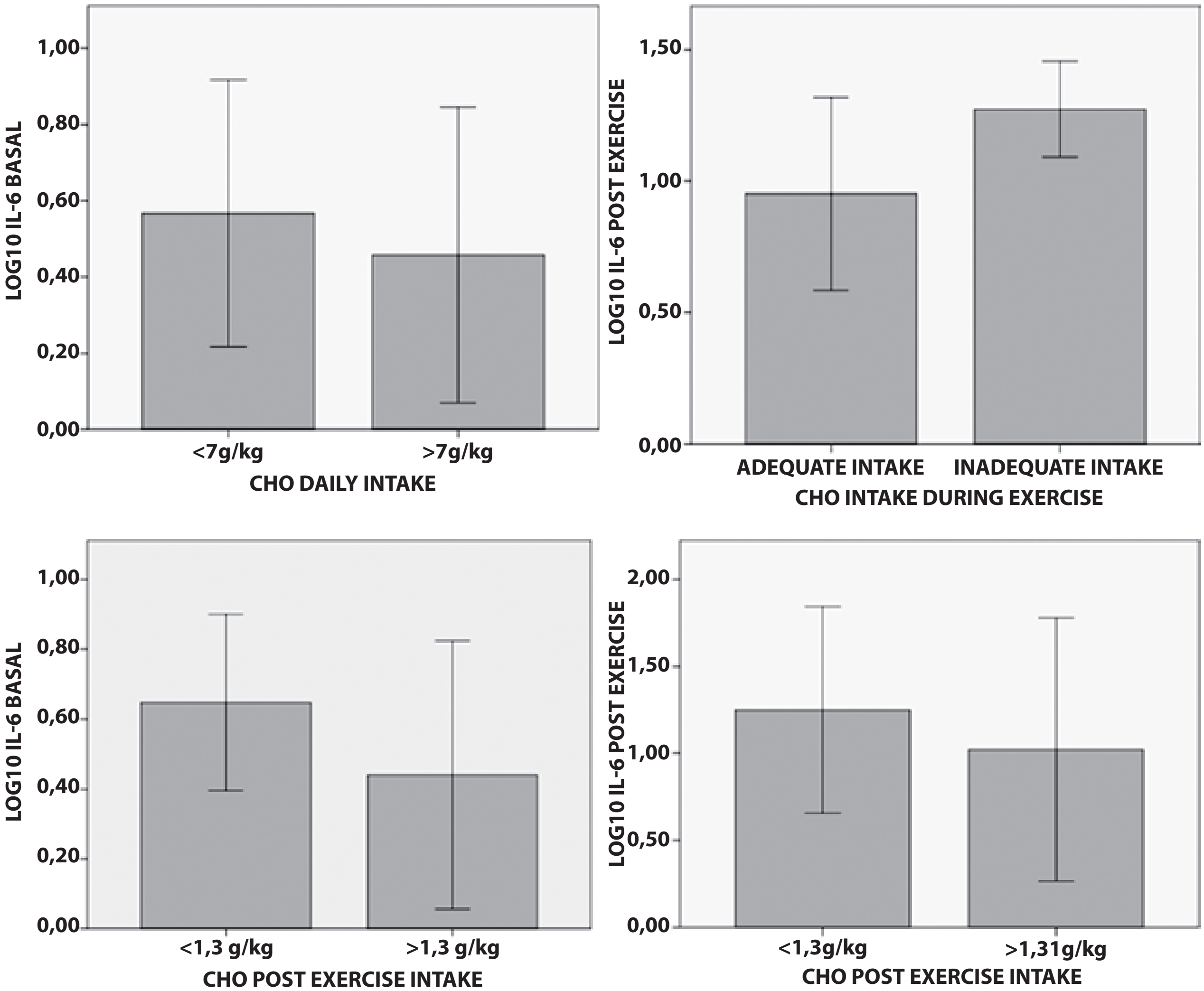

Figure 1. Basal and post-exercise serum interleukin-6 (IL-6) levels in cyclists and CHO intake (daily, during exercise and post exercise).
Within group, CHO daily intake varied namely: <7 g/kg/day (6 cyclists), >7 g/kg/day (4 cyclists); CHO during exercise: adequate intake (5 cyclists), inadequate intake (5 cyclists); CHO post exercise: <1,3 g/kg (4 cyclists), >1,3 g/kg (6 cyclists). The values are shown as mean ± SD.

particularly suboptimal energy intake and are at risk of low EA (20). In the present study, subjects' reported daily energy expenditure was 17% higher than their daily energy intake, while EA (35 kcal/kg FFM) was well below the recommended levels. Energy expenditure and energy intake vary greatly between training periods. Male competitive cyclists tend to have large energy deficits during training camps (21) and races (22,23), as well as during entire cycling seasons (5), which is consistent with our results showing that only 30% of cyclists recorded adequate energy intake. On the other hand, a positive energy balance has been observed on cyclists' rest days (21) and on their rest days during stage races (23). Low energy intake leads to low EA. Most studies (5,21) have shown that the EA values of cyclists are far below the EA threshold for low EA classification (30 kcal/kg FFM/day). We found that most of our participants had subclinical EA

Table 4. Associations between pro-inflammatory and anti-inflammatory biomarkers, biochemical parameters and macronutrients intake.

	Log_{10} IL-6_{post}	APN_{post} (μg/ml)	Ferritin (μg/l)	EA (kcal/kg FFM)
CHO_{daily} (g)	-0.137 0.705	0.434 0.210	0.743 0.014*	0.701 0.024*
CHO_{during} (g/h)	-0.443 0.199	-0.409 0.240	0.049 0.893	-0.036 0.921
CHO_{post} (g/kg)	-0.013 0.971	-0.184 0.416	0.182 0.843	0.048 0.894
$Fructose_{daily}$ (g)	-0.137 0.706	0.409 0.240	0.901 0.000**	0.166 0.648
EA (kcal/kg FFM)	0.287 0.421	0.252 0.482	0.462 0.179	1

All associations are presented as Pearson's correlation coefficients (*r*). CHO_{daily}, carbohydrates daily intake; CHO_{during}, carbohydrates intake during exercise; CHO_{post}, carbohydrates intake in first meal post exercise; EA, energy availability; $Fructose_{daily}$, fructose daily intake; APN, adiponectin; IL-6, interleukine-6. *$p < 0.05$; ** $p < 0.001$.

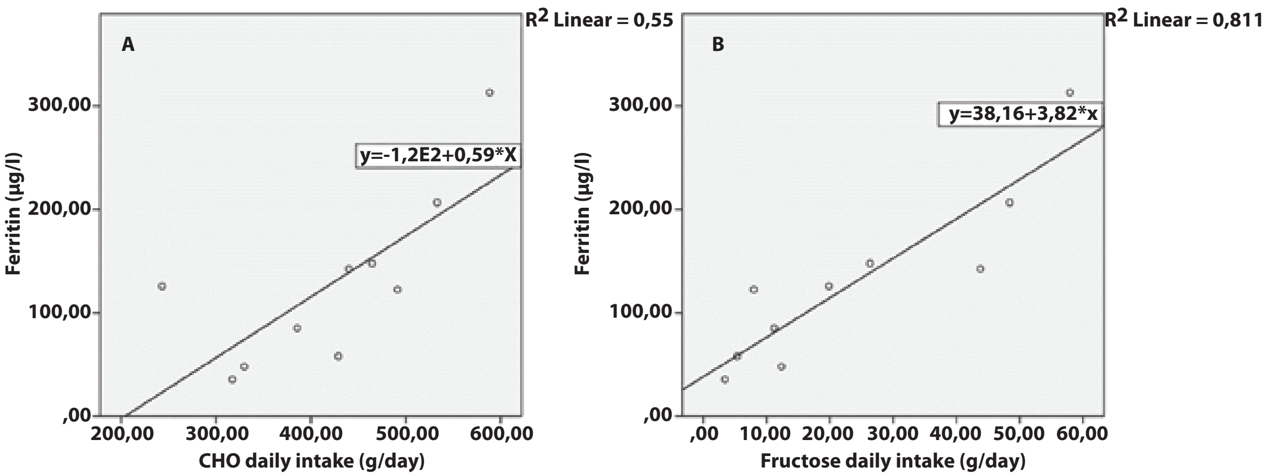

Figure 2. Correlation analysis between carbohydrate (CHO) and fructose intake with ferritin.

(30-45 kcal/kg FFM). Long-term low EA and energy deficits can have negative effects on health and performance, and they also increase the risk of injury (20). Low energy intakes, and specifically low CHO intakes, appear to be the main cause of low EA in cyclists. Similar to the study by Viner et al. (5), we found that participants with high EA recorded the highest daily CHO intake (p= 0.024).

CHO availability describes the balance between the CHO requirements of the muscle and central nervous system during an exercise session relative to the endogenous and/or exogenous CHO supply (24). There is ample evidence suggesting that strategies for reaching high CHO availability are associated with improvements in exercise capacity and sports performance (10, 25). The sports nutrition literature provides guidelines for CHO intake before, during, and after specific training sessions, as well as for total daily intakes (7, 26). While general sports nutrition guidelines recommend a daily CHO intake of 7-10 g/kg (10), in the present study only 40% of young cyclists met or exceeded the recommended amounts.

Similar results have been reported in other studies on young endurance athletes (4) and national-level cyclists (5). On the other hand, the intake of CHO seems to be higher in high-level cyclists (>12 g/kg), who more often meet macronutrient intake recommendations (6, 21, 27, 28).

Although daily CHO intake is an important component of sports nutrition, athletes also require exogenous carbohydrates during exercise to meet their energy needs, delay fatigue, and improve their performance during prolonged continuous and intermittent high-intensity activities (10,12). In our study, the majority of athletes (50%) consumed less CHO than recommended during exercise; similar results have also been observed in young team athletes (3), while professional cyclists have generally been reported to reach adequate CHO intake levels (>90 g/h) (29). In terms of post-exercise nutrient intake, we found that athletes consumed sufficient CHO (1.2 g/kg). Current sports nutrition guidelines recommend that athletes consume CHO (1.0–1.2 g/kg/h) to optimise their muscle glycogen replenishment during recovery from endurance exercise (10, 30).

The lower CHO intake levels reported in the present study could be attributed to increased protein and fat consumption (31). In our study, cyclists' daily fat intake was at the upper limit of the recommendations for athletes (29% of total energy intake, with recommendations stating 20-30% of total energy intake), and similar results have been reported for other elite cyclists (28,31,32). Fat intake during exercise was at the upper limit, while fat intake after exercise exceeded the recommended levels (32% of energy intake from meals); similar results have been reported for other elite cyclists for fat intake during exercise, while fat intake after exercise was around 17-22% (28,32). There is evidence that a high-fat meal after exercise contributes to a slow replenishment of glycogen stores (33).

Consistent with other studies (4, 5, 6), the protein intake of our participants was in line with the recommended values. During periods of intense training, protein requirements may be higher even in well-trained athletes, leading to higher intakes in professional athletes (6,8). However, more important than total daily protein intake is the distribution of protein intake throughout the day (20 g in 3-4 hour intervals) (9, 34). Consumption of up to 20 g (0.2-0.5 g/kg) of protein within 30-60 min of endurance training, along with CHO (1-1.3 g/kg), has been shown to help stimulate protein synthesis and attenuate any breakdown that may occur (34). In addition, protein intake decrease lactate dehydrogenase and creatine kinase levels and may protect athletes from exercise-induced muscle damage(35). Therefore, adequate protein intake after exercise support rapid recovery from endurance training and glycogen synthesis (34). In terms of post-exercise macronutrient intake, we found that male cyclists consumed adequate CHO and protein amounts, similar to the results reported by Baker et al. (3).

Our finding that the daily CHO intake of young male cyclists was lower than expected and similar to values previously reported in the literature (3, 4, 5) deserves some attention. Studies with acute (36) and chronic (37) low CHO availability in athletes' diets have shown a significant increase in the post-exercise IL-6 response compared to conditions with high CHO availability. Consistent with these studies, cyclists with adequate CHO daily intake and adequate CHO intake during exercise in the present study exhibited lower IL-6 post-exercise levels; however, the differences were not statistically significant. Factors affecting the release of IL-6 during exercise include exercise intensity, participant training status, and also muscle glycogen stores (38, 39). Diets with a low total daily CHO intake may be insufficient to replenish muscle and liver glycogen stores which have been depleted during training or competition (10) and may negatively impact health and performance, as well as increase inflammation and the risk of injury (39). Recent evidence suggests that adequate pre-exercise energy intake is an important factor in attenuating exercise-induced IL-6 by preserving muscle glycogen (39). Although increased production of IL-6 has been demonstrated as a response to running or cycling for ≥2 hours at moderate to high intensities, this response may be attenuated if CHO is consumed during exercise to maintain blood glucose concentrations and muscle glycogen stores (38). On the other hand, CHO intake during exercise has been shown to have a minimal effect on IL-6 levels when exercise lasts 2 hours; unless exercise starts with low muscle glycogen stores

(38). In the present study, young cyclists likely had low muscle glycogen stores, which is why CHO ingestion during exercise bouts of less than 2 hours had an impact on the IL-6 response to training.

As shown by Peeling et al. (38), a post-exercise increase in IL-6 levels contributes significantly to the subsequent post-exercise hepcidin increase. Given the relationship between IL-6 and hepcidin activity, an increased IL-6 response resulting from training with low CHO availability and/or low muscle glycogen stores may increase post-exercise hepcidin levels, which could, in turn, have a negative effect on iron regulation in athletes.

Fructose intake plays an important role in the replenishment of glycogen stores. Recent evidence suggests that a combination of fructose-based CHO sources leads to higher total glycogen stores (30). Indeed, in the present study we found a significant positive correlation between daily CHO intake and ferritin and daily fructose intake and ferritin, but, unfortunately, we did not measure serum hepcidin levels. We should also not neglect the fact that pre-exercise iron status (serum ferritin < 30 $\mu g \cdot L^{-1}$) is another important regulator of exercise-induced hepcidin (37840).

Limitations

Limitations of the present study include the inherent risk of bias due to reliance on self-reports of food intake (41). However, we conducted detailed monitoring of dietary intake to determine what the young cyclists consumed before, during, and after training. The simultaneous use of different methods, such as food records, digital food records and interviewing applied in the present study, help to overcome these limitations. This study examined a relatively small sample, which consisted only of young male elite cyclists. In addition, hepcidin levels were not analysed in the present study; therefore, no associations between CHO intake, iron status, inflammation, and hepcidin levels could be presented.

Conclusion

Despite the aforementioned limitations of our study, our results indicate that low CHO intake is associated with low EA, high inflammation, and low ferritin concentration. These findings may have both short- and long-term implications for the health, performance, and physical development of young cyclists.

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