Section: Original Investigation

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Journal: *International Journal of Sports Physiology and Performance*

Acceptance Date: February 14, 2019

DOI: https://doi.org/10.1123/ijspp.2018-0842

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International Journal of Sports Physiology and Performance

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Running head: Nutrition during single-day cycle racing

Word count: 3514 words
Abstract count: 250 words
Number of references: 30 references
Number of figures and tables: 1 figure and 5 tables

ABSTRACT

Purpose: To assess energy and carbohydrate availability and changes in blood hormones in 6 professional male cyclists over multiple single-day races. **Methods**: We collected weighed food records, powermeter data and morning body mass across 8 days. Carbohydrate intakes were compared to contemporary guidelines. Energy availability (EA) was calculated as energy intake minus exercise energy expenditure, relative to fat-free mas (FFM). Skinfold thickness and blood metabolic and reproductive hormones were measured pre- and post-study. Statistical significance was defined as $p \leq 0.05$. **Results**. BM ($p=0.11$) or skinfold thickness ($p=0.75$) did not change across time, despite alternate-day low EA (14±9 vs 57±10 $kcal \cdot kg\ FFM \cdot d^{-1}$, race vs rest days, respectively; $p<0.001$). Cyclists with extremely low EA on race days (<10 $kcal \cdot kg\ FFM \cdot d^{-1}$; n=2) experienced a trend towards decreased testosterone (-14%) and insulin-like growth-factor-1 (-25%), despite high EA (>46 $kcal \cdot kg\ FFM \cdot d^{-1}$) on days in-between. Carbohydrate intakes were significantly higher on race vs rest days (10.7±1.3 vs 6.4±0.8 $g \cdot kg \cdot d^{-1}$, respectively;$p<0.001$). The cyclists reached contemporary pre-race fueling targets (3.4±0.7 $g \cdot kg \cdot 3h^{-1}$ carbohydrates; $p=0.24$), while the execution of CHO guidelines during race (51±9 $g \cdot h^{-1}$; $p=0.048$) and within acute (1.6±0.5 $g \cdot kg \cdot 3h^{-1}$; $p=0.002$) and prolonged (7.4±1.0 $g \cdot kg \cdot 24h^{-1}$; $p=0.002$) post-race recovery was poor. **Conclusions**: We are the first to report day-by-day periodization of energy and carbohydrate in a small sample of professional cyclists. We have also examined the logistics of conducting a field study under stressful conditions in which major cooperation of subjects and team management is needed. Our commentary around these challenges and possible solutions is a major novelty of the paper.

Key words: professional cycling, nutrition periodization, energy availability, hormones, single-day racing

INTRODUCTION

Historically, professional road cyclists have been defined by their lean physiques as well as a high aerobic capacity [1]. Morphological differences exist between different cyclists which usually dictate the main specialty of each cyclist in the racing environment (or vice versa); flat terrain specialists and sprinters have higher muscularity to achieve highest absolute power (e.g. watts [W]), while hill climbers are focused on high relative power ($W \cdot kg^{-1}$) due to their greater need to perform against gravity, and are therefore lighter and also leaner [2]. However, the most recent information provided on social media by professional teams or companies producing power meters/heart rate monitors suggests that there has been further refinement from these published data of what is considered "podium physiology" and "optimal physique" from within the peloton. These reports describe the phenotype of the successful stage racer/climber as having a very low BMI (< 18 kg/m^2) and extremely low levels of body fat (< 35 mm total skinfold thickness for 7 sites, according to ISAK methodology, < 10% body fat via Dual X-ray Absorptiometry [DXA] assessment of body composition, personal observations, L.M. Burke and J.P. Morton) and little upper body musculature Furthermore, testimonials from the sport's most decorated cyclists have often associated significant weight loss to the dominant phases of their careers [3,4]. Although these cyclists typically refine their (very) light and lean physiques as a by-product of large training volumes and restricted energy intake [3,4], success in many endurance and ultra-endurance cycling races is also determined by their ability to consume substantial amounts of carbohydrate and energy during the event [5]. Therefore, many cyclists switch between low and high energy intakes relative to their exercise energy expenditure (i.e. periodize nutrition) depending on the desire for fat/mass loss and the performance benefits of being well fueled.

Another recent and related update in sports nutrition is the recognition of the Relative Energy Deficiency in Sport syndrome in male athletes [6,7]. The underpinning cause of this issue is low energy availability (LEA); a mismatch between energy intake and the energy committed to the athlete's daily training/event program, such that the energy costs of maintenance of health and wellbeing are no longer met. The outcomes of this scenario include impairment of bone health, metabolic rate, endocrine function and cardiovascular system, leading to increased risk of illness and injury as well as a direct impairment of performance [6]. Typically, the factors underpinning LEA are considered to range from an inadvertent failure to meet the high energy costs of training/competition, to well-intentioned yet often misguided practices to manipulate body mass or composition, to clinical disordered eating/eating disorders [8,9]. In addition, a suite of factors within the culture, regulation, and performance drivers of a sport/event may predispose an athlete to adopting eating and/or exercise patterns that result in LEA [10].

Road cycling is, therefore, recognized to provide a number of risk factors for LEA. The Grand Tours, which are composed of 3 weeks of (almost) consecutive daily racing with the inclusion of many mountainous sections, can superimpose two of the three risk factors for LEA in the form of extremely high energy expenditure on certain days and the benefit to General Classification cyclists from being extremely light/lean. Scientific reports on energy and macronutrient intakes during Grand Tours have shown high intakes of energy (5415-7815 $kcal \cdot d^{-1}$) and carbohydrate (~12.6 $g \cdot kg \cdot d^{-1}$) as well as considerably high energy expenditures (6070-7815 $kcal \cdot d^{-1}$) during these events (Table 1). A recently published report by BBC Sport describes nutrient intakes of a professional male cyclist during two stages (moderate intensity hilly stage and high intensity mountain stage) of Giro d'Italia 2018 [11]. Of note is a significant variation in day-to-day nutrition, including 2.7-fold and 3.3-fold higher energy and carbohydrate intakes, respectively, during the mountain stage compared to the hilly stage (Table 1). This finding, highlights an important shortcoming of the current Grand Tour literature, where nutritional intakes have been described as an average across 3 weeks of racing, thereby ignoring the likely significant day-by-day variation in nutrition across stages. Another interesting theme across current scientific and anecdotal reports from the Grand Tours is the lack of decrease in body mass despite extreme energy expenditures. While limited data on EA during Grand Tours exists, this finding suggests that professional cyclists typically manage to achieve adequate EA during these races, as a result of good understanding of physiological and energy requirements of racing, sophisticated nutrition support including the involvement of mobile kitchens and professional chefs, and aggressive feeding while riding for 4-8 h each day [12]. Maintenance of body mass is not always achieved, and even when it is, it can sometimes be a poor signal of adequate EA [13].

Notwithstanding the unresolved issues in stage-race cycling, it is important to recognize that other competition formats in professional cycling present different challenges.Single-day races (the Classics) are characterized by one day of racing followed by one or more days of rest in between. Here, while fueling remains a key focus of race nutrition support, it does not require the same aggressive approach that is necessary with daily racing. Indeed, a minimum of 40 hours of recovery (considering one rest day) should provide adequate opportunity for complete replenishment of fuel stores. In fact, cyclists may risk a body mass (BM) gain if they overcompensate for race day energy expenditure on rest days (personal communication with M. Quod and N. Strobel). Therefore, EA during single-day racing may need to be even more carefully periodized to support performance on race days while preventing unwanted weight gain on rest days by utilizing a "fueling for the work required" concept [14].

We have highlighted a few important gaps in the current scientific literature on professional cycling, namely: (1) lack of estimates/measures of EA across short/long-term racing, (2) a failure to approach nutrition day-by-day to account for the varying physiological requirements of different stages, and (3) lack of research overall on cycling single-day racing. Our aim was to (1) assess energy and carbohydrate availability and changes in blood hormones as a consequence of changes in EA in professional male cyclists over four separate single-day races within an 8-day period, and (2) to investigate the robustness of our methodology to collect data in a challenging group (world-class athletes) in a challenging environment (field research) at a challenging time (in the middle of a series of races). While our intention was to investigate a group of 11 professional male cyclists, due to last minute dropouts from illness/injuries, we ended up with a final cohort of n=6. Therefore, the outcomes will be reported in the form of a pilot study with a special focus on the methodology observations and experiences of the authors. These observations, including pre-determined best practice protocols for testing, our experience from the field, and finally, future suggestions, are presented in Table 2.

METHODS

Participants and Study design

Six professional male cyclists from the Mitchelton-Scott UCI World Tour (Road Cycling) team participated in the study (Table 3). All participants were healthy and fully informed of the study design before signing the informed consent. To characterize the current fitness level, maximal mean power (MMP) over 1, 5 and 20 minutes of racing were collected across a 6-week period around the Classics. These field-based values reflect laboratory-based testing outcomes of anaerobic capacity (1' MMP), maximal aerobic power (5' MMP), and threshold power (20' MMP) [23].

The study protocol was built around an 8-day window of racing (four single-day races interspersed with 1-2 recovery days), part of a larger period of Spring Classics 2018. The races took place in Belgium and included the following: Driedraagse de Panne (Day 2: 202.4 km); E3 Harelbeke (Day 4: 206.1 km including 15 steep [mostly cobbled] climbs); Gent-Wevelgem (Day 6: 250.8 km including 11 climbs [the steepest sector 23%] and sections of cobblestones); Dvaars door Vlaanderen (Day 9: 180.1 km, including 12 climbs and cobble sections).

Dietary and training data were collected from day 1 until day 9. Venous blood samples were collected on the morning of days 2 (baseline) and 10 (post) and skinfold thickness on days 1 (baseline) and 10 (post). Morning body mass (BM) and urine specific gravity (USG; data not shown) were measured daily. The study design was approved by the Ethics Committee of Australian Catholic University and conformed to the Declaration of Helsinki.

Hematology and anthropometry

Venous blood samples were obtained between 0800 and 0900 in an overnight fasted state. The bloods were drawn into sealed tubes by the team doctor and transported at room temperature to a university laboratory in Ghent, Belgium, for analysis of hemoglobin (Hb), hematocrit (Hct), ferritin (baseline only), thyroid-stimulating hormone (TSH), free thyroxine (T4), free triiodothyronine (T3), cortisol, total testosterone, insulin-like growth-factor-1 (IGF-1), luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The testosterone/cortisol ratio (T/C-ratio) was calculated for baseline and post values of these markers.

Hb and Hct were analyzed with an XN-9000 (Sysmex), IGF-1 with an LBS (Diasorin), and the rest of the markers with Cobas 8000 (Roche). SLS hemoglobin method and impedance/hydrodynamic focusing were used for the analysis of Hb and Hct, respectively. IGF-1 analysis followed the sandwich chemiluminescence immunoassay method, and ferritin analysis was done with the particle enhanced immunoturbidimetric assay. For the rest of the blood samples, electrochemiluminescence immunoassays were used. Actual intra-assay coefficients of variation (CV%) were 1.3% (Hb), 1.4% (Hct), 2.3% (ferritin), 3.6% (TSH), 3.8% (T4), 3.0% (T3), 3.2% (cortisol), 3.0% (LH), 3.5% (FSH), and 4.3% (testosterone). Skinfold thickness, body mass and USG were assessed according to standardized protocols (Table 2).

Analysis of nutrient intake, energy expenditure and energy availability

Exercise energy expenditure. During training and racing, duration, distance, exercise energy expenditure (EEE), average power and heart rate (HR) were recorded/estimated using powermeters (Schoberer Ran Mebtechnic, Julich, Germany) and HR monitors (Garmin International, Kansas, USA) (Table 2).

Dietary intakes. The cyclists were able to freely choose the type, quantity and timing of food and drink consumption, with the exception of main meals (set times) (see Table 2 for details). For data analysis, recipes and special race foods were first entered into a food analysis software (FoodWorks 8 Professional program; Xyris Software Australia Pty Ltd, Australia), followed by individual diet record entry and analysis. Dietary records were analyzed for total daily energy and macronutrient intakes (absolute and relative) using a 24h period that may better reflect the nutrition philosophy of professional cycling (1900 until 1900: i.e. race nutrition starts at dinnertime the night before the race and ends at 1900 on race day). In addition, carbohydrate and protein intakes within 3h pre-race, during and 3h post-race were calculated. Finally, the immediate 24-hour post-race period was analyzed for total carbohydrate intake to estimate whether muscle glycogen replenishment following the race and in preparation for the next race was successful.

Energy availability. Short-term EA was estimated based on dietary and training records following Loucks formula [24] with a cutoff of 30 kcal·kg FFM·d^{-1}being considered as low EA. On this basis, cyclists were divided into two crude subgroups: those whose mean estimated EA was below this cutoff (low EA [LEA]) and those who were above (moderate EA [ModEA]). This division resulted in cyclists 1, 3 and 6 being classified as achieving ModEA and cyclists 2, 4 and 5 as LEA.

Statistical analysis

Statistical analyses were conducted using SPSS Statistics 24 software (INM, Armonk, New York, USA). Data are presented as individual data points as well as means and standard deviations. Statistical significance was set at $p \leq 0.05$ and normality of data was checked using Shapiro-Wilk goodness-of-fit test, although given the small sample size of our cohort, we have used these analyses to illuminate our observations rather than declare definite outcomes. Differences between race vs recovery days in BM, USG, nutrition and exercise parameters were analyzed using Student's t-tests for paired samples, while repeated-measures analysis of variance (ANOVA) was used to analyze the variation in BM and dietary parameters across time. To compare actual intakes daily and around the races to contemporary nutrition guidelines, paired t-tests were used with the following "optimal" target intakes: CHO intake within 3h pre/post-race: 3 $g \cdot kg^{-1}$; CHO intake within 24 h post-race: 10 $g \cdot kg^{-1}$; CHO intake during the race: minimum 60 $g \cdot h^{-1}$ and maximum 90 $g \cdot h^{-1}$ [25].

RESULTS

Daily nutrition, exercise and BM and skinfolds across the Classics

Table 3 summarizes exercise energy expenditure and mean power outputs for race and rest day activities (race vs training sessions, respectively). Group mean EA and total daily carbohydrate intake were significantly higher and protein intake significantly lower on race compared to rest days (Table 4). LEA athletes (overall EA 28.2 ± 2.1 $kcal \cdot kg\ FFM \cdot d^{-1}$) had lower race and rest day

EA (7 ± 3 vs 49 ± 3 kcal·kg FFM·d^{-1}, respectively) compared to ModEA (overall EA 43.1 ± 3.4 kcal·kg FFM·d^{-1}; 22 ± 3 vs 64 ± 8 kcal·kg FFM·d^{-1} on race vs rest days, respectively; mean, race and rest day EA p=0.050 compared to LEA). There were no significant changes in BM (p=0.11, Table 3) or skinfold thickness (p=0.75, Table 3) across time. BM fluctuated across the 9 day period within 1.6 ± 0.5 kg (range 1.1–2.2 kg or 1.4 to 2.9 % change in BM).

Timing of carbohydrate and protein intake around the Classics races

Carbohydrate intakes around racing are shown in Table 3. Overall, pre-race intakes were in line with contemporary sports nutrition recommendations (p=0.24), while post-race intakes were significantly less than recommendations (p=0.002). During-race, carbohydrate intake was significantly less than the recommended bottom value of the targeted range (p=0.048), and well below the top value (p=0.002). Race nutrition included solids (first half of the race), and liquids (sports drinks/gels; second half of the race). Due to the nature of the Classics, the cyclists found it very challenging to memorize timing and type of food and drink consumption throughout races. However, we managed to get data from cyclists 1 and 4 who were able to memorize their patterns of food/drink consumption during Gent-Wevelgem, which enabled analysis of within-race timing of carbohydrate. Subsequent analysis showed that while cyclist 1 maintained a continuous carbohydrate supply (26-31 g·$30min^{-1}$) throughout the race, carbohydrate intake for cyclist 4 varied much more (3-35 g·$30min^{-1}$) and was nearly absent (6 g·h^{-1}) in the last hour. Mean CHO intake in the 24 h post-race period for all cyclists was 7.4 ± 1.0 g·kg^{-1}, which was significantly lower than the recommended 10 g·kg^{-1} (p=0.002).

Blood hormone concentrations at baseline and after the Classics

Statistically significant changes were observed for Hct (3% decrease; p=0.028), TSH (39% increase; p=0.028) and T3 (17% increase; p=0.008; Figure 1), while other blood markers showed no effect over time (Table 4). Hb decreased in LEA (-7.5%) while no change was seen in ModEA (-0.7%; difference to LEA p=0.023), while TSH increased more in ModEA (+65%) compared to LEA (+16%; p=0.049). The trend of change for testosterone, T3, IGF-1 and cortisol was different between LEA and ModEA (Figure 1). There was a mean decrease of 14% in testosterone in LEA compared to a mean increase of 7% in ModEA. Similar magnitudes of differences in changes were also seen in T3 (+12% vs +20% for LEA and ModEA, respectively) and IGF-1 (-25% vs +5% for LEA and ModEA, respectively) concentrations. The magnitude and direction of change in T/C-ratio (-14% vs +11% for LEA and ModEA, respectively) followed the same pattern. Cyclist 4 experienced significant drops in testosterone (-27%), T/C ratio (-28%) and IGF-1 (-25%).

DISCUSSION

To our knowledge, this study is the first to-date to investigate energy availability and hormone concentrations in professional male cyclists across single-day racing (the Classics). Our findings suggest that: (1) Professional cyclists periodize energy and carbohydrate intakes day-by-day, as shown by low EA (14 vs 57 kcal·kg FFM·d^{-1}) and high carbohydrate intakes (10.7 vs 6.4 g·kg·d^{-1}) on race vs rest days; this appears to be different to stage racing where considerable effort is focused on increasing energy intake and EA on race days; (2) Alternate-day low EA (<10 kcal·kg FFM·d^{-1}) lead to a trend towards decreased testosterone (-14%) and IGF-1 (-25%) after only 8 days, despite high EA (>46 kcal·kg FFM·d^{-1}) on days in-between; (3) These cyclists reached contemporary pre-race fueling targets (3.4 g·kg^{-1} carbohydrates), while the execution of acute (0.5 g·kg·h^{-1}) and prolonged (7.4 g·kg·24h^{-1}) post-race carbohydrate fueling guidelines was poor and contributed to the reduction in EA on race days. Finally, our pilot study provides important insights into the research methodology needed to investigate real world practice within professional cycling

(Table 2), including best practice protocols and their successful application in the field for most reliable outcomes.

Due to the small sample size and short duration of our study, we feel that it is unwise to draw major conclusions from this study. However, individual behavior and the overall cycling culture around nutrition support for one-day cycling Classics appears different to that of stage racing. Even though the modern approach to stage racing is to periodize energy and CHO intake according to the anticipated needs of the stage, we note that stage racing includes a more aggressive approach to nutrition support, including greater intake during the race [11]. This approach considers not only the cyclists' fuel requirements for the present stage, but also the potential carryover to the next day's stage.Reports from Grand Tours include high energy (5415-7815 kcal·d^{-1}) and carbohydrate (~12.6 g·kg·d^{-1}) intakes daily as well as around racing in professional male cyclists (Table 1). By contrast, the cyclists in the present study chose to consume less energy and carbohydrate while riding the race as well as a lower post-race intake in consideration of the upcoming rest day. The reduction in during-race feeding in one-day races compared with stage events or current nutrition guidelines might reflect the more aggressive riding style of the former format, which distracts or interferes with the cyclists' opportunities for food/fluid intake. In addition, team or individual tactics for the one-day race might require them to ride aggressively for the first part of the race before reducing their workload or withdrawing from the race; thus reducing the need for nutrition support. Further exploration of the culture and practical considerations of one-day racing is warranted. Despite the sample size, there are indications that intermittent days of LEA, due to inadequate intake on race day, are associated with interruptions to normal hormonal function. However, further investigation is required.

Finally, this study has provided an opportunity to examine the logistics of conducting a field study under stressful conditions in which major cooperation of subjects and team management is needed, and logistical considerations may hamper the implementation of best practice protocols. Here, several challenges emerged and have been discussed in detail in Table 2. Our biggest challenge was the small sample size, due to several cyclists not being available for the study at a late stage in the planning process. Therefore, our findings should be considered as trends worthy of further exploration rather than extrapolating these outcomes to all professional cyclists. Furthermore, due to the stressful and hectic environment of professional cycling racing, where key focus is on performance, we were unable to complete BM testing within the immediate time period around the races. Whilst not ideal, this was a purposeful compromise to remove any additional stress on the cyclists around the races. Finally, in terms of estimating exercise energy expenditure, powermeter data was a key measure in our study. Due to accidents and subsequent change of bikes, we lost half the racing data during one race for two cyclists. Nevertheless, we believe that our extrapolations represent this missing data accurately enough. Despite several challenges, we are satisfied with the dietary recording process and are confident this data closely approximates actual EA of our athletes.

PRACTICAL APPLICATIONS

Professional cyclists may need to pay special attention to adequate EA and post-exercise CHO recovery on race days, as ignorance of these factors may impair recovery, subsequent performance and impair health outcomes in the long-term. Research with professional cycle racing poses challenges including logistics and athlete/staff availability. Our commentary and suggested solutions to manage research within this sport aim to support and enhance the quality of future work within this space of sports science.

CONCLUSIONS

We investigated day-by-day periodization of nutrition and changes in hormone concentrations in professional male cyclists across single-day racing. Our findings suggest that professional cyclists periodize energy and carbohydrate intakes day-by-day. Alternate-day low EA led to a trend towards decreased testosterone and IGF-1 after only 8 days, despite high EA on days in-between. Finally, we have provided important insights into the research methodology needed to investigate real world practice within professional cycling, including best practice protocols and their successful application in the field for most reliable outcomes. Our commentary around the challenges and solutions is a major novelty of the paper and should provide future researchers with a blue print for the successful completion of subsequent work on this topic.

ACKNOWLEDGEMENTS

The authors would like to thank Australian Catholic University and Mitchelton-SCOTT for funding this study, and the amazing team of Mitchelton-SCOTT staff and most importantly, the cyclists, for their invaluable efforts to collaborate and participate in this research.

CONFLICTS OF INTEREST

The authors and funding agents do not have any conflicts of interests.

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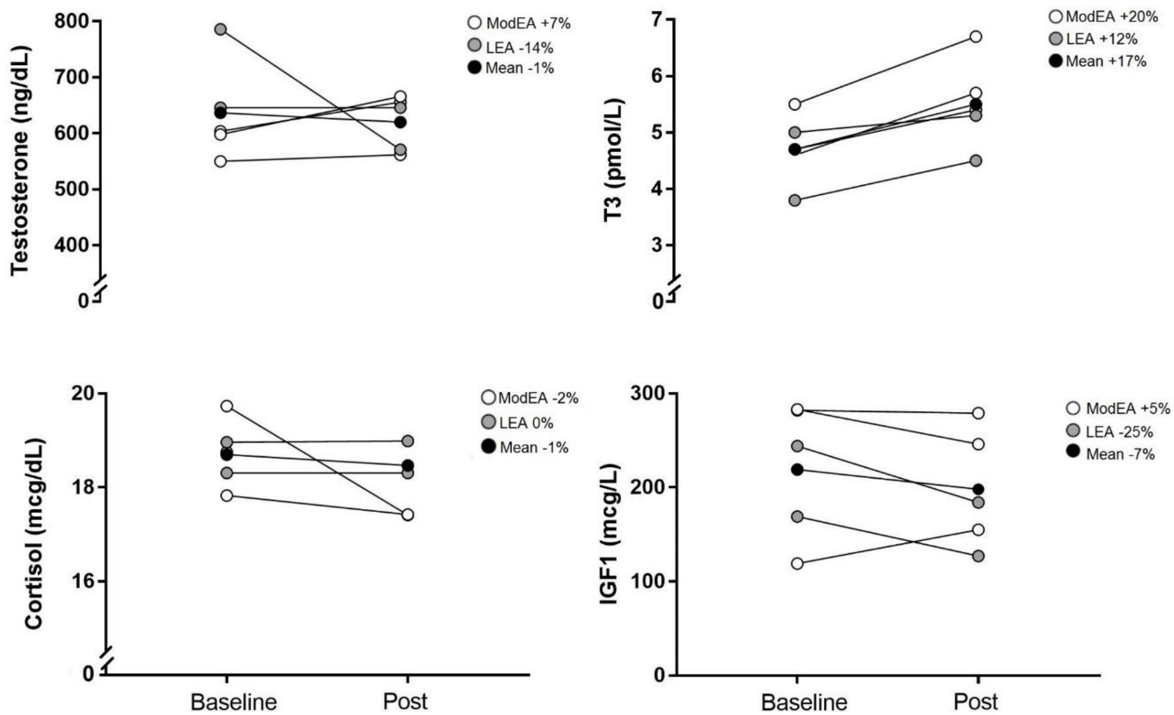

Figure 1. Blood concentrations of testosterone, triiodothyronine (T3), cortisol, and insulin-like growth-factor 1 (IGF-1) at baseline and after Spring Classics. Data are shown as individual cyclists grouped into low (LEA [n=2]; *gray dots*) or moderate (ModEA [n=3]; *white dots*) energy availability (EA; cutoff of 30 kcal·kg FFM·d^{-1}, based on dietary/exercise characteristics during the Classics) and as means (*black dots*). Percentage (%) of change has been calculated for the whole group (mean) as well as separately for LEA and ModEA.

Table 1. Available literature on nutrition during stage racing (4 days up to 3 weeks of consecutive-day racing) in male professional cyclists. A non-peer review case report published on BBC website has also been included for comparison.

Reference	**Participants**	**Race period**	**Dietary assessment**	**Daily nutrient intakes**	**Race nutrition**	**Other measures**
Muros et al. [15]	Male professional (UCI World Tour team) cyclists (n = 9): 31.3 ± 3.0 years 1.79 ± 0.07 m 69.1 ± 7.3 kg	Tour of Spain 2015: A 3-week stage race; Total distance of 3356.1 km; 6 flat, 8 mid-mountain, 5 high-mountain, 1 team TT, 1 individual TT; 2 rest days	Daily for the whole Tour	Energy: 5415±567 $kcal \cdot d^{-1}$ CHO: 12.5 ± 1.8 $g \cdot kg \cdot d^{-1}$ Protein: 3.3 ± 0.3 $g \cdot kg \cdot d^{-1}$ Fat: 1.5 ± 0.5 $g \cdot kg \cdot d^{-1}$	During the race: CHO: 91 ± 15 $g \cdot h^{-1}$ After the race (between race finish and dinner): CHO: 147 ± 33 g Fat: 16 ± 18 g Protein: 55 ± 17 g	HR: 128-159 bpm depending on stage PO: 216-329 W EEE: 374-4707 kcal/stage BM: 69.1 ± 7.3 kg (baseline) to 68.1 ± 7.1 kg (post) Sum of 8 skinfolds: 42.8 ± 4.3 mm (baseline) to 38.3 ± 3.6 mm (post)
Saris et al. [16]	Male professional cyclists (n = 4): 1.78 m 69.2 kg VO2max: 79.4 $ml \cdot kg \cdot min^{-1}$	Tour de France: A 3-week stage race; Total distance of ~4000 km; 30 mountain passages (up to 2700 m altitude); 1 rest day	Daily for the whole Tour	Energy intake: Overall mean: 24.7 ± 2.4 $MJ \cdot d^{-1}$ Highest (mountain stage): 32.4 ± 4.4 $MJ \cdot d^{-1}$ Lowest (rest day): 16.1 ± 3.9 $MJ \cdot d^{-1}$ CHO: 61% total energy intake (~900 $g \cdot d^{-1}$) Protein:	During the race: CHO: 94 $g \cdot h^{-1}$	Energy expenditure: Overall mean: 25.4 ± 1.4 $MJ \cdot d^{-1}$ Highest (mountain stage): 32.7 ± 1.6 $MJ \cdot d^{-1}$ Lowest (rest day): 12.9 ± 0.9 $MJ \cdot d^{-1}$ BM: 69.2 kg (baseline) to 68.9 kg (post)

Reference	**Participants**	**Race period**	**Dietary assessment**	**Daily nutrient intakes**	**Race nutrition**	**Other measures**
				217 ± 47 g·d^{-1} Fat: 147 ± 39 g·d^{-1}		Sum of 4 skinfolds estimation of body fat %: 11.6 (baseline) to 11.4 (post)
Garcia-Rovez et al. [17]	Male professional cyclists (n = 10): 27.6 ± 2.0 years 1.79 ± 0.04 m 66.9 ± 3.2 kg VO2max: 71.0 ± 6.2 ml·kg·min^{-1}	Tour of Spain: A 3-week stage race; Total distance of 3600 km Average distance of 170 km per stage; Range altitude of 10-2520 m above sea level; No rest days	Weighed food records (by RD) for three separate 24-hour periods: 1 flat stage (day 2, 178 km) and 2 mountain stages (day 14, 174 km; day 16, 148 km)	Energy: 23.5 ± 1.8 MJ·d^{-1} (352 ± 33 kJ/kg/d) CHO: 12.6 ± 1.1 g·kg·d^{-1} Protein: 3.0 ± 0.3 g·kg·d^{-1} Fat: 2.4 ± 0.3 g·kg·d^{-1}	During the race: 25 g·h^{-1} CHO After the race (between race finish and dinner): CHO: 2.0 ± 0.5 g·kg^{-1} Fat: 0.2 ± 0.1 g·kg^{-1} Protein: 0.3 ± 0.1 g·kg^{-1}	NR
Ebert et al. [18]	Male professional cyclists (n = 8): 25 ± 5 years 1.77 ± 0.05 m 71.4 ± 7.4 kg VO2max: 71.0 ± 6.2 ml·kg·min^{-1}	Tour Down Under: A 6-day stage race; Total distance of 719 km (stages between 50-152 km)	Recall immediately after each stage.	NR	During the race: CHO: 48 g·h^{-1}	BM pre- and post-race for each stage

Reference	**Participants**	**Race period**	**Dietary assessment**	**Daily nutrient intakes**	**Race nutrition**	**Other measures**
Ross et al. [19]	Male international level cyclists (n = 10): 19.7 ± 0.8 years 1.80 ± 0.05 m 72.0 ± 6.1 kg	*Tour of Gippsland* (n=5): 9 stages over 5 days *Tour of Geelong* (n=5): 6 stages over 5 days	Recall immediately after each stage.	NR	*Gippsland*: CHO: 40.5±24.2 $g \cdot h^{-1}$ *Geelong*: CHO: 64.2±23.7 $g \cdot h^{-1}$	Hydration, change in BM during stages
Sanches-Munoz et al. [20]	Male professional cyclists (n = 6): 25.5 ± 1.5 years 1.76 ± 0.06 m 67.7 ± 3.6 kg	Tour of Andalucia 2009: A 4-day stage race; Total distance of 647.6 km	Weighed food records collected by investigators	Energy: 5644±593 $kcal \cdot d^{-1}$ CHO: 12.8 ± 1.7 $g \cdot kg \cdot d^{-1}$ Protein: 3.0 ± 0.3 $g \cdot kg \cdot d^{-1}$ Fat: 2.1 ± 0.2 $g \cdot kg \cdot d^{-1}$	During the race: CHO: 278 ± 91 g After the race (between race finish and dinner): CHO: 74 ± 20 g Fat: 14 ± 2 g Protein: 42 ± 9 g	Mean PO: 246 ± 22 W Mean HR: 134 ± 5 bpm BM: 67.6 kg (baseline) to 67.5 kg (post) Sum of 8 skinfolds: 49.9 ± 7.7 mm (baseline) to 47.0 ± 8.1 mm (post)
Rehrer et al. [21]	Male elite cyclists (n = 4): 20 ± 3 years 1.91 ± 0.06 m 84.1 ± 8.2 kg	Tour of Southland 2005: A 6-day race with 10 stages; Total distance of 883 km	Weighed food records collected for the 6-day period	Energy: 27.3 ± 3.8 $MJ \cdot d^{-1}$ CHO: 12.9 ± 1.4 $g \cdot kg \cdot d^{-1}$ Protein: 2.9 ± 0.3 $g \cdot kg \cdot d^{-1}$		TEE (via DLW): 27.4 ± 2.0 $MJ \cdot d^{-1}$ EE: 16.9 ± 0.2 $MJ \cdot d^{-1}$ DXA lean mass: 68.8 ± 6.2 kg (baseline)

Reference	Participants	Race period	Dietary assessment	Daily nutrient intakes	Race nutrition	Other measures
	VO2peak: 57.6 ± 3.9 $ml \cdot kg \cdot min^{-1}$ PPO: 415 ± 35 W			Fat: 128 ± 61 $g \cdot d^{-1}$ (17.3 ± 2.3 E%)		DXA fat mass: 11.3 ± 2.9 kg (baseline) RMR: 11.5 ± 0.7 $MJ \cdot d^{-1}$
Pfeiffer et al. [22]	Male professional cycling teams at Dauphine Libere (n = 7) and at Tour of Spain (n = 8): *Dauphine Libere:* 31 ± 5 years 1.81 ± 0.05 m 70 ± 5 kg *Tour of Spain*: 29 ± 3 years 1.81 ± 0.05 m 71 ± 7 kg	*Dauphine Libere 2009:* An 8-day stage race; this study focused on two flat stages (228 km and 182 km) *Tour of Spain 2009:* A 3-week stage race; this study focused on two mountain stages (204.7 km and 188.8 km) and one flat stage (171.2 km)	Self-report retrospective questionnaire	NR	During the race: CHO: 64 ± 20 $g \cdot h^{-1}$ Caffeine: 21 ± 29 $mg \cdot h^{-1}$ Sodium: 208±183 $mg \cdot h^{-1}$	NR
BBC Sport [10]	Male professional cyclist (n = 1: Chris Froome).	Giro d’Italia 2018: A 3-week stage race;	Weighed food records/ recall?	*Stage 11:* Energy: 2466 kcal CHO:	*Stage 11:* CHO: 57 $g \cdot h^{-1}$	*Stage 11:* Morning BM: 69.3 kg

Reference	**Participants**	**Race period**	**Dietary assessment**	**Daily nutrient intakes**	**Race nutrition**	**Other measures**
	Data provided by Team Sky	Total distance of 3572.4 km across 21 days 3 rest days. This publication focused on two stages: *Stage 11* on May 16, 2018 (156 km / 4 h, hilly, EEE 3635 kJ) *Stage 19* on May 25, 2018 (185 km / 6 h, summit finish, EEE 6180 kJ)		5.8 $g \cdot kg \cdot d^{-1}$ Protein: 2.0 $g \cdot kg \cdot d^{-1}$ Fat: 0.5 $g \cdot kg \cdot d^{-1}$ *Stage 19:* Energy: 6663 kcal CHO: 18.9 $g \cdot kg \cdot d^{-1}$ Protein: 2.1 $g \cdot kg \cdot d^{-1}$ Fat: 1.3 $g \cdot kg \cdot d^{-1}$	*Stage 19:* CHO: 96 $g \cdot h^{-1}$ CHO	Post-stage BM: 67.1 kg EEE: 3635 kJ *Stage 19:* Morning BM: 68.9 kg EEE: 6180 kJ

UCI, Union Cycliste Internationale; TT, time-trial; CHO, carbohydrate; HR, heart rate; PO, power output; EEE, exercise energy expenditure; BM, body mass; VO2max, maximal oxygen uptake; NR, not reported

Table 2. Methodological goals, current best practice protocols, final study outcomes and future suggestions.

Goal	Best practice protocol	Outcomes in the current study	Commentary and future suggestions
To measure baseline and post blood hormone concentrations	Venous samples should be collected in the morning fasted state with standardized preceding conditions, including hydration level. Repeated measures should be conducted in similar conditions (time of day, preceding exercise and nutrition). For certain blood markers, the circadian rhythm and variability should be considered. Blood sample storage, transport and analysis should follow guidelines specific to each analyzed biomarker.	Due to race and camp schedules, fasted venous blood samples were measured as follows: *Baseline*: On the morning of the first race (Day 2: between 0800 and 0900; preceding 24h included light activity only). *Post*: On the morning after the last race (Day 10: between 0800 and 0900; preceding 24h included an intense, 5-hour race). One subject was taking TUE-supported medication that might have interfered with the interpretation of blood analysis; his data were excluded from this analysis	Professional cyclists train and compete under World Anti-Doping Association regulations and thus, are used to frequent blood testing. Therefore, cyclists are usually easy to collaborate with for the collection of samples. If time allows, future studies should aim to obtain blood samples under matched conditions (time of day, preceding 24h activity). One option would be to schedule baseline and post blood tests on day -1 before racing and on day +2 after racing to allow standardization of hydration status and preceding 24h exercise load.
To measure baseline and post skinfold thickness	Skinfold measures in the morning in the fasted state before any activity, hot showers or massage. Repeat measures should be standardized (time of day, preceding meals and activity, etc.)	Due to race and camp schedules, skinfolds were taken as follows: *Baseline*: On the afternoon of the day before the first race (Day 1: no hot showers or physical activity in the 2-3h period before the measurements,	It should be possible to implement best practice protocol in future studies.

Goal	Best practice protocol	Outcomes in the current study	Commentary and future suggestions
	Measurements should follow ISAK guidelines [26].	adequate hydration throughout the day). *Post*: On the morning of the last race (Day 10: fasted conditions with no showers before the measurements). Calibrated skinfold calipers (CMS Weighing Equipment Ltd, London, UK) were used. An ISAK accredited level 1 anthropometrist completed the measurements according to the ISAK guidelines. Body fat percentage and FFM were estimated from predicted body density calculations using the Durnin & Womersley equation [30].	
To measure morning body mass (BM) and USG to control for hydration	Morning urine samples should be collected in the morning upon wakening (mid-stream) and analyzed for USG by using a refractometer. Morning BM should be measured after emptying the bladder, in standardized	Morning urine samples and BM were collected each morning according to best practice protocols. Due to confusion between team doctor and the researchers, urine samples were missed on the mornings of days 2 and 3.	Clear communication between the researchers and team staff is required to avoid miscommunication. However, it should be possible to undertake such measurements under best practice protocols in future studies.

Goal	Best practice protocol	Outcomes in the current study	Commentary and future suggestions
	conditions, with a calibrated scale, before consumption of food/drinks.	USG was measured using a hand-held refractometer (Exacta and Optech, San Prospero, Modena, Italy). Body mass was recorded to the nearest 0.1 kg.	
To measure BM before and after races to determine changes in hydration status	BM should be measured just before and immediately after the race, in minimal clothing (e.g. underwear) and with the same set of calibrated scales. If BM is measured with race kits on, any added/removed clothing needs to be taken into consideration when comparing pre and post values. The change in BM can be estimated by use of the equation below: $(BM_{pre} - BM_{post})/BM_{pre}$ Due to effects of eating, drinking and possible toilet stops and weather (rain), on BM changes, these factors should be	After conversations with the cyclists and team staff, we abandoned the goal of measuring pre/post-race BM as the usefulness and accuracy of this measure seemed very questionable due to the following facts:. 1. Strict time schedule around racing (travel, change into race kits, team presentation, etc.) might have disturbed the race preparation of the cyclists. 2. Several uncontrollable factors have the potential to influence BM changes during race, including: (a) Change in the amount of clothing and/or rain that would affect the weight of clothing.	Measurement of BM in the immediate time period around the race can be challenging. We propose guidelines for time-efficient weighing that should minimize cyclist burden while maximizing measurement validity: *Pre-race:* Cyclists should weigh themselves in the team bus before changing into race kits (e.g. wearing only underwear). Any food/drink consumed after this measurement should be recorded. *Post-race*: Cyclists should be weighed with the same set of scales immediately upon returning to the team bus, before showering (e.g. wearing only underwear) but possibly drying themselves after sweating or riding in the rain.

Goal	Best practice protocol	Outcomes in the current study	Commentary and future suggestions
	considered and included in the calculations.	(b) Unknown amounts of body fluid losses due to urination. (c) Only estimated amounts of fluid intakes due to drinking. (d) Unknown changes in muscle glycogen stores due to race and interaction of this with race carbohydrate intake. In addition, the cyclists felt that this measurement would have disturbed their racing by confusing already strict time schedules.	**Interpretation of BM change pre-post:** Use of the equation proposed by the best practice protocol, with special consideration for factors including: Race nutrition (food and drinks: self-reported by the cyclists). Urination during the race.
To record dietary intake daily and around the race	Several methods which each have their pros and cons [27]. The prospective weighed food records (chosen for use for the current study) should be used to record all food and fluid intake by use of calibrated kitchen scales. Recording recipes, brand names and product details (fat content, type of product, etc.) will	The team chef prepared all the meals for the cyclists (breakfast, lunch, dinner, as well as race and recovery foods), while a separate snack area was provided with varying snacks for consumption in between meals. The chef provided the research team with detailed recipes, which were entered in daily meal sheets to enable efficient recording at meal times. Two researchers attended all meals times and weighed/helped cyclists	The methods used in this study were able to be implemented to achieve best practice and subject co-operation and can be recommended for future studies: 1. It resulted in less participant burden (weighing and recording for the most part done by the researchers). 2. It resulted in a high level of accuracy (most of the food was prepared by team chef, brand names were available for all products, recording was done by the researchers to a standardized method, post-

Goal	Best practice protocol	Outcomes in the current study	Commentary and future suggestions
	increase the quality of food records. In addition, retrospective interviews will strengthen self-reported data by revealing any missed items and/or quantities of foods/drinks consumed. If data is recorded by an investigator on behalf of the athlete, the same investigator(s) should record all meals. For data analysis, data entry should be completed by one investigator to improve reliability of data [28].	weigh all food and fluid consumed using calibrated kitchen scales (to the nearest 1 g). Food and fluid intake was then recorded using sheets individual to each cyclist and meal time. For snacks, the cyclists self-reported food and fluid consumption (weight and timing) using sets of kitchen scales provided to them in the separate snack area. For race nutrition (pre-race in the bus, during race, post-race in the bus), cyclists self-reported intakes (kitchen scales were provided) on individual recording sheets. Apart from drinks (bottles), race foods were pre-packed and weighed, therefore number of units (cakes, bars, gels) was recorded. Retrospective interviews immediately post-race were used to cross-check race nutrition records.	race interviews improved accuracy of race nutrition records). 3. It resulted in a highly reliable data set (it minimized typical errors of recording such as underreporting of actual portion sizes or foods considered unhealthy, over-reporting of foods considered as healthy; it reduced the likelihood of subjects altering usual intake due to burden of recording it). Data entry and analysis were completed by one investigator, which should have minimized errors arising from having multiple people working on the same data set.

Goal	**Best practice protocol**	**Outcomes in the current study**	**Commentary and future suggestions**
		The cyclists were encouraged to take photos of race nutrition (snacks inside the pockets) before the start of the race and again post-race (for what was consumed/left) to assist them in remembering what was consumed. Between races (from post-race until the night before the next race), one of the cyclists went home and was given a kitchen scale and detailed instructions on dietary recording.	
To record training/race energy expenditure	Calibrated powermeters can be used to acquire information on the mean power output (MPO) for each race. This can be used to calculate the mechanical work for each race as follows: MPO * time (s) = mechanical work (kJ) Gross efficiency (GE) can be derived from individual testing data.	Power meters were factory calibrated and zero-offset was checked prior to each ride according to the manufacturer's recommendations. EEE was estimated using the equations for mechanical work and EEE as described by the best practice protocol. Cyclist 6 did not have a powermeter in his bike, therefore, heart rate monitor was used to get an estimate of his EEE.	Crashes and subsequent changes in bikes are a part of professional cycling racing, and cannot be avoided in real-life studies.

Goal	Best practice protocol	Outcomes in the current study	Commentary and future suggestions
	Alternatively, a common GE value of 20.7 % for cyclists [29] can be used. EEE can be estimated by multiplying mechanical work by GE. Units can be converted to kilocalories for reporting purposes. Use of calibrated machinery will assist in collecting reliable information.	**Acute race challenges:** Two cyclists had a crash and subsequent change of bike during racing (cyclist 2 on day 6 after 178km; cyclist 4 on day 4 after 146km of racing), which resulted in missing powermeter data for the final part of the race. For these cyclists, the EEE for the final part of the race was estimated from powermeter data (average EEE as kcal/min) during the early part of the race (total race EEE = EEE for the early part of the race + EEE (kcal/min) x min racing in the final part of the race).	

Table 3. Cyclist characteristics at baseline and post-Classics. Values are means and standard deviations (SD).

	Mean	**SD**
Age (yr)	30.0	5.7
Height (m)	1.87	0.04
Baseline BM (kg)	77.4	2.7
Post BM (kg)	77.1	3.0
Baseline sum of 7 skinfolds (mm)	37.2	4.0
Post sum of 7 skinfolds (mm)	37.2	3.3
1' MMP [W ($W \cdot kg^{-1}$)]	646 (8.3)	50 (0.5)
5' MMP [W ($W \cdot kg^{-1}$)]	470 (6.1)	14 (0.2)
20' MMP [W ($W \cdot kg^{-1}$)]	399 (5.2)	33 (0.4)
UCI rank 2018 (15/10/2018)	948	408
UCI rank 2017 (10/05/2018)	562	382

BM, body mass; MMP, maximal mean power for 1, 5 and 20 minutes of continuous work during racing, averaged over a 6-week period around the Classics; UCI, Union Cycliste Internationale

Table 4. Race and rest day exercise (mean power output, MPO; exercise energy expenditure, EEE) and nutrition (energy and macronutrient intakes; energy availability, EA) characteristics for each cyclist as well as means and standard deviations (SD).

Cyclist	**1**	**2**	**3**	**4**	**5**	**6 #**	**Mean**	**SD**
Race variables								
MPO [W (W·kg^{-1})]) **	228 (3.0)	269 (3.4)	254 (3.3)	217 (2.9)	263 (3.2)	147 bpm	246 (3.2)	23 (0.2)
EEE (kcal/race) ***	4777	5855	5365	4421	5920	4766	5184	624
Race day intakes								
Energy (kcal) **	6131	6239	7196	4831	6686	6215	6216	789
EA (kcal·kg FFM^{-1}) ***	19.4	5.2	25.3	5.9	9.9	20.6	14.4	8.5
CHO (g·kg^{-1}) ***	11.7	10.8	12.3	8.5	11.1	10.0	10.7	1.3
Protein (g·kg^{-1}) *	2.8	2.6	3.3	2.5	2.7	3.0	2.8	0.3
Fat (g·kg^{-1})	2.5	2.6	3.3	2.0	2.7	3.2	2.7	0.5
Race carbohydrate intakes								
3h pre-race (g·kg^{-1})	3.1	3.2	4.6	3.4	3.8	2.4	3.4	0.7
3h pre-race (g·kg·h^{-1})	1.0	1.1	1.5	1.1	1.3	0.8	1.1	0.2
During race (g·kg^{-1}) #	60.0	57.0	56.0	36.0	50.0	47.0	51.0	9.0
3h post-race (g·kg^{-1}) ##	1.3	1.8	2.5	0.9	1.6	1.7	1.6	0.5
3h post-race (g·kg·h^{-1})	0.4	0.6	0.8	0.3	0.5	0.6	0.5	0.2
24h post-race intakes								
CHO (g·kg^{-1}) ##	7.1	7.9	8.7	5.7	7.2	8.0	7.4	1.0
Rest day exercise variables								
MPO [W (W·kg^{-1})])	152 (2.0)	229 (2.9)	177 (2.3)	175 (2.3)	186 (2.3)	113 bpm	184 (2.4)	29 (0.3)
EEE (kcal/session)	657	1420	987	778	1060	821	954	271
Rest day intakes								
Energy (kcal)	4948	4813	5233	4430	4909	5968	5050	519
EA (kcal·kg FFM^{-1})	61.4	46.3	58.6	52.2	49.6	73.2	56.9	9.8
CHO (g·kg^{-1})	6.1	7.1	7.1	5.1	5.9	7.2	6.4	0.8
Protein (g·kg^{-1})	3.5	2.7	3.6	3.1	3.0	4.0	3.3	0.5

Cyclist	**1**	**2**	**3**	**4**	**5**	**6 #**	**Mean**	**SD**
Fat ($g \cdot kg^{-1}$)	2.8	2.2	2.5	2.8	2.4	3.6	2.7	0.5

CHO, carbohydrate; * $p<0.05$, ** $p<0.01$, *** $p<0.001$ significant difference between race and rest day # Cyclist 6 did not have powermeter data so HR data has been reported here; # $p<0.05$, ## $p<0.01$ significant difference to contemporary sports nutrition guidelines on 3h pre-race (3 $g \cdot kg^{-1}$), 3h post-race (3 $g \cdot kg^{-1}$), during race (60 $g \cdot h^{-1}$) and 24 h post-race (10 $g \cdot kg^{-1}$) CHO targets.

"Alternate-Day Low Energy Availability During Spring Classics in Professional Cyclists" by Heikura IA et al.
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Table 5. Blood concentrations of hormones at baseline and after the 10-day racing period for individual cyclists and as mean and standard deviations (SD).

		Blood concentrations of hormones and iron markers							
Cyclist	***Time point***	**Hb ($g \cdot dL^{-1}$)**	**Hct (%)**	**Ferritin ($mcg \cdot L^{-1}$)**	**TSH ($mU \cdot L^{-1}$)**	**T4 ($pmol \cdot L^{-1}$)**	**LH ($U \cdot L^{-1}$)**	**FSH ($U \cdot L^{-1}$)**	**T/C ratio**
Cyclist 1	*Pre*	14.8	44.7	179	1.2	15.2	3.6	3.8	30.6
	Post	15.0	44.3		1.9	15.1	6.0	4.3	37.7
Cyclist 2	*Pre*	14.7	44.3	108 #	2.4	13.8 #	5.6	5.3	35.3
	Post	13.4	41.8		2.8	13.4 #	4.2	5.0	35.3
Cyclist 3	*Pre*	13.7	41.7	187	1.5	17.1	5.3	2	29.3
	Post	13.6	41.2		2.8	18.2	5.6	2.1	27.8
Cyclist 4	*Pre*	15.5	44.3	252	2.7	18.5	3.8	2.7	41.5
	Post	14.5	42.4		3.1	17.8	2.8	2.7	30.1
Cyclist 6	*Pre*	15.3	45.9	97 #	1.5	15.8	3.7	7.6	33.5
	Post	15.0	45.0		2.1	16.1	7.4	7.2	38.2
Mean	*Pre*	14.8	44.2	165	1.8	16.1	4.4	4.3	34.0
	Post	14.3	42.9		2.5	16.1	5.2	4.3	33.8
SD	*Pre*	0.7	1.5	63	0.7	1.8	1.0	2.2	4.8
	Post	0.8	1.6		0.5	2.0	1.8	2.0	4.7

Hb, hemoglobin; Hct, hematocrit; TSH, thyroid-stimulating hormone; T4, thyroxine; T3, triiodothyronine; LH, luteinizing hormone; FSH, follicle-stimulating hormone; TC ratio, testosterone/cortisol ratio.
values within lowest quartile of reference range
Note: one cyclist was taking medication for a medical condition, supported by a therapeutic use exemption, which is unrelated to the current study but might have affected the results of some blood tests. His data have been excluded from this table.