Abstract 2019

A year in the life of a Brazilian professional female road cycling team – Part II: Nutritional and clinical outcomes

Patrícia Campos-Ferraz[1], Gabriel Barreto[1], Luana Farias de Oliveira[1], Tiemi Saito[1], Rafael Klosterhoff[1,2], Pedro Henrique Lopes Perim[1], Eimear Dolan[1], Rosa Maria Rodrigues Pereira[4], Fernanda R. Lima[1], Bryan Saunders[1,5]

1 – Applied Physiology and Nutrition Research Group, School of Physical Education and Sport; Rheumatology Division; Faculdade de Medicina FMUSP, Universidade de Sao Paulo, Sao Paulo, SP, BR, University of São Paulo, SP, Brazil.
2 – Department of Biochemistry and Molecular Biology, Federal University of Paraná, Curitiba, PR, Brazil.
3 – Laboratory of Assessment and Conditioning in Rheumatology (LACRE), Rheumatology Division, Universidade de São Paulo, SP, Brazil.
4 – Bone Laboratory Metabolism, Rheumatlogy Division, Hospital das Clínicas HCFMUSP, Faculdade de Medicina da Universidade de Sao Paulo, SP, Brazil.
5 – Institute of Orthopaedics and Traumatology, Faculty of Medicine FMUSP, University of São Paulo, Brazil.

Background: Professional female cyclists undertake intense training, and the demands of this training must be met by adequate nutritional intake. This is particularly relevant for professional teams who train and compete all year round, as they can cover up to 18 000 km (Sanders et al., 2019). Energy availability (EA) refers to the amount of energy available for basic physiological processes, once the demands of training have been met (Loucks et al., 2011). Low EA (often defined as <30 kcal·kgLBM^{-1}·day^{-1}), can lead to a wide range of detrimental physiological and performance consequences (as described in the Relative Energy Deficiency in Sport model (Mountjoy et al., 2014)) with menstrual dysfunction and compromised bone health in particular known to be affected As such, adequate energy (and nutrient) intake is essential to protect the health and performance of top-level cyclists. In this investigation, we monitored the nutritional habits of a professional Brazilian female cycling team throughout a competitive season and measured several outcomes related to REDs.

Methods: Five female Brazilian cyclists (age 26 ± 4 y; body weight 53.6 ± 4.2 kg; height 1.64 ± 0.05 m; VO_{2max} 55.5 ± 4.2 ml·kg^{-1}·min^{-1}) from the same professional cycling team, including the former Brazilian national time-trial champion and two current members of the Brazilian national road-race team, were monitored throughout their competitive season. Dietary analysis was performed at the beginning and end of their competitive year (February and December). Nutritional assessment was performed by a trained sport nutritionist who asked the athletes about their habitual food and supplement consumption by recall and macronutrients and fibre intake were calculated using nutrition software (AVANUTRI, Brazil). Anthropometric measures were performed using a Harpenden calliper and a metric tape at 7 locations (Guedes and Rechenchosky, 2008) to determine percentage body fat and lean mass. Energy availability was calculated from training and competition days using the original Harris–Benedict equation for resting metabolic rate (Harris and Benedict, 1918), considering an average metabolic equivalent of 7.1 METs for training and 9.8 METs for competition (Jette et al., 1990), averaging their energy intake from the two moments of assessment and accounting for each individual athlete's average time spent cycling in each category. Menstrual cycle health was assessed via a questionnaire. Clinical analyses were performed at three moments throughout the year (February, July and December). Blood samples were analysed at a central laboratory (Clinical Hospital of São Paulo) for immunological and haematological parameters (haemoglobin, haematocrit, leukocytes), nutritional status (vitamin B12, vitamin B, folic acid, total protein, albumin and ferritin) and stress markers (uric acid, creatine kinase, free and total testosterone, cortisol, thyroid-stimulating hormone). Bone mineral density (BMD) was determined at the end of the season using an X-ray source dual-emission apparatus (DXA; Discovery A; Hologic Inc., Bedford, USA). Low BMD was defined as a Z score ≤-1 (Mountjoy et al., 2014).

Results: Nutritional intake was similar for all athletes at the start and end of the season (Table 1). Total calorie intake ranged from 42 to 69 kcal/kg per day, protein from 2 to 4 g/kg, carbohydrate between 4 and 9 g/kg and fat from 1 – 2 g/kg. Fibre intake was lower than the daily recommended allowance (25 – 30 g/day) for 3 out of 5 athletes at the start of the season and 3 out of 4 athletes at the end. Estimated energy availability ranged from 32.6 to 57.2 kcal/kg LBM/day during training and from 26.7 to 54.6 kcal/kg LBM/day during competition (Table 1). Athletes reported taking supplements daily including protein bars and whey protein, carbohydrate gels, creatine, potassium, vitamin B and C, folic acid, multi-ingredient powders, resveratrol, chelated magnesium and multivitamins.

Clinical analyses revealed all cyclists were healthy at the start of the season (Table 2), with regular menstrual cycles between 21 and 35 days; three of the five athletes were taking oral contraceptives. Immunological and haematological parameters were maintained throughout the season and there were no cases of any kind of infection. Nutritional and stress markers remained largely unchanged throughout the season for those who repeated the exams, although testosterone levels were low for some individuals at various moments (Table 2). There was a recurrent locomotor complaint of lower back pain from 3 athletes during the year which was treated with physical therapy in all cases.

Bone mineral density at the lumbar spine was between 0.831 and 0.944 g/cm^2 and whole-body Z-scores ranged from -0.6 to 0.5 (Table 3). Athlete 4 became pregnant in September and did not perform the DXA scan or any other assessment.

Discussion: Our results showed that three individuals may have had sub-optimal energy availability during training, while one of these also showed low energy availability during competition. Fibre intake was also low in several of the athletes. This indicates that some of these cyclists may have been in energy deficit at some points throughout the year. This may be reflected in some of the low testosterone levels which may be an attempt by the body to conserve energy; low levels of free testosterone may have a mechanistic role in the development of low BMD (Almeida et al., 2017). However, regular use of the contraceptive pill can also lead to low levels of testosterone (Zimmerman et al., 2014). Therefore, caution should be taken when interpreting these data since there are several issues associated with the calculation of energy availability in an applied setting (Burke et al., 2018) while oral contraceptive use may alter the hormonal profile.

Athletes are susceptible to low energy availability due to several reasons including poor nutritional knowledge (Burke et al., 2018). At the start of the season, two athletes reported being afraid to eat carbohydrate at night but were instructed by the nutritionist to include it at dinner. One of them did not use any nutritional supplements, such as carbohydrate gels and whey protein, during training or competition and was advised to use them. All of them were advised to use carbohydrates during training, recovery drinks and whey protein after training and prescribed creatine and beta-alanine throughout the season. It is possible that the recommendations of the nutritionist helped to maintain their health and performance throughout the season despite possible inadequate or low energy availability at certain moments. Indeed, there were no cases of infection, no large weight fluctuations or changes in menstrual cycle across the season. The importance of a nutritionist should not be underestimated, and further research should evaluate the true impact of this role for professional cycling.

Evidence suggests that cyclists may be at risk for low BMD due to the non-weight-bearing nature of the sport which does not adequately stimulate bone formation. Although no athlete reached a clinical level of significance, all athletes were below the mean of the reference population. This could be a consequence of low testosterone levels which play a mechanistic role in low BMD development (Almeida et al., 2017). Unfortunately, we could only measure BMD at the end of the season, meaning we could not monitor changes over time. Previous studies have shown contradictory findings regarding BMD loss over a season, with reports of losses in BMD (Sherk et al., 2014) and no change (Viner et al., 2015) in female cyclists. Interestingly, the two athletes with the lowest BMD were also those that sustained fractures throughout the year, namely a compound fracture of clavicle (Athlete 1) and a fracture of the wrist (Athlete 2). These cannot be causally linked using the available data, but seeing as BMD is associated with fracture risk, we recommend that these athletes undergo continuous monitoring, and that strategies are implemented to protect their bone health.

Conclusions: Several members of this professional female cyclists may have been exposed to sub-optimal energy availability during training and competition. This may have resulted in low testosterone levels in several athletes, although no further alterations in the hormonal profile, menstrual cycle or incidences of infection were shown. Further longitudinal studies on top-level female cyclists are warranted.

References:

ALMEIDA, M., LAURENT, M. R., DUBOIS, V., CLAESSENS, F., O'BRIEN, C. A., BOUILLON, R., VANDERSCHUEREN, D. & MANOLAGAS, S. C. 2017. Estrogens and Androgens in Skeletal Physiology and Pathophysiology. *Physiol Rev,* 97**,** 135-187.

BURKE, L. M., LUNDY, B., FAHRENHOLTZ, I. L. & MELIN, A. K. 2018. Pitfalls of Conducting and Interpreting Estimates of Energy Availability in Free-Living Athletes. *Int J Sport Nutr Exerc Metab,* 28**,** 350-363.

GUEDES, D. P. & RECHENCHOSKY, L. 2008. Comparison of predicted body fat from anthropometric methods: Body mass index and skinfold-thickness. *2008,* 10**,** 7.

HARRIS, J. A. & BENEDICT, F. G. 1918. A Biometric Study of Human Basal Metabolism. *Proc Natl Acad Sci U S A,* 4**,** 370-3.

JETTE, M., SIDNEY, K. & BLUMCHEN, G. 1990. Metabolic equivalents (METS) in exercise testing, exercise prescription, and evaluation of functional capacity. *Clin Cardiol,* 13**,** 555-65.

LOUCKS, A. B., KIENS, B. & WRIGHT, H. H. 2011. Energy availability in athletes. *J Sports Sci,* 29 Suppl 1**,** S7-15.

MOUNTJOY, M., SUNDGOT-BORGEN, J., BURKE, L., CARTER, S., CONSTANTINI, N., LEBRUN, C., MEYER, N., SHERMAN, R., STEFFEN, K., BUDGETT, R. & LJUNGQVIST, A. 2014. The IOC consensus statement: beyond the Female Athlete Triad--Relative Energy Deficiency in Sport (RED-S). *Br J Sports Med,* 48**,** 491-7.

SANDERS, D., VAN ERP, T. & DE KONING, J. J. 2019. Intensity and Load Characteristics of Professional Road Cycling: Differences Between Men's and Women's Races. *Int J Sports Physiol Perform***,** 1-7.

SHERK, V. D., BARRY, D. W., VILLALON, K. L., HANSEN, K. C., WOLFE, P. & KOHRT, W. M. 2014. Bone loss over 1 year of training and competition in female cyclists. *Clin J Sport Med,* 24**,** 331-6.

VINER, R. T., HARRIS, M., BERNING, J. R. & MEYER, N. L. 2015. Energy Availability and Dietary Patterns of Adult Male and Female Competitive Cyclists With Lower Than Expected Bone Mineral Density. *Int J Sport Nutr Exerc Metab,* 25**,** 594-602.

ZIMMERMAN, Y., EIJKEMANS, M. J., COELINGH BENNINK, H. J., BLANKENSTEIN, M. A. & FAUSER, B. C. 2014. The effect of combined oral contraception on testosterone levels in healthy women: a systematic review and meta-analysis. *Hum Reprod Update,* 20**,** 76-105.

Acknowledgments

Bryan Saunders (2016/50438-0 and 2017/04973-4), Gabriel Barreto (2017/15314-1) and Pedro Perim (2018/01594-5) have been financially supported by Fundação de Amparo à Pesquisa do Estado de Sao Paulo. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Table 1. Dietary intake and anthropometric of the athletes at the start and end of the season.

	Athlete 1		Athlete 2		Athlete 3		Athlete 4		Athlete 5	
	Start	**End**	**Start**	**End**	**Start**	**End**	**Start**	**End**	**Start**	**End**
Total calories (kcal)	2789	2744	2920	2910	2411	-	2385	2273	3610	3217
Total calories (kcal/kg)	47.2	44.8	61.3	56.8	42.5	-	44.7	42.0	68.6	61.6
Total protein (g)	176	170	123	195	130	-	108	154	210	165
Total protein (g/kg)	2.97	2.77	2.58	3.81	2.29	-	2.03	2.85	4.00	3.16
Total carbohydrate (g)	311	348	443	425	282	-	340	212	499	456
Total carbohydrate (g/kg)	5.26	5.68	9.31	8.30	4.97	-	6.38	3.93	9.49	8.70
Total fat (g)	93	74	73	47	85	-	66	90	86	81
Total fat (g/kg)	1.58	1.21	1.52	0.93	1.49	-	1.23	1.67	1.63	1.55
Fibre (g)	14	18	15	19	26	-	9	12	32	23
Body weight (kg)	59.1	61.3	47.6	51.2	56.8	-	53.3	54	52.6	52.2
%Body fat	15.6	15.3	11.3	14.8	23.5	-	12.5	15.4	17.9	17.2
%Lean mass	84.3	84.7	88.7	85.2	76.5	-	87.5	85.6	82.0	82.7
Body Mass Index (kg/m^2)	20.2	21.0	19.2	20.6	20.9	-	19.1	19.3	19.9	19.7
Estimated training EA (kcal/kg LBM/day)	39.8		50.5		43.1		32.4		56.9	
Estimated competition EA (kcal/kg LBM/day)	32.6		45.3		38.1		26.7		54.6	

*EA = energy availability; LBM = lean body mass

Table 2. Clinical analyses performed at the start, middle (Mid) and end of the season.

Laboratory Tests	Normal range	Athlete 1			Athlete 2			Athlete 3			Athlete 4			Athlete 5		
		Start	**Mid**	**End**	**Start**	**Mid**	**End**	**Start**	**Mid**	**End**	**Start**	**Mid**	**End**	**Start**	**Mid**	**End**
Leukocytes (mil/mm³)	4.00 - 11.00	7.31		4.84	6.45	-	5.53	3.69	-	-	5.41	-	5.4	8.24	-	-
Haemoglobin (g/dl)	12.0 - 16.0	13.4	14.5	13.8	13.7	12.7	13.0	12.2	14.1	-	12.5	12.3	12.1	13.5	13.2	-
Haematocrit (%)	35.0 - 47.0	40.5	42.9	41.2	43.3	38	39.4	35.7	41.5	-	37.9	36.4	36.1	40.3	40.1	-
Vitamin D (ng/mL)	30 - 100	45.7	36.9	-	41.9	29.8	-	24.1	19	-	48.6	38.6	-	38.4	38	-
Vitamin B12 (pg/mL)	197 - 771	799	931	716	463	433	790	605	701	-	1010	1212	814	1204	1494	-
Folic Acid (ng/mL)	3.1 - 17.5	10.9	17.9	13.9	11.1	9.2	18.0	13.8	11.6	-	26.4	11.9	10.3	15.3	18.9	-
Ferritin (ng/mL)	13 - 150	268	259	201	73	46	52	124	87	-	431	385	356	154	149	-
Total Protein (g/dL)	6.0 - 8.0	6.9	-	-	7.4	-	-	7.0	-	-	7.4	-	-	6.7	6.95	-
Albumin (g/dL)	3.2 - 5.0	3.8	-	-	3.6	-	-	3.9	-	-	3.9	-	-	3.7	4.51	-
Cortisol (µg/dL)	6.7 - 22.6	11.4	15	20.8	17.7	25.9	26.0	7.7	12.8	-	13.1	13.3	20.7	6.1	9.7	-
Creatine kinase (U/L)	<167	153	310	123	86	83	89	77	-	-	142	558	133	95	127	-
Uric Acid (mg/dL)	2.4 - 5.7	-	2.5	-	2.7	2.5	3.6	3.6	3.7	-	3.7	3.5	2.5	2.9	3.5	-
Free Testosterone (pmol/L)	2.4 - 37.0	1	3	1	2	1	2	7	12	-	1	8	4	5	2	-
Total Testosterone (ng/dL)	<48	< 12	16	< 12	<12	<12	< 12	19	28	-	< 12	24	24	18	< 12	-
Thyroid-stimulating hormone (mU/L)	0.4 – 4.0	0.92	1.40	2.46	4.63	5.96	7.32	2.96	3.04	-	3.45	1.87	3.98	1.72	2.65	-

Table 3. Bone mineral density (BMD) at lumbar spine and total body Z-scores.

	BMD (g/cm^2)	Z-score
Athlete 1	0.831	-0.6
Athlete 2	0.870	0.0
Athlete 3	-	-
Athlete 4	0.944	0.2
Athlete 5	0.926	0.5