Dietary periodisation for health and performance in world-class endurance athletes

Submitted by

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BSc, MSc

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

Ph.D. with Publication

Submitted 3rd January 2020

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DECLARATION OF AUTHORSHIP AND SOURCES

This thesis contains no material that has been extracted in whole or in part from a thesis that I have submitted towards the award of any other degree or diploma in any other tertiary institution.

No other person's work has been used without due acknowledgement in the main text of the thesis. All research procedures reported in the thesis received the approval of the relevant Ethics/Safety Committees.

[redacted]

Ida A. Heikura Date: 3rd January 2020

ACKNOWLEDGEMENTS

To dare is to lose one's footing momentarily. Not to dare is to lose oneself.

– Søren Kierkegaard

When my dad unexpectedly passed away in March 2015, it broke my heart. Life was not the same anymore, yet I knew it had to go on. Seven months later I arrived in Australia for the first of many Supernovas. Here, on the other side of the world, I found a family – the wonderful team Supernova with its amazing athletes, staff, and volunteers.

Deeply impressed by the quality of work by Louise and her team, I knew I wanted to relocate to Australia for a PhD. A couple months later, in July 2016, I packed my bags and left Finland. I took a leap of faith. Although moving to the other side of the world was scary, taking the leap has been more than worth it. It has changed my life.

I have met some of the most wonderful people during this journey.

To my supervisors, **Louise**, **John** and **Trent**. Thank you. The past four years have been amazing. I came to Australia as I believed this was the best place to learn more. My expectations have been by far exceeded and I am leaving with more experience and skills than I ever dared to wish for. Thank you, Louise and John, for giving me this opportunity. Louise and Trent, thank you for all the wisdom, support and encouragement along the way. Thank you for always pushing me a bit further and for making this the most comprehensive PhD experience – I feel very lucky to have had the opportunity to expand my skills and expertise in such a broad way. The three of you have been my biggest role models in academia and an ongoing inspiration to keep learning.

I want to thank the ENRP team at the MMIHR/ACU in Melbourne, where I had the pleasure to spend the first year of my PhD. A special thank you to **Jill**, **Ev** and **Brooke** for welcoming me in Melbourne with open arms.

Ever since I first stepped through the doors of the AIS, this is where I always felt most at home. Thank you to the whole team, and especially to **Meg**, **Laura** and **Julia** for guidance and company during the process. I also want to thank the extended team: **Alannah** and **Beccy** – my "partners in crime" in the lab and behind the diet scenes during all the Supernovas; **Nicolin Tee** for help in the biochem lab; **Greg Cox** for all the cool talks and guidance over the triathlon study; The fantastic **athletes** I had the honor to work with; Coaches **Brent Vallance** and **Jamie**

Turner; **Team Mitchelton-SCOTT** and **Marc Quod**, for the opportunity to collaborate around the hectic Classics – I learned a lot and enjoyed the experience.

Throughout this journey I have been fortunate to make some life-long friends. **Nicki Strobel** and **Rita Civil** – you were the main reason I not only survived but truly enjoyed the long Supernova days back in 2015-17 (followed by a fun reunion at the 2018 Classics). I am grateful for your friendship.

To friends and extended family in Finland and all over the world, thank you. Thank you to **Vilho Ahola** for the help over the last 2.5 years. I am finally starting to feel like myself again.

Life can only be understood backwards; but it must be lived forwards. **Noah**, you continue to inspire me with your knowledge of anything and everything; in a relatively short period of time you have taught me more than most will during a lifetime. I have learned to let go of the expectations of tomorrow to make the most of today; to have faith in the unexpected, against the odds. Simple, but complex. Damn.

Finally, thank you to everyone I have not mentioned including colleagues, funding agents, and collaborators that have helped make this thesis and the studies within possible.

I may have found a family at the AIS, but I have a family of my own back in Finland. And they mean the world to me. Therefore, I would like to dedicate this thesis to my family: Mum, Dad, Enni and Kaisa.

Thank you, **Mum**, for being there for me whenever I needed it (throughout my 31 years of life!). You are my rock and role model. Thank you **Enni** and **Kaisa** for being the best sisters one could hope for, and for reminding me there is life outside of the PhD bubble. Leaving home was a lot easier knowing there was always a place and people to go back to. I want to thank my family for the constant support and encouragement throughout this journey. Thank you for giving me a reason to never give up. Thank you for believing in me when no one else did. I love you to the moon and back.

Dad, you showed me that you can do anything if you have a passion for it. You were kind yet extremely persistent. There were so many days I would have given anything to be able to call you, but I know you have been with me all this time. I love and miss you.

Life is unpredictable and there would be no highs without the lows. Ironically, amidst the fading grief, the past four years turned out to be some of the best in my life so far. And for that I am forever grateful to every single person that was part of this journey – you know who you are. Thank you for walking by my side, whether it was a couple blocks or the whole way.

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1. **Heikura, IA.**, Stellingwerff, T., & Burke, LM. (2018). Self-reported periodization of nutrition in elite female and male runners and race walkers. *Front Physiol*. Dec 3;9:1732. doi: 10.3389/fphys.2018.01732. eCollection 2018

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2. **Heikura, IA.**, Quod, M., Strobel, N., Palfreeman, R., Civil, R., & Burke, LM. (2019). Alternate-Day Low Energy Availability During Spring Classics in Professional Cyclists. *Int J Sports Physiol Perform*. Mar 12:1-35. doi: 10.1123/ijspp.2018-0842. [Epub ahead of print]

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4. **Heikura, IA.,** Ross, ML., Tee, N., McKay, AKA., Sharma, AP., & Burke, LM. (2020). Effects of low energy vs low CHO availability on markers of bone modelling at rest and during exercise in elite race walkers *In preparation for Bone*

   This manuscript is currently under preparation to be submitted to *Bone*:

LIST OF CONFERENCE PRESENTATIONS

2018	**Heikura, IA.**, Stellingwerff, T. & Burke, LM. Self-reported periodisation of nutrition in elite female and male runners and race walkers. *Poster presentation at International Sports and exercise Nutrition Conference, Newcastle Upon Tyne, UK. December 2018.*
2018	**Heikura, IA**., Cox, GC, McKay, AKA., Burke, LM. Acute Implementation of train/recovery with low carbohydrate availability does not compromise bone metabolism in world-class triathletes. *Mini-oral presentation at European College of Sport Sciences conference, Dublin, Ireland. July 2018.*
2017	**Heikura, IA.**, Burke, LM., Bergland, D., Uusitalo, ALT., Mero, AA. & Stellingwerff, T. Impact of elite runner's energy availability on bone mineral density, health, injuries and haemoglobin responses at altitude. *Poster presentation, SPIN Summit 2017, Vancouver, Canada. October 2017.*

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2019	Burke, LM., Sharma, AP., **Heikura, IA.**, Forbes, S., Holloway, M., McKay, AKA., Bone, JL., Leckey, J., Welvaert, M., & Ross, ML. (2019). Crisis of confidence averted: impairment of exercise economy and performance in elite race walkers by ketogenic Low Carbohydrate, High Fat (LCHF) diet is reproducible. *Plos ONE.* In review
2019	Stellingwerff, T., Peeling, P., Garvican-Lewis, LA., Hall, R., Koivisto, AE., **Heikura, IA.,** & Burke, LM. (2019). Nutrition and altitude: strategies to enhance adaptation, improve performance and maintain health. *Sports Med.* 2019 Nov 6. doi: 10.1007/s40279-019-01159-w. [Epub ahead of print] Review.
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2017	**Heikura, IA.**, Stellingwerff, T., Mero, AA., Uusitalo, ALT. & Burke, LM. (2017). A Mismatch Between Athlete Practice and Current Sports Nutrition Guidelines Among Elite Female and Male Middle- and Long-Distance Athletes. *Int J Sports Nutr Exerc Metab,* 27(4):351-360.
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2016	Burke, L., Ross, M., Garvican-Lewis, L., Welvaert, M., **Heikura, IA.**, Forbes, S., Mirtschin, J., Cato, L., Strobel, N., Sharma, A. & Hawley, J. (2016). Low Carbohydrate, High Fat diet impairs exercise economy and negates the performance benefit from intensified training in elite race walkers. *J Physiol* 595(9), 2785-2807.

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ABSTRACT

Periodisation – defined as the systematic planning and sequencing of training blocks across macro, meso and micro cycles of training and racing as a means to optimise athletic adaptation and performance – has been implemented by coaches to athletes across most sports for several decades. Similarly to training, nutrition should also be periodised to support training and racing goals. For example, the diets of endurance athletes may move along the spectrum from low to high energy availability (EA) or low to high carbohydrate (CHO) availability (e.g. body composition management with brief periods of low EA or CHO vs optimised adherence to sports nutrition guidelines via high energy and CHO availability). Despite robust evidence behind current guidelines, the literature lacks systematic information on the knowledge and implementation of periodised nutrition strategies across various levels of training and racing by world class endurance athletes. Another popular dietary approach – chronic or long-term ketogenic low-CHO, high fat (LCHF) diet – has become popular among some athletes and scientists as a means to enhance endurance performance and to support health. Notably, current literature does not support the former claim of enhanced performance, and the majority of existing evidence in favour of the latter claim comes from research in clinical populations (such as obesity, cancer, and epilepsy). Since endurance athletes are at an especially high risk for low bone mineral density (BMD) and stress fractures, and as available evidence suggests that both acute low EA and CHO availability may have independent and negative effects on bone modelling process in active individuals, this is an important topic that has been largely unexplored in the literature to-date. Therefore, this thesis will investigate dietary periodisation approaches in elite endurance athletes as well as the effects of low energy and low CHO availability on direct and indirect outcomes of markers of bone modelling and bone health in this population.

Study 1: Self-reported periodization of nutrition in elite female and male runners and race walkers

Study 1 (described in Chapter 4) examined self-reported dietary periodisation practices across macro, meso and micro levels of annual training and racing program in elite female and male middle/distance runners and race walkers (n=104, 50 % Major championship qualifiers). We detected a number of key repeated themes across various levels of training periodization, including: (1) Road athletes reported different nutritional practices to middle- and track-distance athletes, by including strategies of training with both low and high CHO availability

within the annual training plan; (2) Middle-distance athletes were the most conscious about the effects of nutrition strategies on physique management goals; (3) Females seemed to be more conscious of and/or reluctant to consume extra energy/CHO compared to males; (4) Overall, training and race performance appeared key factors influencing nutrition choices, while themes such as body composition manipulation, health, and practicality were less important; (5) Many athletes within this cohort of high level athletes were unaware of the use of nutrition to manipulate training adaptations, or felt that there were side-effects or challenges that prevented their use.

Study 2: Alternate-Day Low Energy Availability During Spring Classics in Professional Cyclists

Study 2 (described in Chapter 5) measured dietary energy and CHO availability, metabolic and reproductive hormone concentrations and physique outcomes across an 8-d period of single-day road racing in 6 male professional road cyclists. Our findings suggest that: (1) Professional cyclists periodise 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; (2) Extreme 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 d, despite high EA (>46 kcal·kg FFM·d^{-1}) on days in-between; (3) These cyclists reached contemporary pre-race fuelling 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 fuelling guidelines was poor and contributed to the reduction in EA on race days. Finally, our pilot study 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.

Study 3: A short-term ketogenic diet impairs markers of bone health in response to exercise

Study 3 (described in Chapter 6) examined the effects of 3.5-week LCHF diet and subsequent acute CHO feeding on markers of bone modelling during a prolonged, intense exercise bout in elite male and female race walkers. Our data revealed novel and robust evidence of acute (negative) changes to bone modelling/remodelling after the LCHF diet, including increased markers of resorption [at rest and post-exercise; area under curve (AUC) mean (95 % confidence interval) +81 % (54, 109) around exercise] and decreased markers of formation [at rest and across exercise; AUC for P1NP and OC -19 % (-25, -12) and -29 % (-35, -23), respectively, around exercise], with only partial recovery (bone resorption) of these effects following acute restoration of CHO availability.

Study 4: Effects of low energy vs low CHO availability on markers of bone modelling at rest and during exercise in elite race walkers

Study 4 (described in Chapter 7) investigated the effects of 5 d of either 1) high energy and CHO availability (HEA: ~40 kcal·kg FFM·d^{-1} and ~9.5 g·kg·d^{-1} CHO), 2) high energy but extremely low CHO availability (LCHF: ~40 kcal·kg FFM·d^{-1} and <0.5 g·kg·d^{-1} CHO), or 3) low energy and moderate CHO availability (LEA: ~15 kcal·kg FFM·d^{-1} and ~5.0 g·kg·d^{-1} CHO) on markers of bone modelling during a prolonged, intense exercise bout in elite male race walkers. Compared to continued HEA or brief exposure to LEA, LCHF was associated with significant decreases in the AUC around exercise for a marker of bone formation P1NP [-20 % (-31, -9)], as well as markers of bone modelling, OC [-26 % (-32, -20)] and Glu-OC [-31 % (-50, -11)]. Meanwhile, CTX did not change for any of the treatment groups.

Summary and future directions

This series of research studies has addressed several gaps in the literature and contributed novel insights into the nutrition periodisation practices of elite endurance athletes and into the interactions between acute and prolonged dietary energy and CHO restriction and bone modelling in world-class endurance athletes. The key findings can be summarised as: 1) World-class track and field endurance athletes periodise nutrition across all levels of training and racing, where key incentive appears to be performance optimisation; 2) Professional cyclists micro-periodise energy and CHO availability to match fuel intakes to the day-by-day demands of training and racing; 3) Prolonged, extreme CHO restriction (the LCHF diet) impairs markers of bone formation and resorption during a prolonged intense exercise bout, and these effects are not fully recovered with acute CHO feeding; and 4) Short-term CHO restriction in the form of a LCHF diet impairs markers of bone formation during a prolonged exercise bout, while no effects are seen with a diet providing low energy but moderate CHO availability. In general, athletes should to aim to match fuel intakes to the goals and demands of training and racing across a periodised program. Care should be taken with extreme CHO restriction as this appears to lead to impairments in bone modelling markers at rest and during exercise even beyond low EA, at least over a 5 d period. Future research should aim to assess whether the effects of energy or CHO restriction on bone modelling could be avoided by the use of stepwise reductions in energy or CHO availability (as opposed to a single, sudden, massive drop), as well as by manipulating the length of exposure and by optimising timing of nutrient intakes around exercise.

LIST OF ABBREVIATIONS AND NOMENCLATURE

ACTH	Adrenocorticotropic hormone
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
ATP	Adenosine triphosphate
BAP	Bone alkaline phosphatase
BM	Body mass
BMD	Bone mineral density
BMI	Body mass index
CHO	Carbohydrate
CRH	Corticotropin-releasing hormone
CT	Calcitonin
CTX	C-terminal telopeptide of type 1 collagen
DXA	Dual-energy x-ray absorptiometry
E2	Estrogen
EA	Energy availability
EEE	Exercise energy expenditure
EHMC	Exercise hypogonadal male condition
EI	Energy intake
FAT	Female athlete triad
FFM	Fat-free mass
FHA	Functional hypothalamic amenorrhea
FM	Fat mass
FSH	Follicle-stimulating hormone
GE	Gross efficiency

GH	Growth hormone
GHRH	Growth hormone-releasing hormone
GIP	Glucose-dependent insulinotropic polypeptide
Glu-OC	Undercarboxylated octeocalcin
GLP-1	Glucagon-like peptide-1
GnRH	Gonadotropin-releasing hormone
Hb	Hemoglobin
HCHO	High CHO diet
Hct	Hematocrit
HR	Heart rate
IAAF	International Association of Athletics Federations
IGF-1	Insulin-like growth-factor-1
IL-6	Interleukin-6
IMTG	Intramuscular triacylglycerol
ISAK	International Society for the Advancement of Kinanthropometry
LEA	Low energy availability
LCHF	Low carbohydrate-high fat
LH	Luteinising hormone
LT_1	Lactate threshold 1
LT_2	Lactate threshold 2
LV	Left ventricular
Mb	Myoblogin
MidD	Middle-distance athlete (800 m and 1500 m)
MMP	Maximan mean power
MPO	Mean power output
O_2	Oxygen

OC	Osteocalcin
OPG	Osteoprotegerin
P	Progesterone
p38MAPK	p38 mitogen activated protein kinase
PDH	Pyruvate dehydrogenase
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
P1NP	Procollagen type 1 N propeptide
PPO	Peak power output
PTH	Parathyroid hormone
PV	Plasma volume
PYY	Peptide YY
RANK	Receptor activator of nuclear factor κB
RANKL	RANK ligand
RBC	Red blood cells
RED-S	Relative energy deficiency in sport
RER	Respiratory exchange ratio
RoadD	Road-distance athlete (marathon and 20 km and 50 km race walks)
RPE	Rating of perceived exertion
T3	Triiodothyronine
T4	Thyroxine
T/C-ratio	Testosterone/cortisol-ratio
TEE	Total daily energy expenditure
TPR	Total peripheral resistance
TrackD	Track-distance athlete (3000 m to 10 000 m)
TRH	Thyroid-releasing hormone
TRIMP	Training impulse

TSH	Thyroid-stimulating hormone
UCI	International Cycling Union (Union Cycliste Internationale)
VE_{max}	Maximal minute ventilation
VO_{2max}	Maximal oxygen uptake
VO_{2peak}	Peak oxygen uptake
W	Watts
WR	World record

GLOSSARY OF TERMINOLOGY

The list below includes some of the key glossary of terminology utilised in the current thesis. The majority of the terminology has been derived from the current and most recent sports nutrition and exercise science literature. However, both in academia and in the field, it is not uncommon to come across several terms that have been used interchangeably to describe a single phenomenon. Alternatively, scientists or athletes may at times refer to a single term that has different meanings to different people or in different contexts. Therefore, the main purpose of this list is to describe what these terms are in reference to in the context of the current body of work.

Athlete availability, the ability of the athlete to undertake their optimal training program without modification or absences due to fatigue, illness or injury.

Bone modelling process, the balance between the breakdown (resorption) and formation of bone tissue (also known as *bone turnover*).

Bone turnover, the balance between the breakdown (resorption) and formation of bone tissue (also known as *bone remodelling process*).

CHO availability, the match between the timing and amount of CHO that is consumed compared with the fuel requirements of a training session/event or daily training/competition workload. Low CHO availability notes that body CHO (glycogen) stores or CHO intake during exercise are less than the fuel requirements of exercise whereas high CHO availability denotes that glycogen stores and/or exogenous CHO intake were sufficient to provide the fuel requirements of exercise.

Elite athlete, an athlete competing at an international (Major Championship) level in his/her sport; this may be further defined according to their event/sport (e.g. membership of a UCI pro-cycling team, threshold of points awarded by IAAF).

Energy availability, the energy that remains after exercise energy expenditure for other body systems to use (including reproduction and cellular maintenance), calculated as energy intake minus exercise energy expenditure, expressed relative to fat-free mass.

Endurance athlete, an individual training for and competing in sports lasting from 2 minutes (middle-distance running) to 1-3 hours (marathon, Olympic distance triathlon, road cycling) and beyond (road cycling, 50 km race walk).

Exercise economy, the oxygen cost of movement at any given submaximal power or speed.

Exercise energy expenditure, energy expended during exercise only (i.e. excluding sedentary energy expenditure over the same time period).

Exercise session/workout, a single exercise session (e.g. a 60 min run).

Low energy availability, energy availability that causes perturbations to the physiological and psychological homeostasis, usually defined as <30 kcal·kg FFM·d^{-1}.

Macro-cycle, a training cycle of several months to years in duration (e.g. base training phase from November to February)

Maximal oxygen uptake, the maximal rate of oxygen consumption during maximal exercise.

Meso-cycle, a training cycle lasting several weeks (e.g. a 4-week altitude training camp).

Micro-cycle, a training cycle lasting several days to one week (e.g. a single day of training that includes two workouts, or a 7 d training micro-cycle).

Periodisation of nutrition, targeted periodisation of nutrition (e.g. carbohydrate intake) and/or dietary strategies (e.g. training with low carbohydrate availability) to best support the goals of each training cycle.

Periodisation of training, the purposeful sequencing of different training units or blocks (e.g. micro-, meso- and macro-cycles) to reach the desired physiological, tactical, technical and psychological adaptations for optimised sports performance.

Physique, a combination of body mass and composition.

Power-to-weight ratio, the highest possible power output that the athlete is able to produce relative to his/her body mass.

Training, the chronic accumulation of exercise sessions to achieve long-term adaptations.

Training load, the product of training volume and intensity.

1 INTRODUCTION

The term periodisation is frequently utilised in sports science to refer to the systematic planning and sequencing of specialised training blocks across various phases of training and racing. The underlying theory behind periodisation is to stimulate a few key physiological (or psychological, technical, tactical, etc.) systems across a block, before moving the focus to the development of another outcome, eventually leading to optimised athletic adaptation and performance. Athletes in both endurance and strength sports have been implementing a periodised approach to training and racing for several decades. However, until early 21st century, nutrition was mainly considered as a static component of an athlete's preparation. For example, early guidelines from the 1990's encouraged endurance athletes to maintain high carbohydrate (CHO) intakes at all times for maximal performance and recovery. Also, adequate energy availability (EA) was considered a key requirement for optimal athlete health. Indeed, at the time, periodisation of nutrition was not acknowledged or emphasised (at least in academia) to the extent it currently is, even though athletes may have been implementing it by accident for much longer. In contrast, contemporary sports nutrition guidelines are characterised by a dynamic understanding of the importance of periodised nutrient availability based on training/racing phase as well as individual goals and sport-specific performance determinants. For endurance athletes, depending on the specific goal and training/racing phase, dietary approaches can range from low to high energy or CHO availability, where rather distinct approaches can be utilised depending on whether the goal is to target body composition management (brief periods of low EA or CHO restriction), adaptation (alternating between low and high CHO intakes on a session-by-session basis), or athletic performance (where optimal energy and CHO availability is usually a prerequisite). It is important to note that despite robust evidence behind current guidelines, the literature lacks systematic information on the knowledge and implementation of periodised nutrition strategies across macro, meso and micro levels of training and racing by world class endurance athletes. While any nutrient can be periodised, the main focus of research in endurance athletes has been on energy and CHO, which are also the main focus of this thesis.

Another popular dietary approach promoted to and by some endurance athletes has been the chronic or long-term ketogenic low CHO-high fat (LCHF) diet. Such diets provide adequate EA but are extremely limited in dietary CHO availability (less than $50\ g\cdot d^{-1}$) and high in dietary fat in order to develop and maintain ketosis. The LCHF diet is claimed to enhance endurance performance via increased reliance on fat-based fuels during exercise, thus sparing muscle glycogen use and prolonging the time to fatigue. Despite these claims, current evidence does not support the benefits of LCHF diets for endurance performance, in fact recent research shows that a 3-week LCHF diet impairs exercise economy in elite race walkers. Another reason for increasing interest towards LCHF diets has been their proposed health benefits, mainly from the point of view of the general population and individuals with an underlying clinical condition (such as obesity, cancer, and epilepsy). Here, claimed positive effects of the LCHF diet include enhanced weight loss and metabolic health (reversal of Type 2 diabetes, improved lipid profiles, etc.) as well as better management of diseases. However research on other health outcomes especially important for athletes including the bone has been lacking.

Indeed, in addition to optimal adaptation and performance, a key consideration of athletic preparation is maintenance of health which has a direct effect on athlete availability, i.e. availability of the athlete to undertake full training and racing according to their individualised program. Athlete availability, on the other hand, has been shown to be directly linked to success on the competition day. Therefore, strategies that support optimal athlete health and thereby, training ability, remain an important consideration from the nutritional perspective. Endurance athletes are at a relatively high risk for low BMD and stress fractures, in part due to the nature of the sport (often involving low to no impact activity) and in part due to a high prevalence of dietary restraint and low EA. While chronic (months to years) exposure to low EA is a clear and significant risk factor for poor bone health, the effects of acute (within-day and around exercise) and/or short-term (a couple of weeks) low EA on disturbances of bone modelling have been less studied. Available evidence in sedentary and moderately active females suggests that only 5 d of low EA (<30 kcal·kg FFM·d^{-1}) has negative effects on hormones that impact bone health and on the bone modelling process itself. Another potential nutrient that impacts on bone is CHO. Here, studies have reported negative changes in markers of bone modelling around an acute exercise bout completed in the fasted state or without additional CHO intake during exercise, as opposed to completing the same session with high CHO availability. Whether these findings are present in highly trained elite athletes, and whether the length of exposure to low CHO availability has an interactive effect, is unknown. Given the high prevalence of poor bone health in endurance athletes, this thesis will focus on the effects of low energy or low CHO availability on direct and indirect outcomes of bone modelling markers and bone health in elite endurance athletes.

This thesis will address several knowledge gaps in the literature, and is comprised of four independent but related studies to determine (1) Self-reported dietary periodisation practices in world-class endurance athletes across macro-, meso- and micro-cycles of training and competition; (2) Day-by-day periodisation of energy and CHO availability in professional cyclists across a series of single-day races and concomitant effects of these strategies on physique and endocrine system; (3) The effects of a 3.5-week LCHF diet and acute CHO feeding on markers of bone modelling in world-class race walkers; and (4) The effects of 5 d of low energy and moderate CHO availability vs high energy and high CHO availability (optimal EA diet) vs high energy and low CHO availability (LCHF diet) on markers of bone modelling in world-class race walkers.

A review of the literature can be found in Chapter 2, and chapters 4, 5, 6 and 7 are comprised of the experimental studies of the thesis listed below. Chapter 8 will discuss the novel findings of these studies as well as provide directions for future research.

- **Study 1:** Self-reported periodization of nutrition in elite female and male runners and race walkers
- **Study 2:** Alternate-Day Low Energy Availability During Spring Classics in Professional Cyclists
- **Study 3:** A short-term ketogenic diet impairs markers of bone health in response to exercise
- **Study 4:** Effects of low energy vs low CHO availability on markers of bone modelling at rest and during exercise in elite race walkers

2 LITERATURE REVIEW

2.1 Periodisation of nutrition integrated into a periodised training program

Training for endurance sports involves a periodised program which manipulates the duration, intensity and frequency of exercise sessions across macro (months to years), meso (several weeks) and micro (within-day to between-day) cycles to stress various physiological systems for optimal training adaptations and performance outcomes (Mujika et al., 2018; Sylta et al., 2016). Although there are several different approaches to periodisation of training, most methods share commonalities including: 1) progressive overload, 2) periodised emphasis on the development of physiological, psychological, technical and tactical outcomes, 3) inclusion of recovery periods within the program, and 3) basing decisions of the design of the training program on the main performance goal of the season (Mujika et al., 2018). To avoid confusion regarding the various ways in which words can be used, hereafter, this work will consistently use the terms “session” or “workout” to describe a single bout of exercise within a training program, while “training” will be used to describe the chronic accumulation of exercise sessions to achieve long-term adaptations.

Periodisation has traditionally been regarded as the cornerstone of planning training programs, although more recently, the concept has received some criticism. Most of the discussion has revolved around the apparent mismatch between the amount (lack of) scientific evidence behind the concept, in contrast with the (high) popularity of practical applications of training periodisation (Mujika et al., 2018). Others have noted that the traditional model of periodisation ignores the need for a flexible and reflective approach in the planning of individual training programs (Kiely, 2012). Here, ideally, decision-making should be based on how each individual responds to the current training stress and monitored via a combination of internal [e.g. heart rate (HR), rating of perceived exertion (RPE)] and external (e.g. speed/power, intensity) signals. Therefore, periodisation should be seen as a framework that is continuously adjusted based on the background and characteristics of each individual athlete, rather than a set plan.

While the periodisation of training has underpinned the preparation of endurance athletes for decades, the concept of a holistic approach to athletic preparation, including strategic and targeted periodisation of dietary strategies to best support the goals of each training cycle, is a much more recent theme in sports nutrition (Mujika et al., 2018; Thomas et al., 2016). Although nutrition plays a key role in athletic preparation, it has mostly been viewed as a static or constant contributor. However, newer paradigms around the interaction of diet and exercise note that the presence or absence of various nutrients can modulate the adaptive response or performance outcome of an exercise session. Therefore, nutritional strategies can be arranged around the training program to support and amplify its effects. Indeed, decisions on nutrition strategies at any time should be preceded by a thorough examination and understanding of the generic, sport-specific determinants of success as well as individual, athlete-specific performance gaps (Stellingwerff et al., 2019). Therefore, nutrition strategies should not be used randomly nor in isolation, but instead strategically in combination with training strategies to yield optimal performance outcomes.

In addition to the periodisation of nutrition and dietary strategies based on the goals of training/racing, current sports nutrition guidelines also promote the importance of individualisation, where the implementation of a specific nutrition strategy is likely to depend on factors such as: 1) competition demands; 2) sport-specific body composition; 3) training/event volume; 4) environmental conditions (Stellingwerff, 2012; Stellingwerff et al., 2007a; Melin et al., 2019; Stellingwerff et al., 2019); and 5) individual responsiveness to trialled strategies. Although “dietary periodisation” can be used as a generic term to describe specific changes in nutrition as a whole, or any specific nutrient/component within it (e.g. caffeine, protein intake), the focus of the current thesis is on the periodisation of energy and carbohydrate (CHO). Therefore, key terminology and available evidence of periodised energy and CHO availability in elite/sub-elite athletes as well as effects of energy and CHO availability on health will be discussed.

2.1.1 Endurance athletes and periodised endurance training programs

For the purposes of the current thesis, the term "endurance athlete” refers to an individual who competes in sports lasting anywhere from 2 min (middle-distance running, sprint races in cross country skiing) to 1-3 h (marathon, Olympic distance triathlon, cross country skiing, road cycling) and beyond (ultra-marathons, Ironman triathlon, road cycling, etc.). Meanwhile, elite is used to refer to an athlete that competes at an international (Major Championship) level in their event/sport. Although the duration of these sports may seem very different, high calibre athletes in these events must be able to sustain exercise intensities, throughout or at critical times of the race at intensities above 75 % maximal oxygen uptake (VO_{2max}), where the body is reliant on mostly CHO-based fuel sources. In addition, the training regimens of these athletes generally incorporate large amounts of aerobic or submaximal training, performed below or around lactate threshold 1 (LT_1) interspersed with high intensity training and strength training over a periodised training program (Fiskerstrand & Seiler, 2004; Munoz et al., 2014; Seiler &

Kjerland, 2006; Stellingwerff, 2012; Tonnessen et al., 2014; Tonnessen et al., 2015). Of course, training is heavily influenced by the time of the year and main goals of each specific phase and thus, differences do exist between and within sports and training cycles. While sports such as rowing or swimming also involve substantial crossover with the periodised training cycles of endurance athletes, these will not be considered within this body of work due to differences in requirements based on form of locomotion, skill execution and race duration. Therefore, this thesis focuses on elite endurance athletes within track & field (middle- and long-distance running and race walking) and road cycling.

Within endurance sports, success is dependent on the athlete’s capacity to sustain a given velocity or power output for as long as possible, requiring the regeneration of adenosine triphosphate (ATP) via aerobic pathways, underpinned by the delivery of environmental oxygen to the mitochondria located within the skeletal muscle (Hawley, 2002; Jones & Carter, 2000; Jones, 2006). Endurance performance is mainly determined by VO_{2max} (provides a “ceiling’ for aerobic capacity), economy of movement (defines the cost of movement at any power or speed) and the second lactate threshold (LT2; defines the ceiling at which prolonged high intensity exercise can be maintained) (Jones & Carter, 2000; Jones, 2006; Midgley et al., 2006). As physique (i.e. body mass and composition) has influence on the economy of movement and on the relative values of VO_{2max}, it has indirect effects on endurance performance and is of particular relevance to especially gravitational (e.g. weight-bearing) endurance sports such as middle- and long-distance running. Section 2.2 will continue discussion around the topic of optimal physique, or race weight, within these events.

The purpose of endurance training is to stress pulmonary, cardiovascular and neuromuscular systems in the body (Figure 2.1) to induce several beneficial adaptations including increased stroke volume, skeletal muscle capillarisation and mitochondrial content, via a mixture of training concepts to maximise the athlete’s training adaptation and performance (Midgley et al., 2006). These concepts include high-volume low-intensity sessions (e.g. a 2 h long run below LT_1), lactate threshold sessions (e.g. a 40 min tempo run between LT_1 and LT_2), high intensity interval sessions (e.g. 5*5 min at or above LT_2) and polarised training [a combination of large volumes of low intensity (below LT_1) and high intensity (above LT_2) training, with less time in the zone between LT_1 and LT_2] training (Stöggl & Sperlich, 2014). For optimal adaptation and performance outcomes, a program should incorporate most or all of these training concepts in varying quantities within different macro-, meso- and micro-cycles for each individual for optimal training and performance outcomes. Figure 2.2 shows an example of training

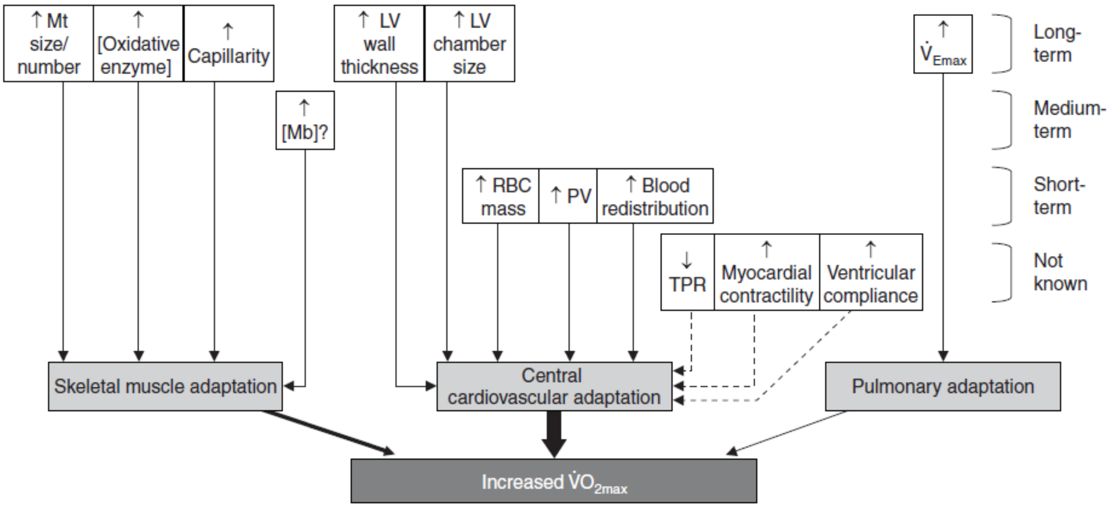

↑ Mt size/ number
↑ [Oxidative enzyme]
↑ Capillarity
↑ [Mb]?
↑ LV wall thickness
↑ LV chamber size
↑ V̇Emax
Long-term
Medium-term
Short-term
Not known
↑ RBC mass
↑ PV
↑ Blood redistribution
↓ TPR
↑ Myocardial contractility
↑ Ventricular compliance
Skeletal muscle adaptation
Central cardiovascular adaptation
Pulmonary adaptation
Increased V̇O2max

Figure 2.1. Physiological adaptations following endurance training lead to the improvement of maximal oxygen uptake (VO_{2max}). The arrows with broken lines indicate that the time course of those adaptations is currently unknown. The width of the three shaded arrows at the bottom of the figure broadly represents the total contribution of each of those adaptations in the long-term improvement of VO_{2max}. Maximum period of adaptability for myoglobin concentration is based on rat studies. *LV*, left ventricular; *Mb*, Myoglobin concentration; *Mt*, mitochondrial; *Oxidative enzyme*, oxidative enzyme concentration; *PV*, plasma volume; *RBC*, red blood cell; *TPR*, total peripheral resistance; VE_{max}, maximal minute ventilation; ↑ indicates increase; ↓ indicates decrease; ? indicates presently unknown in humans. (Midgley et al., 2006)

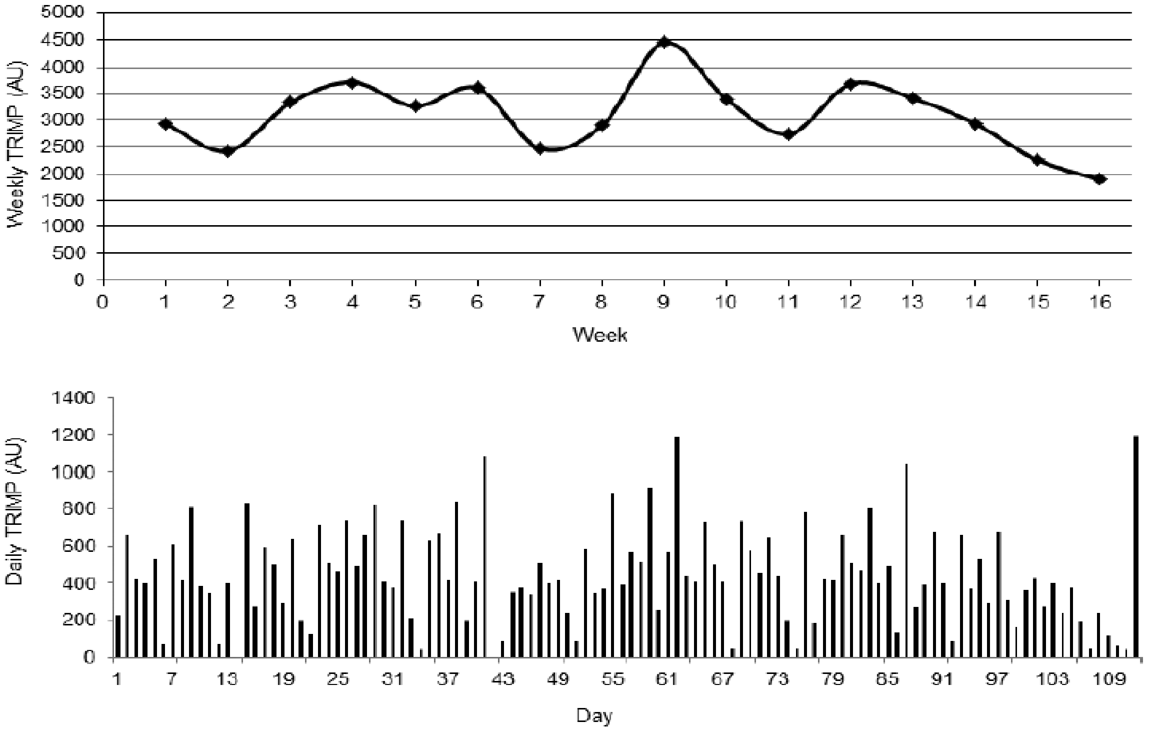


Figure 2.2. Average weekly (above) and daily (below) training impulse (*TRIMP*) in an elite marathon runner during a 16-week preparation period for a marathon. (Stellingwerff, 2012)

periodization (daily and weekly training load) in an elite marathon runner over a 16-week training period prior to the key race.

2.2 Commentary on physique issues related to success in endurance sports

Power-to-weight ratio refers to the power output that the athlete is able to produce relative to his/her body mass (BM) and is one of the key performance determinants of endurance runners and cyclists. Logically, there are two factors that can be manipulated within this equation: power and BM. Typically, the athlete aims to develop as high power-to-weight ratios as possible via increased power, decreased BM, or a combination of both. While one focus of the training programs of endurance athletes is to improve their absolute power or speed, nutrition is undoubtedly a powerful tool in manipulating BM; it is much easier to reduce dietary intake by 200 kcal than to increase energy expenditure by the same amount when training volumes are already high. The following sections will focus on the relevance of BM in elite running and cycling performance, along with evidence for and against physique manipulation in these sports.

2.2.1 Body mass and running performance

Running economy is key for success in middle- and long-distance events. As explained in the previous section, economy of movement is one of the key factors regulating successful sports performance in elite middle- and long-distance running events (Saunders et al., 2004). Indeed, according to Di Prampero et al. (1993), a 5 % increase in running economy yields a performance improvement of ~3.8 % in distance running. For example, in the female's marathon at the Rio de Janeiro Olympic Games in 2016, this margin (5:28 min) was the difference between the winner and the runner who placed 12th in the event, although the co-efficient of variation in daily performance needs to be included in any model before such a change in performance can be translated into the likely change in event outcomes (Malcata & Hopkins, 2014). In addition, a report spanning 5 years of training of the current women's world record (WR) holder in the marathon showed that enhanced running economy (from 53 to 48 $\text{ml}\cdot\text{kg}\cdot\text{min}^{-1}$ at 16.0 $\text{km}\cdot\text{h}^{-1}$, i.e. a 9.4 % improvement) and a higher lactate threshold (from 15.0 to 18.0 $\text{km}\cdot\text{h}^{-1}$) were the main reasons behind an improved performance in the 3000 m event (Jones, 1998). Therefore, enhanced economy alone has great influence on running performance and accordingly, should be one of the key targets of the training of distance runners.

Running economy is partially dependent on BM. It is important to note that running economy is a very complex issue and is affected by several factors including biomechanical (stride length, elasticity of the muscle-tendon unit), neuromuscular (muscle fibre type), metabolic (mitochondrial efficiency, thermoregulation) and cardio-respiratory (HR, minute ventilation) systems (Tawa & Louw, 2018). In addition, anthropometry [i.e. height, BM, body composition and body mass index (BMI)] has a significant impact on running economy, as is evident from the fact that maximal and submaximal running capacity are often expressed as VO_2 relative to BM. These notions are further supported by the fact that some of the best distance runners in the world tend to share similarities in body shape (including a small body mass, slender body type and small lower legs) (Larsen, 2003).

Is the East-African physique optimal for running? Since the 1968 Olympic games, East-African runners have dominated the distance running scene. Indeed, as of November 2018, the top 20 lists of the all-time best male and female marathon performances consisted of 20 males and 16 females of East-African origin (Larsson, 2018). Several studies have aimed to explain the superiority of these runners compared to their Western counterparts. One of the key explanations relates to exercise economy. For example, Saltin and colleagues (1995) reported that while there was no difference in VO_{2max} between elite Kenyan and Scandinavian runners, the economy of running was significantly greater in the Kenyan runners. This, in turn, can be influenced by anthropometry. Indeed, Kenyan male and female runners are typically small and lean (BMI 18.6 and 16.9 kg/m^2 for males and females, respectively) (Mooses & Hackney, 2017). Furthermore, Joyner et al. (2011) reported that only 1 out of 30 male runners who have broken 27 min in the 10 000 m were >1.78 m and >70 kg, highlighting the likely importance of a combination of being small in stature and light in weight. Later analyses have suggested that the optimal BMI for males and females at maximal speeds (equating to current marathon WR times) of 5.7 $m \cdot s^{-1}$ (20.5 $km \cdot h^{-1}$) and 5.19 $m \cdot s^{-1}$ (18.7 $km \cdot h^{-1}$) would be 19.8 and 18.2 kg/m^2 for males and females, respectively (Marc et al., 2014). Accordingly, an investigation among the top 100 male international athletes reported that the most successful distance runners were the ones with the lowest BMI (Sedeaud et al., 2014). In addition to benefits to exercise economy, an additional benefit to being light and small is improved thermoregulation (Cheuvront & Haymes, 2001). Finally, a study in Ethiopian male and female runners showed a significant inverse relationship between the sum of four skinfolds and the International Association of Athletics Federations (IAAF) points score in the 800 m and 1500 m distances (Legaz-Arrese et al., 2009), suggesting that in addition to BM, body composition may also be important for race performance. The evidence for and against an optimal race weight or physique will be discussed in the next sections.

Evidence for the importance of race weight for running performance. Running is a weight-bearing sport in which the athlete is required to carry his/her BM throughout the session/event. Indeed, BM accounts for 80 % of the metabolic cost of running (Hoogkamer et al., 2017). Previous studies have shown that the addition of extra (dead) weight in the form of weight vests increases the metabolic cost of running, thereby reducing economy of movement. Meanwhile, similar experiments have shown that when BM is artificially reduced, the energy cost of running is decreased (i.e. increasing economy) (Ackerman & Seipel, 2016). Therefore, assuming metabolically inactive mass [i.e. fat mass (FM)] is lost, the physics of running suggest that a lower BM would improve running performance.

In light of this, a recent study by Zacharogiannis and colleagues (2017) investigated the effects of artificial BM reduction via the use of a rope system on 3 km performance time. The purpose of the rope system was to lift the runner to induce 5 and 10 % reduction in BM without affecting running technique. It was shown that 3 km performance time improved by 3.1 and 5.2 % with 5 and 10 % BM reduction, respectively. This translates into a 1.4% improvement in performance time with each reduction of a kg of inactive BM lost. For example, if the current male's marathon WR holder Eliud Kipchoge of Kenya (WR 2:01:39 h in Berlin in 2018), were to lose 1 kg of metabolically inactive BM (i.e. FM), theoretically, he would improve his marathon time to 1:59:59 h – a time below the magical 2-hour barrier he has recently broken, albeit by unconventional means (Keh, 2019). However, whether he actually "has" the metabolic inactive tissue to lose, given how apparently lean he already visually presents, is questionable; as without a doubt he would also lose lean mass while dropping BM.

Previously, Cureton et al. (1978) showed that adding a load of 5 % of BM decreased VO_{2max} by 2.4 ml and a 12 min run distance by 89 m. In this study, the participants completed on average of 3230 m during the 12 min run test when no extra weight was carried. However, with extra 5% attached to the body, the distance during this test was only 3141 m (i.e. a ~2.8 % reduction in distance covered). Here, running speed was reduced from 3:43 to 3:49 $min \cdot km^{-1}$ (a ~2.6 % decrement in speed) per each kilometre with a 5 % gain in (dead) BM. Since this 5% BM gain represented, on average, an extra 3.2 kg, it can be calculated that running speed was reduced by ~1.9 $sec \cdot km^{-1}$ (or ~0.8 %) per each additional kg of (inactive) BM added. The authors concluded that these changes were a direct consequence of impaired exercise economy at submaximal speeds (Cureton et al., 1978). If the current male's marathon WR holder gained 1 kg of FM, this would, in theory, slow him down to a finishing time of 2:02:37 h (considering a 0.8 % decrease in running speed) – nearly 1 min slower than the current WR time.

Another landmark study investigated the difference in the location of extra weight on running economy. In this experiment it was shown that when extra weight is added to the torso, for every 1 kg added weight, the aerobic demand is increased by 1 %. However, when the mass is added to the ankles/shoes, the same mass increases aerobic demands by 10 %. This lead the authors speculate that this might explain the benefits of the relatively slender legs of some of the best distance runners in the world (Myers & Steudel, 1985).

Finally, Stellingwerff (2018) proposed a theory of the potential benefits of the long-term adaptation to a higher BM (i.e. during the base training phase) that might then be useful during the competition period (i.e. when a lower race weight has been achieved). This theory is based on the findings by Carmelo Bosco and colleagues (1985, 1986) who showed that training 3 to 5 times a week for 3 weeks using weight vests (11-13 % of BM, worn at all times excluding during sleep) improved neuromuscular performance (including an improvement in the vertical jump test of from 44.3 to 54.9 cm). Accordingly, Stellingwerff (2018) suggests that, in theory, these neuromuscular benefits might be even greater for a distance runner, when higher BM is maintained over several months of running training, highlighting the usefulness of body composition periodisation across the year. However, this theory requires further research to be confirmed.

The relevance of body composition for running performance. In terms of running, fat tissue is relatively useless as it does not contribute to the to the energy cost of exercise but is extra mass that needs to be carried against the force of gravity (i.e. the muscle will have to lift a greater mass over the ground, especially in running events involving a vertical component such as hilly road or cross country courses, or the steeplechase) (Legaz-Arrese et al., 2009). Furthermore, high levels of body fat provide insulation which is useful in cold environments, but impairs thermoregulation in the heat; which represents a challenge in the environments in which the majority of the Championships races are held. Therefore, in addition to a low overall BM and BMI, a low body fat level may benefit performance of endurance running event with additional physiological advantages. On the contrary, while a high lean mass to FM-ratio certainly seems of benefit to running performance, absolute levels of lean mass do not necessarily need to be higher than what is absolutely necessary to transport the body, especially over long distances (Fletcher & MacIntosh, 2017). Indeed, studies of Kenyan runners have consistently shown that these athletes have slender figures and less muscle mass in the legs compared to Scandinavian runners (Saltin et al., 1995). In addition to the total BM, it is possible that a high mass in the (distal parts of the) limbs may actually impair exercise economy. Therefore, losing extra muscle mass from the upper body (arms) or from the lower legs (calf muscles) might be able to further improve economy (Fletcher & MacIntosh, 2017), if it does not impair the relative leg power for running.

Evidence against the concept of race weight. Based on the available evidence, it does appear that from the muscle energetics point of view, extra weight especially in the form of inactive (fat) tissue and/or large amounts of mass distributed towards the distal parts of the limbs impair running economy due to increased oxygen requirements (relative to BM) at any given speed. However, it is important to note that physique is only one of the many factors influencing running economy (Mooses and Hackney, 2017). Indeed, several other (perhaps even more important) factors include training stimulus (strength training, plyometrics, speed training), environmental stimulus (such as altitude training) as well as physiology (e.g. VO_{2max}) and biomechanics (e.g. the ability to store elastic energy), as explained in detail in (Saunders et al., 2004). Finally, it is important to emphasise that targeting a reduction in BM or FM may come at a cost. Indeed, too severe and/or prolonged periods of low energy availability (EA) may lead to a range of health and performance impairments related to Relative Energy Deficiency in Sport (RED-S; Mountjoy et al., 2018). These outcomes, which may impair "athlete availability' (the number of days in which the athlete may undertake their optimal training program), as well as directly impair performance and recovery, may negate the positive effects of decreased BM. This is especially true if the weight loss consists of lean tissue that contributes to the power generated for running movement. It is likely that the negative impact of RED-S is much greater in weight-bearing sports (e.g. running) in terms of increased injury risk; meanwhile, the consequences of RED-S in non-weight bearing sports (e.g. cycling, swimming, rowing) are most likely to manifest as poor BMD and increased traumatic fracture risk later in life. The background and risks of these outcomes will be discussed in detail in sections 2.3 and, with regards to bone health, in section 2.4.

2.2.2 Body mass and cycling performance

Cycling performance is dependent on the power output. Compared to running, economy of movement has a much smaller impact on cycling performance. Here, typical gross mechanical efficiency can vary between 18.5 and 23.5 % and is mostly related to the muscle fibre type distribution. As Type I slow twitch fibres possess greater mechanical efficiency at cadences of 60-120 rpm, a high proportion of these fibres becomes an important characteristic of a successful professional cyclist (Joyner & Coyle, 2008). However, cycling performance is closely related to the absolute (flat terrain and time trials) or relative (hill climbs) power output (watts, W). For example, uphill cycling specialists have been reported to produce peak power outputs (PPO) of ~404 W compared to 461 W in flat terrain cyclists (Mujika & Padilla, 2001). However, relative to BM, the former (6.5 W·kg^{-1} PPO) outweigh the latter (6.0 W·kg^{-1} PPO) and are therefore superior during racing that involves work against gravity (e.g. hill climbs). Therefore, there is a BM component to professional cycling, whereby especially hill specialists and general classification riders will benefit from a light physique (i.e. a high power-to-weight ratio) for optimised performance. In light of this, if a road cyclist weighing 60 kg and with an absolute PPO of 380 W (6.3 W·kg^{-1}) were to lose 5% of BM (i.e. 3 kg from the FM), this would improve his relative power output by 6% (i.e. to 6.7 W·kg^{-1}). Therefore, he would either require less oxygen to climb a mountain at the same power output absolute, or he could go faster with the same rate of oxygen consumption as before the weight loss. However, if this weight loss were to come from critical lean tissue (muscle mass), this might negate the improvements to the relative power output as in this case, absolute power would be likely to decrease along with the lean tissue. Accordingly, professional cyclists need to carefully weigh out the benefits and risks of such strategies.

Issues of BM in professional cycling. The issues relating to BM were recently highlighted in a review by Burke et al. (2018a) which described the current trend for professional male cyclists to be extremely lean [<35 mm sum of 7 skinfolds, or <10 % body fat via Dual-energy X-ray Absorptiometry (DXA)] and light (BMI <18 kg/m^2) with little upper body musculature. While these physiques might at least for some be a result of genetic predisposition, it was noted by the authors that the culture within road cycling might in itself promote behaviours intended to reduce BM to new targets. Indeed, one of the targets of these practices is an even higher power-to-weight ratio than previously considered necessary for success in stage racing as described two decades ago by Olds et al. (1995). Furthermore, these authors highlighted several anecdotes whereby success in professional cycling usually follows significant weight loss periods. According to Mujika and colleagues (2016), overall, it appears that muscle mass in legs may be beneficial for cycling performance, however minimization of extra mass (lean or fat) from the upper body is likely ideal. Similarly to runners, there might be a sweet spot for the cyclists beyond which decreases in BM impair health and performance. For example, Haakonssen et al. (2013) showed that in competitive female cyclists, 1 kg of added muscle gave an extra 35 W during a 30 sec sprint exercise. Therefore, functional muscle mass especially in the lower extremities may be more important than being as light as possible. Finally, as with runners, cyclists are also at risk for low EA and a whole host of symptoms related to the RED-S. However, due to the non-weight bearing nature of cycling (no osteogenic stimulus from exercise), the threat of poor bone health is exponentially greater among these athletes. Of course, the manifestation of RED-S in athletes is highly variable due to the complexity of interactions between the various forms of characteristics such as EA, training stimulus (including the benefits of bone loading but the disadvantages of overloading via excessive training load), dietary factors and genetics. The topic of bone health in relation to nutrition is discussed in section 2.4.

2.3 Manipulation of energy and CHO availability in elite endurance athletes

2.3.1 Periodisation of energy availability across the annual training program

Optimal EA at the macro level of training. EA considers the difference between dietary energy intake and the energy committed to exercise [exercise energy expenditure (EEE)], and represents the energy that is left to support all other physiological processes in the body, including reproduction, immunity and bone health (Loucks et al., 2011). Since different tissues of the body have different physiological characteristics and requirements, it is usual to express EA relative to the amount of metabolically active tissue, or fat-free mass (FFM), yielding the final calculation:

$$EA = (EI - EEE) / FFM.$$

When energy intake is reduced and/or energy expenditure from exercise is increased such that EA is reduced below a critical level, typically defined as low EA = ~30 $kcal \cdot kg\ FFM \cdot d^{-1}$, the resultant “energy deficiency” fails to support the normal healthy physiological and psychological processes. Low EA has recently been identified as the dietary factor with potentially the greatest impact on several health and performance outcomes (Mountjoy et al., 2014; Mountjoy et al., 2018). Indeed, impairments have been reported across a range of health and performance outcomes (Ackerman et al., 2018).

It is important to note that the threshold for low EA was derived from several laboratory-based studies by Prof. Anne Loucks. While sophisticated in methodology, these studies focused almost solely on sedentary females and observed outcomes within a relatively small time frame (i.e. 4 to 5 days). Subsequent studies have challenged the idea of a threshold for low EA. For example, Lieberman and colleagues (2018) determined the effects of three months of low (23–34 $kcal \cdot kg\ FFM \cdot d^{-1}$), moderate (35–41 $kcal \cdot kg\ FFM \cdot d^{-1}$) and high (41–50 $kcal \cdot kg\ FFM \cdot d^{-1}$) EA on menstrual function in 35 healthy females. While the study showed that menstrual dysfunction increased with decreasing EA (likelihood of menstrual dysfunction increased by 9% per every unit of decrease in EA), these changes were not related to the low EA threshold (30 $kcal \cdot kg\ FFM \cdot d^{-1}$) itself. Of course, the methodology of estimating EA is highly prone to errors and the period of assessment may not be related to the period of time during which the metabolic and health adaptations occurred (Burke et al., 2018c). Furthermore, it is likely that the threshold for low EA and the subsequent impairments to health and performance is slightly different across each individual and is likely to depend on their age (younger athletes may be more prone to low EA; Loucks, 2006), sex (males may have a lower threshold of low EA; Fagerberg, 2018) and genetics. However, this topic needs further research.

Another topic of interest that remains reasonably unexplored in the literature is the time-course that it takes for the low EA (and the severity of low EA) to cause more permanent negative adaptations in the body as well as the time-course of recovery of symptoms/outcomes of low EA. Based on the limited available research, prolonged lack of menstruation (amenorrhea) prolongs the time it takes to restore menstrual function (Cialdella-Kam et al., 2014). In addition, recovery of lost bone mineral content may take years (Joy et al 2014), or in the worst case, be irreversible (Barrack et al., 2011). Accordingly, future studies should assess the maximal time period that can be spent in low EA without causing more severe, prolonged or irreversible impairments to health or performance. Finally, research should focus on the time-course of reversal of outcomes/symptoms of low EA, although as mentioned this is likely to depend on the length and severity of low EA. Notwithstanding individual variability and differences in the robustness of various body systems, adequate EA for optimal long-term athletic adaptation and preparation is typically considered to occur at ~45 kcal·kg FFM·d^{-1}, while impairments to body systems, and therefore a typical "threshold" for low EA, is identified and referred to in this thesis as below ~30 kcal·kg FFM·d^{-1} (Loucks et al., 2011).

Meso-periodisation of EA. One of the key aspects of a periodised training program is the within-individual variability in the training load between micro-, meso- and macrocycles. For example, a middle-distance runner may require more calories during a heavy general preparation training phase (weekly volumes of ~150 km), meanwhile caloric needs may be much less during a competition week (weekly volumes of ~70 km; Stellingwerff et al., 2007a; Table 2.1). On the contrary, a road cyclist may require less energy during a training period or during individual sessions compared to a 3 week stage race. Here, if daily energy intake is not adjusted to track the changes in EEE across different training competition/cycles, it can lead to chronic scenarios of low or surplus EA, both of which may cause health or performance decrements over time. For example, in some sports there may be benefits from reducing BM or FM in the initial stages of an energy deficit before metabolic adjustments occur. However, sustained low EA can cause perturbations in hormones and markers of various body systems in as little as 5 d (Loucks et al., 2011), although it is not clear whether this always manifests in clinical changes and if so, within what time frame. Alternatively, unnecessarily high energy availability can lead to a gain in BM and unfavourable change in body composition.

Periodisation of low EA within a meso/microcycle. Successful endurance performance often requires the benefits of a light and powerful physique (a high power-to-weight ratio, low energy cost of movement). Accordingly, there may be benefits to reducing BM or FM, as outlined in sections 2.2.1 and 2.2.2. However, achieving "race shape/physique" (i.e. optimal body composition) often requires the athlete to reduce their BM and/or FM below levels that represent healthy function of several body systems. While it is neither safe nor feasible for health or performance to maintain an extremely lean physique over the entire year, at times careful periods of low EA may be required to optimise body composition (i.e. decrease FM). This strategy was recently defined as "the strategic manipulation of energy intake and energy expenditure between various training phases to reach a targeted body composition range that is optimal for performance (e.g. peak power-to-weight ratio), while minimizing risk to short-term and long-term health." (Stellingwerff, 2018).

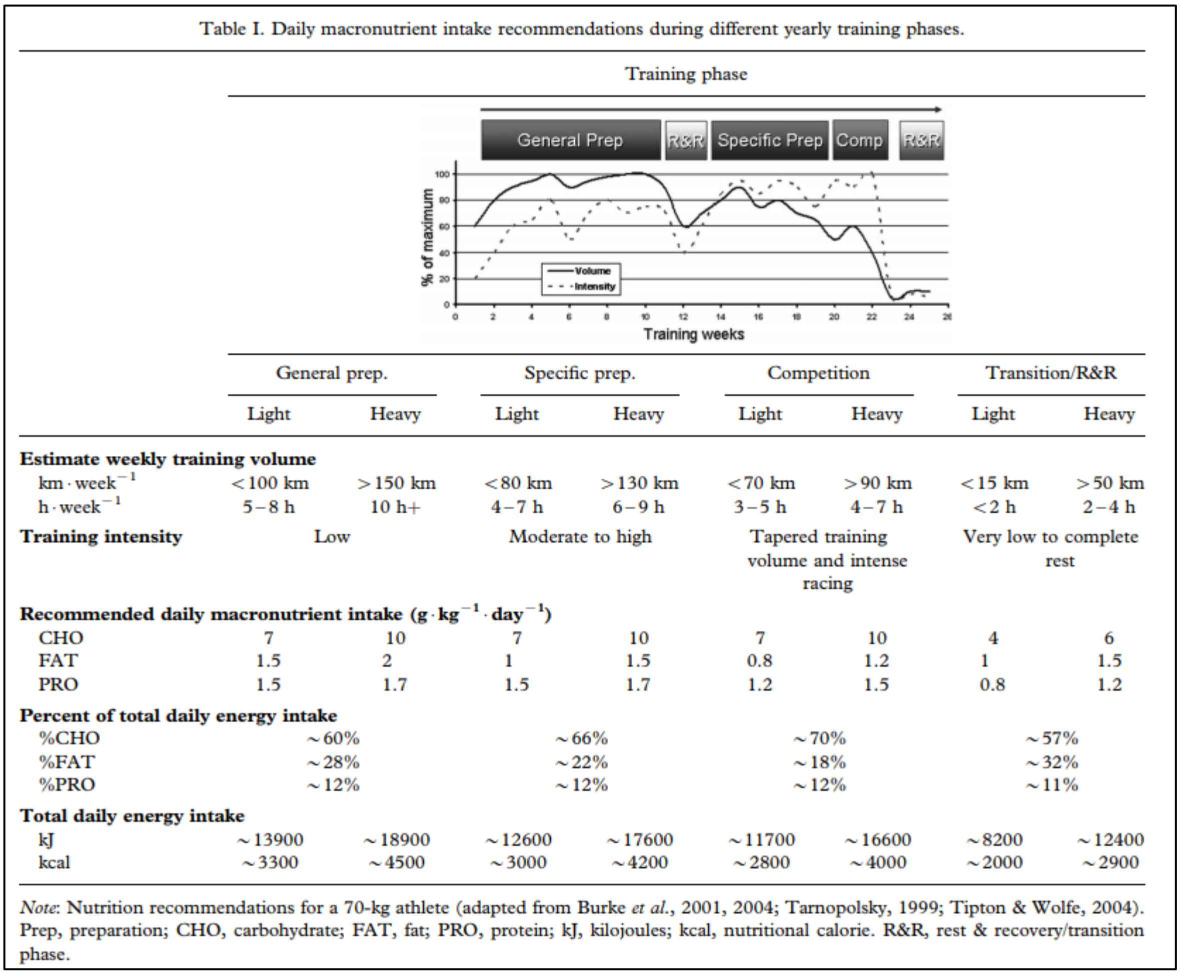

General Prep
R&R
Specific Prep
Comp
R&R
% of maximum
100
80
60
40
20
0
Volume
Intensity
0
2
4
6
8
10
12
14
16
18
20
22
24
26
Training weeks

Table I. Daily macronutrient intake recommendations during different yearly training phases.

	Training phase							
	General prep.		Specific prep.		Competition		Transition/R&R	
	Light	Heavy	Light	Heavy	Light	Heavy	Light	Heavy
Estimate weekly training volume								
km · week^{-1}	< 100 km	> 150 km	< 80 km	> 130 km	< 70 km	> 90 km	< 15 km	> 50 km
h · week^{-1}	5–8 h	10 h+	4–7 h	6–9 h	3–5 h	4–7 h	< 2 h	2–4 h
Training intensity	Low		Moderate to high		Tapered training volume and intense racing		Very low to complete rest	
Recommended daily macronutrient intake (g · kg^{-1} · day^{-1})								
CHO	7	10	7	10	7	10	4	6
FAT	1.5	2	1	1.5	0.8	1.2	1	1.5
PRO	1.5	1.7	1.5	1.7	1.2	1.5	0.8	1.2
Percent of total daily energy intake								
%CHO	~ 60%		~ 66%		~ 70%		~ 57%	
%FAT	~ 28%		~ 22%		~ 18%		~ 32%	
%PRO	~ 12%		~ 12%		~ 12%		~ 11%	
Total daily energy intake								
kJ	~ 13900	~ 18900	~ 12600	~ 17600	~ 11700	~ 16600	~ 8200	~ 12400
kcal	~ 3300	~ 4500	~ 3000	~ 4200	~ 2800	~ 4000	~ 2000	~ 2900

Note: Nutrition recommendations for a 70-kg athlete (adapted from Burke *et al.*, 2001, 2004; Tarnopolsky, 1999; Tipton & Wolfe, 2004). Prep, preparation; CHO, carbohydrate; FAT, fat; PRO, protein; kJ, kilojoules; kcal, nutritional calorie. R&R, rest & recovery/transition phase.

Table 2.1. One of the first peer review publications highlighting the importance of a periodised nutrition plan aligned with periodised training across the annual training/racing cycle. (Stellingwerff et al., 2007a)

Evidence for successful periodisation of body composition was presented by Stellingwerff (2018) who showed that an elite female middle-distance runner was able to reach target race body composition annually while maintaining a healthy hormone function across a 9 year career. This was done by implementing a strategic, periodised manipulation of body composition at key time points, whereby targeted 6-8 week periods in the competition season were used to reduce FM by reductions in CHO and energy (-300 $kcal \cdot d^{-1}$) intake. These interventions resulted in decreased BM and FM during the competition season compared to the rest of the year, while lean mass gradually increased throughout the athletes international career (Stellingwerff, 2018). While this is the first well-documented report in an elite endurance athlete, there is a larger body of literature on body composition periodisation around bodybuilding/fitness competitions. In summary, these studies generally report extreme weight (~10 %) and body fat (~50 %) reductions within a 4 to 6 month preparation period, usually as a result of extreme energy deficits and high volumes of strength and endurance exercise. These extreme practices result in extremely lean physique outcomes but often come at a price of suppressed hormone function (Halliday et al., 2016; Hulmi et al., 2017) and reduced fitness levels (Tinsley et al., 2018), reflective of prolonged low EA (Mountjoy et al., 2018). Further discussion on physique athlete-focused studies will be omitted from the current thesis as these studies are characterised by particularly extreme and longer-term low energy and CHO availability that is generally not applicable to endurance and/or Olympic based sports. Furthermore, such sports feature different training (strength or concurrent training) and performance (primary performance measure is appearance, i.e. leanness and muscularity, not physical performance capacity per se) characteristics compared to endurance sports. Therefore, based on limited available evidence, it appears that in terms of changes in hormone function, brief periods of moderate reductions in EA (e.g. weeks to ~ 2 months) are safe and helpful in targeting optimal competition period body composition range. Strategies to support hormonal health and body composition optimisation during these periods may include consideration of the timing of energy and CHO intake around exercise (Melin et al., 2019), as shown by studies in which large hourly energy deficits were associated with poor health outcomes despite similar total daily EA (Deutz et al., 2000; Fahrenholtz et al., 2018; Torstveit et al., 2018). In other words, regardless of total daily EA, consuming a meal in a close proximity to an exercise session may support hormonal and metabolic health better than delaying a meal until several hours post-exercise, or completing a session in the fasted state.

When EA is decreased, there is usually a simultaneous reduction in CHO availability. This is mainly because daily protein and fat requirements do not drastically change with training load, whereas targets for CHO are more tied to the energetic cost of daily training and individual sessions (Helms et al., 2014; Stellingwerff et al., 2007a; Thomas et al., 2016). Therefore, it follows that low EA is often characterised by low CHO availability, and low CHO availability sessions may also be low EA sessions (unless compensated for with increased intake of other macronutrients). The topics of high and low CHO availability will be discussed in detail in the following sections.

2.3.2 General CHO guidelines for elite endurance athletes

Competitive high-intensity endurance performance is dependent on CHO stores that are considered either endogenous [skeletal muscle (~400-600 g) and liver (~100-120 g) glycogen stores, blood glucose (~5 g)] or exogenous CHO (from food/drinks consumed immediately before or during exercise) (Hawley & Leckey, 2015). Indeed, CHO oxidation from muscle glycogen stores and blood glucose dominates with (1) increasing exercise intensity (>75 % VO_{2max}), (2) increasing pre-exercise muscle glycogen concentration and (3) higher CHO intakes pre/during exercise (Hearris et al., 2018). For example, half-marathon running capacity (intensity: ~78-80 % VO_{2max}; time to exhaustion: ~1:20-1:26 h) was dependent on CHO, but not fat, availability (Leckey et al., 2016). Indeed, body fat stores [intramuscular triacylglycerol (IMTG, ~350 g) and adipose tissue triacylglycerol (~ 5000 g)] contribute to ATP production *predominantly* at lower exercise intensities and during prolonged, submaximal activities. However, there is evidence to show that IMTG does contribute to energy metabolism during high intensity exercise and especially at the onset of exercise. For example, Type I fibre IMTG content decreased by 27 % (along with a 23-44 % decrease in type I, IIa and IIx fibres) after a 45 min resistance exercise session (Koopman et al., 2006). In addition, Stellingwerff et al. (2007b) showed that during 3 h of cycling at ~63 % VO_{2max} type I fibre IMTG content decreased by 76-78 % (~20 % contribution to fuel utilisation during exercise) regardless of whether exercise was completed with or without CHO. However, CHO remain the main fuel source for high intensity exercise and therefore, given the fact that all current Olympic endurance events are completed at or above these intensities, it is clear that adequate CHO is crucial for elite endurance performance.

The importance of high CHO availability is emphasised in the current sports nutrition guidelines for endurance athletes, which provide generalised recommendations for dietary intakes to achieve the various goals of training and competition (Thomas et al., 2016). These guidelines have evolved over the past couple of decades from a one-size-fits-all high CHO diet to a more individualised and periodised approach where the intake of energy and macronutrients changes between and during the various micro-, meso- and macrocycles of an athlete's training and racing program (Thomas et al., 2016). While the concept may seem relatively recent, more than a decade ago Stellingwerff et al. (2007a) described a theoretical framework for a periodised nutrition approach for elite middle-distance runners to match nutrition needs based on each training phase across the annual training cycle. The key message was that nutrition, but especially CHO, targets should be based on training loads and altered across the annual training program based on the goal and fuel requirements of each exercise session and training cycle. Here, in a sample 70 kg athlete, daily CHO targets can range from 280 to 700 $g \cdot d^{-1}$ (4 to 10 $g \cdot kg \cdot d^{-1}$) based on time of the year, while protein (56 to 119 $g \cdot d^{-1}$ or 0.8 to 1.7 $g \cdot kg \cdot d^{-1}$) and fat (56 to 140 $g \cdot d^{-1}$ or 0.8 to 2.0 $g \cdot kg \cdot d^{-1}$) targets vary much less. Indeed, the difference in total energy intake can be explained largely by changes in CHO intake (1680 kcal), compared to changes in protein (252 kcal) or fat (756 kcal) intakes (Stellingwerff et al., 2007a). The diagram of this framework is shown in Table 2.1.

Several new themes around CHO have been incorporated into the evolution of the sports nutrition guidelines. First is the concept of "CHO availability" (Burke et al., 2011) where CHO intake is based on the fuel needs of an athlete's specific training program. This concept emphasises the fact that two substantially different CHO intakes (differing in amounts or timing of intake) might be equally successful in delivering "high" CHO availability if they were matched to an exercise task with compatible fuel requirements. More recently, this topic has been referred to as "fuelling for the work required" (Impey et al., 2016). Thus, guidelines need to be interpreted within the context, considering the different and changing fuel needs of an athlete (Burke et al., 2011). This theme will be discussed in detail in section 2.3.3. A second theme is the recognition that it is not necessary to promote CHO intakes achieving high CHO availability every day or for every workout/event within the athlete's program. Therefore, for some sessions within the training cycle, CHO intake targets are more flexible. Finally, there is emerging awareness that body CHO stores have a role beyond that of providing muscle substrate: indeed, they evoke a variety of hormonal and metabolic consequences that can attenuate or stimulate adaptations within the muscle (Hawley et al., 2018; Hearris et al., 2018) as well as influence a range of systems underpinning health and function (Badenhorst et al., 2015; Sale et al., 2015). In this regard, there is growing evidence of the concept of occasionally completing an exercise session with low endogenous and/or exogenous CHO availability ("train low") to further enhance training stimulus (Bartlett et al., 2014). Discussion around this concept will follow in section 2.3.4. Thus, the basis for new guidelines for CHO intake for endurance athletes is on a personalised and periodised approach to support a much broader range of goals (Bartlett et al., 2014).

The following sections will discuss the underlying theory along with health and performance outcomes of chronic and acute manipulations in CHO availability. Indeed, although the main focus is on a periodised low or high CHO availability, due to its recent popularity, chronic low CHO high fat (LCHF) diets will also be discussed in terms of the underlying theory and current scientific evidence for athlete health and performance.

2.3.3 High CHO availability

Chronic high CHO (availability) diet. Guidelines from the 1990s were based on a robust literature (Coggan & Coyle, 1987; Coggan & Coyle, 1988; Coggan & Coyle, 1989; Coggan & Coyle, 1991; Coyle et al., 1983; Coyle & Coggan, 1984; Coyle, 1991; Coyle, 1992; Ivy et al., 1988) which demonstrated that the performance of prolonged moderate and/or intermittent high-intensity exercise is enhanced when various dietary strategies are undertaken before, during or between exercise bouts to provide sufficient CHO to meet the body's need for this important substrate during exercise. Such observations produced both the principle of sports nutrition guidelines for the training and competition practices of endurance athletes (repletion of body CHO stores) and an approach to their implementation (static and universal targets for high CHO intake daily and around exercise sessions) (Coyle, 1991). The benefits of this diet include: (1) consistent high-quality training, performance, and recovery, and (2) optimal function of several body systems important for health and performance (immune function, central nervous system, endocrine system). Table 2.2 discusses the concept of chronic high CHO availability diet (across macro and meso levels of training periodisation), recently defined by Burke et al. (2018b). Contemporary sports nutrition guidelines promote manipulation of daily and within-day CHO availability according to the characteristics of the immediate exercise load, the specific goals of the session, and the larger context of the athlete's nutrition plan (Thomas et al., 2016). These topics of CHO micro-periodisation will be discussed in the following section.

Acute high CHO availability around exercise. In the case of competition, where optimal performance is desired, there is plentiful evidence supporting the benefits of dietary strategies that promote high CHO availability – i.e. that provide sufficient CHO to meet muscle fuel needs for a race or other competitive event. These strategies include consuming adequate CHO between or before events to achieve sufficient muscle glycogen stores and consuming CHO just prior to and/or during a race to provide additional exogenous CHO fuel (Costill, 1985; Costill, 1991; Coyle et al., 1983; Coyle & Coggan, 1984; Hargreaves et al., 1984; Hargreaves et al., 1987). Indeed, based on studies from the 1960's, prolonged and/or high-intensity endurance performance and capacity can be improved by 2-3 % and 20 %, respectively, with the use of CHO loading (Hawley et al., 1997) to maximise muscle and liver glycogen stores. For optimal loading, a high CHO diet (8-12 $g \cdot kg \cdot d^{-1}$ for ~2 d) is required (Bergstrom et al., 1967; Sherman et al., 1981) and ideally combined with a pre-exercise meal of 1-4 $g \cdot kg^{-1}$ CHO 1-4 h pre-exercise (Thomas et al., 2016).

In addition to providing a growing contribution to the muscle's fuel needs endogenously, CHO consumed during exercise enhances exercise capacity or performance via liver glycogen sparing and prevention of hypoglycemia (Cermak & van Loon, 2013). The amount of CHO required during exercise is dependent on factors such as duration and mode of exercise but most likely intakes between 30-90 $g \cdot h^{-1}$ are beneficial (Stellingwerff & Cox, 2014), where multiple transportable CHO such as glucose and fructose are required when the amount exceeds 60 $g \cdot h^{-1}$ to maintain high intestinal CHO absorption rates. More recently, there has been recognition of further centrally derived benefits from CHO ingestion, whereby the sensing of CHO by receptors in the oral cavity activates reward centres in the central nervous system to reduce sensation of fatigue and enhance pacing strategies (Burke & Maughan, 2015).

Post-exercise recovery nutrition is also an important consideration, especially for athletes who have limited recovery time in between exercise bouts (i.e. twice a day training or frequent racing). After exercise, nutrition should focus on replenishment of muscle and liver glycogen stores, replacement of fluid losses, and rebuilding of body proteins (structural and metabolic). With regards to glycogen resynthesis post-exercise, CHO should be provided at a rate of ~1 $g \cdot kg \cdot h^{-1}$, accompanied by adequate EA (Tarnopolsky et al., 2001), in the first four hours to enable rapid resynthesis rates (Burke et al., 2017b; Jentjens & Jeukendrup, 2003), after which resumption of daily CHO targets is recommended.

Taken together, high CHO availability is absolutely central for optimal endurance performance and recovery. While the benefits of optimal application of these strategies to competition scenarios are self-evident, they are also likely to be valuable in the case of key exercise sessions within the athlete's program where high-intensity workloads, and simulation of race pace, technique or race nutrition practices are important. As well as practicing feeding behaviours that may be challenging under race conditions, CHO intake during exercise sessions may assist with "gut adaptation" to enhance the tolerance and intestinal absorption of CHO (Stellingwerff & Jeukendrup, 2011). Table 2.2 discusses some of these strategies in detail.

2.3.4 Low CHO availability

Low CHO high fat (LCHF) diets. Another popular dietary approach promoted to and by some endurance athletes has been the LCHF diet (Volek et al., 2015; Burke, 2015), with adequate EA but limited CHO availability to develop and maintain ketogenesis/ketosis. High fat diets can be divided into ketogenic and non-ketogenic diets (Table 2.2). Ketogenic diets contain very little ($<50\ g\cdot d^{-1}$) CHO and moderate amounts (15-20 % of energy, %E) of protein, meanwhile the remainder of the energy contents (~75-80 %E) comes from fats (Paoli et al., 2015). The intake of CHO and protein is restricted as the production of ketones only occurs when the body is derived of glucose and is forced to turn to alternative fuel sources (fats) for energy production (Cox & Clarke, 2014). On the contrary, non-ketogenic high fat diets are typically ~60–65 %E fat, meanwhile CHO and protein intake is less rigidly controlled, as long as the CHO content is below daily fuel requirements and above that associated with chronically high levels of circulating ketone bodies. In free living conditions, due to a misunderstanding of the dietary principles and the general difficulty of constructing diets with a substantially restricted dietary range, many individuals who believe they are practising a strict ketogenic diet, are probably achieving a non-ketogenic high fat and/or high protein diet.

Short-term LCHF and exercise performance. The cellular adaptations to high fat diets are achieved in as little as 5 d, during which the number of fatty acid transporters and the activity of enzymes required for fat oxidation increase (Sherman & Leenders, 1995). Short-term (<3 d) high fat diets (>60 % energy) are detrimental for high-intensity/sustained performance, mainly due to the body's inability to adequately utilise fats as an energy source in the absence of glycogen. However, by ~5 d of adaptations, rates of fat oxidation during submaximal exercise can be doubled in comparison to typical rates (Burke & Hawley, 2002; Helge, 2002; Cameron-Smith et al., 2003). However, there are limits to the absolute ATP rates/power outputs that can be supported solely by fat oxidation, potentially due to the requirement for oxygen delivery to the muscle. Furthermore, there appears to be a downregulation of CHO oxidation and utilisation in athletes who are fat-adapted, even when endogenous and exogenous CHO availability is high. In fact, 5 d of a high fat diet reduced glycogenolysis and suppressed the active form of a key enzyme involved in CHO metabolism, pyruvate dehydrogenase (PDH), even when high CHO availability was restored by glycogen loading and pre-exercise intake of CHO (Stellingwerff et al., 2006). The practical implications of this were shown by another study of similar design, in which bouts of high intensity exercise embedded into an endurance cycling protocol were compromised after a fat-adaptation and CHO restoration (Havemann et al., 2006). Indeed, these studies show the importance of training and sustaining the capacity of the

CHO oxidation pathway for successful performance in competitive endurance sports in which all or critical components of the race involve intensities >75 % VO_{2max} (Stellingwerff et al., 2006).

Chronic LCHF and exercise performance. Studies on longer-term (>14 d; so-called "chronic") high fat diets are less clear (Helge, 2002). While some claim that the body's increased reliance on fats as a fuel and the subsequent sparing of muscle glycogen during exercise is an advantage (Noakes et al., 2014; Volek et al., 2015), others have noted that despite the higher rates of fat oxidation, no study so far has been able to show actual performance benefits following such diets (Burke, 2015; Hawley & Leckey, 2015). Equally noteworthy is the fact that the oxidation of CHO yields ~5.5 % more energy per litre of oxygen consumption (i.e. a more economical fuel source) compared to the oxidation of fats, and therefore, fats are an ineffective source of energy at high exercise intensities (Krogh & Lindhard, 1920). Indeed, as most endurance athletes train several times a week at high intensities (Hawley & Leckey, 2015), these effects would clearly be a disadvantage to the elite athlete. In fact, it was recently shown that pharmacological blocking of the body's ability to oxidise fats for energy during a half marathon run did not impair performance, in other words showing evidence for fat independence during high intensity endurance exercise (Leckey et al., 2016). The first study on the effects of a LCHF diet on endurance exercise performance is frequently cited in support of this dietary approach for enhancing endurance performance. In this study by Phinney and colleagues (1983), 5 male cyclists implemented a LCHF diet (<20 $g \cdot d^{-1}$ CHO) for 4 weeks, preceded and followed by a cycling test at ~63 % VO_{2max} in an overnight fasted state. The same cyclists also participated in a high CHO availability trial 4 weeks prior to the LCHF intervention, thus making comparisons of order effect impossible. While the mean outcomes showed no overall impairment (nor improvement) to exercise performance by the LCHF diet, these results were skewed by a single participant who showed a significant improvement from pre to post testing. Furthermore, it should be highlighted that the exercise test and dietary control applied (moderate intensity exercise with low CHO availability) are not representative of real world competition or competition preparation and in fact are of the kind that would most benefit from fat adaptation – yet no clear performance benefits were shown (Phinney et al., 1983). A more recent investigation aimed to examine the effects of a 3-week LCHF diet compared to a high CHO diet on several aspects of health and performance outcomes in world-class male race walkers (Burke et al., 2017a). In this study, athletes were divided into three dietary treatments of which two followed a high CHO or a periodised CHO approach (both providing ~8.5 $g \cdot kg \cdot d^{-1}$ CHO). Meanwhile, the LCHF group implemented a strict ketogenic approach with <50 $g \cdot d^{-1}$ CHO and

~75-80 % of energy from fat. Despite highest rates of fat oxidation reported to-date in the literature, the athletes following the LCHF diet showed significant impairments to exercise economy and subsequent 10,000 m race performance (Burke et al., 2017a). Furthermore, a study by Shaw et al. (2019) recruited 8 trained male endurance athletes to investigate the effects of a 31 d LCHF diet on submaximal exercise capacity (run to exhaustion at 70 % VO_{2max}). The authors reported impaired exercise efficiency, particularly at intensities at or just below 70% VO_{2max}, following the ketogenic diet, meanwhile, no changes were seen in efficiency at intensities below 60 % VO_{2max} (Shaw et al., 2019). Therefore, it is highly unlikely that LCHF diets would benefit elite endurance performance where intensities above 75 % VO_{2max} are important to training or racing outcomes. Indeed, current sports nutrition guidelines do not support the use of LCHF diets for the general elite athlete population (Thomas et al., 2016).

Chronic LCHF and health. In addition to performance, LCHF diets have gained attention in terms of their potential benefits to health, especially among the general population and as a means to treat or manage clinical conditions. Some of these benefits include improvement of markers of cardiovascular health (blood glucose, insulin, triglycerides, cholesterol, glycated haemoglobin, blood pressure and BM) and reversal of conditions such as non-alcoholic fatty liver disease and Type 2 Diabetes (Noakes & Windt, 2017). The mechanisms remain unclear but might be related to suppressed appetite, which results in lower ad libitum energy intake, or to reductions in hyperinsulinemia, which remains a controversial theory. Indeed, studies in which these benefits are seen following a LCHF diet typically involve weight loss in previously overweight subjects with pre-existing metabolic impairments (Hall et al., 2016). Furthermore, whether the BM loss associated with LCHF in some individuals is achieved by a metabolic or appetite advantage, or reflects a reduction in food intake associated with the difficulty of constructing the diet has not been studied. In any case, the benefits to apparently healthy endurance athletes remain unclear, with reports showing neither benefits nor risks of such diets in terms of cardiovascular health (O’Neal et al., 2019). It is possible, however, that the endurance athlete might face several other health-related impairments as a consequence of chronic high fat diets. First, CHO restriction compromises the immune system and may lead to illness and injury (Pedersen et al., 2000), which may affect performance directly or indirectly via lower training capacity (Raysmith & Drew, 2016). On the contrary, the relationship between low CHO or low energy availability and impaired immune function has recently been challenged in an excellent review by Neil Walsh (2019), who shows that the evidence behind these claims is currently very limited, while proposing an intriguing, potentially more meaningful link between protein-energy malnourishment and impaired immunity (Walsh, 2019). Second, low CHO intakes tend to lead to higher ratings of perceived fatigue during exercise compared to high CHO diets (Stepto et al., 2002). Indeed, a recent case study reported increased and persistent fatigue along with significant performance impairments in a world-class male triathlete even after 21-32 weeks of a LCHF diet (Mujika, 2018). Third, low CHO intakes appear to suppress the reproductive and metabolic hormonal function in as little as five days, where the effects of low CHO availability may be a more powerful determinant of impairments as opposed to low EA per se (Hilton & Loucks, 2000; Spaulding et al., 1976). LCHF diets may also challenge iron status, as evidenced by a reduction in corpuscular haemoglobin levels in athletes after 12 weeks of LCHF diet (McSwiney & Doyle, 2019). Ketogenic diets are not only low in CHO but are also characterised by a high dietary fat intake. High intakes of fat may further impair health, as was shown in a recent study where post-exercise high-fat feeding led to a suppression in muscle protein synthesis (Hammond et al., 2016). Finally, there is emerging evidence on the interaction between bone health and LCHF diets, where likely impairments have been reported at least in animals (Bielohuby et al., 2010) and children (Simm et al., 2017). This topic will be discussed in detail in section 2.4.5.

Chronic LCHF and physique. Athletes may also be interested in the LCHF diets as a means to decrease BM or manipulate physique (Manninen, 2004). However, some of this hype might be related to a rapid initial BM loss, which can be attributed to a decrease in body water due to water released from depleting glycogen stores (Burke et al., 2017a). In addition, according to the “carbohydrate-insulin” hypothesis, LCHF diets improve weight loss by increasing lipolysis via reduced insulin concentrations (Petterson et al., 2013). However, recent analyses clearly demonstrate that when energy and protein content of the diet is matched, rates of weight loss are not different between low fat high CHO and LCHF diets (Hall, 2017). On the other hand, speculations exist on the role of LCHF diets in increasing energy expenditure via increased metabolic rate. Indeed, this effect has been shown in a few investigations. For example, Hall et al. (2016) reported an increase of ~151 kcal·d^{-1} in total daily energy expenditure [measured via doubly labelled water (DLW)] after 1 month of ketogenic diet. However, a recent report contradicts these claims by showing that the calculations using DLW fail to account for respiratory quotient differences between habitual high vs low CHO diets and as such, estimate EE to be higher than it actually is (Hall et al., 2019). Indeed, this is a key application of such diets among the clinical population, where studies suggest that a ketogenic diet may be beneficial in terms of weight loss (Noakes & Windt, 2017). Reports in athletes have shown decreases in BM, FM and FFM as a consequence of LCHF diets. For example, a study in 8 elite artistic gymnasts reported ~1.6 kg reduction in BM and a significant, ~1.9 kg reduction in FM, following 30 d of LCHF diet (Paoli et al., 2012). Compared to a high CHO intake, a 12-week exercise program in the military with LCHF diet resulted in a significant loss of BM (-7.7 kg) and percentage body fat (~5.1% decrease) (LaFountain et al., 2019). Furthermore, 5 endurance athletes reported reductions in BM (~4 kg) and sum of 8 skinfolds (~25.9 mm) following a 10-week LCHF dietary intervention (Zinn et al., 2017). Meanwhile, a recent review suggests that ketogenic diets have the potential to lead to a loss of FFM (Tinsley & Willoughby, 2016), where this was reported to be in the range of ~1 and 3.5 kg (of which some may obviously reflect a change in body water stores as a result of decreased muscle glycogen stores). On the contrary, elite race walkers exposed to 3 weeks of either LCHF (<0.5 $g \cdot kg \cdot d^{-1}$ CHO), periodised (~8.6 $g \cdot kg \cdot d^{-1}$ CHO) or high CHO (~8.6 $g \cdot kg \cdot d^{-1}$ CHO) diet reported similar, albeit modest, losses of BM across the training camp (~1.8 kg vs ~1.6 kg vs ~0.6 kg, respectively; no difference between the groups) (Burke et al., 2017a). Table 2.2 summarises the main theory, mechanisms and outcomes of LCHF diets.

Acute low CHO availability and training adaptation. Many of the exercise sessions undertaken by endurance athletes neither challenge their body CHO stores nor require optimised performance (e.g. a 30 to 60 min easy running session at an intensity <75 % VO_{2max}). Therefore, these sessions may not require a proactive approach to CHO availability. Furthermore, there is now evidence that when exercise is undertaken with low CHO availability, there is an amplification of the cell signalling responses to endurance exercise (Hawley & Burke, 2010), with a potential to over time, increase training adaptation and exercise capacity (Marquet et al., 2016a; Marquet et al., 2016b). The main outcomes of such sessions are enhanced mitochondrial biogenesis and increases in mitochondria-related enzymes, protein kinases and signalling factors such as the cellular energy sensor AMP-activated protein kinase (AMPK), as well as p38 mitogen-activated protein kinase (MAPK), and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1a) (Hawley & Burke, 2010). While the changes in fuel metabolism and cellular signals related to this occur acutely, e.g. after a single exercise session (AMPK, p38 MAPK), the metabolic adaptations (including increased muscle glycogen content, enzymes involved in fat metabolism) are seen after ~3–10 weeks of training (for review, see Impey et al., 2018b). These adaptations are similar to typical endurance training-induced adaptations with the exception that *train low* appears to amplify the cellular stress and adaptations within, thereby potentially requiring less training for the achievement of similar cellular adaptations compared to completing the same session with high CHO availability (Impey et al., 2016). Therefore, less exercise may be required for the same metabolic adaptations to occur. It is noteworthy that while muscle metabolic adaptations might be enhanced with this strategy, other adaptations important for the endurance athlete such as pulmonary and cardiovascular adaptations, require high volumes of exercise training and may be compromised when low CHO availability limits the intensity and/or duration of exercise (Impey et al., 2018b). Therefore, a balanced approach may be required to optimise the different adaptation responses to training.

Different models for creating low CHO availability around exercise include: (1) training in the fasted state (low exogenous CHO availability), (2) twice a day training with minimal CHO between sessions (low endogenous and exogenous CHO availability), (3) prolonged training without exogenous CHO (low exogenous CHO availability and a decrease in muscle glycogen concentration below the glycogen threshold), and (4) withholding CHO during acute or prolonged recovery after a quality session to prolong the period of adaptation ("recover low" or, in the case of sessions undertaken in the evening, "sleep low'; low exogenous and eventually, low endogenous CHO availability) (Hawley & Burke, 2010; Burke et al., 2018b). Recent literature suggests that the adaptations associated with low CHO availability training may occur below the so-called glycogen threshold, initially theorised to be muscle glycogen concentrations of ≤300 mmol·kg dry weight $(dw)^{-1}$ (Impey et al., 2018b). Therefore, ideally, such an exercise session is either commenced with muscle glycogen concentrations of less than 300 mmol·kg dw^{-1} or depleted beyond this level during exercise. Since training intensity may suffer with extremely low glycogen concentrations (less than 100 mmol·kg dw^{-1}), the optimal window of opportunity may lie somewhere between 100-300 mmol·kg dw^{-1}. Indeed, a recent investigation showed that further reductions in muscle glycogen concentrations below 300 mmol·kg dw^{-1} provided no additional benefits in terms of cell signalling, while significantly impairing exercise capacity (Hearris et al., 2019). In this study, 8 males completed three conditions of a sleep low regimen, where CHO feeding between an evening glycogen depletion session (an intermittent cycling protocol for a total of 120 min) and a morning exhaustive exercise session (8 x 3 min at 80 % PPO followed by 1 min efforts at 80 % PPO until exhaustion) was either 0, 3.6 or 7.6 g·kg^{-1} to create a difference in muscle glycogen content prior to the morning exercise. The authors reported equal $AMPK^{Thr172}$ phosphorylation and PGC-1a mRNA expression in all treatments, therefore suggesting that beyond a certain glycogen threshold, there are no further benefits in terms of cell signalling. On the contrary, exercise capacity was significantly reduced in the low (~18 min) and moderate (~36 min) CHO treatments compared to high CHO (~44 min), highlighting the importance of maintaining adequate CHO feeding in relation to the demands of subsequent training (Hearris et al., 2019). Although the framework of a glycogen threshold is useful in theory, it may be difficult to achieve in practice due to lack of knowledge actual glycogen utilization rates of several training modalities (Impey et al., 2018b; Areta & Hopkins, 2018). It is important to emphasise that not all sessions should be conducted with reduced CHO availability. Instead, this strategy should be utilised based on the adaptive goal of an exercise session. Therefore, potentially some low intensity sessions that target fat oxidation and mitochondrial adaptations could be combined with low CHO availability training for maximal cellular adaptations. Furthermore, according to current emerging approaches, this strategy appears to be best utilised over 2 to 3 sessions per week, ideally during the base training phase (Impey et al., 2018b; Stellingwerff, 2012). Finally, it is important to differentiate training with low CHO availability from a low CHO diet. Indeed, the former refers to a strategic manipulation of the timing of CHO intake within the day, while daily intakes remain unaffected. Meanwhile, the latter refers to a low daily CHO intake irrespective of how this intake is spread across the day.

Acute low CHO availability and performance. Notwithstanding the improvements in cellular characteristics often associated with these strategies, however, few studies have shown that training with low CHO availability leads to a benefit to exercise/sports performance. Although this may be attributed to the failure to implement study protocols with sufficient sensitivity to detect small but meaningful changes in performance, it may also be due to an incorrect application of such “train low” strategies. For example, several studies (Hulston et al., 2010; Yeo et al., 2008) have implemented a chronic program of “train low” protocols which was shown to interfere with the athlete’s ability to train at high-intensities during quality/key workouts (e.g. interval training sessions); in such scenarios, it is likely that benefits achieved from one aspect of training (amplified response to sub-maximal sessions) are negated by the drawbacks of others (reduction in capacity for high-intensity sessions). Therefore, it appears that strategies to create low CHO availability around individual exercise sessions need to be carefully integrated into the periodised training program, so that all aspects of athletic preparation can be maintained. Indeed, a case study by Stellingwerff (2012) documented programs undertaken by three elite marathon runners which implemented strategic placement of train low (emphasised during the general preparation phase) and train high (emphasised during the specific preparation and tapering phases) sessions over a 16 week period before a race day. This resulted in noteworthy improvements in marathon personal best times for two of the marathoners, and a successful debut for the third marathoner. Nevertheless, it is important to note that this study was observational and did not include a control group. However, this study does provide preliminary peer-reviewed evidence of the usefulness of practical implementation of different dietary strategies within the athlete’s training program. In addition, recent intervention studies involving the current research team were able to incorporate periodic use of some of these strategies within one and 3 weeks of training undertaken by sub-elite triathletes and cyclists to achieve performance gains of ~3 % (i.e. a 3 % improvement in a 35 min 10 km time for a recreational runner would result in a 1 min 3 sec faster race finishing time) not seen with a control group (Marquet et al., 2016a; Marquet et al., 2016b). Meanwhile, strictly controlled intervention studies in elite endurance athletes have failed to show improvements in performance after several weeks of periodic train low/recovery low implementation within the athlete’s personal training program (Burke et al., 2017a; Gejl et al., 2017; Riis et al., 2019). Possible explanations include that the high volumes of training undertaken by elite endurance athletes (e.g. some elite rowers, cyclists, swimmers and triathletes already training > 25 h per week, and in triple days and >6 h training days) may already lead to some training being performed with suboptimal/low CHO availability, even without the need to include a “micro-periodisation” in which dietary CHO is withheld between training sessions. For example, elite race walkers may complete a total of 50 km of training per day, and therefore, despite a high CHO diet, the second/last training session of the day may (unintentionally) be completed with low glycogen availability (a train low session) regardless of dietary CHO intake. Due to discrepancies between studies in elites and sub-elites, further research is needed of actual practices of elite endurance athletes as well as effects of train low on performance. Table 2.2 highlights the key underlying mechanisms, adaptation and performance outcomes as well as possible challenges of the train low concept.

2.3.5 Evidence of periodisation of energy and CHO availability in elite athletes

While there is a reasonable body of literature on the dietary habits of elite endurance athletes (Burke et al., 2001), few of these dietary surveys have been recently conducted, to reflect the extent to which the current sports nutrition guidelines have been integrated into practice, and even fewer have involved world class athletes. Importantly, most of the available literature presents information on mean daily intakes of energy and macronutrients of athletes, usually as group data, rather than exploring patterns of intake between and within days, or at different times of the annual training/competition calendar. Therefore, despite the growing scientific support for such deliberate and strategic manipulations of energy and macronutrient intake, termed “dietary periodisation” and their incorporation into sports nutrition guidelines and athlete education pieces, whether elite athletes understand these guidelines and whether they transfer this knowledge into practice, is less clear. In addition, the inadvertent implementation of dietary periodisation strategies or barriers to putting such guidelines into effect are unknown but of interest to characterise, since these factors may contribute towards the achievement of an optimal plan.

While the latest sports nutrition guidelines emphasise a strategic manipulation of energy and macronutrient intake within and between the various cycles of the training and racing program (Mujika et al., 2018; Thomas et al., 2016); Stellingwerff et al., 2019), very little is known about actual dietary periodisation practices among elite athletes. Indeed, although Burke and colleagues (2001) prepared a comprehensive summary of the available dietary surveys of serious athletes published from 1970-1999, this literature was published thirty to nearly fifty years ago, in the period prior to the development of the idea of dietary periodisation, and hence, lacks data or commentary on between- and within-day eating practices.

Despite the growing interest towards dietary periodisation in the last decade, a review of the current literature shows that only 20 papers exist on actual or reported practices of dietary energy and/or CHO periodisation (defined as comparison of intakes within/between-days or training cycles) in elite and sub-elite athletes (Table 2.3). These studies have focused on micro- (n=10), meso- (n=11), and/or macro- (n=3) periodisation of nutrition during training and competition in elite (n=6 peer-reviewed publications and n=1 anecdotal/non peer-review publication) and sub-elite (n=5) endurance, elite team-sport (n=7), and athletes in other sports (n=1). Importantly, of the 20 papers, 16 were published after 2012, highlighting the emerging interest towards the topic. Based on available literature, it appears that most (17 out of 20 studies) elite and sub-elite endurance and team-sport athletes practice some form of periodisation of dietary CHO between days (micro-periodisation: Anderson et al., 2017b; Anderson et al., 2019; Bradley et al., 2015; Carr et al., 2018; Erdman et al., 2013; Fordyce, 2018) and between training/competition phases (meso-periodisation: Barr & Costill, 1992; Carlsohn et al., 2012; Clark et al., 2003; Fogelholm et al., 1992; Kopetschny et al., 2018; Kuzuhara et al., 2018; Stellingwerff, 2018; Viner et al., 2015) based on training/racing load (i.e. evidence for “fuelling for the work required”; Impey et al., 2016; Impey et al., 2018b). Indeed, it appears that CHO intake is emphasised on competition vs training days (Carr et al., 2018; Anderson et al., 2017a; Anderson et al., 2019; Bradley et al., 2015), and on demanding vs moderate effort race days (Fordyce, 2018). Thus, an overview of available evidence suggests that elite endurance athletes periodise especially CHO intake on micro and meso levels of training and competition. In addition to this literature, there are anecdotal reports that elite Kenyan runners commonly undertake morning sessions in a fasted state (Stellingwerff, 2013). This is supported by evidence from elite Western marathon runners, which suggests that at least some athletes practice purposeful training with low or high CHO availability to improve training adaptations and race performance (Stellingwerff, 2012). However, whether elite athletes implement periodisation of CHO availability systematically, and intentionally, in their training program, and whether these strategies are based on scientific evidence, chance or practical considerations remains unknown.

Before conclusions can be made that current high performance athletes understand and practice periodisation of energy and CHO intake systematically and intentionally, the limitations of this literature must be acknowledged. The frailty of dietary survey methodology in terms of validity and reliability must always be taken into account (Capling et al., 2017; Burke et al., 2001). Furthermore, most of the available studies have focused on a single microcycle (within days or one week of training) and, even if precise, these analyses only reflect nutrition practices of athletes within a small window of time [i.e. one week of records against the rest of the year (51 weeks)] and therefore fail to characterise the overall nutrition philosophy that is (or should be) largely dictated by training and performance goals of each individual athlete. Having athletes complete dietary records at various time points of the year, or even daily throughout the year, might provide a near-complete reflection of dietary practices and periodisation across several training cycles. Nevertheless, this approach is neither practical (athlete burden is high with increasing days of recording) nor reliable (athlete compliance decreases with increased number of recording days and several challenges exist with the use of dietary records, e.g. see (Capling et al., 2017). Therefore, questionnaires spanning the annual training/competition program with specific questions on various levels of dietary periodisation may be a better option due to less subject burden (15-20 min to complete a single questionnaire) compared to time-consuming dietary records. An additional benefit of the questionnaires is access to a larger pool of (likely higher calibre) athletes and the opportunity to assess reasons behind nutrition practices and the underlying knowledge of emerging themes in sports nutrition. Indeed, it is possible that a number of different iterations of knowledge and practice might be observed among athletes with examples including 1) excellent insight and implementation of dietary periodisation strategies, 2) accidental, unintentional and potentially sub-optimal achievement of some periodisation strategies - such as restricted intake of CHO before and during individual sessions due to practical issues such as gut discomfort during exercise or lack of time to eat rather than specific desire to train with low CHO availability, 3) good insight but lack of opportunity to implement strategies due to practical challenges - such as poor availability of appropriate food/drinks in the training environment, and 4) lack of knowledge about dietary periodisation strategies. Understanding athlete practices and the circumstances that unpin them is an important piece of evidence in the development of targeted education activities and further research questions.

Finally, the majority of observational or survey studies have focused solely on dietary practices of athletes, while ignoring the effects of these practices on health outcomes. The issues of within-day periodisation of EA and CHO availability on general health and performance outcomes have been briefly discussed earlier, however further discussion with regards to the effects of reduced energy and CHO availability on bone health will follow in the next section.

Table 2.2. Chronic and acute dietary strategies to manipulate carbohydrate (CHO) availability for training adaptation and performance, as well as health consequences of such strategies.

	CHRONIC HIGH AND LOW CHO DIETS		**ACUTE LOW AND HIGH CHO AVAILABILITY**	
	High CHO availability diet	**LCHF diet**	**Train Low**	**Train High**
Explanation (Burke et al., 2018b)	A diet which provides high CHO availability for optimised muscle glycogen stores and availability of exogenous CHO during exercise. Daily targets range from 3-12 $g \cdot kg \cdot d^{-1}$ and depend on the total training load.	A moderate (nonketogenic LCHF: <2.5 $g \cdot kg \cdot d^{-1}$ CHO) to extremely low (ketogenic LCHF: <50 $g \cdot d^{-1}$ CHO) CHO diet. Dietary fat constitutes the majority of dietary energy intake (60-65 % up to 75-80 % for nonketogenic and ketogenic LCHF, respectively). Utilised by some athletes to increase fat oxidation rates and enhance moderate-intensity (<75 % VO_{2max}) performance.	Training and/or recovering with low endogenous (muscle and liver glycogen) and/or exogenous (no CHO before/during/after exercise) CHO availability to provide additional metabolic stress to enhance training adaptation. Manipulates the timing of CHO intake, meanwhile overall daily CHO availability is maintained at adequate levels. Can be utilised occasionally for specifically targeted sessions as part of a periodised CHO availability diet.	Training with high endogenous (muscle and liver glycogen) and/or exogenous (CHO before/during exercise) CHO availability to support high quality sessions/events. Can be utilised occasionally for specifically targeted sessions as part of a periodised CHO availability diet.
Exercise-diet strategy (Burke, 2010; Burke et al., 2018b; Thomas et al., 2016)	• CHO is supplied based on the fuel needs of training and racing to optimise glycogen stores. • Special focus on CHO around training sessions with adequate intakes before and after (see “Train High”).	• All training is completed with low CHO availability. • Some athletes may supplement with CHO during select key sessions or racing. • Adaptations may occur in as little as 5 d of nonketogenic LCHF diet and have been shown to persist after 2 d of CHO restoration.	• Training after an overnight fast. • Twice-a-day training: the first session of the day is followed by minimal CHO, whereby the second session of the day is completed with low CHO availability. • No exogenous CHO during a prolonged training session. • Restricting CHO intake in the acute recovery period after exercise ("*recover low*"). • Completing afternoon HIT session with high CHO availability, followed by CHO restriction overnight and a morning fasted session ("*sleep low*").	• Training after a meal (fed state) . • Ingesting CHO during training ("*training the gut*"). • Ingesting CHO during recovery following exercise.

Physiological adaptations	•Optimised/maximised CHO availability from endogenous and/or exogenous sources. •Enhanced reliance on CHO fuels due to high CHO availability. •Higher insulin and lower cortisol and catecholamine concentrations. •Maximised absorption of CHO from the gut due to up-regulation of transport proteins (Cox et al., 2010). •Maximised CHO oxidative capacity (Jeukendrup, 2017; Costa et al., 2017), including enhanced activity and function of key enzymes hexokinase and PDH.	•Increased rates of fat oxidation. •Increased IMTG stores. •Increased fatty acid mobilisation and transport. •Increased enzyme activity (fat metabolism). •Reductions in mitochondrial respiration. •Downregulation of CHO metabolism including PDH activity, glycogenolysis and glucose oxidation.	•Reduced endogenous (muscle and liver glycogen stores) and/or exogenous (CHO foods/drinks) CHO availability. •Increased reliance on fats for fuel source (Van Proeyen et al., 2011; Yeo et al., 2008; De Bock et al., 2008). •Increased cortisol and catecholamine and reduced insulin concentrations. •Activation of AMPK and p38 MAPK pathways leads through a number of steps to enhanced mitochondrial biogenesis and other training adaptations (Burke, 2010), including: a) Enhanced activity and number of key proteins, enzymes and transcription factors involved in CHO and fat metabolism (Van Proeyen et al., 2011; Hansen et al., 2005; Hulston et al., 2010; Morton et al., 2009; Bartlett et al., 2013; Psilander et al., 2013) b) Increased resting muscle glycogen concentration (Yeo et al., 2008; Hansen et al., 2005).	•Optimised/maximised CHO availability from endogenous and/or exogenous sources. •Enhanced reliance on CHO fuels due to high CHO availability. •Higher insulin and lower cortisol and catecholamine concentrations. •Maximised absorption of CHO from the gut due to up-regulation of transport proteins (Cox et al., 2010). •Maximised CHO oxidative capacity (Jeukendrup, 2017; Costa et al., 2017), including enhanced activity and function of key enzymes hexokinase and PDH.
Performance outcomes	•Acutely enhances endurance performance due to a combination of increased capacity to absorb and oxidise CHO for fuel and full glycogen stores leading into a capacity to maintain high exercise intensities (Cermak & van Loon, 2013; Stellingwerff & Cox, 2014; Costa et al., 2017).	•Decreased economy (Burke et al., 2017a, Shaw et al., 2019) •Initial fatigue and lethargy that takes several weeks to disappear (Burke et al., 2017a). •Reduced endurance performance (Mujika, 2018; Zinn et al., 2017).	•Potential for enhanced endurance performance due to a combination of increased capacity to store glycogen in the skeletal muscle and increased capacity to oxidise fuels aerobically (Burke, 2010). •Enhanced performance in trained athletes (Hulston et al., 2010; Marquet et al., 2016a; Marquet et al., 2016b).	•Acutely enhances endurance performance due to a combination of increased capacity to absorb and oxidise CHO for fuel and full glycogen stores leading into a capacity to maintain high exercise intensities (Cermak & van Loon, 2013; Stellingwerff & Cox, 2014; Costa et al., 2017).

	•Delayed fatigue during submaximal prolonged exercise (Coyle et al., 1983).	•Reduced anaerobic performance (Wroble et al., 2018) •Increased sprint peak power and critical power (McSwiney et al., 2018) •No difference in strength and power performance compared to a high CHO diet (Wilson et al., 2017; Paoli et al., 2012) •Persistent fatigue (Mujika, 2018). •Unaltered exercise capacity (Phinney et al., 1983) •May support moderate intensity performance (<75 % VO_{2max}) but less able to support performance at higher intensities (>75 % VO_{2max}) (Burke et al., 2017a)	•No effect on performance in elite endurance athletes (Gejl et al., 2017, Burke et al., 2017a) •Reduced training quality and power outputs and increased perceived effort (Burke, 2010; Yeo et al., 2008; Hulston et al., 2010).	•Delayed fatigue during submaximal prolonged exercise (Coyle et al., 1983).
Health outcomes	•Attenuates secretion of IL-6 from the skeletal muscle (Febbraio et al., 2003), which has the potential to: a) Suppress hepcidin response to exercise, supporting good iron status b) Decrease osteoclastogenesis, leading to decreased bone resorption •Attenuates stress hormone and inflammatory response, supporting immune function (= reduced risk of illness/injury → better performance on the competition day; Raysmith & Drew, 2016). •Maintaining high CHO availability during high training	•Initial fatigue and lethargy that takes several weeks to disappear. •Persistent fatigue (Mujika, 2018). •Restricted food variety reduces nutrient density (Mirtschin et al., 2018). •No effect on mucosal immunity responses to exercise in elite athletes (McKay et al., 2018) •Possible impairment of digestibility of calcium may impair bone health (Frommelt et al., 2014) • Impairments to bone health have been shown in rats (Bielohuby et al., 2010; Ding et al., 2019; Wu et al., 2017) and in	•Increased secretion of IL-6 from the skeletal muscle (Keller et al., 2001; Steensberg et al., 2001), which has the potential to: a) Upregulate hepcidin response to exercise (Badenhorst et al., 2015), leading to poor iron status (Peeling et al., 2008), b) Increase osteoclastogenesis (Guo et al., 2017), leading to increased bone resorption when CHO is restricted before (Scott et al., 2012), during (Sale et al., 2015; de Sousa et al., 2014) and after (Townsend et al., 2017) exercise as well within the 24 hour period around twice-a-day training (Hammond et al., 2019).	•Attenuates secretion of IL-6 from the skeletal muscle (Febbraio et al., 2003), which has the potential to: a) Suppress hepcidin response to exercise, supporting good iron status b) Decrease osteoclastogenesis, leading to decreased bone resorption •Attenuates stress hormone and inflammatory response, supporting immune function (= reduced risk of illness/injury → better performance on the competition day; Raysmith & Drew, 2016). •Maintaining high CHO availability during high training loads has the

	loads has the potential to support adequate daily CHO intake, which can help in: a) Maintaining body mass and lean body mass b) Maintaining good training quality c) Reducing the risk of illness and overtraining.	children (Bergqvist et al., 2008; Simm et al., 2017; Draaisma et al., 2019) however evidence from obese adults does not support these findings (Brinkworth et al., 2016; Athinarayanan et al., 2019) • Possible benefits to body composition (Zinn et al., 2017; Heatherly et al., 2018; Greene et al., 2018; McSwiney et al., 2018; Paoli et al., 2012; LaFountain et al., 2019) • Possible negative effects on lean mass (decrease; Tinsley & Willoughby, 2016) and muscle protein synthesis (Hammond et al., 2016) • May impair thyroid function (Kose et al., 2017), which would have implications for metabolic rate and bone health • May challenge iron status (McSwiney & Doyle, 2019) • In non-athletes, proposed benefits include enhanced markers of cardiovascular (CV) health and reversal of conditions such as non-alcoholic fatty liver disease and Type 2 diabetes (Noakes & Windt, 2017) • In athletes, neither benefits or risks in terms of CV health (O'Neal et al., 2019)	• Increased stress hormone and inflammatory cytokine response reduces immunity and increases the risk of illness (Gleeson et al., 2004). • Suppression of muscle protein synthesis, which can be rescued by protein ingestion before/after exercise (Impey et al., 2018a) • Increased daily protein requirements (Gillen et al., 2019) • If periodic CHO restriction compromises daily CHO intakes, this can lead to: a) Loss of body mass and/or lean body mass (Helms et al., 2014) b) Poor training quality and inadequate recovery (Yeo et al., 2008; Hulston et al., 2010; Achten et al., 2004; Halson et al., 2004) c) Increased risk of injury/illness, fatigue and overtraining (Gleeson et al., 2004; Achten et al., 2004; Halson et al., 2004)	potential to support adequate daily CHO intake, which can help in: a) Maintaining body mass and lean body mass b) Maintaining good training quality c) Reducing the risk of illness and overtraining.

Current sports nutrition recommendations (Thomas et al., 2016)	• A high CHO availability diet is recommended for most athletes as it allows for a range of CHO intakes based on individual needs and the training load.	• Based on current evidence, LCHF diets are not recommended for the majority of elite endurance athletes due to a risk of adverse health and performance outcomes.	• Recommended to integrate in the training program with care. • Utilise periodically, under guidance of a sports scientist. • Need to balance adaptations and performance and manage adverse health outcomes.	• Consistent with guidelines for optimal endurance performance. • High intensity sessions require adequate CHO fuelling and recovery. • Adopt this strategy with key sessions, preparation for competition and during competition.
Practical applications	• Use nutrition to support the goals of a periodised, individualised training program on an athlete-by-athlete (i.e. different gaps between different athletes) and day-by-day (i.e. different requirements based on training load and performance goals) basis (Stellingwerff et al., 2019). • Assess individual athlete's training load on a day-by-day basis to create individual targets for CHO intake to match needs of daily training. • Usually targets may be somewhere between 3 and 12 g·kg·d^{-1}; however extremes do exist especially in racing environments (Fordyce, 2018) • Spread CHO intake across the day. • Focus on CHO around exercise for optimal fuelling (1-4 g·kg^{-1} in the 1-4 h before exercise; 30-90 g/h during exercise) and recovery (~1 g·kg·h^{-1} in the first 4 hours after which return to	• Use nutrition to support the goals of a periodised, individualised training program on an athlete-by-athlete (i.e. different gaps between different athletes) and day-by-day (i.e. different requirements based on training load and performance goals) basis (Stellingwerff et al., 2019). • Make sure the diet is nutrient-dense and provides adequate energy. • Consume caffeine before workouts to rescue training intensity. • Trial high CHO availability training or racing occasionally after the adaptation period to test how this affects performance.	• Use nutrition to support the goals of a periodised, individualised training program on an athlete-by-athlete (i.e. different gaps between different athletes) and day-by-day (i.e. different requirements based on training load and performance goals) basis (Stellingwerff et al., 2019). • Remember that training low refers to the manipulation of timing of CHO intake, i.e. the daily intake should still be adequate. • Practice training with low CHO availability when quality is less important and overall stress of training is low. • Do not train low for more than 2-3 sessions per week. • Ingestion of caffeine and/or protein before/during/after train low sessions may benefit perceived exertion/power output (Lane et al., 2013) and maintenance of LBM (Taylor et al., 2013), respectively. • Ingestion of calcium-rich foods before exercise could potentially	• Use nutrition to support the goals of a periodised, individualised training program on an athlete-by-athlete (i.e. different gaps between different athletes) and day-by-day (i.e. different requirements based on training load and performance goals) basis (Stellingwerff et al., 2019). • Practice high CHO availability during prolonged and/or high intensity training to optimise training quality and recovery. • Practice gut training by ingesting CHO during exercise as the competition season approaches (note: this will depend on your discipline). • Provide a mixture of CHO sources during exercise when exercise duration increases to >60-90 min and/or when high CHO oxidation rates are targeted for competition (marathons and race walks). • Maintain high CHO availability when: a) energy intake is compromised, b) risk of illness/injury is especially high, c)

	normal eating practices to maintain high daily CHO availability (Burke et al., 2018b)		attenuate increase in bone resorption (Haakonssen et al., 2015). • Limit this nutrition strategy for experienced athletes only (after training volume and intensity have been maximised). • Use of a mixture of train low strategies may be beneficial. • Do not use this strategy if injured/recovering from injury. • Carefully plan this and other nutrition strategies based on an athlete's background and goals.	training at altitude/in hot temperatures, d) recovering from illness/injury, e) engaged in a strenuous training program and f) an athlete is a developing athlete. • Ingest CHO before/during exercise when the goal is to support immunity, iron and bone health.

Table 2.3. Existing studies of self-reported (surveys) or recorded (food and training records) dietary energy and carbohydrate (CHO) periodisation in elite endurance and team sport athletes as well as in sub-elite/trained athletes from endurance or aesthetic sports. Each study has been assigned level of periodisation (micro, meso and/or macro) and study outcomes have been reported as providing evidence for or against dietary (CHO) periodisation.

Reference	Participants	Assessment method	Assessment length and target period [periodisation level]	Practices
Elite endurance athletes				
Carr et al., 2018	Elite female and male cross-country skiers (n=31)	Dietary records	One day of training and one day of competition. **[Micro-periodisation]**	Significantly higher CHO intakes on the competition day (males: 8.9 g·kg·d^{-1}; females: 8.5 g·kg·d^{-1}) compared to the training day (males: 8.2 g·kg·d^{-1}; females: 7.0 g·kg·d^{-1}). **→ Evidence for micro-level (between-d) CHO periodisation**
Fogelholm et al., 1992	Elite female and male cross-country skiers (n=17)	Dietary records	7 d records at 3-month intervals to assess season changes in nutrient intakes. **[Meso/macro-periodisation]**	Seasonal changes in energy and CHO intake reflected changes in EE in male athletes (CV 19.1 %) particularly. **→ Evidence for meso/macro-level (between training phases) energy and CHO periodisation**
Fordyce, 2018	Professional male cyclist (n=1)	Dietary records	Two separate stages during Giro d'Italia: medium intensity hilly stage (4 h, EEE 3635 kJ); high intensity summit finish (6 h, EEE 6180 kJ). **[Micro-periodisation]**	Higher CHO intake on a more demanding mountain stage (18.9 g·kg·d^{-1}) compared to an easier stage (5.8 g·kg·d^{-1}). Evidence of CHO periodisation during a stage race based on fuel demands. **→ Evidence for micro-level (between-day) CHO periodisation**

Garcia-Roves et al., 2000	Professional male cyclists (n=6)	Dietary records	3 d during high intensity training at a training camp and 3 d during competition (Tour of Spain): similar energy expenditure during training and competition. **[Meso-periodisation]**	No difference in energy or CHO intake between training and racing (logical, as no difference in load either). **→ Evidence against meso-level (training vs competition phase) energy and CHO periodisation**
Stellingwerff, 2018	Elite female middle-distance runner (n=1)	Observations and reflections of dietary strategies.	Observations across 9 years of training and competition. **[Meso-periodisation]**	Competition weight and body composition periodisation achieved by a moderate decrease in extra energy/CHO (sugar) foods in the 6-8 week period before competition season. **→ Evidence for meso-level (training vs competition preparation) energy and CHO periodisation**
Stellingwerff, 2012	Elite male marathon runners (n=3)	Self-report on two main nutrition strategies	16-week preparation for a marathon, including focus on 1) training with low HCO availability, and 2) training the gut. **[Micro/meso-periodisation]**	Athletes reported practicing train low ~2.5 times weekly, mainly during the first 8 weeks of preparation. The frequency of CHO fuelling practices increased towards the race week (mean 19 sessions across 16 weeks). **→ Evidence for micro/meso-level (between-week and within-day) CHO periodisation**
Viner et al., 2015	Competitive female and male road and off-road cyclists (n=10)	Dietary records	3 d during preseason, competition season, and off-season. **[Meso/macro-periodisation]**	Low EA in 70 %, 90 % and 80 % of cyclists during preseason, competition, and off-season, respectively. Daily CHO intake decreased significantly from competition season (4.3 g·kg·d^{-1}) to off-season (3.7 g·kg·d^{-1}). **→ Evidence for meso/macro-level (between training phases) energy and CHO periodisation**

Sub-elite athletes				
Barr et al., 1992	Male collegiate swimmers (n=24)	Dietary records	2 d during the early season (4 weeks), during the period of increased or stable training volumes (6 weeks), and during the late season with moderate training (15 weeks). Comparison of increased vs stable training volume on dietary intakes. **[Meso-periodisation]**	Significantly higher CHO intakes in the increased training volume group (from 500 g to 600 g). **→ Evidence for meso-level (between training phases) CHO periodisation**
Brown et al., 2017	Pre-professional female ballet dancers (n=25)	Dietary records and 24-hr recalls	7 d including 5 week days (scheduled training) and 2 weekend days (no training). **[Micro-periodisation]**	Lower energy intake and EA on week days compared to weekends. Lower CHO intake on week days (4.8 $g \cdot kg \cdot d^{-1}$) compared to weekend days (5.4 $g \cdot kg \cdot d^{-1}$). **→ Evidence for micro-level (between-day) energy and CHO periodisation**
Carlsohn et al., 2012	Male junior triathletes (n=7)	Dietary records	7 d during a moderate and an intensive training period. **[Meso-periodisation]**	CHO intake was higher during the intensive period (9 $g \cdot kg \cdot d^{-1}$) vs moderate period (7.9 $g \cdot kg \cdot d^{-1}$). **→ Evidence for meso-level (between training phases) CHO periodisation**
Drenowatz et al., 2012	Male endurance athletes (n=15)	An online food frequency questionnaire at the end of each week of data collection	7 d during a high volume and a low volume training week. **[Meso-periodisation]**	No difference in energy or CHO intake between low or high volume weeks. **→ Evidence against meso-level (between training weeks) CHO periodisation**

Kopetschny et al., 2018	Long-distance triathletes (n=74)	An online survey of dietary practices	Questions on a training macrocycle. **[Meso/macro-periodisation]**	36 % planned to reduce CHO intake at some point in training, mainly early (29 %) and toward the end (22 %) of the macrocycle. **→ Evidence for meso-level (between training phases) CHO periodisation**
Kuzuhara et al., 2018	Male collegiate rowers (n=15)	Dietary records	For 7 d at the start of each training phase: off season, pre-season, in season. **[Meso-periodisation]**	CHO intake was higher during pre-season (7 g·kg·d^{-1}) than off season (6.8 g·kg·d^{-1}), and during in season (7.8 g·kg·d^{-1}) than pre-season or off-season. **→ Evidence for meso-level (between training phases) CHO periodisation**
Elite team-sport athletes				
Anderson et al., 2017b	Male English Premier League soccer players (n=6)	Dietary records suppored by photographs and 24-h recalls	7 d including 5 training days and 2 match days. **[Micro-periodisation]**	Greater CHO intake on match (~6.4 g·kg·d^{-1}) vs training (~4.2 g·kg·d^{-1}) days. **→ Evidence for micro-level (between-days) CHO periodisation**
Bradley et al., 2015	Male European rugby union players (n=14)	Dietary records and sensewear armband data	6 d during in-season: 5 days pre-game and one day after the game. **[Micro-periodisation]**	Low EA earlier in the week and high EA before and after game day. CHO intake was significantly higher on the day before the game (~5 g·kg·d^{-1}) compared to other time points (~3.5 g·kg·d^{-1}). **→ Evidence for micro-level (between-days) energy and CHO periodisation**
Clark et al., 2003	NCAA division 1 female soccer players (n=14)	Dietary records	3 d during preseason and post-competitive season training. **[Meso-periodisation]**	Significantly higher CHO intakes during pre-season (~5.2 g·kg·d^{-1}) compared to post-competitive season (~4.3 g·kg·d^{-1}) period. **→ Evidence for meso-level (between training phases) CHO periodisation**

Erdman et al., 2013	Elite Canadian athletes (n=324)	Dietary records	3 d, single assessment. **[Micro-periodisation]**	No difference between training day and rest day meal frequency; fewer snacks (fewer CHO) on rest day. **→ Evidence for micro-level (between-days) CHO periodisation**
Naughton et al., 2016	Male Elite Youth Academy soccer players (n=59)	Dietary records	7 d training period. **[Micro-periodisation]**	Lower CHO intake at breakfast compared to lunch and snacks. **→ Evidence for micro-level (within-days) CHO periodisation**
Anderson et al., 2019	Professional male goalkeeper from the English Premier League	Dietary records (remote food photographic method and 24 h recalls) and DLW	7 d in-season microcycle **[Micro-periodisation]**	Daily energy (3034 vs 3475 kcal·d^{-1}) and CHO (2.3 vs 3.3 g·kg·d^{-1}) intakes were higher on game vs training days. **→ Evidence for micro-level (between-days) energy and CHO periodisation**
Jenner et al., 2019	Female Australian football league players (n=23)	Dietary records	3 d during a preseason training week **[Micro-periodisation]**	No significant difference in energy or CHO intake between main training, light training, or rest days. **→ Evidence against micro-level (between-days) energy and CHO periodisation**

2.4 Endurance athletes are at risk for low bone mineral density and poor bone health

While the desired training adaptations and performance are achieved via a sophisticated and targeted utilisation of a mixture of training and nutrition strategies (described previously in sections 2.1 and 2.2), another key goal of an endurance training program is to minimise the number of days lost to illness and injury to support training availability of an athlete (i.e. “athlete availability”). Yet it is known that injuries related to poor bone health are a major cause of interrupted training, lack of availability for competition and sub-optimal race performance among endurance athletes (Raysmith & Drew, 2016). Indeed, low bone mineral density (BMD) is a common issue among endurance athletes (Hind et al., 2006), especially since it increases the risk of stress fractures (Tenforde et al., 2016). As many as 40-45 % of female (Barrack et al., 2008; Melin et al., 2015; Pollock et al., 2010) and 24-40 % of male runners (Barrack et al., 2017; Tam et al., 2018) and between 63 and 82 % of male road cyclists (Scofield & Hecht, 2012; Rector et al., 2008) have been reported to suffer from low BMD. Although weight-bearing physical activity is known to promote bone heath, and confers some protection to the bone health of runners over endurance athletes in non-weight bearing activities (e.g. cyclists) (Rector et al., 2008), it is clear that dietary factors play an essential and interactive role in bone modelling that must be considered (Banfi et al., 2010). The following chapter will provide an overview of bone modelling and its disruptions related to imbalances in diet and/or exercise.

2.4.1 Bone physiology and metabolism

Bone is a metabolically active tissue that undergoes constant remodelling to adapt to changing conditions, to remove old bone and to replace it with new one. Indeed, around 5-10 % of bone tissue is renewed each year. The process of formation of new bone tissue is referred to as ossification or osteogenesis, and includes the following phases: quiescent phase, activation phase, resorption phase, reversal phase, formation phase and mineralization phase (Figure 2.3). Osteoblasts and osteoclasts are responsible for bone formation and bone resorption (the balance of these is known as bone modelling), respectively. The function of these two depends mainly on physical activity and diet, which influence bone via several hormones, growth factors and cytokines. In addition, osteocytes, which are formed from osteoblasts that get trapped within the matrix, are responsible for supporting bone structure and metabolism (Kini & Nandeesh, 2012).

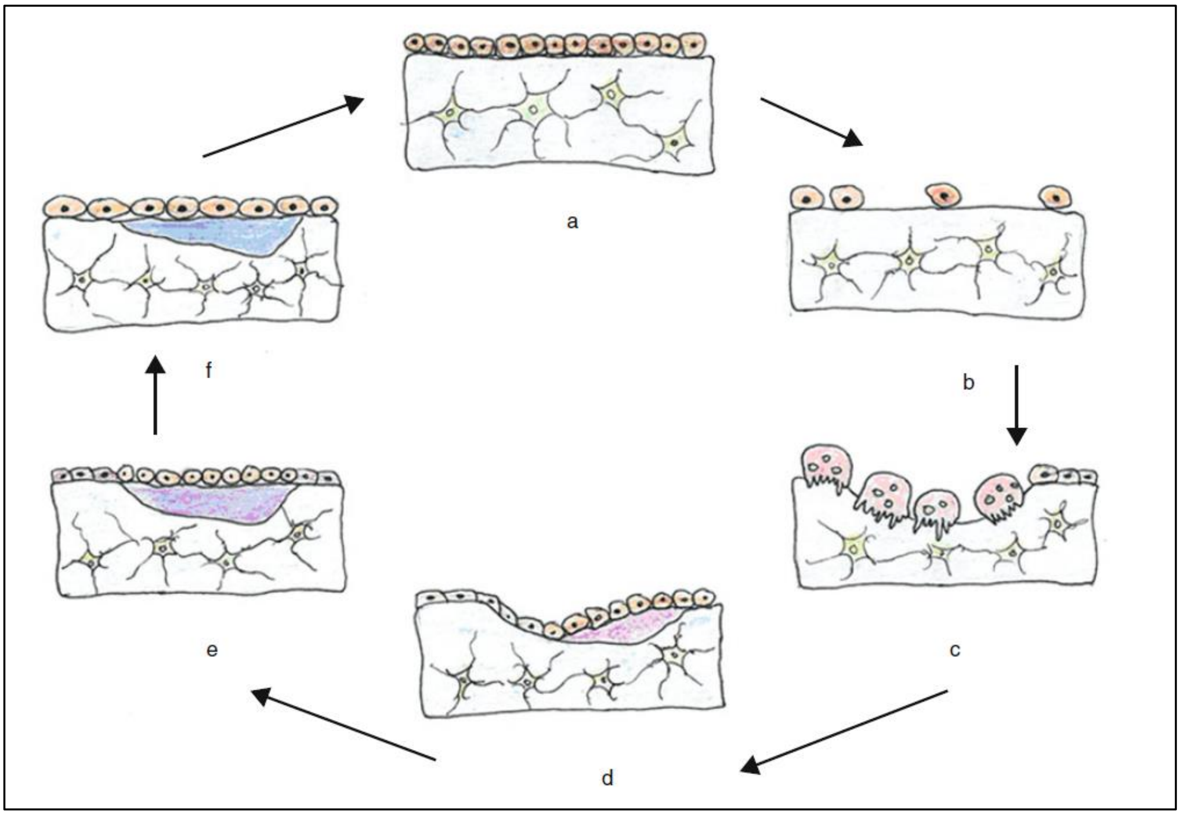


Figure 2.3. The phases of bone turnover. Bone turnover starts with a quiescent phase (a; ‘rest’ phase), followed by activation phase (b), characterised by cell retraction. Next phases include resorption (c) where osteoclasts break down bone tissue, and formation (d), where osteoblasts build new bone tissue. Finally, osteoid matrix mineralises (e), a result of which is a newly formed bone structure unit (f) that will enter the quiescent phase again (Kini & Nandeesh, 2012).

The key functions of bone tissue are (1) structural support to the body, (2) a mineral reservoir, and (3) hemapoiesis. Indeed, 99 % of body calcium, 85 % of body phosphorus and 65 % of body magnesium are located in bones. Although acting as a structure to support locomotion is an important task, the metabolic processes that require minerals take priority over this role. Therefore, if there is a shortage of calcium, phosphorus or magnesium, the body mobilises these from bones to maintain systemic homeostasis. Long-term, this may lead to a decrease in BMD (Kini & Nandeesh, 2012). In addition, bone has been recently recognised as an endocrine tissue. Among the hormones the bone secretes is osteocalcin (OC), a protein synthesised by osteoblasts and chondrocytes, that is responsible for not only bone formation, but has also a role in fat and glucose metabolism, insulin secretion as well as proliferation of pancreatic B cells (Banfi et al., 2010).

Bone turnover. During the resorption phase, which precedes bone formation, osteoclasts break down bone tissue. This phase is stimulated by parathyroid hormone (PTH), glucocorticoids [which inhibit insulin-like growth factor-1 (IGF-1) synthesis by osteoblasts], hyperthyroidism, and hypoestrogenism, as well as an imbalance in physical activity and/or dietary intakes of calcium and vitamin D. In contrast, bone formation is inhibited by calcitonin and estrogens, while osteoprotegerin (OPG), produced by osteoblasts, and thus, the bone resorption process. It is, therefore, an essential factor regulating the bone resorption process. Finally, interleukin-6 (IL-6) acts on bone directly through receptor activator of nuclear factor κB ligand (RANKL), which enhances osteoclast differentiation, and indirectly through osteoblast-derived prostaglandin estrogen-dependent osteoclast activation (Lombardi et al., 2016). Osteoblasts, which are responsible for bone formation, have receptors for several hormones including PTH and estrogens. Main factors stimulating osteoblast activity are thyroid hormones (in physiologically normal levels), insulin, growth hormone (acts directly on osteoblasts and indirectly on IGF-1), androgens, estrogens (favour bone formation and reduce bone resorption), and progesterone. In addition, PTH regulates serum calcium and phosphorus levels: when serum calcium levels drop, more PTH is produced, which then acts to mobilise calcium from bones to maintain systemic calcium levels. Furthermore, vitamin D acts in three different ways to support bone formation: 1) it increases intestinal absorption of calcium, 2) it promotes bone mineralization, and 3) it regulates OC activity. Calcium plays an important role in bone health as it is needed for mineralization of new bone (Kini & Nandeesh, 2012). In healthy young adults, bone resorption and formation equal each other in that bone mass is relatively stable. However, when bone resorption exceeds formation, long-term result is a decrease in BMD (Banfi et al., 2010).

Markers of bone modelling. While densitometries such as DXA provide information on BMD (long-term balance), biochemical markers are safe and useful in determining the acute state of bone modelling. The most commonly used markers for measurement of bone resorption and formation are c-terminal telopeptide of type 1 collagen (CTX) and procollagen type 1 N propeptide (P1NP), respectively. The formation of P1NP has been shown to closely follow the collagen synthesis of bone, and hence this marker can be considered useful in determining bone formation status. Similarly, CTX is a breakdown product of collagen degradation and has been widely used to reflect bone resorption. Bone alkaline phosphatase (BAP), an enzyme involved in bone mineralization, and OC, a protein synthesised by osteoblasts, are also markers of bone formation (Banfi et al., 2010) – although it should be noted that the exact role of OC in bone remodelling process is yet to be determined (Lin et al., 2018). OC undergoes carboxylation in osteoblasts, however a small amount remains stable and is referred to as undercarboxylated OC (Glu-OC). Glu-OC plays a role in glucose homeostasis and cardiometabolic health, as well as muscle function and glucose uptake (Lin et al., 2018). Approximately half (40-60 %) of total

OC released into circulation is Glu-OC, where higher levels indicate low vitamin K bioavailability and increased fracture risk (Lin et al., 2018; Szulc et al., 1993). Circulating Glu-OC also regulates whole-body energy metabolism (via increased insulin secretion and enhanced insulin sensitivity) (Lin et al., 2018). Since markers of bone modelling show a circadian variation, measurements should be done at the same time of the day, and in similar conditions (fasted vs fed, etc.) to allow comparison between trials (Bjarnason et al., 2002). Table 2.4 summarises the use and interpretation of key markers of bone remodelling process within research setting.

Table 2.4. Key markers of bone modelling in terms of available evidence on the interaction between reduced energy or CHO availability and concentrations of bone markers.

Marker	Biological material	Biochemistry	What it measures	Effects of reduced energy and/or CHO availability
P1NP	Serum	Collagen precursor; follows the synthesis of new Type I collagen that accounts for >95 % of bone collagen	Bone collagen (type I) synthesis (i.e. bone formation)	Energy restriction has been shown to reduce P1NP after 3 d (Zanker & Swaine, 2000) and 5 d (Papagergiou et al., 2017)
OC	Serum	Synthesised by osteoblasts, odontoblasts and hypertrophic chondrocytes; Involved in osteoid mineralisation and regulation of energy, glucose and lipid metabolism	Marker of osteoblastic function (i.e. bone formation)	No apparent change in OC levels in response to altered energy or CHO availability 9
Glu-OC	Serum	A product of undercarboxylation of OC (i.e. the remaining OC that does not go through carboxylation)	Participates in glucose homeostasis; high proportions have been linked to low vitamin K bioavailability and increased fracture risk	Increase in the relative proportion of Glu-OC of total OC over a 3-week cycling stage race (Lombardi et al., 2012)
CTX	Serum	End product of the enzymatic degradation of the carboxy-terminal collagen cross-links terminal regions of Type I collagen	Collagen degradation (i.e. bone resorption)	Low EA (Ihle & Loucks, 2004; Papageorgiou et al., 2017) and low CHO availability with (Sale et al., 2015; de Sousa et al., 2014) and without (Hammond et al., 2019) energy restriction increase CTX concentrations acutely (within 24 h) or after a short-term (5 d) exposure

Compiled from Banfi et al., 2010; Lin et al., 2018

2.4.2 Exercise and bone metabolism

Bone is continuously being broken down and formed in response to two main factors: physical activity and various dietary influences. Indeed, bone tissue reacts to the forces under which it is placed, in that it changes size and shape, removes damaged tissue and repairs this tissue to maintain and improve bone strength. Thus, high levels of mechanical loading (weight bearing activities such as jumping and running) improve bone strength, while low levels of loading (non-weight bearing activities, such as swimming and cycling) or lack of physical activity decrease BMD. Also, muscle contractions exert large loads on the bone and indeed, there has been shown to be a linear relationship between muscle contraction force and bone strength. There is evidence that the so-called Wnt/β-catenin pathway is a key factor in changes in bone mass. This pathway appears to be regulated by sclerostin – a marker that is stimulated by lack of mechanical loading and that contributes to the inhibition of the Wnt pathway, thereby impairing bone modelling (Qin et al., 2013).

Indeed, one year of cycling training and competition led to a loss of BMD in female (Sherk et al., 2014) and male (Barry & Kohrt, 2008) cyclists, highlighting the lack of skeletal benefit from non-weight bearing exercise (Olmedillas et al., 2012). Meanwhile, current evidence suggests that dynamic loading which induces high bone strains and is rapid in nature is most beneficial for bone health and strength (Tenforde & Fredericson, 2011). It appears that frequency of loading is less important than intensity. Furthermore, short bouts with rest periods in between are more useful compared to longer continuous bouts as bone cells become desensitised to repetitive loading (Beck et al., 2016). For example, for prevention of osteoporosis in low-risk individuals (i.e. BMD T score >-1.0), 3-5 sets of 10-20 repetitions of vertical jumps (drop jumps, skipping rope, hopping; exerting forces > 4 times BM) interspersed with 1-2 min rest, completed 4 to 7 times a week is recommended by Exercise and Sports Science Australia Position Statement (Beck et al., 2016). In addition to jumping, it is known that multidirectional activities (team sports) provide a better stimulus for the bone instead of monotonous types of activities (running, walking) (Beck et al., 2016). Indeed, a 12-month prospective study in adolescent male athletes reported greater improvements in bone mineral content and stiffness among footballers compared to cyclists and swimmers (Vlachopoulos et al., 2018). Furthermore, male cyclists who incorporated weight training within their weekly cycling program had a higher BMD compared to those who did not lift weights (Mathis & Caputo, 2018). In addition to the increased mechanical strain towards the bone, weight training supports increase or maintenance of lean body mass, which has been shown to be a strong determinant of BMD (Vlachopoulos et al., 2017). Indeed, risk factors for low BMD in females

(Gibbs et al., 2014) and in males (Barrack et al., 2017), include low BMI and low lean mass. While exercise is certainly beneficial for the bone, dietary factors may impair bone health even when adequate mechanical stimulus is present. For example, a study on female athletes showed that the beneficial effects of high impact exercise on bone disappeared in the presence of menstrual dysfunction (Fredericson et al., 2005), highlighting the importance of adequate EA for bone health. Therefore, the next sections will discuss some of the key dietary factors with regards to healthy bones.

2.4.3 Overview of dietary factors in bone metabolism

Markers of bone modelling appear to be relatively responsive to acute feeding, where slightly differential effects are seen depending on the macronutrient content of the meal and the bone marker in question. Although several theories have been formed to explain the mechanisms behind these feeding-induced changes in markers of bone modelling, these preliminary ideas remain largely speculative and further research is warranted in the area. For the purpose of the current thesis, the acute effects of the macronutrient and energy content of the meal *in general* (i.e. in the absence of exercise stimulus) will be reviewed briefly below, followed by a discussion of other dietary factors. The remainder of the section will provide a more thorough review of investigations where the effects of an exercise stimulus on bone modelling have been investigated in parallel to dietary manipulations.

Acute effects of feeding on bone metabolism. Studies have reported acute changes in resting concentrations of markers of bone formation and resorption following feeding. For example, Henriksen et al. (2003) reported that ingestion of glucose, protein or fat led to a 52%, 52%, and 39% decrease in CTX concentrations, respectively. It is possible that CTX is mainly mediated by the caloric content, and only somewhat by the macronutrient composition, of the meal. Likewise, habitual breakfast consumption was reported to decrease concentrations of CTX (-17.8%), P1NP (-3.8%) and OC (-4.1%) at 1 h post-meal compared to fasting concentrations (Clowes et al., 2002). The authors hypothesised this difference in change between resorption and formation markers to be related to different clearance rates. However, the exact mechanisms remain unknown (Clowes et al., 2002). Another emerging theory provides a possible link between gastrointestinal hormones, incretins, and bone health. According to this concept, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) – incretins that are stimulated and released in response to (especially glucose) feeding – act on osteoblasts and osteoclasts to induce bone formation and suppress bone resorption (Mabilleau, 2016). Although the exact mechanisms are not known, reports of lower bone mineral mass in animal studies utilising double incretin knock-out models, as well as reports of lower BMD in perimenopausal women with a single-nucleotide polymorphism resulting in decreased GIP receptor activity, suggest the absence of both GIP and GLP-1 is associated with decreased bone mineral mass and impaired microstructure (Mabilleau, 2016).

Effects of other dietary factors on bone metabolism. Other dietary factors such as vitamin D, calcium and protein are also important in overall bone health and have the ability to support or suppress bone formation (Ihle & Loucks, 2004; Ogan & Pritchett, 2013; Sale et al., 2015). The consumption of dairy products (Thorning et al., 2016) or calcium/vitamin-D fortified products (Whiting et al., 2016) has been shown to have beneficial effects on bone density. In addition, single nutrients such as protein are possibly beneficial for bone health (Mangano et al., 2014a) by increasing intestinal calcium absorption (Pasiakos, 2015). On the contrary, there is a theory that a high acidity of the diet would increase the release of calcium from the bone, thereby leading to poor bone health. This idea is supported by some studies in the elderly and in the presence of low dietary calcium intakes (Mangano et al., 2014b). On the contrary, other studies have suggested that at least in healthy, younger humans, the kidneys are able to buffer the acid load enough to prevent deleterious effects on the bone (Garcia et al., 2015; Frassetto et al., 2018). Of course, the pattern and amount of dietary protein intake may also assist with muscle protein synthesis; and may play a permissive role in the synthesis of the bone scaffolding which is largely made from collagen protein. The mechanisms and actions of these and many other nutrients in bone health are beyond the scope of the current work. Instead, the next paragraphs will focus on the interactions between bone and low energy or CHO availability around an exercise setting and/or in exercising individuals.

2.4.4 Low energy availability and bone metabolism

RED-S affects bone health both by direct effects on bone modelling as well as indirect effects on reproductive hormones (Mountjoy et al., 2018). Indeed, when the hypothalamus senses that EA is less than what is required for optimal health, it signals the pituitary to decrease stimulus to the peripheral tissues/organs as a means to conserve energy. Therefore, suppression in the secretion of estrogen, testosterone, triiodothyronine (T3), leptin, insulin, and IGF-1, as well as an increase in cortisol, are usually seen within days (Figure 2.4). As mentioned earlier, these hormones are key regulators of bone modelling process and therefore, impairments in hormone concentrations are responsible for a suppression in bone anabolism (Ackerman & Misra, 2011).

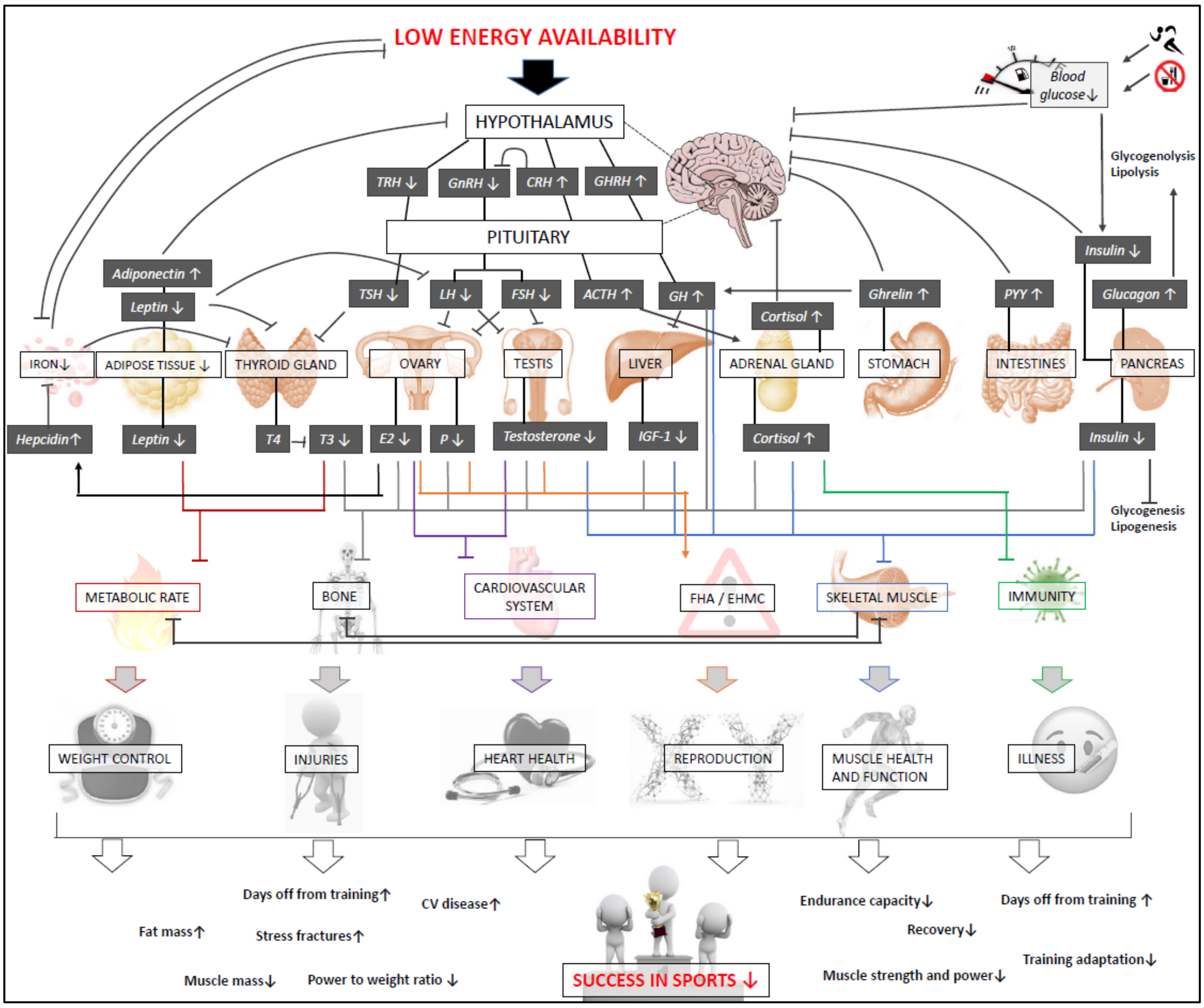

LOW ENERGY AVAILABILITY
Blood glucose↓
HYPOTHALAMUS
TRH↓
GnRH↓
CRH↑
GHRH↑
Glycogenolysis
Lipolysis
PITUITARY
Insulin↓
Adiponectin↑
Leptin↓
TSH↓
LH↓
FSH↓
ACTH↑
GH↑
Cortisol↑
Ghrelin↑
PYY↑
Glucagon↑
IRON↓
ADIPOSE TISSUE↓
THYROID GLAND
OVARY
TESTIS
LIVER
ADRENAL GLAND
STOMACH
INTESTINES
PANCREAS
Hepcidin↑
Leptin↓
T4
T3↓
E2↓
P↓
Testosterone↓
IGF-1↓
Cortisol↑
Insulin↓
Glycogenesis
Lipogenesis
METABOLIC RATE
BONE
CARDIOVASCULAR SYSTEM
FHA / EHMC
SKELETAL MUSCLE
IMMUNITY
WEIGHT CONTROL
INJURIES
HEART HEALTH
REPRODUCTION
MUSCLE HEALTH AND FUNCTION
ILLNESS
Days off from training↑
CV disease↑
Endurance capacity↓
Days off from training↑
Fat mass↑
Stress fractures↑
Recovery↓
Training adaptation↓
Muscle mass↓
Power to weight ratio↓
SUCCESS IN SPORTS↓
Muscle strength and power↓

Figure 2.4. Overview of the endocrine effects of low energy availability, that translate into impairments to bone health. *TRH*, thyroid-releasing hormone; *GnRH*, gonadotropin-releasing hormone; *CRH*, corticotropin-releasing hormone; *GHRH*, growth hormone-releasing hormone; *TSH*, thyroid-stimulating hormone; *LH*, luteinising hormone; *FSH*, follicle-stimulating hormone; *ACTH*, adrenocorticotropic hormone; *GH*, growth hormone; *T4*, thyroxine; *T3*, triiodothyronine; *E2*, estrogen; *P*, progesterone; *IGF-1*, insulin-like growth-factor-1; *PYY*, peptide YY; *FHA*, functional hypothalamic amenorrhea; *EHMC*, exercise hypogonadal male condition.

Acute effects of low EA on hormones. Over the last three decades, several excellent laboratory studies have investigated the effects of short-term (i.e. less than one week) exposure to low EA on reproductive and metabolic hormone concentrations. Most of these studies have been conducted by Prof. Anne Loucks and focused on healthy sedentary females. In these studies, EA has been decreased in a step-wise manner from the optimal 45 kcal·kg FFM·d^{-1} down to 30, 20 and 10 kcal·kg FFM·d^{-1}, to characterise the level at which perturbations to different body systems are seen. It was shown that while the fasting concentrations of P1CP (markers of bone formation), and 24 h mean concentrations of insulin and leptin declined linearly with decreasing EA (from 45 kcal·kg FFM·d^{-1}), the changes in the 24 h pulsatility of LH, 24 h mean glucose, and fasting concentrations of IGF-1, T3, OC occurred only after 30 kcal·kg FFM·d^{-1}. On the contrary, 24 h mean estrogen and urinary NTX (marker of bone resorption) did not decline until after EA decreased to 10 kcal·kg FFM·d^{-1} (Loucks & Thuma, 2003) (Ihle & Loucks, 2004). Despite differing thresholds, a generic level of low EA was defined as <30 kcal·kg FFM·d^{-1}.

The only study of deliberate exposure to low EA in male athletes reported reductions in fasting concentrations of leptin (~55%) and insulin (~36%) after 4 d of low (15 kcal·kg FFM·d^{-1}) or optimal EA (40 kcal·kg FFM·d^{-1}), while no changes were seen in testosterone or T3 concentrations (Koehler et al., 2016). Meanwhile, studies in male soldiers have shown large decreases in fasting concentrations of testosterone (-35%) and leptin (-67%) following only 5 d of extreme energy restriction (Gomez-Merino et al., 2002). In this study, male soldiers were reported to expend more than 5000 kcal·d^{-1} (total daily energy expenditure) while consuming only 3200 kcal·d^{-1}. This study did not report EA nor does it include information of participant BM or FFM. However, if we consider that the average sample male soldier was 80 kg with a body fat percentage of 15%, this would yield a FFM of 68 kg. With the use of the Cunningham equation, his resting metabolic rate would then be ~2000 kcal·d^{-1}, therefore, EEE would account for the remaining 3000 kcal·d^{-1}. Accordingly, EA can be estimated as (3200 kcal – 3000 kcal) / 68 kg = ~3 kcal·kg FFM·d^{-1} (i.e. extremely low EA). It should be noted that these studies were conducted largely in the field with free-living athletes and focused on changes to fasting concentrations of hormones; this contrasts with the studies of Anne Loucks on female athletes conducted in metabolic ward conditions to allow constant monitoring of study parameters and elegant control of diet and exercise. The pros and cons of such study designs include considerations of the importance of investigating mechanisms of change vs ecological validity. In summary, however, there is confusion both over the effect of sex on the interaction between low EA and hormonal function (are males more resilient to a reduction in EA?) and whether shorter periods of low EA that might occur within various strategies of periodisation of EA are detrimental to factors involved in bone health.

Since elite endurance athletes may target periodic reductions in EA to allow changes in body composition, it is important to consider the implications of this strategy for bone health. Indeed, rapid changes (within days) in hormones responsible for maintaining optimal bone health may in the long-term lead to poor BMD and increased risk of stress fractures. The time-course of the effects to take place (from hormonal changes to clear deteriorations in bone health) and reversal of these changes (with refeeding) remains unknown but is likely to depend on the magnitude and duration of energy deficiency, timing of meals with respect to exercise, and the age of an individual.

Acute effects of low EA on markers of bone modelling. In addition to the effects on the endocrine system, low EA has negative effects on bone modelling within less than one week. For example, 5 d of low EA (15 kcal·kg FFM·d^{-1}) decreased fasting concentrations of insulin and leptin as well as decreased fasting bone turnover ratio (P1NP:CTX) in eumenorrheic females but not in males when compared to high EA (45 kcal·kg FFM·d^{-1}) in a study by Papageorgiou et al. (2017). In addition, in healthy sedentary females an EA of 10 kcal·kg FFM·d^{-1} for 5 d increased bone resorption (NTX), while formation (P1NP, OC) decreased linearly when EA decreased below 30 kcal·kg FFM·d^{-1} (Ihle & Loucks, 2004). In male athletes, 3 d of restricted EI (-50% of energy requirements) lead to a decrease in fasting P1NP concentrations as opposed to 3 d of balanced EI (100% of energy requirements) (Zanker & Swaine, 2000). Meanwhile, active females went through 3 d of low EA (15 kcal·kg FFM·d^{-1} for 5 d) achieved by diet (reduced EI) or exercise (increased EEE), it was shown that diet-induced low EA reduced fasting concentrations of bone formation (P1NP), while no effects were shown subsequent to running induced low EA (Papageorgiou et al., 2018). Regardless of these differences, the fasting concentrations of metabolic hormones reduced after both conditions (IGF-1 between -13 and -23%, leptin between -59 and -61%, T3 -15% in diet-induced low EA only). Therefore, as there appears to be a shift towards an imbalance in bone modelling markers (i.e. increased breakdown and reduced formation) measured in the overnight-fasted state after a couple of days of low EA, it is likely that in the long-term, these changes lead to a decrease in BMD and an increased risk for stress fractures. Unfortunately, there is a large gap in the literature with regards to time-course of adaptations (and de-adaptations) in reproductive and metabolic hormone concentrations and bone modelling markers, as well as their functional outcomes, in response to low EA. Indeed, future studies are needed to determine how long and how low of an EA is detrimental to long-term bone health. Importantly, most of the studies around low EA have implemented a large sudden drop in EA (usually from 45 to 15-30 kcal·kg FFM·d^{-1} – in practice this can represent a deficit of ~1000 kcal·d^{-1}!) over a short time period (less than one week). Therefore, these study designs fail to reflect the real-life weight-loss strategies, where gradual weight loss is targeted via very modest (250 to 500 kcal), step-wise caloric deficits per day, usually recommended for and implemented by the athletes over a prolonged time period (i.e. several weeks or months) (Helms et al., 2014; Garthe et al., 2011).

Chronic effects of low EA on bone. Literature on the effects of chronic effects of low EA on bone relies mostly on studies that have examined the prevalence of low BMD and/or stress fractures among athletes who appear to suffer from low EA. These studies have shown that female and male athletes with endocrine signs of low EA have a higher risk for bone stress injuries (Heikura et al., 2018; Ackerman et al., 2015). Furthermore, studies have shown that both females and males show higher rates of stress fractures or low BMD with increasing number of risk factors, or surrogates of low EA, including amenorrhea (females), low BMI, participation in leanness sports, dietary restriction or appreciation of thinness, and high volumes of exercise. For example, female athletes with a moderate or high risk score according to a risk assessment tool associated with the Female Athlete Triad (Joy et al., 2014) were two to four times as likely to sustain a stress fracture compared to athletes with low risk scores (Tenforde et al., 2017). In addition, exercising women who were energy and estrogen deficient had lower fasting levels of bone formation and higher levels of 24 h urinary markers of bone resorption compared to women who were energy and estrogen replete (De Souza et al., 2008). Energy deficiency was related to suppression of OC, while females experiencing estrogen deficiency had lower spine BMD compared to female who were estrogen replete.

Meanwhile, 43% of the male adolescent runners who presented with two of the four risk factors (BMI <18.5 kg/m^2, running >48 km·wk^{-1}, a previous stress fracture, and/or less than 1 serve of calcium rich food per day) suffered from low BMD (Barrack et al., 2017). Tam and colleagues (2018) reported that six (40%) of a cohort of 15 elite Kenyan male runners experienced low BMD, hypothesised to be a consequence of low energy intakes that fail to fuel the high training loads. A few studies exist on longitudinal monitoring of markers of bone modelling in elite endurance athletes. In a study of professional male cyclists across a 3-week stage race, Lombardi et al. (2012) measured fasting concentrations of blood hormones (testosterone, cortisol, leptin and adiponectin) and markers of bone modelling (CTX, P1NP, undercarboxylated OC) before, on day 12 and after a 3-week stage race. They reported that professional cyclists showed signs of low EA (decrease in BM) along with increased bone breakdown at the end of the 3-week period. Meanwhile, in a similar study by Grasso et al. (2015), a 3-week cycling stage race induced increased bone breakdown shown by increased plasma concentrations of sclerostin. Further research is needed especially on the interrelationship between low EA and male athlete health. However, longer-term low EA is very likely to have negative effects on bone density and bone injury rates in elite endurance athletes.

2.4.5 Low CHO availability and bone metabolism

Acute low CHO availability and bone. As previously discussed (section 2.3.4), a current popular theme in sports nutrition is the periodic integration of workouts with low CHO availability into the training program to amplify metabolic adaptations in the muscle for performance improvements. However, in recognition that any diet/training strategy may have both helpful and harmful outcomes, it is important to consider the wider implications of periodization of nutrition involving acute exposure to low CHO availability so that elements such as the frequency and timing of its inclusion or the characteristics of the athlete can be included in decision making. In fact, there is a hypothesis and some evidence that low CHO availability, independently of EA, may affect bone modelling via a complex pathway involving interaction between IL-6 and osteoclasts (Sale et al., 2015). According to the theory, some scenarios of low CHO availability stimulate an increased IL-6 response to exercise. Indeed, this was observed when a running session was undertaken after prior depletion of muscle glycogen, possibly as a consequence of gluconeogenesis in the liver (Badenhorst et al., 2015). Meanwhile, CHO ingestion before exercise was shown to attenuate the exercise-induced rise in IL-6 in another

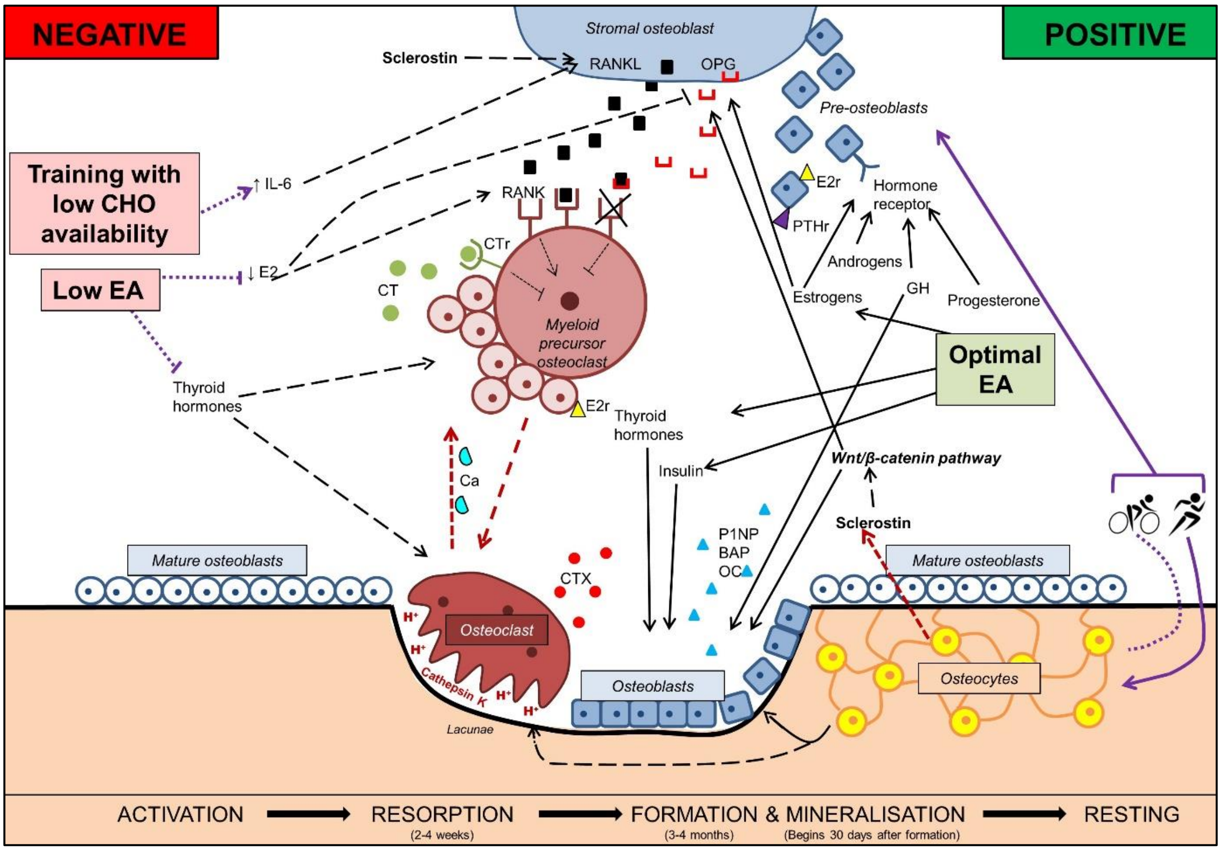

NEGATIVE
POSITIVE
Stromal osteoblast
Sclerostin
RANKL
OPG
Pre-osteoblasts
Training with low CHO availability
↑ IL-6
↓ E2
Low EA
RANK
CTr
CT
E2r
Hormone receptor
PTHr
Androgens
Estrogens
GH
Progesterone
Myeloid precursor osteoclast
Optimal EA
Thyroid hormones
E2r
Thyroid hormones
Insulin
Wnt/β-catenin pathway
Ca
Sclerostin
P1NP
BAP
OC
CTX
Mature osteoblasts
Mature osteoblasts
Osteoclast
H+
Cathepsin K
Osteoblasts
Osteocytes
Lacunae
ACTIVATION
RESORPTION
(2-4 weeks)
FORMATION & MINERALISATION
(3-4 months)
(Begins 30 days after formation)
RESTING

Figure 2.5. Effects mediated by low energy availability (EA) or low CHO availability on bone modelling process. *RANK*, receptor activator of nuclear factor κB; *RANKL*, receptor activator of nuclear factor κB ligand; *OPG*, osteoprotegerin; *E2*, estrogen; *PTH*, parathyroid hormone; *IL-6*, interleukin-6; *CTX*, c-terminal telopeptide of type 1 collagen; *P1NP*; procollagen type 1 N propeptide; *BAP*, bone alkaline phosphatase; *OC*, osteocalcin; *GH*, growth hormone; *CT*, calcitonin. Purple arrows represent effects of nutrition or exercise on bone metabolism. Black arrows represent stimulation. Black lines with a stop at the end represent impairment of a function. Figure compiled based on Lombardi et al. (2016), Banfi et al. (2010), Qin et al. (2013) and Kini & Nandeesh (2012).

investigation (Nieman et al., 1998). Previous studies have shown IL-6 to be linked to osteoclastogenesis and bone resorption (Kwan Tat et al., 2004; Lombardi et al., 2016), providing a possible EA-independent link between CHO availability and bone health. Figure 2.5 presents an overview of these mechanisms.

Evidence of the effects of other strategies such as acute CHO restriction (such as fasted training or CHO restriction in the acute recovery period after exercise) around exercise interventions is limited. However, CHO ingestion in close proximity to exercise may be bone-protective, possibly attenuating negative changes in bone modelling during exercise (de Sousa et al., 2014), or in short-term recovery (Sale et al., 2015). For example, de Sousa and colleagues (2014) measured markers of bone modelling in 24 elite male runners when CHO (a 7% maltodextrin solution) was ingested before, during and immediately after a 10 x 800 m interval session and at 60 min post-exercise (1.2 $g\cdot kg^{-1}$ CHO) compared to a control condition (water only). The concentrations of PTH, OC, P1NP and CTX were collected at baseline and after the 8 d training block (fasting samples) as well as immediately and 80 min after the interval session. CHO ingestion suppressed the increase in bone breakdown (CTX-1) compared to receiving a placebo around the session (de Sousa et al., 2014). However, no difference was found in markers of bone formation (P1NP) between trials. Furthermore, CHO supplementation before, during and after exercise across an 8 d high load running program (including 13 sessions) did not affect the resting levels of bone modelling on day 9 (i.e. no chronic effect was shown).

Meanwhile, Sale and colleagues (2015) investigated the acute effects of CHO feeding during 120 min of running on markers of bone modelling in 10 healthy active males. In this study, participants arrived in the laboratory in the overnight fasted state and completed the exercise session either in the fasted state (placebo), or with exogenous CHO ingestion (intervention) immediately before and throughout exercise. Venous blood was collected before exercise (fasted sample) and immediately, 60 min and 120 min after exercise as well as on three subsequent days (morning fasted samples) for analysis of CTX, P1NP, OC and IL-6. Ingestion of 0.7 $g\cdot kg\cdot h^{-1}$ CHO (~51 $g\cdot h^{-1}$) during running attenuated the acute (within 2 h post-exercise) CTX and P1NP responses to exercise compared to a placebo trial. In addition, concentrations of IL-6 were lower in the CHO trial compared to placebo and hypothesised by the authors to underpin the changes in bone modelling during exercise. However, no differences in bone markers were detected over the 3 follow-up days, suggesting that these effects are transient and quickly reversed. It remains unknown whether such short-term changes in bone modelling could, if repeated over time (e.g. for months), have an effect on bone health.

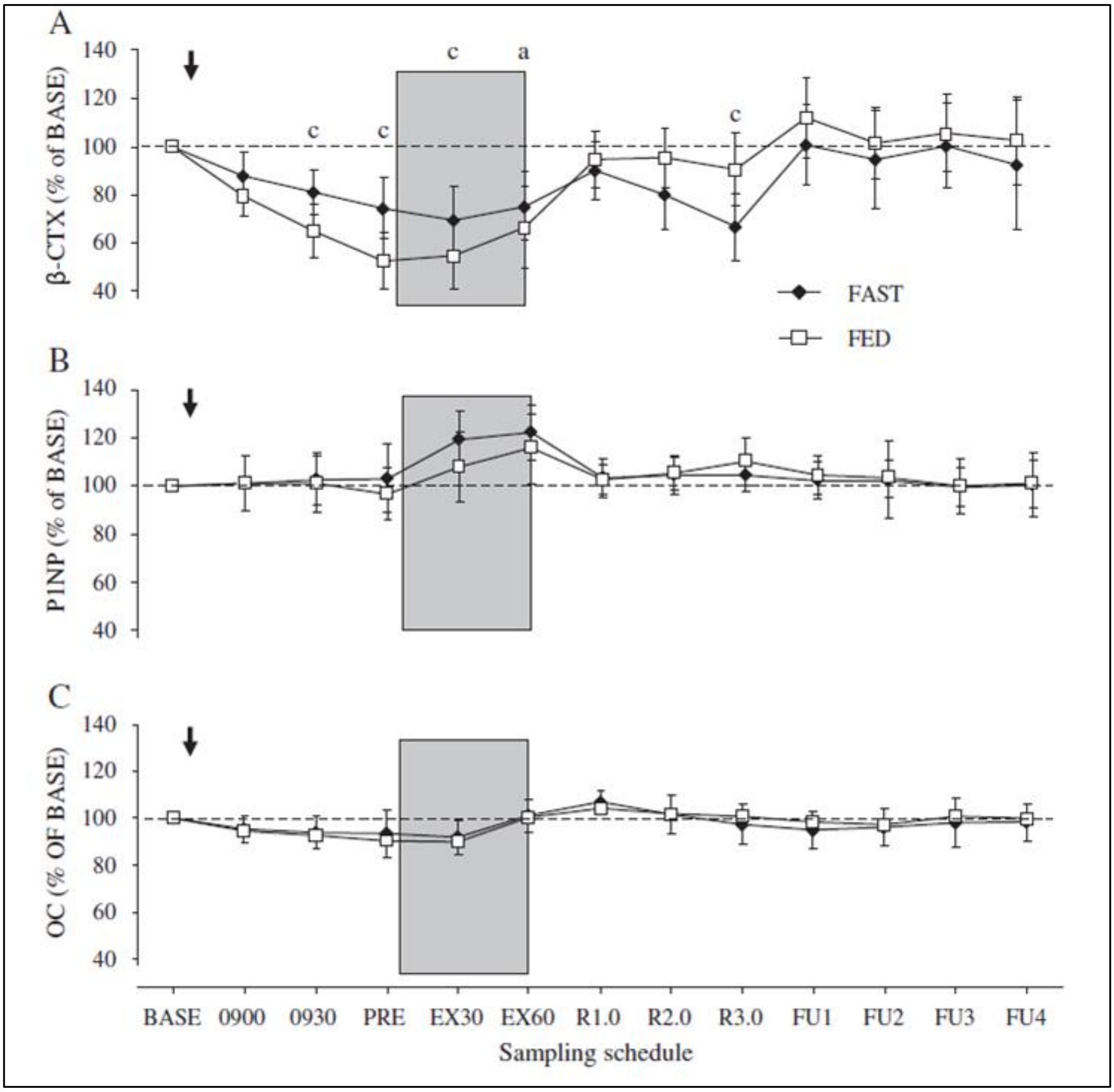


Figure 2.6. Concentrations of CTX, P1NP and OC during, 3h and 4d after exercise in the fed or fasted state. Values are expressed as the percentage of baseline. Open squares, fed state; filled diamonds, fasted state. Vertical arrow denotes meal provision in the fed trial. Grey box denotes exercise. Bone resorption (CTX) was markedly higher in the fasted vs fed trial during, but not after exercise. (Scott et al., 2012)

Similarly, completing an acute exercise session in the fed state as opposed to commencing exercise after an overnight fast attenuated the increase in bone resorption in 10 active males, though only slightly and in the acute recovery period only (Scott et al., 2012). In this study, the participants underwent an acute exercise and dietary intervention where 60 min of running at 65%VO_{2max} was completed with (2.3 MJ, 82 g CHO) or without breakfast. Markers of bone modelling were measured in the fasted state, in-between a meal and exercise session, throughout exercise and after 1, 2 and 3 h of recovery. While the concentrations of CTX increased acutely during exercise in fasted state, there was no difference between treatments in the following days (Figure 2.6). Meanwhile, P1NP showed no differences between groups. These findings suggest that a relatively easy session (i.e. low intensity, less than 1 h in duration) can be completed in the fasted state without complications to the bone. In support of this, we recently showed that a brief 4 day sleep low intervention (high-intensity run/cycle session in the evening of day 1 and 3, followed by CHO restriction and morning fasted low-intensity run/cycle on days 2 and 4) in elite female and male triathletes had no adverse effects on inflammatory response (IL-6 concentrations) to morning low intensity exercise (McKay et al., 2019). Therefore, it can be speculated that this type of acute low CHO availability intervention might not have adverse effects on the bone, at least via the IL-6 pathway.

It is important to note that most intervention studies on low EA and bone modelling markers have implemented matched macronutrient ratios (i.e. % of total energy intake; usually ~50-55% from CHO, 10-20% from protein and 30-35% from fats) for low and optimal EA treatments. Therefore, low EA treatments have often also created a low CHO availability intervention. For example, in Papageorgiou et al. (2017) CHO intakes in females and males were 154-206 $g \cdot d^{-1}$ (or ~2.6-2.8 $g \cdot kg \cdot d^{-1}$) and 302-398 $g \cdot d^{-1}$ (or ~5.0-5.5 $g \cdot kg \cdot d^{-1}$) for low and optimal EA treatments (15 and 45 $kcal \cdot kg\ FFM \cdot d^{-1}$), respectively. As the effects of low energy and low CHO availability become superimposed, it is impossible to determine whether the impairments seen to bone modelling with low EA are linked to a decrease in energy or CHO availability, or a combination of both. Indeed, a recent report by Kojima et al. (2019) showed that only 3 d of LEA (~19 $kcal \cdot kg\ FFM \cdot d^{-1}$) with a fixed macronutrient ratio (~55 % energy from CHO) resulted in significant reductions in muscle glycogen content (from 106.5 mmol/L to 76.7 mmol/L) compared to no change in high EA treatment (~53 $kcal \cdot kg\ FFM \cdot d^{-1}$). A recent study by Hammond et al. (2019) is the first to address this topic. In this investigation, the effects of 24 h of either high energy and high CHO availability (HCHO: ~12 g·kg·d-1 CHO and ~60 $kcal \cdot kg\ FFM \cdot d^{-1}$), low energy and low CHO (LCAL: ~3 $g \cdot kg \cdot d^{-1}$ CHO and ~20 $kcal \cdot kg\ FFM \cdot d^{-1}$) or high energy and low CHO (LCHF: ~3 $g \cdot kg \cdot d^{-1}$ CHO and ~60 $kcal \cdot kg\ FFM \cdot d^{-1}$) diets were investigated around an acute bout of twice-a-day endurance exercise. This study protocol provides a unique opportunity to investigate the independent effects of energy and CHO on bone modelling. The authors reported a lack of suppression in the concentrations of CTX around the second bout of high-intensity exercise with both LCHF and LCAL diets, as opposed to the HCHO diet. The study concluded that acute changes in bone modelling might be more reflective of alterations in dietary CHO, as opposed to energy, availability (Hammond et al., 2019). While these findings are certainly interesting, it is noteworthy that the study implemented an acute 24 h dietary intervention, therefore it does not allow investigation of longer-term effects of energy and CHO on bone. Furthermore, the LCHF diet implemented in Hammond et al. provided ~3 g·kg·d^{-1} CHO, and therefore, was not a strict ketogenic LCHF diet.

Finally, immediate feeding of CHO and protein after an intensive running exercise have been shown to suppress the increase in CTX, while increasing P1NP compared to delayed feeding (Townsend et al., 2017). In this study, healthy males completed a fasted treadmill run to exhaustion at 75% VO_{2max} where the exercise was followed by either: immediate feeding (1.5 g·kg^{-1} CHO and 0.5 g·kg^{-}1 protein at 0 h post-exercise and a non-caloric placebo at 2 h post-exercise); delayed feeding (a non-caloric placebo at 0 h post-exercise and 1.5 g·kg^{-1} CHO and 0.5 g·kg^{-}1 protein at 2 h post-exercise); placebo (a non-caloric placebo at 0 h and 2 h post-exercise). Blood was collected at 0, 1, 2, 3 and 4 h post-exercise as well as on the following morning (fasted sample) for analysis of CTX and P1NP. Immediate feeding suppressed the rise in CTX concentrations at 1 and 2 h post-exercise, whereas delayed feeding lead to the greatest suppression of CTX at 4 h post-exercise. The concentrations of P1NP at 4 h post-exercise were higher with immediate feeding compared to the other conditions. While the authors concluded that the immediate ingestion of a meal may be more beneficial for bone health compared to delayed feeding, it is important to note that at least with CTX, these effects were reversed at 4 h post-exercise, and that the overall effect on bone modelling appears to follow meal ingestion patterns. Therefore, and assuming both immediate and delayed feeding spent an equal amount of time (~2 h) with impaired bone modelling (unfortunately, no areas under curve were provided by these authors), it remains to be seen whether these differences between timing of the post-exercise meal consumption are meaningful for long-term bone health and whether the provision of a pre-exercise meal would have an effect on the bone modelling response with varying timing of post-exercise meal.

Chronic low CHO availability and bone. Another popular dietary approach advocated by some for endurance athletes is a long-term (several weeks to months and beyond) LCHF ketogenic diets, as described earlier in 2.3.4. While the effects of such diets on exercise metabolism appear fairly straight-forward, less attention has been given towards the health consequences of such diets. Indeed, recent research from our lab showed higher post-exercise interleukin-6 concentrations after a 3-week LCHF diet in elite athletes (McKay et al., 2019). If this inflammatory response were to translate into increased bone resorption, this might suggest overall negative effects of chronic CHO restriction on athlete bone health. In support of negative effects of LCHF on bone, 4 weeks of non-ketogenic (66% fat) or ketogenic (94% fat) LCHF diet impaired longitudinal growth, BMD, and mechanical properties in rats, possibly mediated by reductions in insulin-like growth-hormone 1 (IGF-1) (Bielohuby et al., 2010). Further animal studies have shown osteoporosis and delayed fracture healing after 12-20 weeks of non-ketogenic (60% fat) LCHF diet (Scheller et al., 2016), and significant bone loss and reduced biomechanical function in appendicular bones after 12-wk LCHF (Ding et al., 2019). In children, LCHF (6 months to up to 6.5 years) treatment for childhood epilepsy appears to increase bone loss (Bergqvist et al., 2008; Simm et al., 2017). Indeed, according to a recent report, children treated with ketogenic diet due to epilepsy showed a low BMD, and further decreases concomitant with an increase fracture risk during the diet (Draaisma et al., 2019). Meanwhile, limited research in adults indicates no change in bone modelling after 3 months (Carter et al., 2006) but decreased concentrations of bone anabolic hormones triiodothyronine (-16.3%), insulin (-22.2%) and IGF-1 (-20.2%) after 6 weeks of ketogenic LCHF (Urbain et al., 2017). In fact, a recent report of a 2-year open label treatment of Type 2 diabetes with a LCHF diet reported no change in spinal BMD over this period (Athinarayanan et al., 2019). Given the link between these hormones and bone health (as outlined earlier in section 2.4.4), more research is needed on the long-term effects of LCHF diets on bone health in elite athletes before these diets can be recommended for this population.

Overall, available evidence does suggest that alterations to CHO availability around exercise may have an (acute) effect on the markers of bone modelling, and that decisions to integrate training with low CHO availability into a periodised program should be undertaken with full awareness of potential deleterious effects to bone modelling, and if chronically applied, to bone health and ultimately, BMD. Indeed, it is important to note that while one nutrition strategy might be useful for metabolic/muscular adaptations, it might have different, or even opposite (adverse) effects on other body systems (bones and tendons). Available preliminary evidence in mainly non-athletic populations does suggest a link between chronic exposure to LCHF diets and impaired bone health markers, however further research is warranted especially in the athletic population. Should a negative association be found, athletes may need to prioritise (any) acute performance benefits against long-term training consistency and health, or to seek strategies around training with CHO availability to mitigate any impairment of bone modelling.

2.5 Summary and directions for future research

For decades, training programs of endurance athletes have been periodised to stimulate several physiological systems for optimal adaptation, recovery and performance outcomes. Meanwhile, until very recently, nutrition has received less attention as part of an athlete’s preparation process, and the periodisation of nutrition was not acknowledged or emphasised to the extent it currently is. Indeed, the most recent sports nutrition guidelines offer a dynamic understanding of periodised nutrient (and especially CHO) availability on various levels of periodised training based on individual goals and sport-specific performance determinants. For endurance athletes, these goals are (ideally) supported by dietary strategies to enhance adaptation to training (low CHO availability to stimulate cellular signalling pathways; high CHO availability to "train the gut"), strategic periods of periodic low EA to reach target body composition, as well as specific nutrition strategies to enhance race performance (purposeful and targeted high CHO availability, including CHO loading before the race day). While these guidelines are clear and based on profound evidence, current literature lacks systematic information on the knowledge and implementation of these dietary strategies around different types of exercise sessions, over a range of training days, and between training phases by a more representative (i.e. larger cohort) analysis of world class endurance athletes. Such data could be useful in planning nutrition education for the athletes as well as guiding future research.

Bone health is of particular importance to endurance athletes since there is a high risk of low BMD and stress fractures in many cohorts, including runners and cyclists. While there is robust evidence on the role of chronic (months to years) exposure to low EA as a dietary risk factor for poor bone health, the effects of acute (within-day and around exercise) and/or short-term (a couple of weeks) exposure on disturbances of bone modelling require further investigation. Indeed, available evidence suggests that three to five days of low EA (<30 kcal·kg FFM·d^{-1}; depending on daily EI and EEE, this can be ~1000 kcal deficit per day) has deleterious effects on hormones that support bone health and on bone modelling itself, at least in sedentary or moderately active females. Meanwhile, acute exercise in the fasted state or without additional CHO intake during exercise has been shown to have EA-independent effects on bone, whereby bone resorption is higher when exercising without as opposed to with CHO. The mechanisms for the effects of CHO restriction on bone may be mediated by IL-6, which stimulates bone breakdown. Whether these findings are present in highly trained elite athletes, and whether the length of exposure to low CHO availability has an interactive effect, is unknown. Since the prevalence of low EA and the popularity of extreme dietary interventions including the LCHF have increased in recent years, more research is needed on the acute effects of low EA and on the chronic effects of CHO restriction on bone health in elite endurance athletes. Such information is required to allow athletes to make considered decisions around the incorporation, deliberately or accidentally, of workouts or training cycles undertaken with low energy and/or CHO availability as part of a periodised training program.

The experimental chapters in this thesis address several knowledge gaps that have been identified in the literature review. The results from these studies provide novel insights into: (1) Self-reported dietary periodisation in world-class endurance athletes across macro-, meso- and micro-cycles of training and competition; (2) Day-by-day periodisation of energy and CHO availability in professional cyclists across a series of single-day races and concomitant effects of these strategies on physique and endocrine system; (3) The effects of a 3.5-week LCHF diet and acute CHO feeding on bone modelling in world-class race walkers; (4) The effects of 5 d of low energy availability and moderate CHO availability (LEA diet) vs high energy and high CHO availability (optimal EA diet) vs high energy and low CHO availability (LCHF diet) on bone modelling in world-class race walkers.

3 METHODOLOGY AND DESIGN

In keeping with Australian Catholic University guidelines, the methods utilised within each study of this thesis are described in full in the current chapter. In chapters four, five, six and seven, the methods section for each study is written as per the guidelines of the respective journal.

3.1 Study 1

Self-reported periodization of nutrition in elite female and male runners and race walkers

3.1.1 Study design & Participants

Based on a pilot study which tested a survey instrument to gather self-reported information on the manipulation of nutrition practices (Heikura et al., 2017b) we developed an online tool consisting of variously themed questions around dietary micro-, meso- and macro-periodisation across the various annual training phases. Along with strategic changes to the survey, gained from insights from our pilot study, the goal of the online tool was to capture data on a larger (target = 100) and more internationally representative group of athletes. We recruited elite female and male middle/distance athletes using online advertisements as well as direct contacts (via email or word-of-mouth) to athletes, coaches, applied sports practitioners and national sporting organizations (in Canada, US, Australia, Japan and Finland). To be included in the study, the athletes needed to be ≥18 years of age, currently and actively racing in the middle (800m, 1500m), distance (3000m-10000m) or road (half-marathon/marathon, 20km/50km race walk) events under the International Association of Athletics Federations (IAAF) and have a personal best of ≥1043 IAAF points (this corresponds to a 5000m time of 13:47.26 and 16:03.84 in males and females, respectively). Recruitment and completion of the surveys were completed between February 8 and May 21, 2018. The Ethics Committee of Australian Catholic University approved the study protocol (ethics approval # 2017-324E) which conformed to the Declaration of Helsinki.

3.1.2 The survey

The survey consisted of an updated version of our pilot study based on our reflections on the responses from the original cohort and additional feedback from colleagues and athletes. Whereas the pilot survey included a total of 29 questions (7 main questions and 22 sub-questions), the updated survey was expanded to include a total of 59 questions (19 questions on training/racing characteristics, plus 13 main and 27 sub-questions around nutritional practices). The final version of the survey was built online using SurveyGizmo (Boulder, Colorado, USA, 2017). Skip logic was used, building a custom path through the questions according to the respondent's answers, for an improved participation experience (less confusion) and efficiency (less time to complete the survey).

The survey was completed anonymously, and an informed consent (a prerequisite for completing the survey) was completed as part of the online survey by all participants. The first part of the survey included background information, instructions, and general subject information [gender, year of birth, country of birth, country of residence, country of representation, primary and secondary events and personal bests in those events, as well as self-assessment of highest level of achievement as senior athlete (Medallist at the World Championships or Olympic Games; Finalist at the World Championships or Olympic Games; Qualifier at the World Championships or Olympic Games; International level competitor; National Championship medallist; National championship participant]. Thereafter, the athlete was asked to choose one of the two annual training periodization programs [track (e.g. 800m-10000m) vs road (e.g. the marathon and race walks)] that best reflected his/her yearly program. Questions approached annual (macro) periodization of nutrition as a whole (Part A: general principles of annual training/competition diet) and as typically defined separate periodised training phases (meso-cycles): Part B: Base / endurance training phase, Part C: Main competition season (i.e. several months in duration: track athletes) or preparation for competition (i.e. one or more weeks in duration: road athletes), and Part D: Nutrition immediately before and on race day. Part A also included questions on general nutrition principles (e.g. vegetarian, paleo, very high energy, low carb high fat, gluten free) in the overall diet or during specific time periods (e.g. altitude training or during return from illness/injury). Additionally, parts B, C and D asked questions on training volume, key session and race frequency as well as number of race peaks. Across the survey and throughout this manuscript, hard training days were defined as "high volume and/or intensity days" and key sessions as "high intensity and/or high duration (>90 min) sessions or serious gym sessions". Fuelling was defined as eating foods (CHO foods, protein foods, sports foods, etc.) before training. Fasted training was defined as "training first thing in the morning without having eaten any food or consumed any other carbohydrates, or training later in the day without having eaten any carbohydrates for at least 8 h prior". Within the survey, reminders of these terminology were included within each question that targeted nutrition in relation to these themes. Finally, the survey ended with an open, but optional, comment box.

It is important to note that the survey was purposely constructed to apply to the culture, practices and terminology used in endurance events within the sport of track and field. As such it is not directly applicable to other sports, and if used for other populations, even among endurance sports, it will need to be customised to the specific characteristics of these sports. A sample survey has been provided as online supplementary material on Frontiers website.

3.1.3 Data management and statistical analysis

The data were checked and cleaned by excluding duplicate responses (i.e. two responses from the same individual), responses that were clearly false or confusing, and responses from those that did not satisfy the requirement of ≥1043 IAAF points. The answers were classified into clusters for further analysis using groupings based on sex (male vs female), distance (middle distance (MidD; 800 m, 1500 m) vs track distance (TrackD; 3,000 m steeplechase to 10,000 m) vs road distance (RoadD; marathon, race walks)), sex-based training volume groupings based on within sex tertiles of the entire data set (Females: low: ≤100 km/wk, moderate: 101–129 km/wk, high ≥130 km/wk; Males: low: ≤119 km/wk, moderate: 120–155 km/wk, high ≥156 km/wk) and athlete calibre (High: major championship (Olympics or World Championships) medallist, finalist and/or qualifier; Lower: the rest of the data set).

Data were first organised using Microsoft Excel, while further statistical analyses were conducted using SPSS Statistics 22 software (INM, Armonk, New York, USA). Data were presented as means ± standard deviations (SD) and number (n) and percentage (%) of responses. Normality of continuous data was checked with the Shapiro-Wilk goodness-of-fit test. Student's t-test for independent samples was used to test for differences in age, training volume and IAAF scores between subgroups. For YES/NO answers, % was calculated from the n of the total sample; for sub-questions, % was calculated from the remaining n resulting after the main initial question. Chi-square test ($\chi 2$) for independence with Yates Continuity Correction along with phi effect size statistic were used to test for differences between subgroups. Where more than two subgroups were present, Kruskal-Wallis Test was used as a post hoc test. Across the paper, statistical significance is shown when $p \leq 0.05$. In addition to numerical outcomes, relevant quotes provided by athletes (in cursive) were embedded in the results section to provide further qualitative insights into the topic in question. To aid in the interpretation of results from the lengthy survey, aggregation of consistent outcomes across the survey was undertaken by all authors upon visual inspection of figures to develop a series of themes.

3.2 Study 2

Alternate-Day Low Energy Availability During Spring Classics in Professional Cyclists

3.2.1 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 characterise the current fitness level, maximal mean power (MMP) over 1, 5 and 20 minutes of racing was 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) (Quod et al., 2010).

The study protocol was built around an 8 d 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.

3.2.2 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).

Baseline skinfold thickness was measured 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, adequate hydration throughout the day). Post-study skinfold thickness was measured 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 and an ISAK accredited level 1 anthropometrist completed the measurements according to the ISAK guidelines (Marfell-Jones et al., 2012). Body fat percentage and FFM were estimated from predicted body density calculations using the Durnin & Womersley equation (Durnin & Womersley, 1974).

Morning BM was measured after emptying the bladder, in standardised conditions, with a calibrated scale (to the nearest 0.1 kg), before consumption of food/drinks. Morning urine samples were collected in the morning upon wakening (mid-stream) and analysed for USG by using a hand-held refractometer (Exacta and Optech, San Prospero, Modena, Italy). Due to confusion between team doctor and the researchers, urine samples were missed on the mornings of days 2 and 3.

After conversations with the cyclists and team staff, we abandoned the original goal of measuring pre/post-race BM to reflect acute changes in hydration status over the course of the race. Indeed, the usefulness and accuracy of this measure seemed very questionable due to the following factors: 1. Interference with the 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 had the potential to influence BM changes or their accuracy in reflecting fluid balance assessments during the races, including: (a) Change in the amount of clothing and/or rain that would affect the weight of clothing, (b) Unknown amounts of body fluid losses due to urination, (c) Reliance on estimates rather than measured amounts of fluid consumed during the races, and (d) Unknown changes in muscle glycogen stores during the races.

3.2.3 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). 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 following the best practice protocols:

Mechanical work (kJ) for each race = mean power output (MPO) * time (s)

Using a common value of 20.7% for Gross Efficiency (GE) for cyclists (Coyle et al., 1992), we thereafter estimated EEE as follows:

EEE = Mechanical work (kJ) * GE

Notably, an exception was undertaken for Cyclist 6, due to the lack of a powermeter on his bike; here, HR monitor was used to get an estimate of EEE.

Other acute challenges to the methodology were encountered during the races: Two cyclists suffered a crash during a race necessitating a change of bike within the event (cyclist 2 on day 6 after 178 km; cyclist 4 on day 4 after 146 km of racing), and loss of 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 $\text{kcal}\cdot\text{min}^{-1}$) during the early part of the race (total race EEE = EEE for the early part of the race + EEE ($\text{kcal}\cdot\text{min}^{-1}$) * min racing in the final part of the race).

Dietary intakes. The cyclists were able to freely choose the type, quantity and timing of food and drink consumption, with the exception of the timing of the main meals. 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 (IH and RC) with detailed recipes, which were entered in daily meal sheets to enable efficient recording at meal times. Two researchers (IH and RC) attended all meals and assisted with the weighing of all food and fluid consumed by cyclists using calibrated kitchen scales (to the nearest 1 g). Food and fluid intake was recorded using sheets that were individualised 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. 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 maintain accuracy of data entry and analysis, all dietary intake data was prosessed, entered into a software, and analysed, by one researcher (IH) with previous experience in dietary assessment across several studies (Heikura et al., 2017a,b; Heikura et al., 2018a,b; Heikura et al., 2020; Burke et al., 2017a; Mirtschin et al., 2018). 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 analysed for total daily energy and macronutrient intakes (absolute and relative) using a 24 h 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 1859 after the race but before the subsequent dinner on race day). In addition, carbohydrate and protein intakes within 3 h pre-race, during and 3 h post-race were calculated. Finally, the immediate 24 h post-race period was analysed for total carbohydrate intake to estimate whether cyclists met the guidelines for muscle glycogen replenishment following the race and in preparation for the next race.

Energy availability. Short-term EA was estimated based on dietary and training records following Loucks formula (Loucks et al., 2011) and calculated as dietary energy intake minus EEE, divided by FFM, with a cut-off 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 cut-off [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.

3.2.4 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 analysed using Student's t-tests for paired samples, while repeated-measures analysis of variance (ANOVA) was used to analyse 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 $g \cdot h^{-1}$ (Thomas et al., 2016).

3.3 Studies 3 and 4

Study 3: *A short-term ketogenic diet impairs markers of bone health in response to exercise*

Study 4: *Effects of low energy vs low CHO availability on markers of bone modelling at rest and during exercise in elite race walkers*

This section will merge the detailed methodology for studies 3 and 4 as these studies share identical methodology in terms of testing protocols used (preliminary testing and 25 km long walk protocol). Where methodology differences exists (dietary interventions and overall study design), the details have been provided separately and this has been noted in text with subtitles for *study 3* and *study 4*.

3.3.1 Participants

We recruited a total of 50 international world-class race walkers over 3 camps (*Study 3*: Supernova 1 and 2; *Study 4*: Supernova 4); six participants undertook two camps and four study experiences were incomplete due to inadequate sample volumes or injury; thus studies 3 (n = 32; 27 males and 5 females) and 4 (n = 20 males) have been comprised of a total of 52 trials with pre and post treatment data. The participants were expected to be trained at the start of the camp, i.e. to be able to complete the required training intensity and volume during the camp, and be free of injuries prior to the study. The study protocols were approved by the Ethics Committee of the Australian Institute of Sport (AIS) [ethics approval numbers 20150802 (Supernova 1), 20161201 (Supernova 2), and 20181203 (Supernova 4)]. The participants were fully informed about the study protocol prior to signing the informed consent form. All studies adhered to the Declaration of Helsinki.

3.3.2 Study design

Study 3. These two camps consisted of a 3.5-week dietary intervention where intensified training was completed with either a high CHO (HCHO) or a LCHF diet, with a subset continuing to a third test of acute CHO refeed (Figure 3.1). The interventions were preceded and followed by a testing block where the effects of dietary interventions on bone modelling were assessed around 19 km (females) or 25 km (males) long walk tests (Figure 3.1).

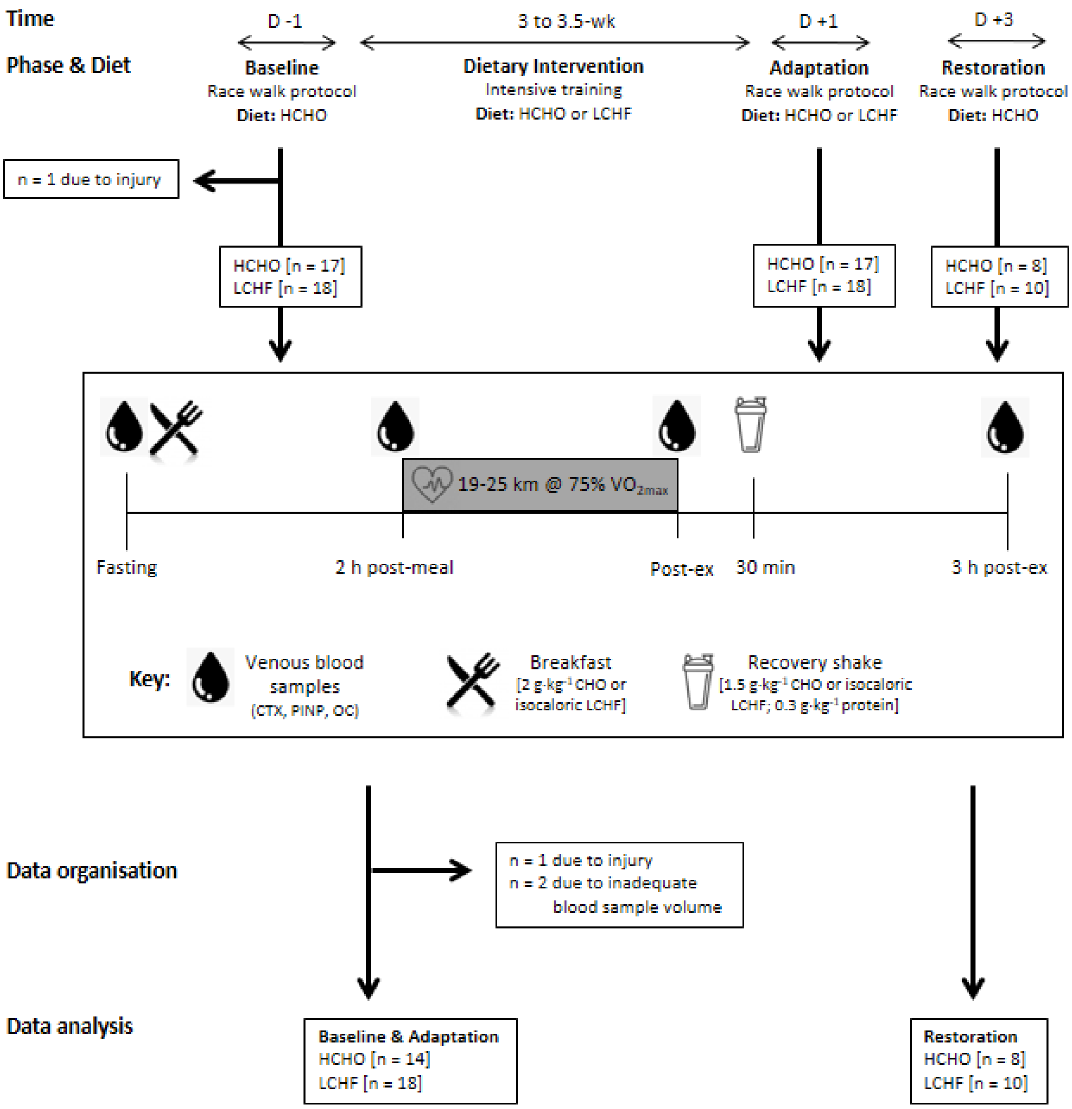

Time
Phase & Diet
D -1
Baseline
Race walk protocol
Diet: HCHO
3 to 3.5-wk
Dietary Intervention
Intensive training
Diet: HCHO or LCHF
D +1
Adaptation
Race walk protocol
Diet: HCHO or LCHF
D +3
Restoration
Race walk protocol
Diet: HCHO
n = 1 due to injury
HCHO [n = 17]
LCHF [n = 18]
HCHO [n = 17]
LCHF [n = 18]
HCHO [n = 8]
LCHF [n = 10]
19-25 km @ 75% VO2max
Fasting
2 h post-meal
Post-ex
30 min
3 h post-ex
Key:
Venous blood samples (CTX, PINP, OC)
Breakfast [2 g·kg-1 CHO or isocaloric LCHF]
Recovery shake [1.5 g·kg-1 CHO or isocaloric LCHF; 0.3 g·kg-1 protein]
Data organisation
n = 1 due to injury
n = 2 due to inadequate blood sample volume
Data analysis
Baseline & Adaptation
HCHO [n = 14]
LCHF [n = 18]
Restoration
HCHO [n = 8]
LCHF [n = 10]

Figure 3.1. Study 3 flowchart and overview. Thirty-two data sets were gathered from 30 participants who participated in one or more training camps. After *Baseline* testing on a carbohydrate-rich (HCHO) diet, they elected to follow a 3.5-week energy-matched dietary intervention of either HCHO or ketogenic low carbohydrate-high fat (LCHF) principles. After *Adaptation*, the participants underwent an acute period of *Restoration* of high carbohydrate availability. At *Baseline* and at the end (*Adaptation*) of this intervention, as well as after acute carbohydrate reintroduction (*Restoration*) they undertook a test block including a 19 (females) or 25 (males) km (~2 h) hybrid laboratory/field race walking protocol at ~75% VO_2max. Venous blood samples were collected after an overnight fast, 2 h after an energy-matched breakfast based on their diet (immediately pre-exercise), immediately post exercise and after 3 h of passive recovery during which an intervention-matched recovery shake was consumed at 30 min. Blood samples were analysed for C-terminal telopeptide of type I collagen (CTX), procollagen 1 N-terminal propeptide (P1NP) and osteocalcin (OC).

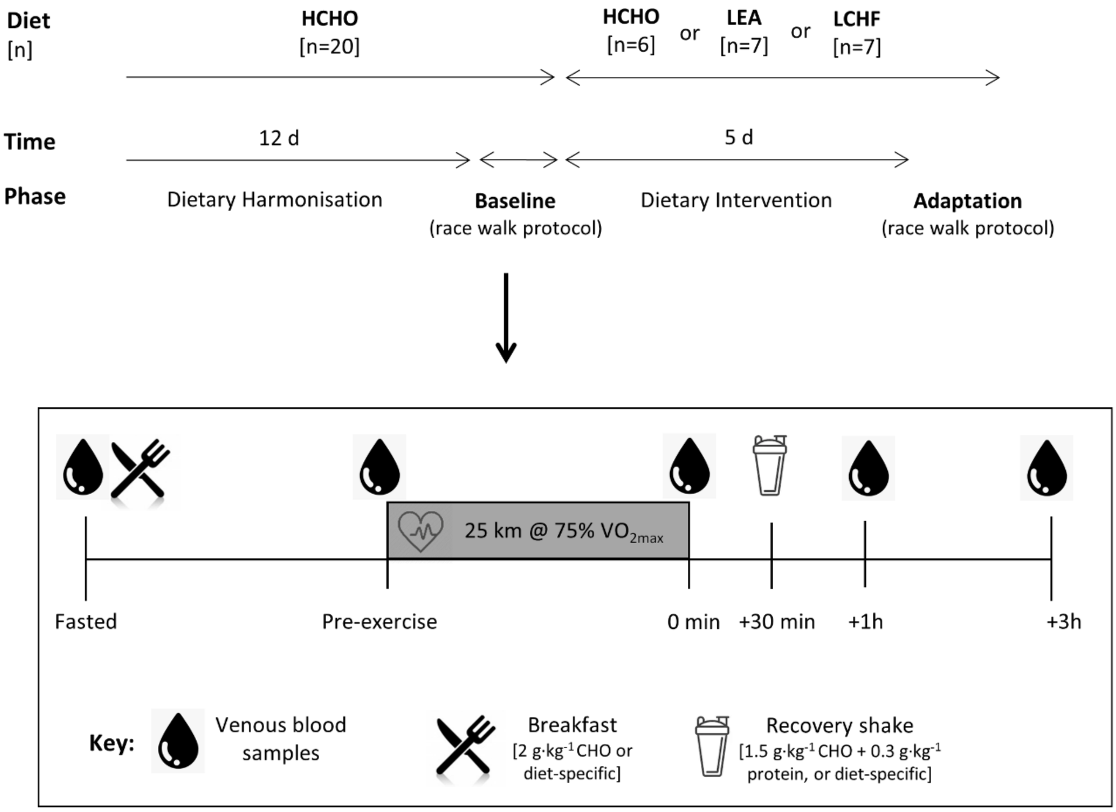

Diet
[n]
HCHO
[n=20]
HCHO
[n=6]
or
LEA
[n=7]
or
LCHF
[n=7]
Time
12 d
5 d
Phase
Dietary Harmonisation
Baseline
(race walk protocol)
Dietary Intervention
Adaptation
(race walk protocol)
25 km @ 75% VO2max
Fasted
Pre-exercise
0 min
+30 min
+1h
+3h
Key:
Venous blood samples
Breakfast
[2 g·kg-1 CHO or diet-specific]
Recovery shake
[1.5 g·kg-1 CHO + 0.3 g·kg-1 protein, or diet-specific]

Figure 3.2. Study 4 overview. After an initial controlled high energy availability (HCHO) period (Dietary standardisation), the participants were assigned to 5 d of either HCHO (high energy and carbohydrate availability), LEA (low energy but high carbohydrate availability) or LCHF (high energy but ketogenic low carbohydrate high fat diet). A standardised 25 km (~2 h) hybrid laboratory/field race walking protocol at ~75% VO_2max was completed at *Baseline* and after dietary interventions (*Adaptation*). On the morning of each test, the participants consumed a standardised diet-specific breakfast and rested for 2 h before commencing the exercise test. After exercise, the participants rested in the laboratory for 3 h, where a recovery shake was consumed at 30 min post-exercise. During *baseline* testing, all participants followed the same (HCHO) diet and received identical meals around the test. During *Adaptation*, diet-specific meals were consumed. Venous blood samples were collected at -120 min and immediately before (Pre-exercise), and immediately (0 min) and 1 and 3 h post-exercise for analysis of concentrations of C-terminal telopeptide of type I collagen (CTX), procollagen 1 N-terminal propeptide (P1NP), osteocalcin (OC) and undercarboxylated OC (Glu-OC).

Study 4. This camp consisted of three separate blocks of dietary standardisation [comprised of *screening* (5 d) and *harmonization* (7 d)] and *intervention* (5 d), with block- and dietary treatment-specific dietary targets (Figure 3.2). The interventions were preceded (*Baseline*) and followed (*Adaptation*) by a testing block where the effects of dietary interventions on bone modelling were assessed around 25-km long walk tests (Figure 3.2).

3.3.3 Preliminary testing

Measurement of FFM (Study 4). FFM was measured in an overnight fasted and hydrated state via a narrow fan-beam Dual-energy X-ray Absorptiometry (DXA, Lunar iDXA, GE Healthcare, USA) following the best practice principles (Nana et al., 2016). Testing was completed during *Screening*.

RMR measurement (Study 4). RMR was measured in an overnight fasted, rested state (Fullmer et al., 2015), during *Screening*. The participants were instructed to walk slowly to the laboratory on the morning of testing. The measurement was done using an indirect calorimetry where respiratory gases were collected into two 120 L gas-impermeable Douglas bags. Testing included a preliminary 10 min resting period, followed by a 15 min period to familiarise the participant with the mouthpiece, and finally two 10 min periods of gas collection via Douglas bags with a nose clip. The participants were instructed to stay rested but remain awake throughout the test protocol. The room was kept dark and quiet. The collection time was recorded to the nearest second for each bag. Respiratory gas concentration and volume were analysed using first principles methods as explained in detail by Woods et al. (2016). The first step included ~1 min analysis of a sample of air from both Douglas bags using AMETEK O_2 and CO_2 analysers (calibrated using two known gas concentrations of 3.989% O_2 and 16.57% CO_2, and 5.487% O_2 and 18.04% CO_2) for determination of fractions of gas and thereafter, the oxygen consumption. The remainder of the Douglas bag was then analysed for volume by emptying the contents of the bag into a water sealed 350 L Tissot Spirometer (Warren Collins, Braintree, MA) using an evacuation pump. Finally, VO_2 and VCO_2 were calculated using the Haldane transformation (Haugen et al., 2007) and converted to $kJ \cdot min^{-1}$ using the Weir equation. RMR was defined as the mean value of the two bags, and expressed as absolute (RMR_{24h}) and relative to FFM values. These values were utilised for calculations of EEE, as explained in detail below.

Incremental exercise test and EEE (Study 4). The incremental test was completed during *Screening*. The exercise test began at a speed of 11 or 12 $km \cdot h^{-1}$ and included a total of four stages. Each stage lasted 4 min and increased in speed by 1 $km \cdot h^{-1}$ per stage, with a 1 min rest between stages. Respiratory gases were collected for 60 sec at the end of each stage, with heart rate (HR) monitored throughout the test. Gas collection was done using a calibrated and custom-built indirect calorimetry system, described in detail by Robertson et al. (2010). EEE was estimated using the Weir equation (Weir, 1949) based on the gas exchange data plotted against speed ($3.94{*}VO_2 + 1.11{*}VCO_2$) which gives an estimate of EEE as $kcal \cdot min^{-1}$. Thereafter, we calculated estimated energy cost for 1 min of exercise excluding RMR for the same period [i.e. EEE (kcal·min^{-1}) – (RMR$_{24h}$ / 1440)]. For the purpose of the current study, where we expected to be required to make acute adjustments to within-day energy intake, a simplified method to prospectively estimate EEE was required. Therefore, we calculated EEE per km race walking at each speed of the treadmill test as follows:

$$EEE\ (kcal/km) = (EEE\ (kcal/min) * 60\,\text{min})\ \ / \, Speed\ (km/h\ )\ )$$

For the final spreadsheet, we averaged the energy cost of all four speeds into a single value that was subsequently used in our prospective calculations. As our participants were elite athletes, we assumed exercise training would account for the majority of daily activity-induced energy expenditure. Therefore, no calculations were made for non-exercise activity thermogenesis. In case of cross-training (biking or swimming) or gym sessions, metabolic equivalents (MET's) were used with a MET of 8 and 4 to estimate energy cost of cross-training and gym, respectively (Ainsworth et al., 2000). In the few occasions that athletes performed a running session, EEE was estimated as BM (kg) * distance (km) (Margaria et al., 1963), excluding RMR for the duration of exercise.

Although this simplified method of estimating EEE was chosen, for the purpose of this paper, we have retrospectively analysed our data using a few alternative approaches, including HR regression (Tomten & Høstmark, 2006) and HR monitor estimates of EEE (a case comparison of those athletes that used HR monitors during the study) and a speed-specific approach where the EEE at the lowest speed of the economy test was used for all training except for high intensity sessions (hills, reps, races) for which the EEE of the highest speed of the economy test was applied in the calculations.

3.3.4 Dietary standardisation

Study 3. The dietary interventions followed a static, pre-determined and dietary treatment-specific daily energy and macronutrient target intake, as detailed below:

1. HCHO: 225 kJ·kg^{-1} for males, 200 kJ·kg^{-1} for females: 65% CHO, 15% protein, 20% fat, with CHO consumed before, during and after training to provide high CHO availability throughout the intervention (n=14).

2. LCHF: 225 kJ·kg^{-1} for males, 200 kJ·kg^{-1} for females: ~78% fat, 15% protein 5% CHO (<50 g·d^{-1}), with low carbohydrate, high fat foods consumed before, during and after training sessions (n=18).

Study 4. During *screening*, all participants followed a standardised diet consisting of a fixed amount of energy (220 $kJ \cdot kg^{-1}$), protein (2.1 $g \cdot kg^{-1}$), CHO (8.5 $g \cdot kg^{-1}$) and fat (1.3 $g \cdot kg^{-1}$) that was used to reflect their current dietary practices. As the screening block started as soon as the participants arrived on campus, it was not possible to design diets based on target EA (as no information on FFM or prospective training was available at this time). Instead, this block acted as a first step of dietary standardization where preliminary testing was undertaken (see 3.3.3) to measure exercise economy, RMR and body composition for subsequent calculations of EEE and EA.

During *harmonisation*, all participants followed a high EA (HCHO) diet with a target EA of 40 (training days), 35 [high volume (>35 $km \cdot d^{-1}$) days] or 45 (rest days) $kcal \cdot kg\ FFM \cdot d^{-1}$, where macronutrient targets were calculated as a percentage (%) of target energy intake (65% of energy from CHO, 15% of energy from protein, 20% of energy from fat). Throughout *harmonisation* and *intervention*, daily targets were adjusted on a day-by-day basis to account for changes in training volume (see section "Energy availability calculations and estimation of daily energy targets" below).

Following *harmonisation*, the participants were split into one of the three dietary treatments for the *intervention* block: HCHO (n = 6), low EA (LEA; n = 7), or LCHF (n = 7) (Figure 1). HCHO continued to follow targets from the harmonisation phase, with the exception that a high-volume day (and subsequent reductions in target EA) was now defined as >30 $km \cdot d^{-1}$ training (this applied to all three dietary treatments). LCHF followed the same target EA as HCHO, however with low CHO (0.5 $g \cdot kg^{-1}$), moderate protein (2.2 $g \cdot kg^{-1}$) and high fat [remainder (~80%) of target energy] intakes. Indeed, with LCHF intervention, dietary CHO and protein remained fixed while fat was adjusted upwards or downwards based on target energy requirements. The LEA treatment was assigned a target EA of 15 (training days), 10 (high volume days) or 20 (rest days) $kcal \cdot kg\ FFM \cdot d^{-1}$, where macronutrient targets were calculated as 60% of energy from CHO, 25% of energy from protein, 15% of energy from fat. Upon finishing their respective intervention diet, all participants completed post-intervention testing (*Adaptation*) with diet-specific nutrition around the tests.

EA calculations and estimation of daily energy targets (Study 4). To prospectively estimate target energy requirements, the following equation was used:

$$\textbf{\textit{Target EI}} = (\text{Target EA} * \text{FFM}) + \text{EEE (excluding RMR)}$$

where target EA was based on the phase of the study and dietary intervention (between 10 and 45 kcal·kg FFM^{-1}). Preliminary testing was completed during screening to gather information on FFM and EEE. These tests have been described in detail in section 3.3.3. Data accrued from this testing was used to create targets for the rest of the study period (i.e. no adjustments were made for changes in FFM or economy throughout the study as we expected these changes to be minimal). However, we adjusted EA and EEE calculations to new FFM retrospectively to compare these values to our chosen method.

To determine individual daily energy targets (and subsequently, macronutrient targets), the participants provided the research team with draft training programs for each week of the study. These plans were entered into individualised spreadsheets which utilised the equations above to create dietary treatment- and block-specific energy and macronutrient targets based on target EA and projected training volume for each day. The process of creating individual meal plans based on these targets has been reported in the next section. To ensure daily EA was maintained within target values, a member of the research team interviewed participants at lunch and dinner times each day throughout the study to check that daily training was completed according to the individual plan. In case of changes to training that exceeded the energy cost equivalent to 2 km of race walking (i.e. a change greater than between 112 and 169 kcal depending on individual – this equals ~ 2.4 kcal deviation from planned EA), a member of the research team adjusted that day's energy intakes to bring actual EA back to the target values. These "real-time" changes to the meal plans were made by reducing / adding (mainly) CHO containing food items to/from the meal plan (usually by modifying a "party bag" containing candy and chocolate).

Studies 3 and 4. The participants were allocated to the diet groups based on preferences to minimise the effect of belief on the efficacy of nutritional treatments. The diets were individualised based on BM and training volume by a group of sports dietitians and physiologists using FoodWorks Professional Edition 8 and 9 (Xyris Software, Brisbane, Australia). All meals and snacks were weighed and provided for the athletes on a daily basis, and recorded by the members of the staff. Any unfinished items were weighed and recorded. Actual dietary intakes were analysed using the same software as during menu planning. The athletes trained according to a fixed schedule which provided opportunities to choose between walking, running, or cross training. However, key training sessions required monitoring (blood metabolites, rating of perceived exertion, HR) and thus were obligatory for everyone.

3.3.5 Hybrid laboratory/field long walk test

The interventions were preceded and followed by a testing block where the effects of dietary interventions on bone modelling were assessed around a 19 (females) or 25 (males) km long walk test (Figures 3.1 and 3.2).

Study 3. Testing before (*Baseline*) and after (*Adaptation*) the intervention and upon acute CHO feeding (*Restoration*) involved a 25 km (males) or 19 km (females) long walk at around 75% VO_2max (Figure 3.1). Upon entering the laboratory in an overnight fasted and rested state, a cannula was inserted into an antecubital vein for collection of blood samples at rest (-120 min), immediately before exercise (Pre-exercise), immediately after exercise (0 min) and at 3 h post-exercise. Blood samples were analysed for concentrations of cross-linked C-terminal telopeptide of type I collagen (CTX), procollagen 1 N-terminal propeptide (P1NP), and osteocalcin (OC) to determine the effects of dietary interventions and exercise on bone modelling. To keep the vein patent, the cannulas were flushed with 5 ml of saline (NaCl) every 30 min throughout the trials. A standardised breakfast (2 $g \cdot kg^{-1}$ CHO for all groups during *Baseline* and *Restoration*, or an isocaloric low CHO option for LCHF during *Adaptation*) was consumed 30 min after the first blood sample, after which the participants rested for 120 min before beginning the session. The session was a combined laboratory and field protocol with regular glucose ingestion (60 $g \cdot h^{-1}$) throughout the test for HCHO (all groups at *Baseline* and *Restoration*, only HCHO at *Adaptation*), or isocaloric high fat snacks for the LCHF group at *Adaptation*. Upon completion of the exercise test, the participants rested in the laboratory for a further 3 h, and received a standardised recovery shake (1.5 $g \cdot kg^{-1}$ CHO for both groups during *Baseline* and *Restoration*, or an isocaloric low CHO option for LCHF during *Adaptation*; both shakes included 0.3 $g \cdot kg^{-1}$ protein) at 30 min post-exercise to improve satiety.

Study 4. Testing before (*Baseline*) and after (*Adaptation*) the intervention involved a 25 km long walk at around 75% VO_2max (Figure 3.2). Upon entering the laboratory in an overnight fasted and rested state, a cannula was inserted into an antecubital vein for collection of blood samples at rest (-120 min), immediately before exercise (Pre-exercise), immediately after exercise (0 min) and at 1 h and 3 h post-exercise. Blood samples were analysed for concentrations of cross-linked C-terminal telopeptide of type I collagen (CTX), procollagen 1 N-terminal propeptide (P1NP), undercarboxylated osteocalcin (Glu-OC) and osteocalcin (OC) to determine the effects of dietary interventions and exercise on bone modelling. The cannulas were flushed with 3 ml of saline (NaCl) every 30 min throughout the trials to maintain the vein patent. A standardised breakfast [2 $g \cdot kg^{-1}$ CHO for all groups during *Baseline*, and an isocaloric low CHO option for LCHF during *Adaptation* and a decreased energy availability (1 g·kg^{-1} CHO) option for LEA during *Adaptation*] was consumed 30 min after the first blood sample, after which the participants rested for 120 min before beginning the session. The session was a combined laboratory and field protocol with regular glucose ingestion (60 g·h^{-1}) throughout the test for HCHO (all groups at *Baseline,* HCHO only at *Adaptation*), or isocaloric high fat snacks for the LCHF group and 30 g·h^{-1} CHO for LEA at *Adaptation*. Upon completion of the exercise test, the participants rested in the laboratory for a further 3 h, and received a standardised recovery shake (1.5 g·kg^{-1} CHO for all groups during *Baseline*, meanwhile an isocaloric low CHO option for LCHF and 0.75 g·kg^{-1} CHO for LEA during *Adaptation*; both HCHO and LCHF shakes included 0.3 g·kg^{-1} protein, while LEA shake protein content was 0.15 g·kg^{-1}) at 30 min post-exercise to improve satiety.

3.3.6 Blood analysis

Blood was collected into a 3.5 ml EDTA BD Vacutainer Plus SST II tube for analysis of CTX, P1NP, OC and Glu-OC (*Study 4*). Blood was allowed to clot by standing at room temperature for 2 hours before centrifuging at 1000 G for 10 min. Serum was aliquoted into four 0.75 ml polypropylene tubes. Blood clotting, centrifuge and freeze time were kept consistent for all samples. Once aliquoted, the samples were immediately stored at -80°C and later used for measurement of CTX, P1NP, Glu-OC and OC.

Study 3. Analysis for CTX, P1NP and OC was undertaken by chemiluminescence on IDS-iSYS (Immunodiagnostic Systems Limited; Boldon, Tyne and Wear, UK). Inter-assay coefficient of variation was 6.2%, 4.6% and 6.1%, respectively.

Study 4. Analysis for CTX, P1NP, OC and Glu-OC was undertaken by enzyme-linked immunosorbent assays (ELISAs) supplied by Cloud-Clone Corp. (CTX: Katy, TX, USA), NovateinBio (P1NP: Woburn, MA, USA) and TaKaRa (OC and Glu-OC: Shiga, Japan). Inter-assay coefficient of variation was <12%, <10.6%, <2.4%, and <9.9% for CTX, P1NP, OC and Glu-OC, respectively.

3.3.7 Statistical analysis

Study 3. Statistical analyses were conducted using SPSS Statistics 22 software (INM, New York, USA) and R (R Core Team, 2018) with a significance level set at $p \leq 0.05$. Normality of data was checked with a Shapiro-Wilk test and visual inspection of residual plots. General Linear Mixed models were fitted using the R package lme4 (Bates et al., 2015) and included random intercepts for Subjects and Camps to account for baseline inter individual heterogeneity and the partial cross-over design. Because the estimated Camp effect variance was 0, this random intercept was subsequently removed to resolve boundary issues in the Restricted Maximum Likelihood estimation. P-values were obtained using Type II Wald F tests with Kenward-Roger degrees of freedom. Initial models included all possible interactions but non-significant interaction terms were dropped for ease of interpretation. Fasting values and exercise area under curve (AUC; Pre-exercise to 3 h post-exercise) for all markers were compared with a two-way mixed analysis of variance (ANOVA), with post hoc tests of Student's t-tests for independent samples (between-groups) and for paired samples (within-groups); where normality was violated, Wilcoxon's test and Mann-Whitney U-test were used. Data are presented as means (95% confidence intervals [CI]).

Study 4. Statistical analyses were conducted using SPSS Statistics 22 software (INM, New York, USA) with a significance level set at $p \leq 0.05$. Normality of data was checked with a Shapiro-Wilk test and visual inspection of residual plots. A three-way mixed ANOVA was used to compare differences in bone markers across exercise, between diets and between tests. Fasting values and exercise AUC (Pre-exercise to 3 h post-exercise) for all markers were compared with a two-way mixed ANOVA, with post hoc tests of Student's t-tests for independent samples (between-groups) and for paired samples (within-groups); where normality was violated, Wilcoxon's test and Mann-Whitney U-test were used. Data are presented as means (95% confidence intervals [CI]).

4 STUDY 1: SELF-REPORTED PERIODIZATION OF NUTRITION IN ELITE FEMALE AND MALE RUNNERS AND RACE WALKERS

Publication statement:

This chapter is comprised of the following paper published in *Frontiers in Physiology*.

Heikura, IA., Stellingwerff, T., & Burke, LM. (2018). Self-reported periodization of nutrition in elite female and male runners and race walkers. Front Physiol. Dec 3;9:1732. doi: 10.3389/fphys.2018.01732. eCollection 2018.

4.1. Abstract

Athletes should achieve event-specific physiological requirements through careful periodization of training, underpinned by individualized and targeted nutrition strategies. However, evidence of whether, and how, elite endurance athletes periodize nutrition is scarce. Accordingly, elite international female (n=67) and male (n=37) middle/long-distance athletes (IAAF score:1129±54, corresponds to 13:22.49 [males] and 15:17.93 [females] in the 5000m) completed an online survey (February-May 2018) examining self-reported practices of dietary periodization for micro (within/between-days), meso (weeks/months) and macro (across the year) contexts. Data are shown as the percentage of all athletes practicing a given strategy followed by the % of athletes reporting various beliefs or practices within this strategy. Differences according to sex, event (middle-distance [800m/1500m] vs track-distance [3000m-10000m] vs road-distance [marathon/race walks]), caliber (high [major championship qualifier] vs lower), and training volume (low/moderate/high male and female tertiles) were analyzed using Chi-square test or Kruskal-Wallis Test and indicated statistically different when $p \leq 0.05$. Most athletes reported eating more on hard training days (92%) and focusing on nutrition before (84%; carbohydrate intake [63%] and timing [58%]) and after (95%; protein goals [59%], timing [55%], carbohydrate goals [50%]) key sessions. Road-distance were the most (62% and 57%), and middle-distance the least (30% and 30%) likely to train fasted (p=0.037) or restrict carbohydrates periodically (p=0.050), respectively. Carbohydrate intake during training (58% of total) was more common in males (79%; p=0.004) and road-distance (90%; p<0.001) than females (53%) or middle/track-distance (48% and 37%). Most athletes (83%) reported following a specific diet before and during race day, with half of the athletes focusing on carbohydrates. Nearly all (97%) road-distance athletes reported following a during-race nutrition plan (carbohydrates/fluids:89%). Only 32% reported taking advice from a dietitian/nutritionist. Based on our analysis: 1) Road-distance athletes periodize carbohydrate availability while track/middle-distance avoid low carbohydrate availability; 2) Middle-distance runners emphasize physique goals to guide their nutrition strategies; 3) Females seem to be more cautious of increasing energy/carbohydrate intake; 4) Among all athletes, nutrition strategies are chosen primarily to improve performance, followed by reasons related to physique, adaptation and health outcomes. Overall, these athletes appear to possess good knowledge of nutrition for supporting training and competition performance.

4.2. Introduction

Despite decades of interest in the periodization of training, it is only recently that a holistic approach to periodization across a range of themes that affect competition preparation has been suggested (Mujika et al., 2018; Burke et al., 2018b; Stellingwerff et al., 2019). In fact, the concept of integrating a periodized nutrition plan within the annual training program was formally proposed in a previous expert panel around nutrition for track and field athletes by Stellingwerff et al., (2007a). The principles, practices and terminology around the periodization of nutrition have been summarized in several recent reviews (Burke et al., 2018b; Jeukendrup, 2017; Stellingwerff et al., 2019). The underlying theme is that strategic and targeted nutritional interventions can be used to augment the outcomes of the various specific training cycles [micro (within-day to days), meso (several weeks) and macro (months to years)]. Thus, decisions on periodization of nutrition should be preceded by a thorough examination and understanding of the sport-specific (general) determinants of success as well as athlete-specific (individual) performance gaps, with strategies (including nutrition interventions) to address the gaps being integrated into the periodized training program (Stellingwerff et al., 2019).

A variety of aspects of nutrition can be periodized in support of different training goals, ranging from fundamental issues such as energy intake through to the more specialized and “fine tuning” aspects of supplement use (Jeukendrup, 2017; Stellingwerff et al., 2019). From a macro perspective, energy intake needs to be manipulated across, and within, training days according to fluctuations in the energy cost of the athlete’s training program, as well as strategic integration of periods of alterations to energy balance to manipulate body mass/composition (Stellingwerff, 2019; Melin et al., 2019). Here, it is important to recognize that energy mismatches due to deliberate efforts to reduce body mass/fat content, or the failure to account for the energy cost of a heavy training loads for prolonged periods are likely to impair health and performance with both short- and long-term consequences (Mountjoy et al., 2018). At the other end of the spectrum, periodized use of supplements may range from the use of iron supplements to ensure adequate iron status during altitude training block (Mujika et al., 2018), to the use of performance aids such as caffeine, creatine, buffers (e.g. beta-alanine or bicarbonate) and nitrate/beetroot juice to practice intended competition strategies, or to provide support for targeted training sessions.

The periodization of macronutrients includes themes of meeting the specific fuel needs of training and competition sessions (micro-periodization: particularly in the case of carbohydrate [CHO], and perhaps, fat intake), practicing event nutrition strategies (meso-periodization:

particularly CHO intake during longer events) and providing both an additional stimulus and the building blocks needed to optimize the synthesis of new proteins as part of the adaptation to training (micro-, meso- and/or macro-periodization: e.g. protein intake). Current guidelines around protein intake for all types of athletes promote the regular intake of modest amounts (e.g. ~25 g every 3-4 hours) of high quality protein over the day, including soon after the completion of key training sessions (i.e. sessions of high intensity and/or high duration [>90 min]) (Phillips & Van Loon, 2011). Meanwhile, there may be advantages in increasing protein intake during periods of deliberate energy manipulation to achieve loss of body fat to assist with the maintenance of lean mass (Hector & Phillips, 2018). The amount and timing of CHO intake between and within days should track the substrate needs of training and events (Areta & Hopkins, 2018), particularly when it is important to optimize performance in competitions or key training sessions (Thomas et al., 2016). There may also be a need to undertake specific strategies to train the gut to tolerate increasing amounts of CHO and fluids during exercise (Cox et al., 2010; Jeukendrup, 2014), in preparation for race nutrition practices during prolonged events, particularly in hot environments, where intake during the event plays a major role in performance success. Taken together, there are numerous examples of macro-, meso- and micro-periodization of nutrition to optimize training adaptation and/or acute sport performance.

Although CHO intake is mostly considered in relation to its role as a key fuel for the muscle and brain, the application of molecular techniques to investigate the muscle response to exercise has created interest in the effects of low CHO availability on cellular signaling and enhanced adaptation to endurance training. According to various reviews (Bartlett et al., 2014; Hearris et al., 2018; Impey et al., 2018b; Philp et al., 2011), undertaking endurance exercise with an environment of low CHO availability during and/or after the session may upregulate the activity of key molecules in the adaptive responses to exercise, leading to an enhanced or prolonged adaptation period. Observations of increases in the acute response to exercise have led to studies of the chronic implementation of periodized CHO availability (i.e. integration of strategies of high CHO availability to "train hard" for optimized performance, and strategies of low CHO availability to "train smart" with enhanced adaptation) to test its effect on performance outcomes. It is important to note that it can be difficult to achieve the right balance between training quality and adaptation within a controlled laboratory study design (Hulston et al., 2010; Yeo et al., 2008). Accordingly, although a few studies have reported superior performance outcomes in cohorts of trained/well-trained individuals (Marquet et al., 2016a; Marquet et al., 2016b), the translation to elite athletes seems more difficult (Burke et al., 2017a; Gejl et al., 2017). Nevertheless, the strategy has been integrated by some elite athletes

(Stellingwerff, 2012) and recognized as an emerging concept in the most recent sports nutrition guidelines (Thomas et al., 2016). One challenge for athletes, coaches and sports scientists is understanding the meanings and nuances of different strategies to periodize CHO availability within training and competition preparation. However, this has been addressed in a recent commentary in which terminology, practices, mechanisms and evidence of different strategies have been summarized (Burke et al., 2018b).

In recognizing the value of a periodized approach to sports nutrition, current guidelines also promote the importance of individualization, which is dependent upon the specific performance demands of the sport/event and the unique athlete response to the intervention. Factors that may influence the individualized implementation of a specific nutrition strategy might include: 1) race distance (e.g. middle-distance athletes may not benefit from training the gut to consume liquids, while this is an important strategy for most road athletes); 2) event specific body composition norms; 3) training/event volume (e.g. higher volume training may require more emphasis on adequate energy/fuel availability); 4) training location (e.g. altitude / heat); and 5) time of the year (e.g. protocols to improve body composition and race performance might be emphasized closer to the competition season) (Stellingwerff, 2012; Stellingwerff et al., 2007; Melin et al., 2019; Stellingwerff et al., 2019). Therefore, it is expected that each athlete will have a unique and constantly changing periodized nutrition plan suited to their specific needs.

In parallel to the growing evidence base for the value of a periodized approach to nutrition, there is interest in understanding whether/how elite athletes practice these strategies within the real-world annual training/racing calendar. The available studies are limited to endurance and team sports athletes who have provided a snapshot of the micro- and meso-periodization of nutrition during training phases (Anderson et al., 2017a; Anderson et al., 2017b; Bradley et al., 2015; Burke et al., 2003; Heikura et al., 2017a; Naughton et al., 2016) or around competition (Stellingwerff, 2012; Stellingwerff, 2018).

More recently, we completed preliminary work to characterize self-reported approaches to periodization of nutrition over the annual training plan (Heikura et al., 2017b). Our pilot project captured an account of practices and the underlying rationale for nutritional periodization across the year (macro-periodization), with special consideration of various micro (between/within-day) and meso (various training/competition phases) cycles in 48 elite distance and middle distance track and field athletes. Having tested and updated this pilot study survey, in the current study we embarked on the investigation of the self-reported practices of dietary periodization across the annual training/racing calendar in a large cohort of world-class track and field endurance athletes Our goal was to characterize periodized nutrition practices across the year (macro cycle), and during specific meso and micro cycles of training/racing in this group, with attention to the effects of sex, athlete caliber, event duration and volume of training on these practices.

4.3. Methods

4.3.1 Study design & Participants

Based on our pilot study using a similar survey self-reported approach (Heikura et al., 2017b) we further developed the current study's survey into an online tool consisting of variously themed questions around dietary micro-, meso- and macro-periodization across the various annual training phases. Along with strategic changes to the survey, gained from insights from our pilot study, we also aimed for this be completed by a larger (target = 100) and more internationally representative group of athletes. We recruited elite female and male middle/distance athletes using online advertisements as well as direct contacts (via email or word-of-mouth) to athletes, coaches, applied sports practitioners and national sporting organizations (in Canada, US, Australia, Japan and Finland). To be included in the study, the athletes needed to be ≥18 years of age, currently and actively racing in the middle (800m, 1500m), distance (3000m-10000m) or road (half-marathon/marathon, 20km/50km race walk) events under the International Association of Athletics Federations (IAAF) and have a personal best of ≥1043 IAAF points (this corresponds to 5000m time of 13:47.26 and 16:03.84 in males and females, respectively). Recruitment and completion of the surveys were completed between February 8 and May 21, 2018. The Ethics Committee of Australian Catholic University approved the study protocol which conformed to the Declaration of Helsinki.

4.3.2 The survey

The survey consisted of an updated version of our pilot study based on our reflections on the responses from the original cohort and additional feedback from colleagues and athletes. Whereas the pilot survey included a total of 29 questions (7 main questions and 22 sub-questions), the updated survey was expanded to include a total of 59 questions (19 questions on training/racing characteristics, plus 13 main and 27 sub-questions around nutritional practices). The final version of the survey was built online using SurveyGizmo (Boulder, Colorado, USA, 2017). Skip logic was used, building a custom path through the questions according to the respondent's answers, for an improved participation experience (less confusion) and efficiency (less time to complete the survey).

The survey was completed anonymously, and an informed consent (a prerequisite for completing the survey) was completed as part of the online survey by all participants. The first part of the survey included background information, instructions, and general subject information. Thereafter, the athlete was asked to choose one of the two annual training periodization programs (track [e.g. 800m-10000m]) vs road [e.g. the marathon and race walks] that best reflected his/her yearly program. Questions approached annual (macro) periodization of nutrition as a whole (Part A: general principles of annual training/competition diet) and as typically defined separate periodized training phases (meso cycles): Part B: Base / endurance training phase, Part C: Main competition season (i.e. several months in duration: track athletes) or preparation for competition (i.e. one or more weeks in duration: road athletes), and Part D: Nutrition immediately before and on race day. Part A also included questions on general nutrition principles (e.g. vegetarian, paleo, very high energy, low carb high fat, gluten free) in the overall diet or during specific time periods (e.g. altitude training or during return from illness/injury). Additionally, parts B, C and D asked questions on training volume, key session and race frequency as well as number of race peaks. Across the survey and throughout this manuscript, hard training days were defined as "high volume and/or intensity days" and key sessions as "high intensity and/or high duration [>90 min] sessions or serious gym sessions". Fueling was defined as eating foods (CHO foods, protein foods, sports foods, etc.) before training. Fasted training was defined as "training first thing in the morning without having eaten any food or consumed any other carbohydrates, or training later in the day without having eaten any carbohydrates for at least 8 h prior". Within the survey, reminders of these terminology were included within each question that targeted nutrition in relation to these themes. Finally, the survey ended with an open, but optional, comment box.

It is important to note that the survey was purposely constructed to apply to the culture, practices and terminology used in endurance events in track and field. As such it is not directly applicable to other sports, and if used for other populations, even among endurance sports, it will need to be customized to the specific characteristics of these sports. A sample survey has been provided in the supplementary material to this paper.

4.3.3 Data management and statistical analysis

The data were checked and cleaned by excluding duplicate responses (i.e. two responses from the same individual), responses that were clearly false or confusing, and responses from those that did not satisfy the requirement of ≥1050 IAAF points. The answers were classified into clusters for further analysis using groupings based on sex (male vs female), distance (middle distance (MidD; 800 m, 1500 m) vs track distance (TrackD; 3,000 m steeplechase to 10,000 m) vs road distance (RoadD; marathon, race walks)), sex-based training volume groupings based on within sex tertiles of the entire data set (Females: low: ≤100 km/wk, moderate: 101–129 km/wk, high ≥130 km/wk; Males: low: ≤119 km/wk, moderate: 120–155 km/wk, high ≥156 km/wk) and athlete caliber (High: major championship (Olympics or World Championships) medalist, finalist and/or qualifier; Lower: the rest of the data set).

Data were first organized using Microsoft Excel, while further statistical analyses were conducted using SPSS Statistics 22 software (INM, Armonk, New York, USA). Data are presented as means ± standard deviations (SD) and number (n) and percentage (%) of responses. Normality of continuous data was checked with the Shapiro-Wilk goodness-of-fit test. Student’s t-test for independent samples was used to test for differences in age, training volume and IAAF scores between subgroups. For YES/NO answers, % was calculated from the n of the total sample; for sub-questions, % was calculated from the remaining n resulting after the main initial question. Chi-square test (X2) for independence with Yates Continuity Correction along with phi effect size statistic were used to test for differences between subgroups. Where more than two subgroups were present, Kruskal-Wallis Test was used as a post hoc test. Across the paper, statistical significance is shown when $p \leq 0.05$. In addition to numerical outcomes, relevant quotes provided by athletes (in cursive) have been embedded in the results section to provide further qualitative insights into the topic in question. To aid in the interpretation of results from the lengthy survey, aggregation of consistent outcomes across the survey was undertaken by all authors upon visual inspection of figures to develop a series of themes.

4.4 Results

4.4.1 General Questionnaire Outcomes

A total of 104 athletes (67 female and 37 male) from middle (n=27), distance (n=34) and road (n=43) groups were included in the final analysis. Fifty-two were classified as high and 52 as lower caliber. Training volume groupings resulted in 33, 31 and 31 athletes classified as low, moderate and high volume, respectively. The majority of responses came from athletes born in USA (25%), Canada (21%) and Japan (13%), while the rest were from Australia and New Zealand (12%), the Nordic countries (10%), Western Europe (14%), South America (4%) and Africa (1%). Training/competition phase specific training and racing characteristics for all athletes pooled, and for specific sex- and event-subgroups, are shown in Table 4.1. It is worth noting that while the difference in training volume was significant between males and females, this difference is likely to disappear if training volume were to be assessed by minutes of total training. For example, most elite males complete training at around 3:30/km pace while most elite females complete their training at around 4min/km pace. Therefore, if this assumption was adjusted for in the results, the difference in total training time between males (~472 min/week) and females (~468 min/week) would be almost equal. MidD and TrackD athletes showed meso-periodization of training volumes, whereby training volume was significantly less during the competition season compared to the base training phase (Table 4.1). This variation in training load was absent among RoadD, who reported equal training volumes between base training and preparation for competition. However, it should be noted that for RoadD athletes, preparation for competition included the ~8 weeks before a key race, where training volumes might be maintained at a relatively high levels until ~2 weeks before the race. This is different to track athletes, whose competition season may be extended over several seeks/months (10-11 weeks; Table 4.1) and where the athlete is likely to take part in frequent racing across this time period.

The results of the survey are summarized in the following figures: Figure 4.1 (Overall dietary practices across the year); Figure 4.2 (Eating on hard training days); Figure 4.3 (Eating on easy training days); Figure 4.4 (Fueling and recovery around key sessions); Figure 4.5 (Training in the fasted state); Figure 4.6 (Periodic CHO restriction); Figure 4.7 (Ingestion of CHO during training); Figure 4.8 (Major nutrition strategies implemented during competition season or preparation for competition); Figure 4.9 (Nutrition in the 24-48 h before the race day); Figure 4.10 (Nutrition on the race day); Figure 4.11 (Nutrition during the race). Key themes that emerged from these data are now discussed.

4.4.2 Theme 1: Road-distance athletes utilize much greater extremes of CHO availability (low to high) during training compared to middle-or track-distance athletes

A chronically high CHO diet was significantly more common among RoadD (46%) compared to TrackD (6%; F(2)=10.195, p=0.009) or MidD (10%) athletes (Figure 4.1). RoadD (62%) were also more likely to train fasted during base training compared to MidD (30%; p=0.032) (Figure 4.5). Similarly, purposeful CHO restriction was more popular among RoadD (57%) compared to MidD (30%; p=0.047) (Figure 4.6). In terms of training with high CHO availability, ingestion of CHO during workouts in the base training phase was more common among RoadD (90%) compared to TrackD (37%) or MidD (48%; p<0.001), mainly to practice race fueling (22% MidD vs 38% TrackD vs 67% RoadD, F(2)=6.534, p=0.038) (Figure 4.7). A higher number of RoadD (97%) and TrackD (85%) compared to MidD (57%) reported following a special diet in the 24-48h period preceding the race (MidD vs RoadD, p<0.001; MidD vs TrackD, p=0.039) (Figure 4.9). During this time, a low residue diet (i.e. low in fiber and whole foods) was more popular among RoadD (34%) compared to TrackD (5%; F(2)=8.546, p=0.021) or MidD (8%). Most RoadD (97%) compared to two-thirds of MidD (67%; p=0.009) reported to follow a special diet on race day (Figure 4.10).

4.4.3 Theme 2: Middle-distance athletes focus on nutrition strategies to manipulate physique

During the base training phase, MidD (32%) were more likely to report eating more on hard training days to prevent weight loss, compared to RoadD (5%; F(2)=7.181, p=0.022), with no difference to TrackD (13%) (Figure 4.2). Furthermore, compared to TrackD (0%) and RoadD (0%), MidD (40%) were more likely to avoid eating less on easy training days because they did not want to lose weight (F(2)=8.512, p=0.023) (Figure 4.3). MidD (80%) were also more likely than TrackD (52%) or RoadD (46%) to focus on eating after key sessions to help retain/build muscle mass (F(2)=6.349, p=0.042 between MidD and RoadD) (Figure 4.4). MidD included individuals (19%) that had never heard of fasted training before (compared to 0% of TrackD and RoadD; F(2)=6.267, p=0.044). During the competition season, MidD were more likely to focus on CHO restriction compared to RoadD (38% vs 9%, F(2)=7.574, p=0.023) (Figure 4.8).

4.4.4 Theme 3: Females are more conscious of intake of extra energy/CHO than males

A higher proportion of males than females (47% vs 16%, X(1)=5.287, p=0.022) reported to follow a chronically high CHO diet (Figure 4.1). In addition, more females (79%; X(1)=7.412, p=0.006) than males (52%) reported eating less on easy days during the base training phase (Figure 4.3). CHO intake during training was more popular among males (79%) than females (53%; p=0.004) (Figure 4.7). In the acute time period preceding the race day, more males than females reported following a high energy diet (36% vs 15%; X(1)=4.151, p=0.042) (Figure 4.9). Also, 76% females vs 94% males (X(1)=4.187, p=0.041) follow a special diet on race day (Figure 4.10). However it should be noted that 65% of males, compared to 29% of females, identified themselves as RoadD, which has most likely influenced the outcomes.

4.4.5 Theme 4: Performance is the main reason behind nutrition strategies; meanwhile less is known about nutrition for adaptation

Overall, the quality of performance during training and racing seem to be the main driving factors behind the decision making when choosing a specific nutrition strategy. This theme is present in nutrition practices overall (Figure 4.1), as well as throughout specific micro, meso and macro levels of training and competition (Figures 4.2-4.11). Other common explanations for choosing a specific nutrition strategy were efforts to manipulate body composition (Figures

4.3, 4.4, 4.5, 4.6), stay healthy/free of injuries (Figure 4.4), practicality (e.g. training before breakfast for time-management purposes; Figure 4.5), and because someone (usually a dietitian) told the athlete to do so. A substantial proportion of athletes seemed to be unaware of the usefulness of specific strategies to enhance training adaptation via periodically training in the fasted state and/or restricting CHO intake around training sessions (Figures 4.5 and 4.6).

4.4.6 Other key findings

Nearly half of the athletes (44%) reported following a periodized CHO diet over the annual training program (Figure 4.1), while ~one fifth of the athletes followed gluten free diets (19%) and vegan/vegetarian diets (17%). Even less common were diets emphasizing low CHO intake such as Paleo (0%), low CHO high fat diet (2%), restricted calorie (6%) and high protein low CHO (10%).

Overall, most athletes (92%) reported eating more on hard training days (Figure 4.2), including more CHO-rich foods (55%) and more in general (54%), while 68% reported adjusting nutrition intake to match lower energy expenditure on easy training days (Figure 4.3), including less in general (51%) and fewer CHO-rich foods (32%). Nutritional strategies to prepare for key training sessions were important to most (84%) athletes with key themes of the choice of CHO-rich foods (63%) and timing (58%). Meanwhile, 95% of athletes prioritized recovery after these sessions with key themes of choosing protein-rich (59%) or CHO-rich (50%) foods or considering the timing of intake (55%) (Figure 4.4). Fasted training (48%; Figure 4.5) and periodic CHO restriction (44%; Figure 4.6) were practiced by almost half of the athlete cohort, with the main rationale being weight loss (42% and 53%, respectively). More than half of the athletes (58%) reported consuming CHO during workouts, with the focus on key sessions 1-2 times a week (56%) and to maintain training intensity (74%) (Figure 4.7). Competition nutrition strategies focused mainly on adequate CHO and fluid intake before (Figure 4.9) and on race day (Figures 4.10 and 4.11) as well as on low residue diet throughout this time period. A number of athletes further explained their dietary choices, A selection of noteworthy athlete quotes are in Table 4.2.

4.4.7 Sources of information

One third (32%) of all athletes relied on a sports dietitian/nutritionist for nutrition advice. Of these, nearly half (43%) were MidD, while only 37% of TrackD and 20% of RoadD relied on this source of information [X(1)=9.751, p=0.008 between MidD and RoadD]. Other sources of information were less popular and included: coach (15%), read it somewhere (13%), training partner/a friend (5%), medical doctor (4%), physiologist (2%), naturopath (2%), and family member (1%), with no meaningful differences between subgroups

4.5 Discussion

This study aimed to characterize self-reported dietary periodization across macro (general practices across the annual cycle), meso (training and racing phases) and micro (between- and within-day) phases of training and competition in a large cohort of elite female and male middle- and long-distance runners and race walkers. We detected a number of key repeated themes across various levels of training periodization, including: (1) Road athletes reported different nutritional practices to middle- and track-distance athletes, by including strategies of training with both low and high CHO availability within the annual training plan; (2) Middle-distance athletes were the most conscious about the effects of nutrition strategies on physique outcomes; (3) Females seemed to be more conscious of intake of extra energy/CHO compared to males; (4) Overall, training and race performance appeared key factors influencing nutrition choices, while themes such as body composition manipulation, health, and practicality were less important; (5) Many athletes within this cohort of high level athletes were unaware of the use of nutrition to manipulate training adaptations, or felt that there were side-effects or challenges that prevented their use.

4.5.1 Theme 1: Road-distance athletes periodize CHO availability across the year

Historically, nutrition guidelines for endurance athletes have focused on strategies to habitually achieve high CHO availability to support performance and recovery around training and races (Coyle, 1991). Protocols that supply sufficient CHO fuels to meet the demands of prolonged and/or high-intensity endurance sessions, such as consuming sufficient CHO to refuel glycogen stores prior to an event, including CHO loading for events > 90 min (Hawley et al., 1997), a CHO rich meal in the hours before exercise (Coyle, 1991) or CHO intake during prolonged exercise according to the duration and mode of exercise (Stellingwerff & Cox, 2014) can enhance performance by ~2-3%. Contemporary recommendations support high CHO availability for competition, as well as for key training sessions in the athlete’s program in which high-intensity performance needs to be completed at the highest quality possible, or in which race nutrition strategies need to be practiced. In the current study, 90% of road-distance athletes (marathon runners and race walkers) reported strategies of ingesting CHO during workouts in the base/endurance training phase (Figure 4.7), while 89% focus on CHO intake during racing (Figure 4.11). Indeed, it seems that this cohort of elite road-distance athletes are aware of, and aim to, follow current sports nutrition guidelines that emphasize optimal CHO intake around key training and racing (Thomas et al., 2016). On the contrary, and as expected, these strategies were less important for athletes competing in shorter distance events where endogenous CHO fuel stores are not limiting.

Meanwhile, more recent studies have focused on the adaptation and performance effects of strategically and periodically implemented low CHO availability before, during, or after exercise (Bartlett et al., 2015; Impey et al., 2018b; Hearris et al., 2018). These studies suggest that occasional and strategic training with low CHO availability increases the cell signaling and gene expression responses that are usually seen after endurance training, thereby leading to further enhanced endurance capacity and performance. Possible strategies, as detailed in a recent commentary of definitions and proposed outcomes (Burke et al., 2018b) include fasted training (Figure 4.5), CHO restriction between the first and the second session of the day (Figure 4.6), CHO restriction during prolonged exercise, and CHO-restricted recovery overnight. While these strategies and their potential outcomes are intriguing, studies in elite athletes have failed to show direct performance benefits (Gejl et al., 2017; Burke et al., 2017a). Furthermore, studies on bone and iron health suggest these strategies may impair bone and iron metabolism, possibly leading into increased bone breakdown (Sale et al., 2015) and decreased iron levels (Badenhorst et al., 2015). Therefore, careful day-to-day periodization is likely required, where low CHO availability is primarily scheduled around low intensity sessions (Hearris et al., 2018). In the current study, 62% of road-distance athletes reported undertaking some training sessions in a fasted state, while this strategy was only half as popular among middle-distance athletes (30%; Figure 4.5). Weight loss was the most popular reason for training fasted (42% of athletes), while only 29% of those who practiced this believed that it might help with training adaptations.

4.5.2 Theme 2: Middle-distance athletes focus on optimal physique

A more recent advancement in the field is periodization of body composition (Stellingwerff, 2018), which refers to the manipulation of body composition (via a mixture of nutrition and training strategies) for optimal health and performance. The underlying idea is that race weight should not be maintained year-round, as this is likely to require chronic periods of low energy availability (EA) and its related impairments of several health and performance related measures (Mountjoy et al., 2018). Therefore, EA may need to be periodized across the year, with emphasis on higher EA levels during heavy training and altitude camps, and lower EA during lower training volumes and closer to the competition season. In addition to this macro and meso periodization of EA, emerging evidence suggests that within-day EA (micro level periodization) has also significant health consequences (Deutz et al., 2000; Fahrenholtz et al., 2018; Torstveit et al., 2018). Indeed, timing of energy intake around exercise (as opposed to “backend loading” with the majority of energy intake consumed in the evening) may be a powerful tool to manipulate physique while maintaining health. In the current study, middle-distance athletes reported more attention to the effects of nutrition strategies on physique outcomes; however, their chief focus was to build and maintain lean mass. For example, 40% of middle-distance athletes reported a maintenance of their food intake on easy training days to avoid weight loss (Figure 4.3). In addition, these athletes focused on nutritional support immediately after key workouts to maintain/build muscle mass (Figure 4.4). During the competition season, however, middle-distance athletes were more likely to report a reduction in CHO intake or use of CHO restriction strategies (Figure 4.8), which may reflect a relative reduction in training volume and/or their efforts to reduce body mass to achieve an optimal race weight.

4.5.3 Theme 3: Females are more conscious of intake of extra energy/CHO than males

Females and males have an equal ability for CHO storage and utilization during exercise if energy availability is adequate (Tarnopolsky et al., 2001; Wallis et al., 2006). However, female distance athletes tend to eat less CHO than males (Burke et al., 2001), although this difference is likely to disappear when CHO intake is adjusted to training volume (Heikura et al., 2017a), as recommended by current guidelines (Thomas et al., 2016). Regardless of equal (relative) energy and CHO needs for female and male athletes, dietary practices of females tend to be more cautious of extra energy/CHO intake. Indeed, females are more likely to suffer from eating disorders (Sundgot-Borgen & Torstveit, 2004). This may be due to a higher frequency of body image issues/concerns over body weight among female athletes (Martinsen et al., 2010). In the current study, we showed similar patterns of calorie/CHO awareness among elite distance athletes as has been previously reported in sub-elite athlete populations. Namely, male athletes were more likely to follow a chronically high CHO diet (Figure 4.1). In addition, a greater proportion of females (79%) than males (52%) reported eating less on easy days during the base training phase (Figure 4.3). Males were also more likely to follow a high energy diet in the acute time period preceding the race day (Figure 4.9). Although qualitative, these outcomes suggest that female athletes may indeed be more concerned about consuming extra energy/CHO however whether the reasons are justified due to a lower fuel requirement, or related to eating disorders/disordered eating, lack of knowledge, or other factors, cannot be concluded based on the current survey.

4.5.4 Theme 4: Nutrition strategies are based on performance rather than adaptation

Contrary to previous guidelines (Coyle, 1991), more recent sports nutrition guidelines have incorporated the value of specialized strategies to optimize adaptations to training, noting that these protocols may often be contradictory for acute performance outcomes or other health goals, and need to be carefully integrated into the various phases of the annual plan (Thomas et al., 2016). We were interested to identify whether these concepts were understood by elite athletes and used to inform their various nutrition strategies. Our results suggest that the most common nutrition strategies reported by this large cohort (n=104) of elite track and field distance athletes (of whom 50% were qualifiers for World Championships and/or Olympic Games) were focused on performance enhancement during training (Figures 4.2, 4.3, 4.4 and 4.7) and competition (Figures 4.9–4.11). Meanwhile less was known about specific strategies to further stimulate cellular adaptations to exercise (Figures 4.5 and 4.6). Indeed, many athletes lacked understanding of the periodization of strategies to train with low CHO availability, furthermore, others were either sceptical of their value, concerned about perceived or actual disadvantages particularly related to illness or injury, or practicing some aspects within their routines by accident. Since several outcomes identified the interest in using nutrition to manipulate body composition and/or to prevent illness/injuries, we conclude that the general priority for decisions around nutrition was performance > health > enhanced adaptation.

4.5.5 Limitations

It is important to note that the current study describes self-reported nutrition practices that are implemented across the training and competition year. We have previously shown that there is a discrepancy between general descriptions of practices (reflecting a macrocycle) and actual self-recorded intakes (collected across a micro cycle) in elite distance athletes (Heikura et al., 2017a). Indeed, it is possible that self-reports such as those found in the current study, reflect either what athletes aspire to achieve or perceive that they follow rather than actual behaviors. However, this potentially perceived versus actual mismatch would hypothetically be equivalent across the various sub-groups of athletes. Furthermore, our survey questions were qualitative (i.e. describing “high” or “low” intakes instead of specific amounts) and it is possible that these relative terms are interpreted differently by different individuals. Nevertheless, our survey was based on the learnings from a pilot study (Heikura et al., 2017b) and we are confident that the expanded and improved survey tool had greater precision and sensitivity, along with more than 100 respondents, in detecting nutrition practices across all levels of training/racing periodization.

4.6 Conclusions

We characterized self-reported dietary periodization across macro (general practices across the annual cycle), meso (training and racing phases) and micro (between- and within-day) phases of training and competition in 104 elite female and male middle- and long-distance runners and race walkers (50% major championship qualifiers). Our key findings suggest that: (1) Road athletes train with both low and high CHO availability within the annual training plan, while track athletes are less likely to incorporate a large spectrum of CHO availability in their training; (2) Middle-distance athletes emphasize physique when choosing a nutrition strategy; and (3) Performance appears to be the key driving factor influencing nutrition choices, while themes such as body composition manipulation, health, and practicality are less important. Overall, our findings indicate that elite track and field distance athletes are aware of and report following the current sports nutrition guidelines in terms of high CHO availability around key training sessions and during racing. However, most of this cohort appears to be unaware of and/or unwilling to aggressively incorporate the more recent strategies of training with reduced CHO availability to support training adaptations.

Acknowledgements

The authors would like to thank colleagues, coaches and athletes for their assistance during the recruitment process. A special thank you goes to all athletes who participated in the study.

Author contributions

Study design and survey development was undertaken by IH, TS and LB. Participant recruitment was done by IH, TS and LB. Data collection, organization and analysis was undertaken by IH. Manuscript preparation was undertaken by IH, TS and LB. All authors approved the final manuscript.

Conflicts of interest

The authors do not have any conflicts of interests.

Table 4.1. Participant background and characteristics of training and competition in elite middle/long-distance athletes (all, sex-based comparisons, distance-based comparisons). Values are means ± standard deviations.

	All (n=104)	**Sex comparison**		**Distance comparison**		
		Females (n=67)	**Males (n=37)**	**MidD (n=27)**	**TrackD (n=34)**	**RoadD (n=43)**
Age (yr)	29.2 ± 5.8	28.9 ± 5.8	29.6 ± 5.9	26.5 ± 3.6 ***	28.3 ± 6.3 #	31.6 ± 5.7
IAAF score	1129 ± 54	1135 ± 54	1119 ± 54	1135 ± 48	1119 ± 45	1133 ± 64
Training/competition background (years)	9.4 ± 4.6	8.5 ± 3.5 £	11.2 ± 5.8	7.1 ± 3.2 ***	9.3 ± 3.6	11.0 ± 5.4
Base training phase						
Training volume (km/wk)	123 ± 39	117 ± 39 £	135 ± 36	92 ± 23 ***	126 ± 41 $	139 ± 34
Number of key sessions per week	3.4 ± 1.3	3.3 ± 1.4	3.4 ± 1.2	3.2 ± 1.0	3.2 ± 1.0	3.6 ± 1.7
Competition season						
Season length (weeks)	9.3 ± 3.8	9.3 ± 3.7	9.5 ± 4.2	10.9 ± 1.6 ***	9.8 ± 3.8 ##	3.8 ± 3.3
Number of serious races within season	5.1 ± 2.2	5.1 ± 2.2	4.9 ± 2.6	5.6 ± 1.6 *	5.2 ± 2.6	3.0 ± 2.1
Preparation for competition						
Season length (weeks)	4.5 ± 2.6	4.2 ± 2.7	4.9 ± 2.6	N/A	3.3 ± 1.8	5.1 ± 2.8
Number of peaks per year	2.0 ± 0.7	2.0 ± 0.6	2.0 ± 0.8	N/A	1.7 ± 0.9	2.1 ± 0.6

Number of serious races within a peak	1.7 ± 0.9	1.9 ± 0.9	1.5 ± 1.0	N/A	2.0 ± 1.3	1.6 ± 0.8
Training volume during competition season/preparation for competition (km/wk)	115 ± 48	109 ± 52	123 ± 41	73 ± 19 *** ^{aaa}	118 ± 46 $$$ ^{aaa}	137 ± 47

IAAF score, International Association of Athletics Federations scoring tables 2017; MidD, middle distance (800m, 1500m); TrackD, track distance (3000m steeplechase to 10000m); RoadD, road distance (marathon, race walks). N/A, not applicable (i.e. these questions were not part of the survey for this population). £ $p<0.05$ significant difference between sexes; * $p<0.05$, *** $p<0.001$ significant difference between MidD and RoadD; # $p<0.05$, ## $p<0.01$, ### $p<0.001$ significant difference between TrackD and RoadD; $ $p<0.05$, $$ $p<0.01$, $$$ $p<0.001$ significant difference between MidD and TrackD; ^{aaa} $p<0.001$ significant difference compared to base training phase.

Table 4.2. Selection of noteworthy athlete quotes regarding why they do, or do not, follow a specific nutrition strategy.

Nutrition strategy	**Reason for following/not following this strategy**
B3: Why do you focus on pre-key session fueling?	*"To help maintain calorie balance and recover from REDS*."
B4: Why do you not train in the fasted state?	*"That will put me so far behind in terms of energy intake which I can't afford."* *"I'm very lean to begin with - I don't think I'd make it through!"* *"I do not believe this approach is scientifically valid*."
B5: Why do you restrict carbohydrate intake periodically?	*"I think there is something to making your body more insulin sensitive*." *"It seems like a bad idea. My understanding is that the body uses carbs as primary fuel source."*
B1: Why do you eat more food/calories on hard training days?	*"I feel like I have earned it*."
B5: Why do you not restrict carbohydrate intake periodically?	*"I like carbohydrate foods*." *"Lack of self*-*control*."
D1: Why do you focus on drinking fluids in the 24-8h before the race day?	*"I am nervous*."
D2: Why do you not follow a specific diet on the race day?	*"I am nervous so it's hard to eat anything*."

Figure legends

Figure 4.1. A1: Overall dietary practices across the year. The prevalence of specific, consistent nutrition practices (e.g. vegetarian) and the reasons for following them in 104 elite female and male track and field endurance athletes. Percentages (%) reflect the % of athletes that chose a specific answer in relation to all athletes (YES/NO answers) or in relation to specific sub-question populations (i.e. % of those that answered YES or NO). In addition, number (n) of athletes per each answer box has been provided. Answer boxes or circles are color coded based on % of all athletes as follows: ≥50%, black box with white font; 30-49%, dark greygray box with white font; 20-29%, light greygray box with black font; <20%, white box with black font. Light gray circles with diagonal stripes indicate zero responses to this option. Symbols have been used to reflect significant ($p<0.05$) between-group differences between sexes (vector sex symbol), athlete caliber (medal symbol), distance event (runner symbol), and reported volume during base training (distance symbol). Where significant differences were detected, answer boxes include a brief description of direction of difference, for example, M>F reflects a higher % of males (M) compared to females (F) for that answer. MidD, Middle Distance (800 m and 1500 m); TrackD, track distance (3000 m steeplechase to 10 000 m); RoadD, road distance (marathon and race walks). High caliber, major championship qualifiers; Lower caliber, those that have not qualified to major championships. Low, Moderate and High-Volume groups as sex-specific tertile cut-offs.

Figure 4.2. B1: Nutrition on hard training days during base training phase. The prevalence of specific nutrition practices on hard training days and the reasons for following them in 95 elite female and male track and field endurance athletes. Please see full description for figure within figure 1.

Figure 4.3. B2: Nutrition on easy training days during base training phase. The prevalence of specific nutrition practices on easy training days and the reasons for following them in 94 elite female and male track and field endurance athletes. Please see full description for figure within figure 1.

Figure 4.4. B3: Nutrition before (3.1) and after (3.2) key training sessions during base training phase. The prevalence of specific nutrition practices around fueling and recovery from key training sessions and the reasons for following them in 93 elite female and male track and field endurance athletes. Please see full description for figure within figure 1.

Figure 4.5. B4: Fasted training during base training phase. The prevalence of training in the fasted state with specific details around timing, frequency and reasons for this strategy in 93 elite female and male track and field endurance athletes. Please see full description for figure within figure 1.

Figure 4.6. B5: Periodic carbohydrate restriction during base training phase. The prevalence of restricting carbohydrate intake periodically with specific details around timing, frequency and reasons for this strategy in 92 elite female and male track and field endurance athletes. Please see full description for figure within figure 1.

Figure 4.7. B6: Gut training during base training phase. The prevalence of training the gut (i.e. ingesting carbohydrates during workouts) with specific details around timing, frequency and reasons for this strategy in 86 elite female and male track and field endurance athletes. Please see full description for figure within figure 1.

Figure 4.8. C. Major nutrition strategies implemented during competition season and preparation for competition as compared to base/endurance phase. Answers to Part C: “Compared to nutrition during base/endurance training phase, how much do you focus on the following dietary strategies during competition season (track athletes) or preparation for competition (road athletes)?” Values are percentages of all athletes (n=83): white bars, less likely; gray bars, equally likely; black bars, more likely to follow this strategy. Symbols have been used to reflect significant ($p<0.05$) between-group differences between sexes (vector sex symbol), and distance event (runner symbol). Where significant differences were detected, the symbols are combined with a brief description of direction of difference, for example, M>F reflects a higher % of males (M) compared to females (F) for that answer. MidD, Middle Distance (800 m and 1500 m); TrackD, track distance (3000 m steeplechase to 10 000 m); RoadD, road distance (marathon and race walks).

Figure 4.9. D1: Nutrition in the 24-48h time period before the race. The prevalence of specific nutrition strategies and the reasons for them in 83 elite female and male track and field endurance athletes within the acute time period preceding the main race. Please see full description for figure within figure 1.

Figure 4.10. D2: Nutrition on race day. The prevalence of specific nutrition strategies and the reasons for them in 83 elite female and male track and field endurance athletes on the day of the main race. Please see full description for figure within figure 1.

Figure 4.11. D3: Nutrition during the race. The prevalence of specific nutrition strategies and the reasons for them in 36 elite female and male track and field endurance athletes during the main race. Only athletes competing in road events replied to this question. Please see full description for figure within figure 1.

Figure 4.1.

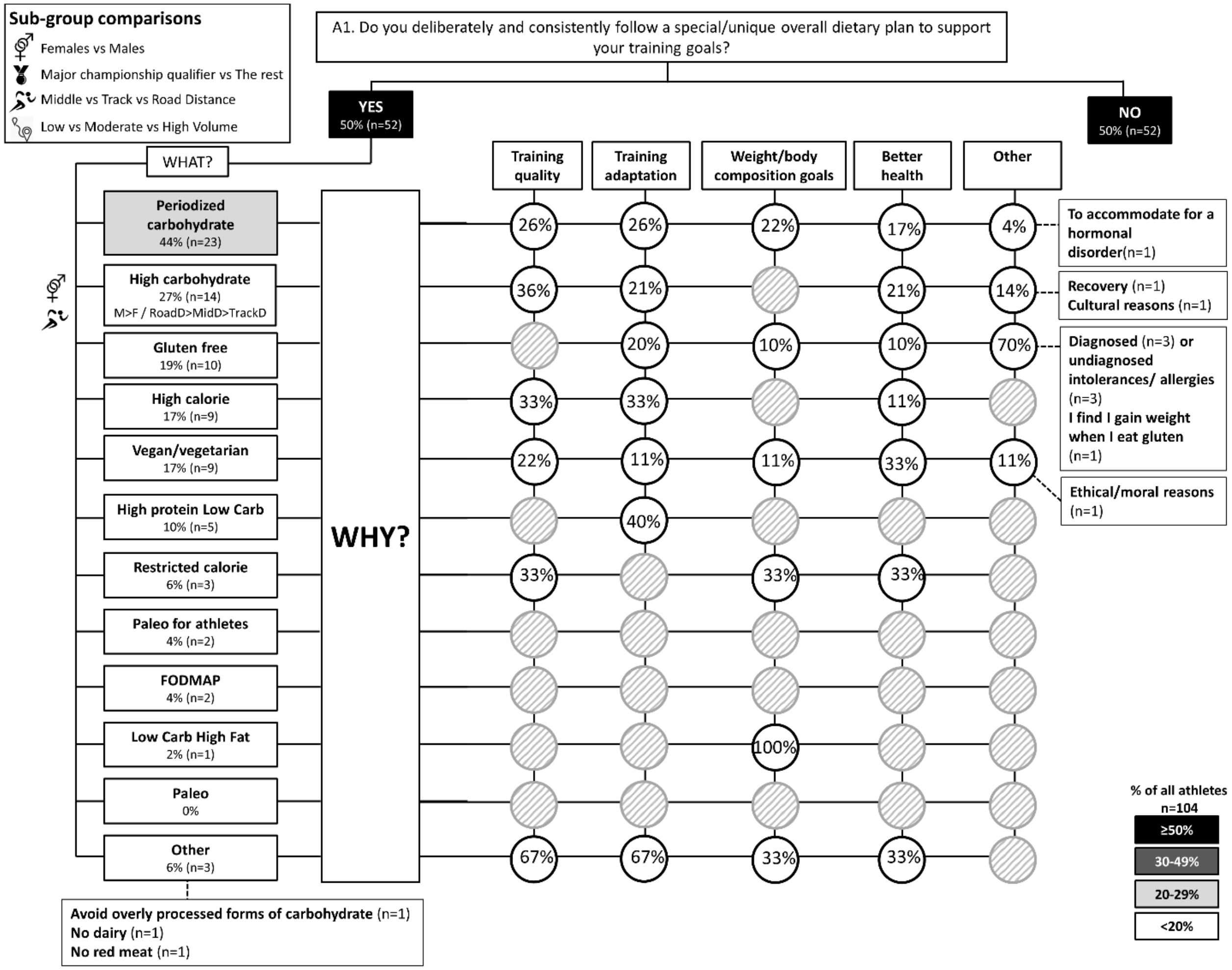
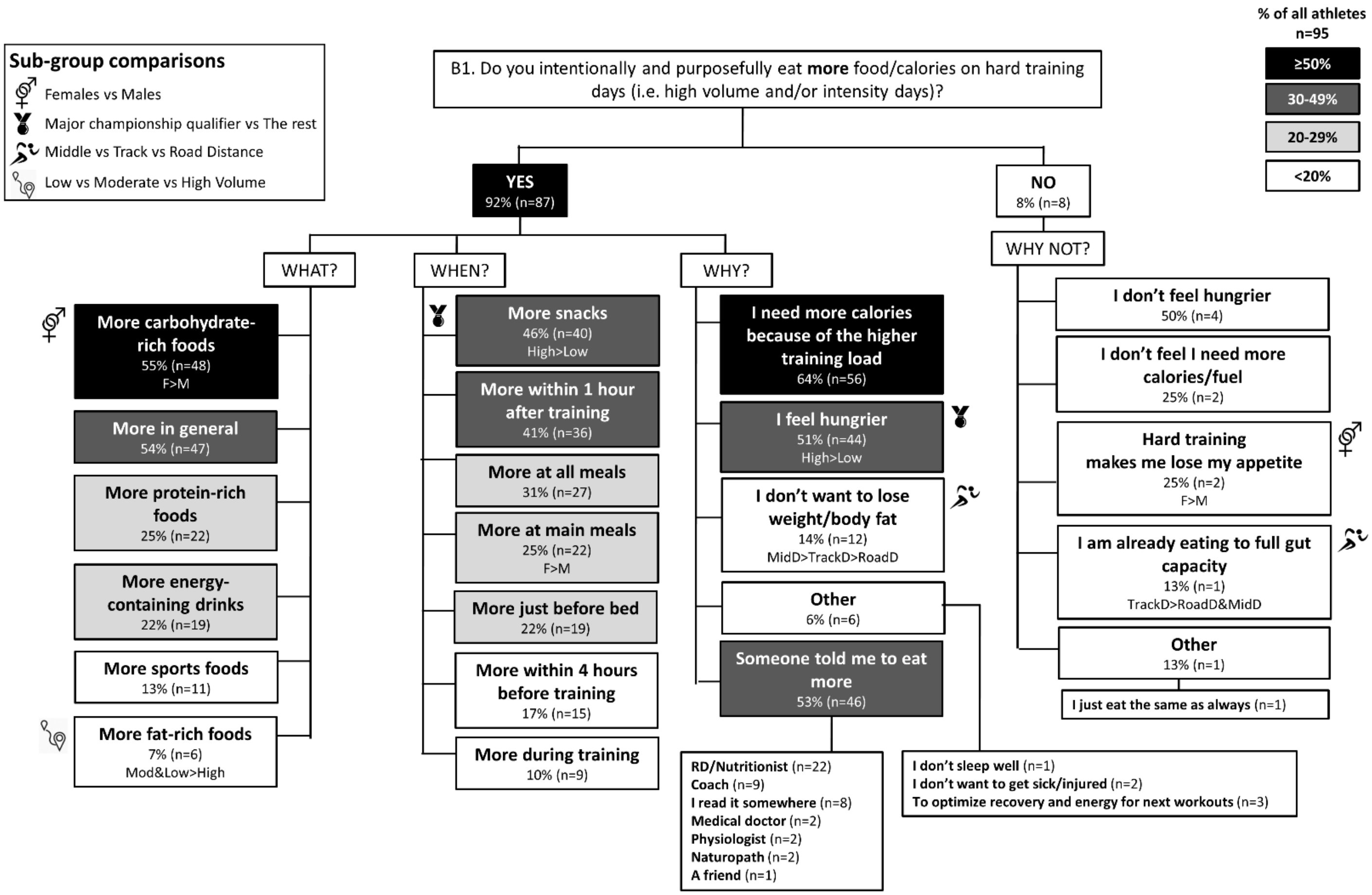

Sub-group comparisons
Females vs Males
Major championship qualifier vs The rest
Middle vs Track vs Road Distance
Low vs Moderate vs High Volume
A1. Do you deliberately and consistently follow a special/unique overall dietary plan to support your training goals?
YES
50% (n=52)
NO
50% (n=52)
WHAT?
WHY?
Training quality
Training adaptation
Weight/body composition goals
Better health
Other
Periodized carbohydrate
44% (n=23)
26%
26%
22%
17%
4%
To accommodate for a hormonal disorder(n=1)
High carbohydrate
27% (n=14)
M>F / RoadD>MidD>TrackD
36%
21%
21%
14%
Recovery (n=1)
Cultural reasons (n=1)
Gluten free
19% (n=10)
20%
10%
10%
70%
Diagnosed (n=3) or undiagnosed intolerances/ allergies (n=3)
I find I gain weight when I eat gluten (n=1)
High calorie
17% (n=9)
33%
33%
11%
Vegan/vegetarian
17% (n=9)
22%
11%
11%
33%
11%
Ethical/moral reasons (n=1)
High protein Low Carb
10% (n=5)
40%
Restricted calorie
6% (n=3)
33%
33%
33%
Paleo for athletes
4% (n=2)
FODMAP
4% (n=2)
Low Carb High Fat
2% (n=1)
100%
Paleo
0%
Other
6% (n=3)
67%
67%
33%
33%
Avoid overly processed forms of carbohydrate (n=1)
No dairy (n=1)
No red meat (n=1)
% of all athletes n=104
≥50%
30-49%
20-29%
<20%

Figure 4.2.



Sub-group comparisons
⚥ Females vs Males
Major championship qualifier vs The rest
Middle vs Track vs Road Distance
Low vs Moderate vs High Volume
% of all athletes n=95
≥50%
30-49%
20-29%
<20%
B1. Do you intentionally and purposefully eat **more** food/calories on hard training days (i.e. high volume and/or intensity days)?
YES 92% (n=87)
NO 8% (n=8)
WHAT?
WHEN?
WHY?
WHY NOT?
More carbohydrate-rich foods 55% (n=48) F>M
More in general 54% (n=47)
More protein-rich foods 25% (n=22)
More energy-containing drinks 22% (n=19)
More sports foods 13% (n=11)
More fat-rich foods 7% (n=6) Mod&Low>High
More snacks 46% (n=40) High>Low
More within 1 hour after training 41% (n=36)
More at all meals 31% (n=27)
More at main meals 25% (n=22) F>M
More just before bed 22% (n=19)
More within 4 hours before training 17% (n=15)
More during training 10% (n=9)
I need more calories because of the higher training load 64% (n=56)
I feel hungrier 51% (n=44) High>Low
I don't want to lose weight/body fat 14% (n=12) MidD>TrackD>RoadD
Other 6% (n=6)
Someone told me to eat more 53% (n=46)
RD/Nutritionist (n=22)
Coach (n=9)
I read it somewhere (n=8)
Medical doctor (n=2)
Physiologist (n=2)
Naturopath (n=2)
A friend (n=1)
I don't sleep well (n=1)
I don't want to get sick/injured (n=2)
To optimize recovery and energy for next workouts (n=3)
I don't feel hungrier 50% (n=4)
I don't feel I need more calories/fuel 25% (n=2)
Hard training makes me lose my appetite 25% (n=2) F>M
I am already eating to full gut capacity 13% (n=1) TrackD>RoadD&MidD
Other 13% (n=1)
I just eat the same as always (n=1)

Figure 4.3.

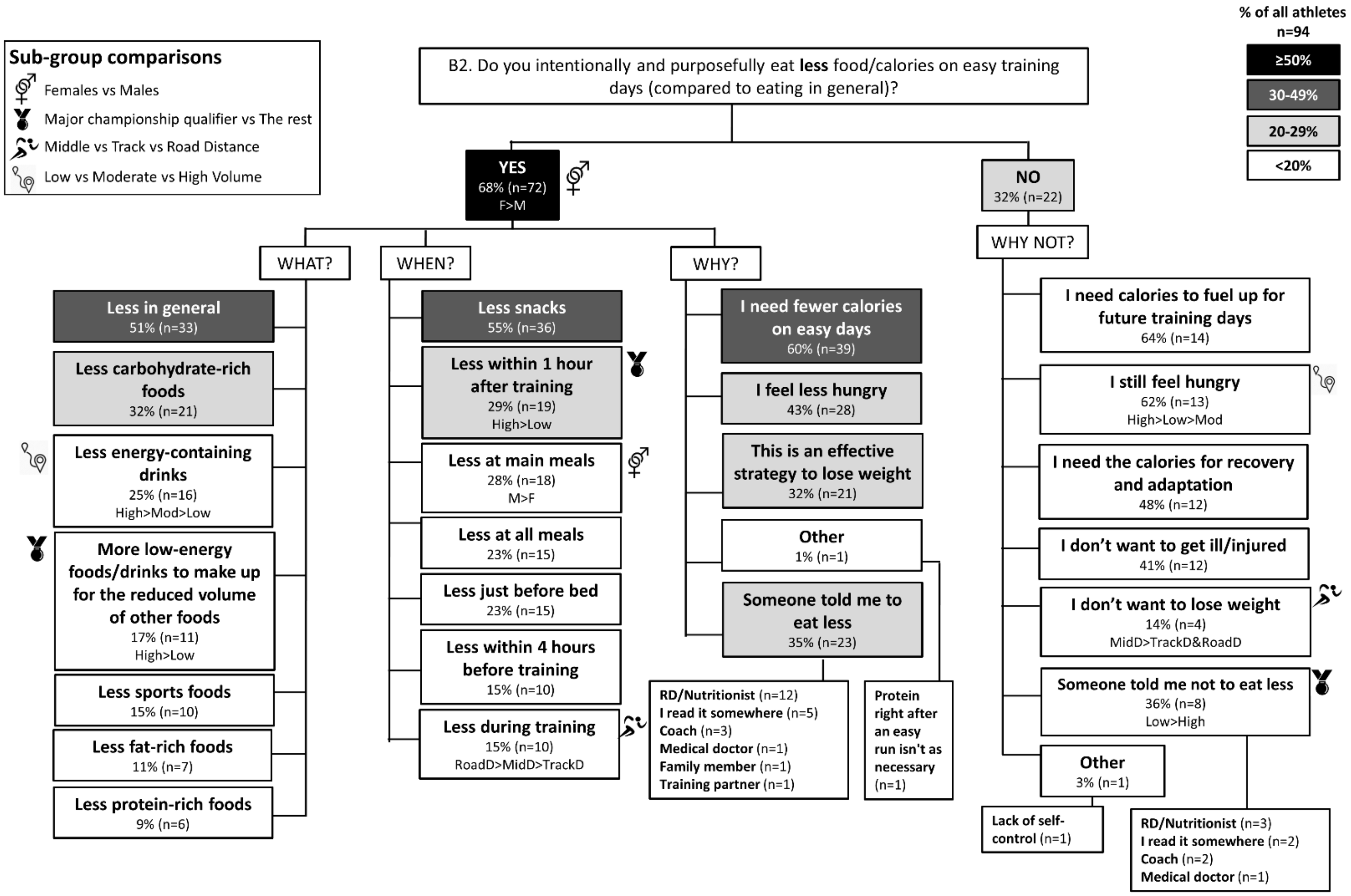

Sub-group comparisons
Females vs Males
Major championship qualifier vs The rest
Middle vs Track vs Road Distance
Low vs Moderate vs High Volume
B2. Do you intentionally and purposefully eat less food/calories on easy training days (compared to eating in general)?
% of all athletes n=94
≥50%
30-49%
20-29%
<20%
YES 68% (n=72) F>M
NO 32% (n=22)
WHAT?
WHEN?
WHY?
WHY NOT?
Less in general 51% (n=33)
Less carbohydrate-rich foods 32% (n=21)
Less energy-containing drinks 25% (n=16) High>Mod>Low
More low-energy foods/drinks to make up for the reduced volume of other foods 17% (n=11) High>Low
Less sports foods 15% (n=10)
Less fat-rich foods 11% (n=7)
Less protein-rich foods 9% (n=6)
Less snacks 55% (n=36)
Less within 1 hour after training 29% (n=19) High>Low
Less at main meals 28% (n=18) M>F
Less at all meals 23% (n=15)
Less just before bed 23% (n=15)
Less within 4 hours before training 15% (n=10)
Less during training 15% (n=10) RoadD>MidD>TrackD
I need fewer calories on easy days 60% (n=39)
I feel less hungry 43% (n=28)
This is an effective strategy to lose weight 32% (n=21)
Other 1% (n=1)
Someone told me to eat less 35% (n=23)
RD/Nutritionist (n=12)
I read it somewhere (n=5)
Coach (n=3)
Medical doctor (n=1)
Family member (n=1)
Training partner (n=1)
Protein right after an easy run isn't as necessary (n=1)
I need calories to fuel up for future training days 64% (n=14)
I still feel hungry 62% (n=13) High>Low>Mod
I need the calories for recovery and adaptation 48% (n=12)
I don't want to get ill/injured 41% (n=12)
I don't want to lose weight 14% (n=4) MidD>TrackD&RoadD
Someone told me not to eat less 36% (n=8) Low>High
Other 3% (n=1)
Lack of self-control (n=1)
RD/Nutritionist (n=3)
I read it somewhere (n=2)
Coach (n=2)
Medical doctor (n=1)

Figure 4.4.

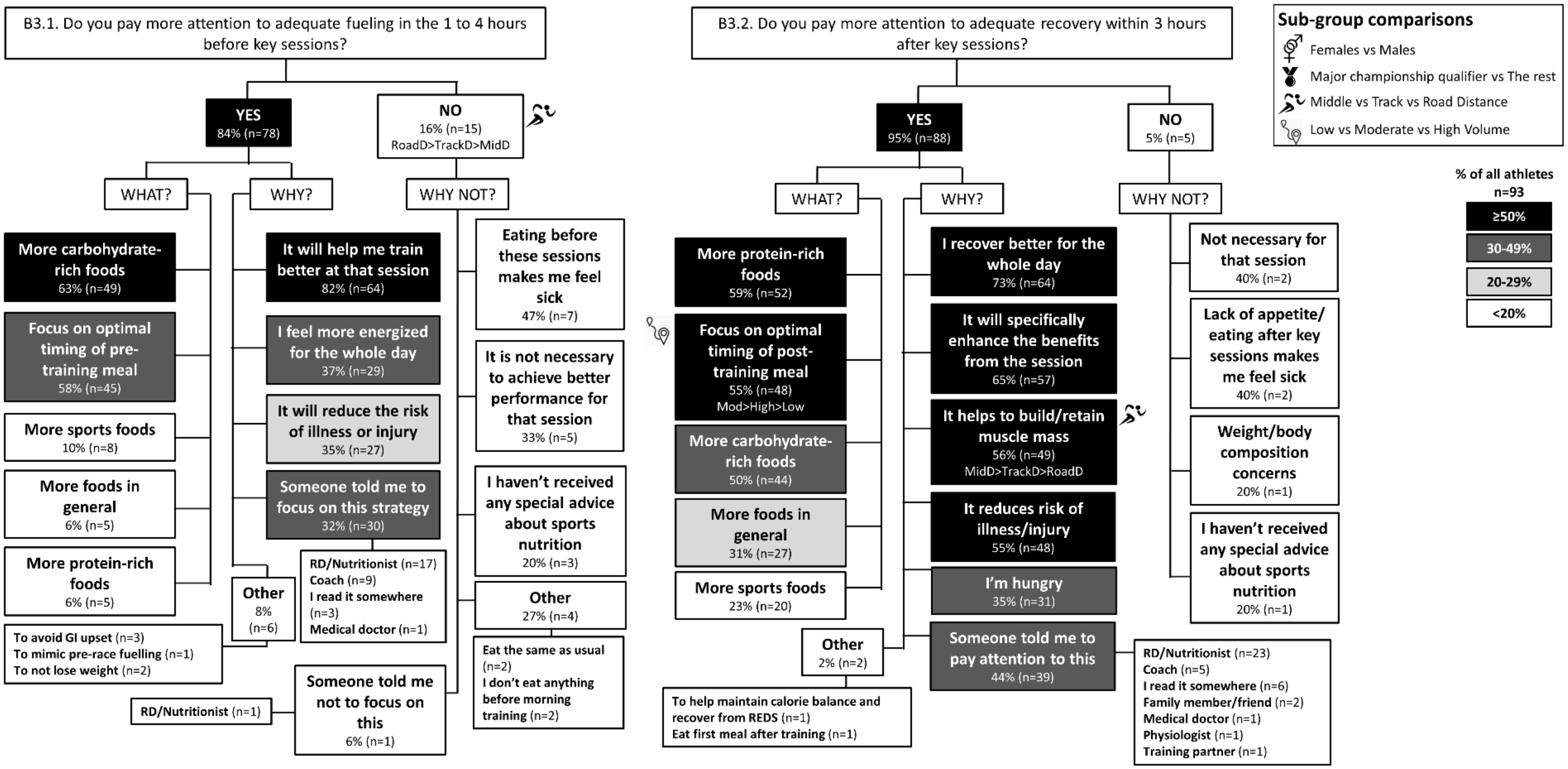

B3.1. Do you pay more attention to adequate fueling in the 1 to 4 hours before key sessions?
YES 84% (n=78)
NO 16% (n=15) RoadD>TrackD>MidD
WHAT?
WHY?
WHY NOT?
More carbohydrate-rich foods 63% (n=49)
Focus on optimal timing of pre-training meal 58% (n=45)
More sports foods 10% (n=8)
More foods in general 6% (n=5)
More protein-rich foods 6% (n=5)
To avoid GI upset (n=3)
To mimic pre-race fuelling (n=1)
To not lose weight (n=2)
It will help me train better at that session 82% (n=64)
I feel more energized for the whole day 37% (n=29)
It will reduce the risk of illness or injury 35% (n=27)
Someone told me to focus on this strategy 32% (n=30)
RD/Nutritionist (n=17)
Coach (n=9)
I read it somewhere (n=3)
Medical doctor (n=1)
Other 8% (n=6)
Eating before these sessions makes me feel sick 47% (n=7)
It is not necessary to achieve better performance for that session 33% (n=5)
I haven't received any special advice about sports nutrition 20% (n=3)
Other 27% (n=4)
Eat the same as usual (n=2)
I don't eat anything before morning training (n=2)
Someone told me not to focus on this 6% (n=1)
RD/Nutritionist (n=1)
B3.2. Do you pay more attention to adequate recovery within 3 hours after key sessions?
YES 95% (n=88)
NO 5% (n=5)
WHAT?
WHY?
WHY NOT?
More protein-rich foods 59% (n=52)
Focus on optimal timing of post-training meal 55% (n=48) Mod>High>Low
More carbohydrate-rich foods 50% (n=44)
More foods in general 31% (n=27)
More sports foods 23% (n=20)
Other 2% (n=2)
To help maintain calorie balance and recover from REDS (n=1)
Eat first meal after training (n=1)
I recover better for the whole day 73% (n=64)
It will specifically enhance the benefits from the session 65% (n=57)
It helps to build/retain muscle mass 56% (n=49) MidD>TrackD>RoadD
It reduces risk of illness/injury 55% (n=48)
I'm hungry 35% (n=31)
Someone told me to pay attention to this 44% (n=39)
RD/Nutritionist (n=23)
Coach (n=5)
I read it somewhere (n=6)
Family member/friend (n=2)
Medical doctor (n=1)
Physiologist (n=1)
Training partner (n=1)
Not necessary for that session 40% (n=2)
Lack of appetite/ eating after key sessions makes me feel sick 40% (n=2)
Weight/body composition concerns 20% (n=1)
I haven't received any special advice about sports nutrition 20% (n=1)
Sub-group comparisons
Females vs Males
Major championship qualifier vs The rest
Middle vs Track vs Road Distance
Low vs Moderate vs High Volume
% of all athletes n=93
≥50%
30-49%
20-29%
<20%

Figure 4.5.

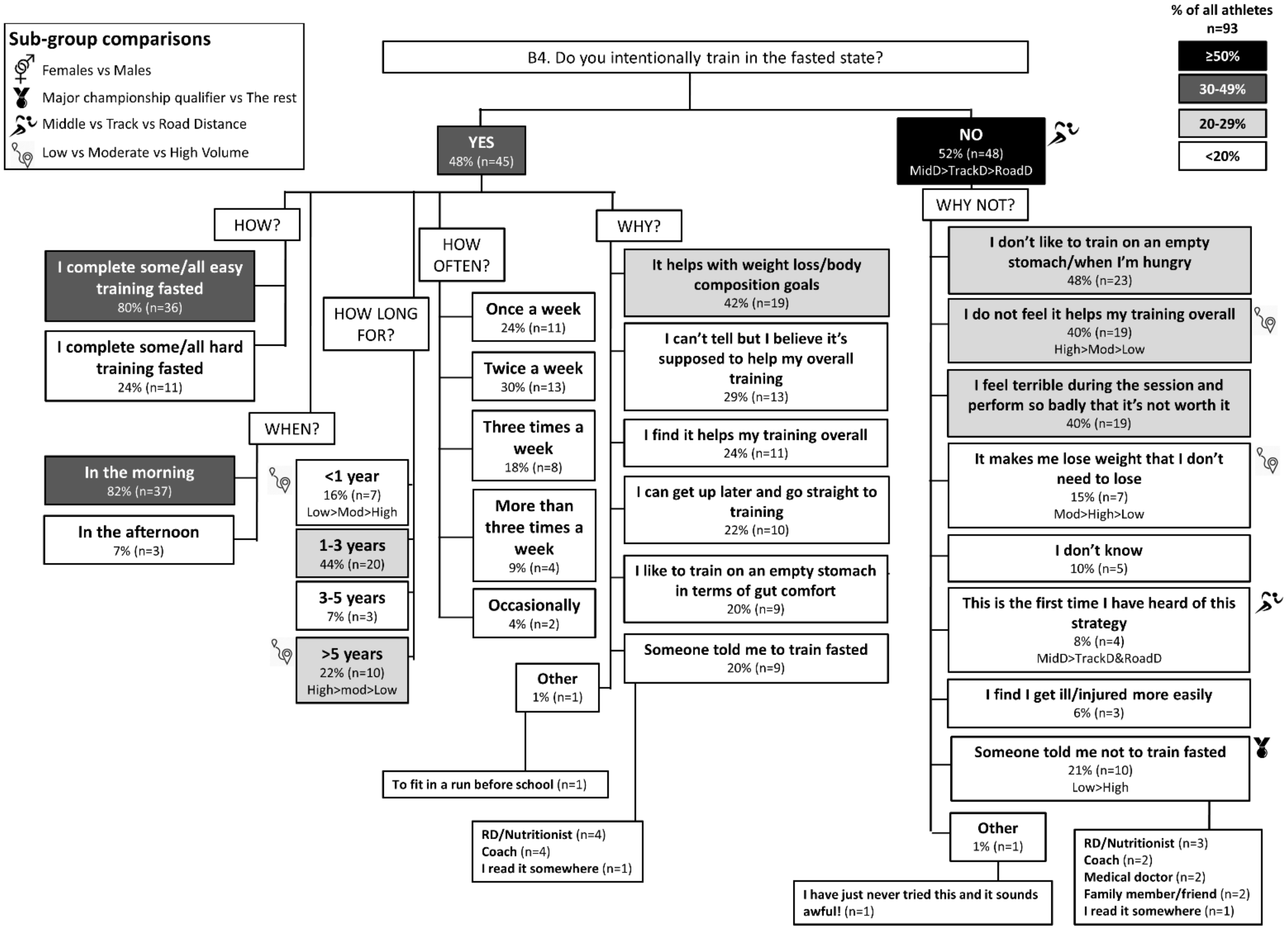

Sub-group comparisons
Females vs Males
Major championship qualifier vs The rest
Middle vs Track vs Road Distance
Low vs Moderate vs High Volume
B4. Do you intentionally train in the fasted state?
% of all athletes n=93
≥50%
30-49%
20-29%
<20%
YES 48% (n=45)
NO 52% (n=48) MidD>TrackD>RoadD
HOW?
I complete some/all easy training fasted 80% (n=36)
I complete some/all hard training fasted 24% (n=11)
WHEN?
In the morning 82% (n=37)
In the afternoon 7% (n=3)
HOW LONG FOR?
<1 year 16% (n=7) Low>Mod>High
1-3 years 44% (n=20)
3-5 years 7% (n=3)
>5 years 22% (n=10) High>mod>Low
HOW OFTEN?
Once a week 24% (n=11)
Twice a week 30% (n=13)
Three times a week 18% (n=8)
More than three times a week 9% (n=4)
Occasionally 4% (n=2)
Other 1% (n=1)
To fit in a run before school (n=1)
WHY?
It helps with weight loss/body composition goals 42% (n=19)
I can't tell but I believe it's supposed to help my overall training 29% (n=13)
I find it helps my training overall 24% (n=11)
I can get up later and go straight to training 22% (n=10)
I like to train on an empty stomach in terms of gut comfort 20% (n=9)
Someone told me to train fasted 20% (n=9)
RD/Nutritionist (n=4)
Coach (n=4)
I read it somewhere (n=1)
WHY NOT?
I don't like to train on an empty stomach/when I'm hungry 48% (n=23)
I do not feel it helps my training overall 40% (n=19) High>Mod>Low
I feel terrible during the session and perform so badly that it's not worth it 40% (n=19)
It makes me lose weight that I don't need to lose 15% (n=7) Mod>High>Low
I don't know 10% (n=5)
This is the first time I have heard of this strategy 8% (n=4) MidD>TrackD&RoadD
I find I get ill/injured more easily 6% (n=3)
Someone told me not to train fasted 21% (n=10) Low>High
Other 1% (n=1)
I have just never tried this and it sounds awful! (n=1)
RD/Nutritionist (n=3)
Coach (n=2)
Medical doctor (n=2)
Family member/friend (n=2)
I read it somewhere (n=1)

Figure 4.6.

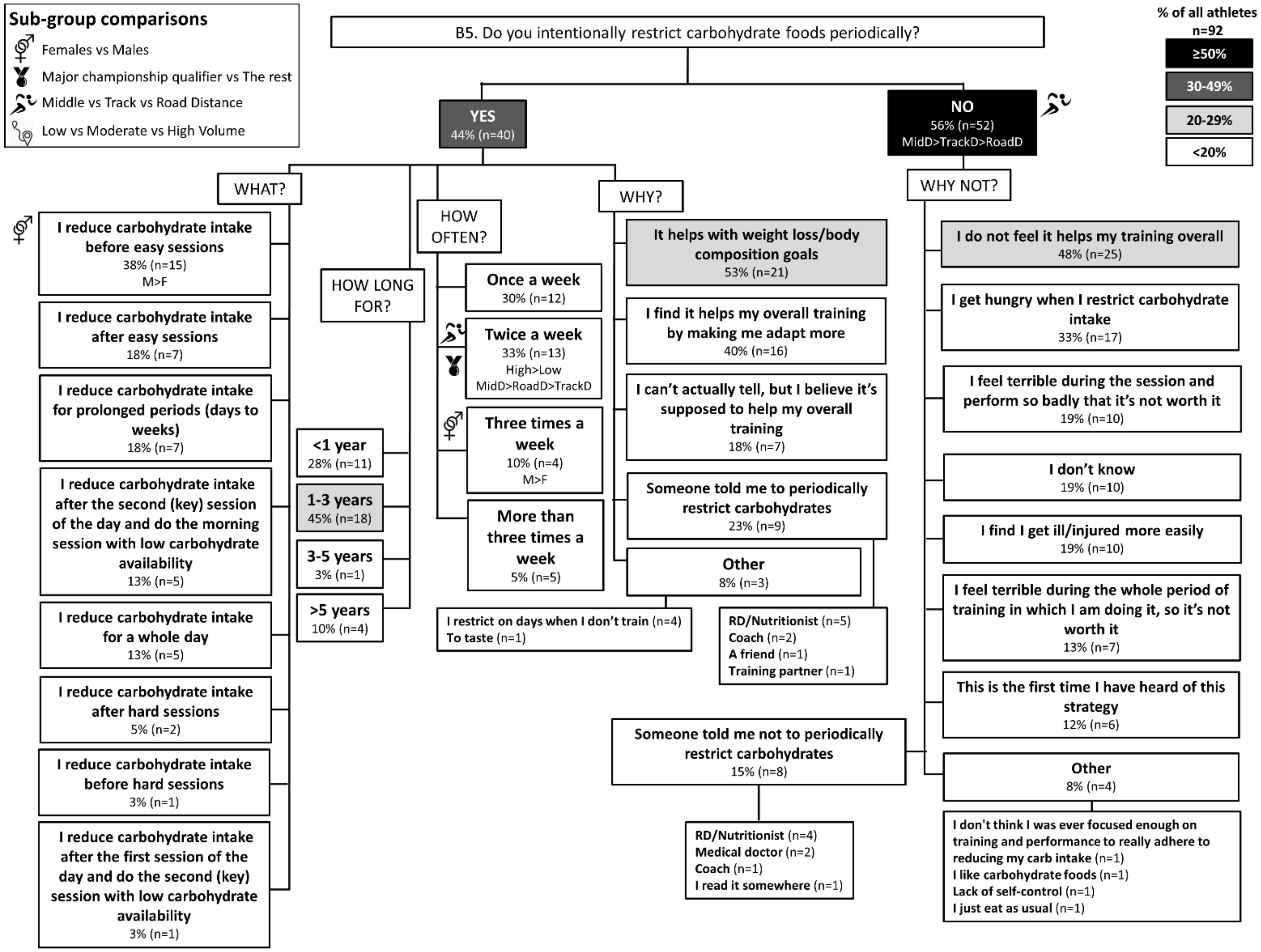

Sub-group comparisons
Females vs Males
Major championship qualifier vs The rest
Middle vs Track vs Road Distance
Low vs Moderate vs High Volume
B5. Do you intentionally restrict carbohydrate foods periodically?
% of all athletes n=92
≥50%
30-49%
20-29%
<20%
YES 44% (n=40)
NO 56% (n=52) MidD>TrackD>RoadD
WHAT?
I reduce carbohydrate intake before easy sessions 38% (n=15) M>F
I reduce carbohydrate intake after easy sessions 18% (n=7)
I reduce carbohydrate intake for prolonged periods (days to weeks) 18% (n=7)
I reduce carbohydrate intake after the second (key) session of the day and do the morning session with low carbohydrate availability 13% (n=5)
I reduce carbohydrate intake for a whole day 13% (n=5)
I reduce carbohydrate intake after hard sessions 5% (n=2)
I reduce carbohydrate intake before hard sessions 3% (n=1)
I reduce carbohydrate intake after the first session of the day and do the second (key) session with low carbohydrate availability 3% (n=1)
HOW LONG FOR?
<1 year 28% (n=11)
1-3 years 45% (n=18)
3-5 years 3% (n=1)
>5 years 10% (n=4)
HOW OFTEN?
Once a week 30% (n=12)
Twice a week 33% (n=13) High>Low MidD>RoadD>TrackD
Three times a week 10% (n=4) M>F
More than three times a week 5% (n=5)
I restrict on days when I don't train (n=4)
To taste (n=1)
WHY?
It helps with weight loss/body composition goals 53% (n=21)
I find it helps my overall training by making me adapt more 40% (n=16)
I can't actually tell, but I believe it's supposed to help my overall training 18% (n=7)
Someone told me to periodically restrict carbohydrates 23% (n=9)
Other 8% (n=3)
RD/Nutritionist (n=5)
Coach (n=2)
A friend (n=1)
Training partner (n=1)
WHY NOT?
I do not feel it helps my training overall 48% (n=25)
I get hungry when I restrict carbohydrate intake 33% (n=17)
I feel terrible during the session and perform so badly that it's not worth it 19% (n=10)
I don't know 19% (n=10)
I find I get ill/injured more easily 19% (n=10)
I feel terrible during the whole period of training in which I am doing it, so it's not worth it 13% (n=7)
This is the first time I have heard of this strategy 12% (n=6)
Someone told me not to periodically restrict carbohydrates 15% (n=8)
RD/Nutritionist (n=4)
Medical doctor (n=2)
Coach (n=1)
I read it somewhere (n=1)
Other 8% (n=4)
I don't think I was ever focused enough on training and performance to really adhere to reducing my carb intake (n=1)
I like carbohydrate foods (n=1)
Lack of self-control (n=1)
I just eat as usual (n=1)

Figure 4.7.

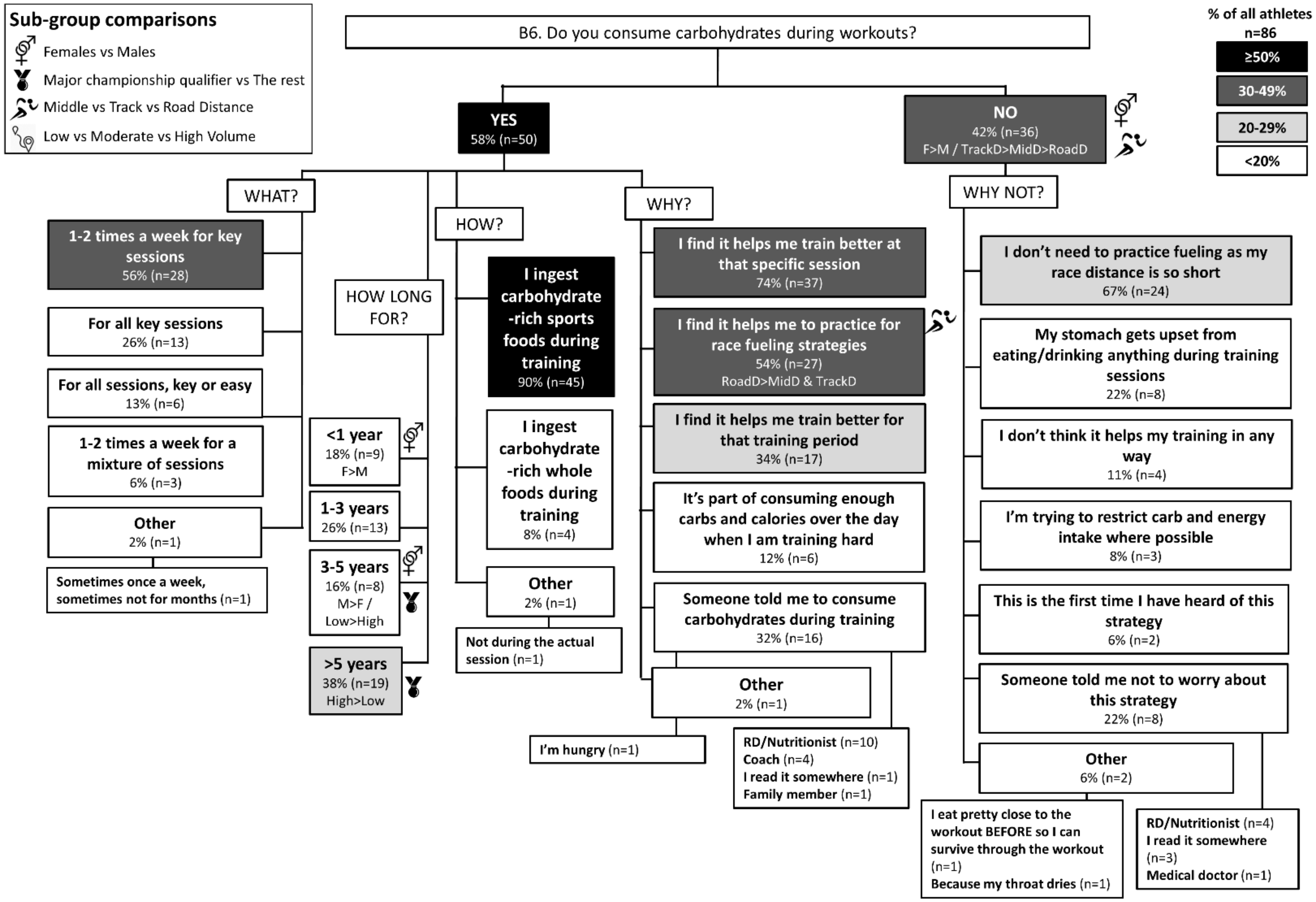

Sub-group comparisons
Females vs Males
Major championship qualifier vs The rest
Middle vs Track vs Road Distance
Low vs Moderate vs High Volume
B6. Do you consume carbohydrates during workouts?
% of all athletes n=86
≥50%
30-49%
20-29%
<20%
YES 58% (n=50)
NO 42% (n=36) F>M / TrackD>MidD>RoadD
WHAT?
1-2 times a week for key sessions 56% (n=28)
For all key sessions 26% (n=13)
For all sessions, key or easy 13% (n=6)
1-2 times a week for a mixture of sessions 6% (n=3)
Other 2% (n=1)
Sometimes once a week, sometimes not for months (n=1)
HOW LONG FOR?
<1 year 18% (n=9) F>M
1-3 years 26% (n=13)
3-5 years 16% (n=8) M>F / Low>High
>5 years 38% (n=19) High>Low
HOW?
I ingest carbohydrate-rich sports foods during training 90% (n=45)
I ingest carbohydrate-rich whole foods during training 8% (n=4)
Other 2% (n=1)
Not during the actual session (n=1)
WHY?
I find it helps me train better at that specific session 74% (n=37)
I find it helps me to practice for race fueling strategies 54% (n=27) RoadD>MidD & TrackD
I find it helps me train better for that training period 34% (n=17)
It's part of consuming enough carbs and calories over the day when I am training hard 12% (n=6)
Someone told me to consume carbohydrates during training 32% (n=16)
Other 2% (n=1)
I'm hungry (n=1)
RD/Nutritionist (n=10)
Coach (n=4)
I read it somewhere (n=1)
Family member (n=1)
WHY NOT?
I don't need to practice fueling as my race distance is so short 67% (n=24)
My stomach gets upset from eating/drinking anything during training sessions 22% (n=8)
I don't think it helps my training in any way 11% (n=4)
I'm trying to restrict carb and energy intake where possible 8% (n=3)
This is the first time I have heard of this strategy 6% (n=2)
Someone told me not to worry about this strategy 22% (n=8)
Other 6% (n=2)
I eat pretty close to the workout BEFORE so I can survive through the workout (n=1)
Because my throat dries (n=1)
RD/Nutritionist (n=4)
I read it somewhere (n=3)
Medical doctor (n=1)

Figure 4.8.

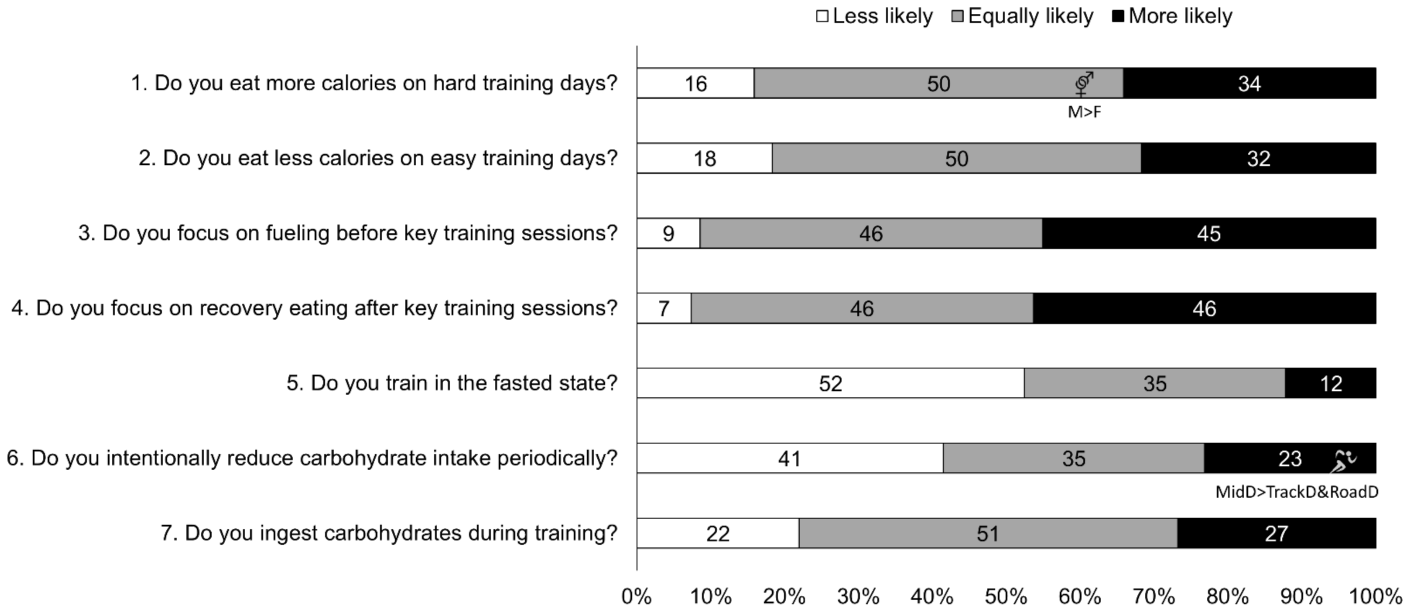
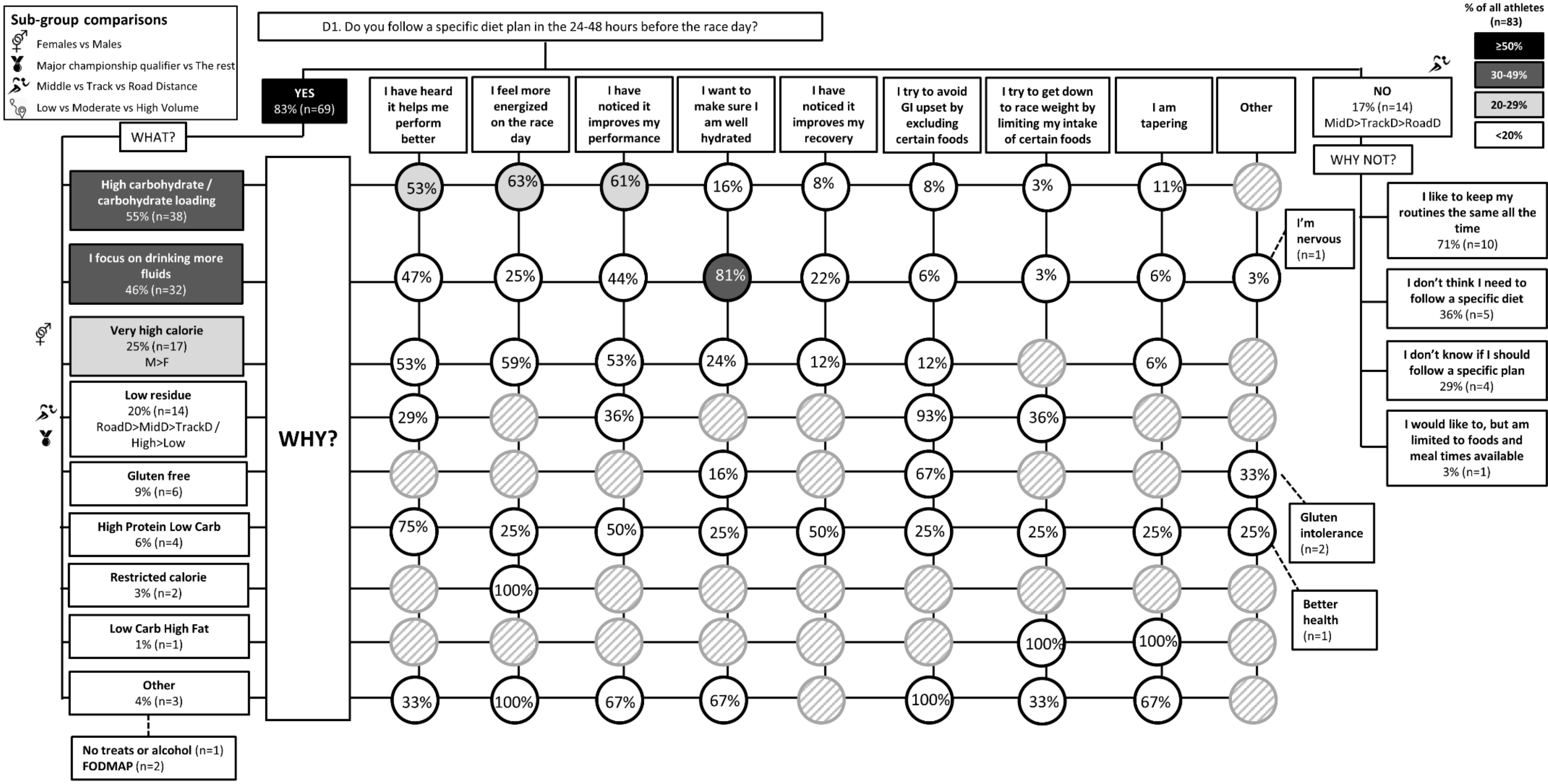

□ Less likely ▨ Equally likely ■ More likely
1. Do you eat more calories on hard training days?
16
50
34
M>F
2. Do you eat less calories on easy training days?
18
50
32
3. Do you focus on fueling before key training sessions?
9
46
45
4. Do you focus on recovery eating after key training sessions?
7
46
46
5. Do you train in the fasted state?
52
35
12
6. Do you intentionally reduce carbohydrate intake periodically?
41
35
23
MidD>TrackD&RoadD
7. Do you ingest carbohydrates during training?
22
51
27
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%

Figure 4.9.



Sub-group comparisons

Females vs Males

Major championship qualifier vs The rest

Middle vs Track vs Road Distance

Low vs Moderate vs High Volume

D1. Do you follow a specific diet plan in the 24-48 hours before the race day?

% of all athletes (n=83)

■	≥50%
▩	30-49%
▒	20-29%
□	<20%

YES 83% (n=69)

WHAT? / WHY?

	I have heard it helps me perform better	I feel more energized on the race day	I have noticed it improves my performance	I want to make sure I am well hydrated	I have noticed it improves my recovery	I try to avoid GI upset by excluding certain foods	I try to get down to race weight by limiting my intake of certain foods	I am tapering	Other
High carbohydrate / carbohydrate loading 55% (n=38)	53%	63%	61%	16%	8%	8%	3%	11%	▨
I focus on drinking more fluids 46% (n=32)	47%	25%	44%	81%	22%	6%	3%	6%	3%
Very high calorie 25% (n=17) M>F	53%	59%	53%	24%	12%	12%	▨	6%	▨
Low residue 20% (n=14) RoadD>MidD>TrackD / High>Low	29%	▨	36%	▨	▨	93%	36%	▨	▨
Gluten free 9% (n=6)	▨	▨	▨	16%	▨	67%	▨	▨	33%
High Protein Low Carb 6% (n=4)	75%	25%	50%	25%	50%	25%	25%	25%	25%
Restricted calorie 3% (n=2)	▨	100%	▨	▨	▨	▨	▨	▨	▨
Low Carb High Fat 1% (n=1)	▨	▨	▨	▨	▨	▨	100%	100%	▨
Other 4% (n=3)	33%	100%	67%	67%	▨	100%	33%	67%	▨

No treats or alcohol (n=1)
FODMAP (n=2)

I'm nervous (n=1)

Gluten intolerance (n=2)

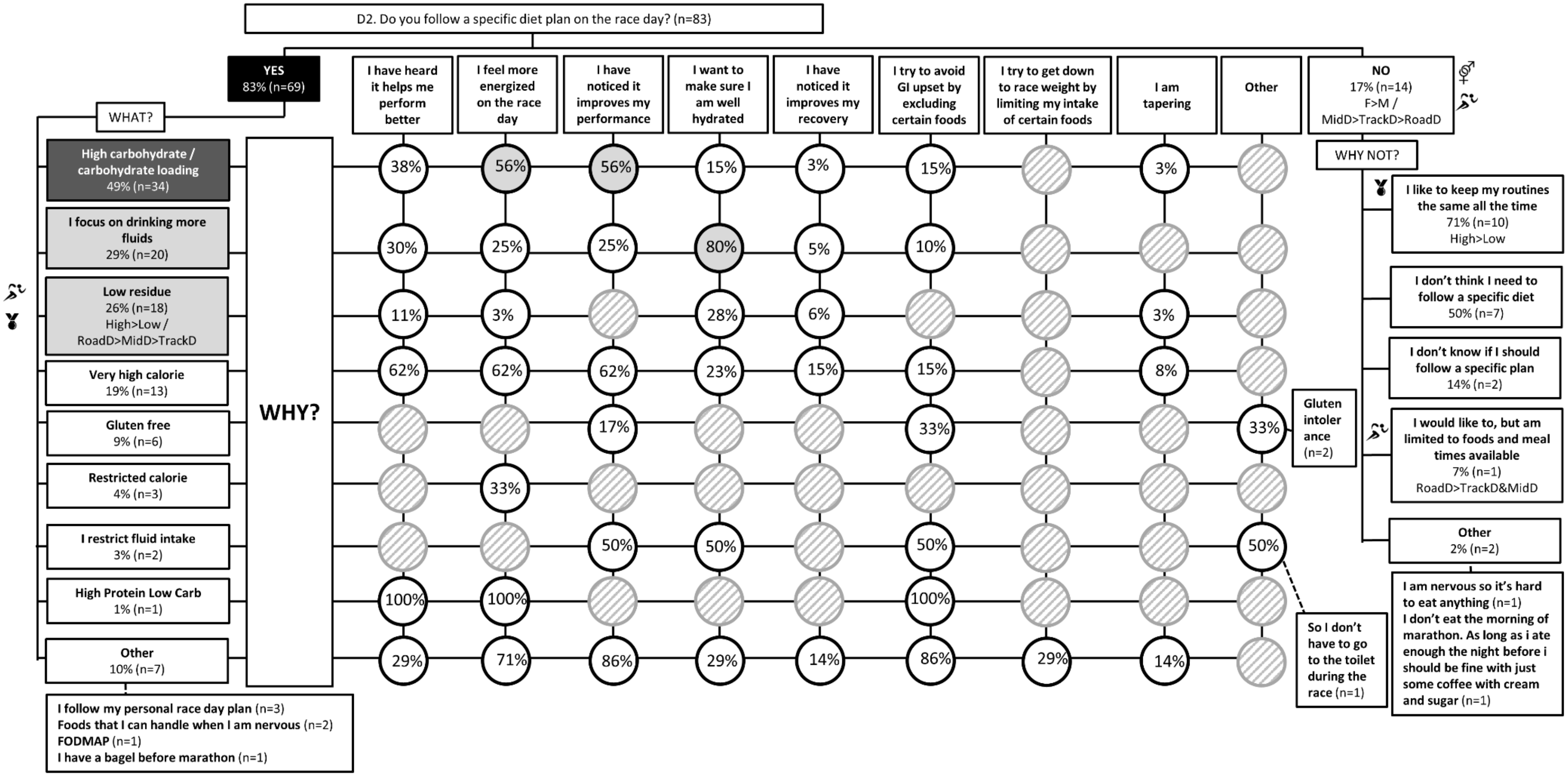
Better health (n=1)

NO 17% (n=14) MidD>TrackD>RoadD

WHY NOT?

- **I like to keep my routines the same all the time** 71% (n=10)
- **I don't think I need to follow a specific diet** 36% (n=5)
- **I don't know if I should follow a specific plan** 29% (n=4)
- **I would like to, but am limited to foods and meal times available** 3% (n=1)

Figure 4.10.



D2. Do you follow a specific diet plan on the race day? (n=83)

YES 83% (n=69) — WHAT? / WHY?	I have heard it helps me perform better	I feel more energized on the race day	I have noticed it improves my performance	I want to make sure I am well hydrated	I have noticed it improves my recovery	I try to avoid GI upset by excluding certain foods	I try to get down to race weight by limiting my intake of certain foods	I am tapering	Other
High carbohydrate / carbohydrate loading 49% (n=34)	38%	56%	56%	15%	3%	15%	▨	3%	▨
I focus on drinking more fluids 29% (n=20)	30%	25%	25%	80%	5%	10%	▨	▨	▨
Low residue 26% (n=18) High>Low / RoadD>MidD>TrackD	11%	3%	▨	28%	6%	▨	▨	3%	▨
Very high calorie 19% (n=13)	62%	62%	62%	23%	15%	15%	▨	8%	▨
Gluten free 9% (n=6)	▨	▨	17%	▨	▨	33%	▨	▨	33%
Restricted calorie 4% (n=3)	▨	33%	▨	▨	▨	▨	▨	▨	▨
I restrict fluid intake 3% (n=2)	▨	▨	50%	50%	▨	50%	▨	▨	50%
High Protein Low Carb 1% (n=1)	100%	100%	▨	▨	▨	100%	▨	▨	▨
Other 10% (n=7)	29%	71%	86%	29%	14%	86%	29%	14%	▨

Gluten intolerance (n=2)

So I don't have to go to the toilet during the race (n=1)

I follow my personal race day plan (n=3)
Foods that I can handle when I am nervous (n=2)
FODMAP (n=1)
I have a bagel before marathon (n=1)

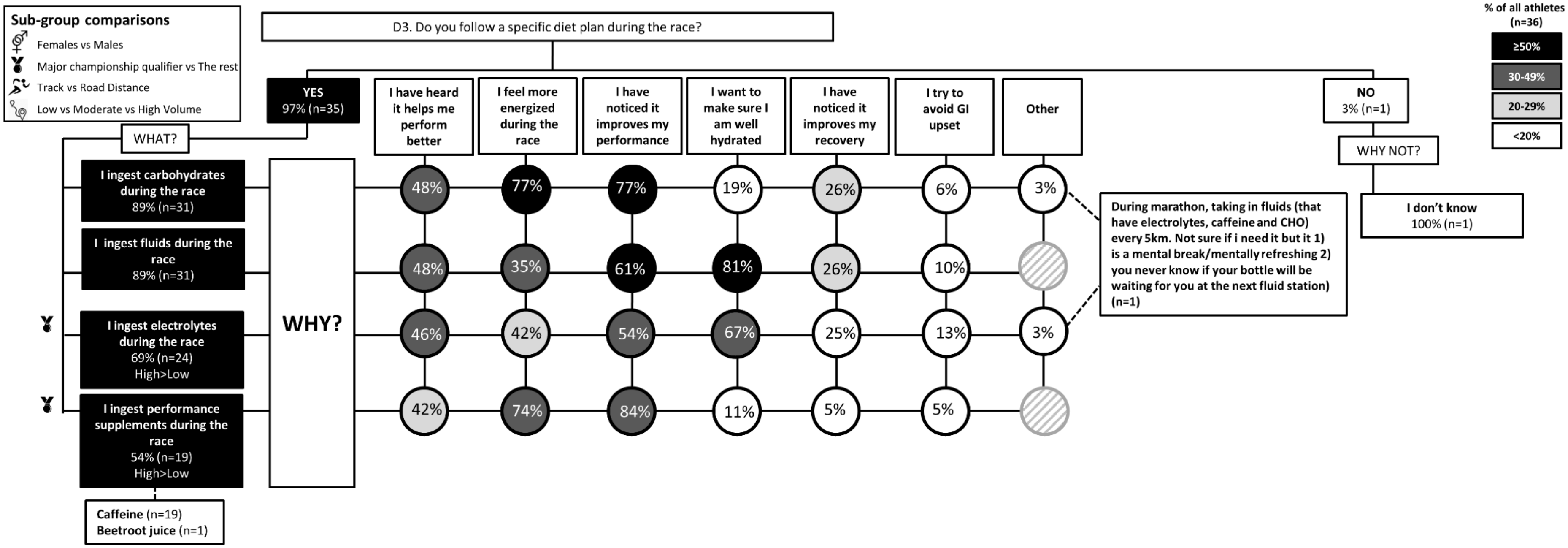
NO 17% (n=14) F>M / MidD>TrackD>RoadD

WHY NOT?

- I like to keep my routines the same all the time 71% (n=10) High>Low
- I don't think I need to follow a specific diet 50% (n=7)
- I don't know if I should follow a specific plan 14% (n=2)
- I would like to, but am limited to foods and meal times available 7% (n=1) RoadD>TrackD&MidD
- Other 2% (n=2)

I am nervous so it's hard to eat anything (n=1)
I don't eat the morning of marathon. As long as i ate enough the night before i should be fine with just some coffee with cream and sugar (n=1)

Figure 4.11.


Sub-group comparisons
Females vs Males
Major championship qualifier vs The rest
Track vs Road Distance
Low vs Moderate vs High Volume
D3. Do you follow a specific diet plan during the race?
% of all athletes (n=36)
≥50%
30-49%
20-29%
<20%
YES
97% (n=35)
WHAT?
WHY?
NO
3% (n=1)
WHY NOT?
I don't know
100% (n=1)
I ingest carbohydrates during the race
89% (n=31)
I ingest fluids during the race
89% (n=31)
I ingest electrolytes during the race
69% (n=24)
High>Low
I ingest performance supplements during the race
54% (n=19)
High>Low
Caffeine (n=19)
Beetroot juice (n=1)
During marathon, taking in fluids (that have electrolytes, caffeine and CHO) every 5km. Not sure if i need it but it 1) is a mental break/mentally refreshing 2) you never know if your bottle will be waiting for you at the next fluid station) (n=1)

	I have heard it helps me perform better	I feel more energized during the race	I have noticed it improves my performance	I want to make sure I am well hydrated	I have noticed it improves my recovery	I try to avoid GI upset	Other
I ingest carbohydrates during the race	48%	77%	77%	19%	26%	6%	3%
I ingest fluids during the race	48%	35%	61%	81%	26%	10%	▨
I ingest electrolytes during the race	46%	42%	54%	67%	25%	13%	3%
I ingest performance supplements during the race	42%	74%	84%	11%	5%	5%	▨

4.7 Interlinking chapter

In the first study of this thesis we reported that world-class endurance athletes periodise their nutrition across macro, meso and micro levels of training and racing. There were some differences between athlete subgroups in terms of nutrition practices based on the event (long vs middle-distance athlete) and sex (male vs female athlete). However, overall, this cohort appeared to focus on nutrition strategies that support elite performance and benefit body composition. Meanwhile, these athletes were less aware of or less concerned about the potential means of manipulating training adaptation with nutrition.

In the next study, we focused on dietary micro-periodisation practices and health outcomes in professional male cyclists across an 8 d single-day racing period.

5 STUDY 2: ALTERNATE-DAY LOW ENERGY AVAILABILITY DURING SPRING CLASSICS IN PROFESSIONAL CYCLISTS.

Publication statement:

This chapter is comprised of the following paper published in *International Journal of Sport Physiology & Performance*.

Heikura, IA., Quod, M., Strobel, N., Palfreeman, R., Civil, R., & Burke, LM. (2019). Alternate-Day Low Energy Availability During Spring Classics in Professional Cyclists. Int J Sports Physiol Perform. Mar 12:1-35. doi: 10.1123/ijspp.2018-0842. [Epub ahead of print]

5.1 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·kg FFM·d-1, race vs rest days, respectively; $p<0.001$). Cyclists with extremely low EA on race days (<10 kcal·kg FFM·d-1; n=2) experienced a trend towards decreased testosterone (-14%) and insulin-like growth-factor-1 (-25%), despite high EA (>46 kcal·kg FFM·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·kg·d-1, respectively;$p<0.001$). The cyclists reached contemporary pre-race fueling targets (3.4±0.7 g·kg·3h-1 carbohydrates; $p=0.24$), while the execution of CHO guidelines during race (51±9 g·h-1; $p=0.048$) and within acute (1.6±0.5 g·kg·3h-1; $p=0.002$) and prolonged (7.4±1.0 g·kg·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.

5.2 Introduction

Historically, professional road cyclists have been defined by their lean physiques as well as a high aerobic capacity (Jeukendrup et al., 2000). 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·kg-1) due to their greater need to perform against gravity, and are therefore lighter and also leaner (Mujika & Padilla, 2001). 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/m2) 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 (Armstrong, 2000; McMahon, 2016). Although these cyclists typically refine their (very) light and lean physiques as a by-product of large training volumes and restricted energy intake (Armstrong, 2000; McMahon, 2016), 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 (Jeukendrup, 2011). 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 (Mountjoy et al., 2018; Burke et al., 2018a). 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 (Loucks, 2004; Mountjoy et al., 2014). 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 (Burke et al., 2018a).

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·d-1) and carbohydrate (~12.6 g·kg·d-1) as well as considerably high energy expenditures (6070-7815 kcal·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 (Fordyce, 2018). 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 5.1). 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 (Tilt, 2018). Maintenance of body mass is not always achieved, and even when it is, it can sometimes be a poor signal of adequate EA (Trexler et al., 2014).

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 (Impey et al., 2018b).

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 5.2.

5.3 Methods

5.3.1 Participants and Study design

Six professional male cyclists from the Mitchelton-Scott UCI World Tour (Road Cycling) team participated in the study (Table 5.3). 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) (Quod et al., 2010).

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.

5.3.2 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 5.2).

5.3.3 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 5.2).

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 5.2 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 (Loucks et al., 2011) with a cutoff of 30 kcal·kg FFM·d-1being 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.

5.3.4 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·kg^{-1}; CHO intake within 24 h post-race: 10 g·kg^{-1}; CHO intake during the race: minimum 60 g·h^{-1} and maximum 90 g·h^{-1} (Thomas et al., 2016).

5.4 Results

5.4.1 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 5.4). LEA athletes (overall EA 28.2 ± 2.1 kcal·kg FFM·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 5.3) or skinfold thickness (p=0.75, Table 5.3) 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).

5.4.2 Timing of carbohydrate and protein intake around the Classics races

Carbohydrate intakes around racing are shown in Table 5.3. 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·30 min^{-1}) throughout the race, carbohydrate intake for cyclist 4 varied much more (3-35 g·30 min^{-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).

5.4.3 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 5.4). 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 5.1). 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%)..

5.5 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 5.2), 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 (Fordyce, 2018). 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 $kcal \cdot d^{-1}$) and carbohydrate (~12.6 $g \cdot kg \cdot d^{-1}$) intakes daily as well as around racing in professional male cyclists (Table 5.1). 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 5.2. 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.

5.6 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.

5.7 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.

Figure legends

Figure 5.1. 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.

Tables

Table 5.1. Available literature on nutrition during stage racing (4 d 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., 2018	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·d^{-1} CHO: 12.5 ± 1.8 g·kg·d^{-1} Protein: 3.3 ± 0.3 g·kg·d^{-1} Fat: 1.5 ± 0.5 g·kg·d^{-1}	During the race: CHO:91 ± 15 g·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., 1989	Male professional cyclists (n = 4): 1.78 m 69.2 kg VO2max: 79.4 ml·kg·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·d^{-1} Highest (mountain stage): 32.4 ± 4.4 MJ·d^{-1} Lowest (rest day): 16.1 ± 3.9 MJ·d^{-1} CHO: 61% total energy intake (~900 g·d^{-1}) Protein: 217 ± 47 g·d^{-1} Fat: 147 ± 39 g·d^{-1}	During the race: CHO: 94 g·h^{-1}	Energy expenditure: Overall mean: 25.4 ± 1.4 MJ·d^{-1} Highest (mountain stage): 32.7 ± 1.6 MJ·d^{-1} Lowest (rest day): 12.9 ± 0.9 MJ·d^{-1} BM: 69.2 kg (baseline) to 68.9 kg (post) Sum of 4 skinfolds estimation of body fat %: 11.6 (baseline) to 11.4 (post)

Garcia-Rovez et al., 1998	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 $ml \cdot kg \cdot min^{-1}$	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 $MJ \cdot d^{-1}$ (352 ± 33 kJ/kg/d) CHO: 12.6 ± 1.1 $g \cdot kg \cdot d^{-1}$ Protein: 3.0 ± 0.3 $g \cdot kg \cdot d^{-1}$ Fat: 2.4 ± 0.3 $g \cdot kg \cdot d^{-1}$	During the race: 25 $g \cdot h^{-1}$ CHO After the race (between race finish and dinner): CHO: 2.0 ± 0.5 $g \cdot kg^{-1}$ Fat: 0.2 ± 0.1 $g \cdot kg^{-1}$ Protein: 0.3 ± 0.1 $g \cdot kg^{-1}$	NR
Ebert et al., 2007	Male professional cyclists (n = 8): 25 ± 5 years 1.77 ± 0.05 m 71.4 ± 7.4 kg VO2max: 71.0 ± 6.2 $ml \cdot kg \cdot min^{-1}$	Tour Down Under: A 6-d stage race; Total distance of 719 km (stages between 50-152 km)	Recall immediately after each stage.	NR	During the race: CHO: 48 $g \cdot h^{-1}$	BM pre- and post-race for each stage

Ross et al., 2014	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 d *Tour of Geelong* (n=5): 6 stages over 5 d	Recall immediately after each stage.	NR	*Gippsland*: CHO: 40.5±24.2 g·h^{-1} *Geelong*: CHO: 64.2±23.7 g·h^{-1}	Hydration, change in BM during stages
Sanches-Munoz et al., 2016	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-d stage race; Total distance of 647.6 km	Weighed food records collected by investigators	Energy: 5644±593 kcal·d^{-1} CHO: 12.8 ± 1.7 g·kg·d^{-1} Protein: 3.0 ± 0.3 g·kg·d^{-1} Fat: 2.1 ± 0.2 g·kg·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., 2010	Male elite cyclists (n = 4): 20 ± 3 years 1.91 ± 0.06 m 84.1 ± 8.2 kg VO2peak: 57.6 ± 3.9 ml·kg·min^{-1} PPO: 415 ± 35 W	Tour of Southland 2005: A 6-d race with 10 stages; Total distance of 883 km	Weighed food records collected for the 6-d period	Energy: 27.3 ± 3.8 MJ·d^{-1} CHO: 12.9 ± 1.4 g·kg·d^{-1} Protein: 2.9 ± 0.3 g·kg·d^{-1} Fat: 128 ± 61 g·d^{-1} (17.3 ± 2.3 E%)		TEE (via DLW): 27.4 ± 2.0 MJ·d^{-1} EE: 16.9 ± 0.2 MJ·d^{-1} DXA lean mass: 68.8 ± 6.2 kg (baseline) DXA fat mass: 11.3 ± 2.9 kg (baseline) RMR: 11.5 ± 0.7 MJ·d^{-1}

Pfeiffer et al., 2012	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-d 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·h^{-1} Caffeine: 21 ± 29 mg·h^{-1} Sodium: 208 ± 183 mg·h^{-1}	NR

Fordyce, 2018	Male professional cyclist (n = 1: Chris Froome). Data provided by Team Sky	Giro d'Italia 2018: A 3-week stage race; Total distance of 3572.4 km across 21 d 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)	Weighed food records/ recall?	*Stage 11:* Energy: 2466 kcal CHO: 5.8 g·kg·d^{-1} Protein: 2.0 g·kg·d^{-1} Fat: 0.5 g·kg·d^{-1} *Stage 19:* Energy: 6663 kcal CHO: 18.9 g·kg·d^{-1} Protein: 2.1 g·kg·d^{-1} Fat: 1.3 g·kg·d^{-1}	*Stage 11:* CHO: 57 g·h^{-1} *Stage 19:* CHO: 96 g·h^{-1} CHO	*Stage 11:* Morning BM: 69.3 kg 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 5.2. 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 24 h 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.) Measurements should follow ISAK guidelines (Marfell-Jones et al., 2012).	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, 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 (Durnin & Womersley, 1974).	It should be possible to implement best practice protocol in future studies.

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 conditions, with a calibrated scale, before consumption of food/drinks.	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. 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.	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.

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 considered and included in the calculations.	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. (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.	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. **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 (Capling et al., 2017). 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 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 (Braakhuis et al., 2003).	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 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. The cyclists were encouraged to take photos of race nutrition (snacks inside the pockets) before	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-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.

		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. Alternatively, a common GE value of 20.7 % for cyclists (Coyle et al., 1992) 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.	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. **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).	Crashes and subsequent changes in bikes are a part of professional cycling racing, and cannot be avoided in real-life studies.

Table 5.3. 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 5.4. 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 ($g \cdot kg^{-1}$) ##	7.1	7.9	8.7	5.7	7.2	8.0	7.4	1.0
Rest day exercise variables								
MPO [W ($W \cdot kg^{-1}$)])	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 ($kcal \cdot kg\ FFM^{-1}$)	61.4	46.3	58.6	52.2	49.6	73.2	56.9	9.8
CHO ($g \cdot kg^{-1}$)	6.1	7.1	7.1	5.1	5.9	7.2	6.4	0.8
Protein ($g \cdot kg^{-1}$)	3.5	2.7	3.6	3.1	3.0	4.0	3.3	0.5
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.

Table 5.5. Blood concentrations of hormones at baseline and after the 10-day racing period for individual cyclists and as mean and standard deviations (SD).

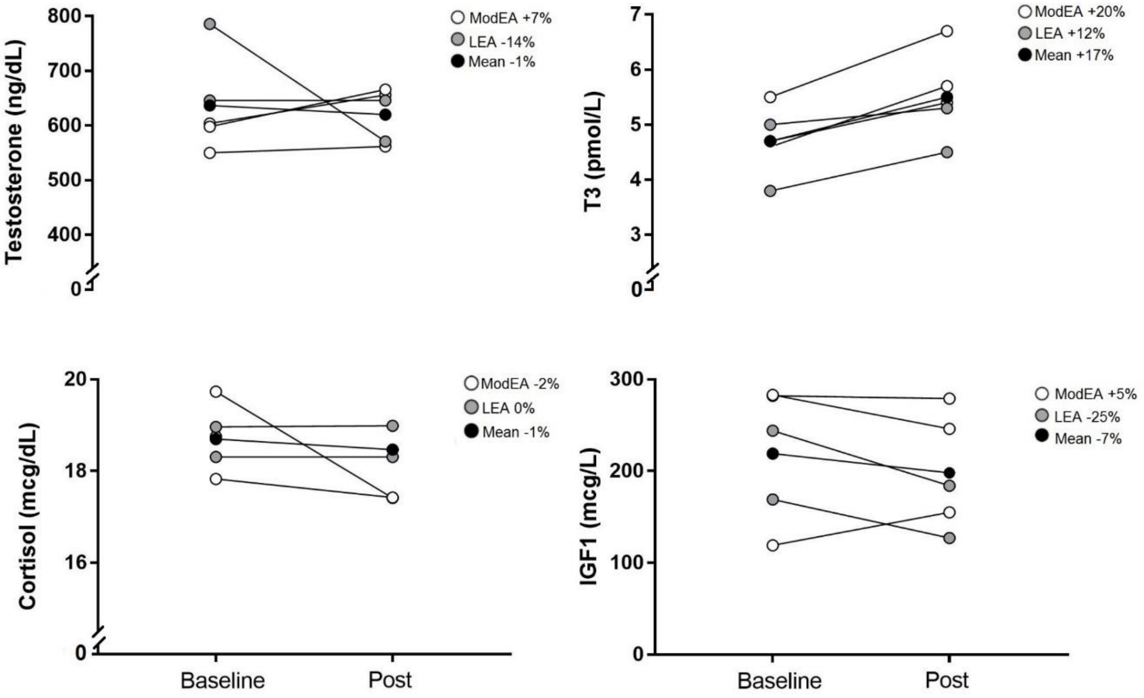
		Blood concentrations of hormones and iron markers							
Cyclist	***Time point***	**Hb (g·dL^{-1})**	**Hct (%)**	**Ferritin (mcg·L^{-1})**	**TSH (mU·L^{-1})**	**T4 (pmol·L^{-1})**	**LH (U·L^{-1})**	**FSH (U·L^{-1})**	**T/C ratio**
Cyclist 1	*Pre*	14.8	44.7	179	1.2	15.2	3.6	3.8	30.6
	Post	15.0	44.3		1.9	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.

Figure 5.1.


Testosterone (ng/dL)
800
700
600
500
400
0
ModEA +7%
LEA -14%
Mean -1%
T3 (pmol/L)
7
6
5
4
3
0
ModEA +20%
LEA +12%
Mean +17%
Cortisol (mcg/dL)
20
18
16
0
ModEA -2%
LEA 0%
Mean -1%
IGF1 (mcg/L)
300
200
100
0
ModEA +5%
LEA -25%
Mean -7%
Baseline
Post
Baseline
Post

5.8 Interlinking chapter

The second study of the thesis showed that professional male cyclists periodise energy and CHO availability across an 8-d single-day racing period, where higher CHO and lower EA occur on race as opposed to rest days. We also showed that in a single rider aiming to lose weight (severe low EA on race days and mean weekly EA below the threshold of low EA), significant drops in blood testosterone and IGF-1 concentrations were seen despite high EA (>45 kcal·kg FFM·d^{-1}) on rest days. On the contrary, overall, the riders maintained good hormonal health despite low EA on race days, likely due to managing to keep overall weekly EA well above the threshold of 30 kcal·kg FFM·d^{-1}.

In the next study, we turned the focus from periodised, acute low CHO availability to the effects of longer-term low CHO (but high energy) availability – 3.5 weeks of LCHF diet – on markers of bone modelling in elite race walkers.

6 STUDY 3: A SHORT-TERM KETOGENIC DIET IMPAIRS MARKERS OF BONE HEALTH IN RESPONSE TO EXERCISE

Publication statement:

This chapter is comprised of the following paper accepted for publication in *Frontiers in Endocrinology*.

Heikura, IA., Burke, LM., Hawley, JH., Ross, ML., Garvican-Lewis, L., Sharma, AP., McKay, AKA., Leckey, JJ., Welvaert, M., McCall, L., & Ackerman, KE. (2019). A short-term ketogenic diet impairs markers of bone health in response to exercise.

6.1 Abstract

Objectives: To investigate diet-exercise interactions related to bone markers in elite endurance athletes after a 3.5-week ketogenic low-carbohydrate, high-fat (LCHF) diet and subsequent restoration of carbohydrate (CHO) feeding. **Methods:** World-class race walkers (25 male, 5 female) completed 3.5-weeks of energy-matched (220 $kJ\cdot kg\cdot d^{-1}$) high CHO (HCHO; 8.6 $g\cdot kg\cdot d^{-1}$ CHO, 2.1 $g\cdot kg\cdot d^{-1}$ protein, 1.2 $g\cdot kg\cdot d^{-1}$ fat) or LCHF (0.5 $g\cdot kg\cdot d^{-1}$ CHO, 2.1 $g\cdot kg\cdot d^{-1}$ protein, 75-80% of energy from fat) diet followed by acute CHO restoration. Serum markers of bone breakdown (cross-linked C-terminal telopeptide of type I collagen, CTX), formation (procollagen 1 N-terminal propeptide , P1NP) and metabolism (osteocalcin, OC) were assessed at rest (fasting and 2 hr post meal) and after exercise (0 and 3 hr) at Baseline, after the 3.5-week intervention (Adaptation) and after acute CHO feeding (Restoration). **Results:** After Adaptation, LCHF increased fasting CTX concentrations above Baseline (p=0.007, Cohen's *d*=0.69), while P1NP (p<0.001, *d*=0.99) and OC (p<0.001, *d*=1.39) levels decreased. Post-exercise, LCHF increased CTX concentrations above Baseline (p=0.001, *d*=1.67) and above HCHO (p<0.001, *d*=0.62), while P1NP (p<0.001, *d*=0.85) and OC concentrations decreased (p<0.001, *d*=0.99) during exercise. Exercise-related area under curve (AUC) for CTX was increased by LCHF after Adaptation (p=0.001, *d*=1.52), with decreases in P1NP (p<0.001, *d*=1.27) and OC (p<0.001, *d*=2.0). CHO restoration recovered post-exercise CTX and CTX exercise-related AUC, while concentrations and exercise-related AUC for P1NP and OC remained suppressed for LCHF (p=1.000 compared to Adaptation). **Conclusion:** Markers of bone modeling/remodeling were impaired after short-term LCHF diet, and only a marker of resorption recovered after acute CHO restoration. Long-term studies of the effects of LCHF on bone health are warranted.

6.2 Introduction

Despite the generally positive effects of exercise in promoting bone health, bone injuries represent a challenge to consistent training and competition in high performance sport (Mountjoy et al., 2018). This, in part, is due to the interaction of dietary factors (e.g., low energy availability, poor vitamin D status, inadequate calcium intake) with unique features of the exercise program (e.g., minimal or excessive bone loading associated with weight- and non-weight-bearing sports, poor biomechanics (Mountjoy et al., 2018; Schofield & Hecht, 2012)). Low energy availability (a mismatch between energy intake and the energy cost of exercise) occurs in both female and male athletes (Schofield & Hecht, 2012) and impairs bone health via direct (uncoupled bone turnover with increased resorption rates) and indirect (mediation by reproductive and metabolic hormones) mechanisms (Mountjoy et al., 2018). In addition, carbohydrate (CHO) availability may also play a role in bone health. Indeed, results from several studies show that commencing endurance exercise with low compared to normal or high glycogen availability stimulates the release of the cytokine interleukin-6 (IL-6) from the exercising muscles (Steensberg et al., 2001; Keller et al., 2001). Among its range of effects, IL-6 has been hypothesized to lead to enhanced activity of the receptor activator of the nuclear factor K B-ligand, which controls bone turnover by increasing osteoclastic activity (thereby increasing bone breakdown) (Lombardi et al., 2016). In support of this contention, bone resorption is acutely increased when CHO is restricted before (Scott et al., 2012), during (Sale et al., 2015) and after (Townsend et al., 2017) prolonged (1-2 h) endurance (running) exercise, and may be linked to concomitant increases in IL-6 concentrations (Sale et al., 2015). However, a recent study has reported that acute reductions in CHO availability around exercise mediated an increase in markers of bone resorption that are independent of energy availability and circulating IL-6 (Hammond et al., 2019). Apparent effects on other markers of bone metabolism, such as osteocalcin (OC) and the bone formation marker procollagen 1 N-terminal propeptide (P1NP) in these models have been small (Scott et al., 2012; Sale et al., 2015; Townsend et al., 2017; Hammond et al., 2019), although a 24 h fast has been reported to reduce blood OC concentrations in lightweight rowers (Talbott et al., 1998).

Whether these changes in markers of bone metabolism persist (or are amplified) after chronic exposure to low CHO availability around exercise remains unknown, but is of relevance in view of the promotion of a ketogenic low CHO-high fat (LCHF) diet to athletes and its putative benefits for endurance performance (Volek et al., 2015). To date, no studies have examined the effects of longer-term restriction of CHO at rest or in relation to exercise, although in animal models and children with intractable epilepsy, chronic adaptation to a ketogenic LCHF diet is associated with poor bone health (Bielohuby et al., 2010; Scheller et al., 2016; Simm et al., 2017; Bergqvist et al., 2008; Scott et al., 2019). In view of our recent observations of increased post-exercise IL-6 concentrations in elite race walkers following a 3.5-week adaptation to a LCHF diet (McKay et al., 2019), we investigated the interaction of this diet and strenuous exercise on markers of bone modeling/remodeling as secondary outcomes of our larger study.

6.3 Methods

6.3.1 Participants

Thirty world-class athletes (25 male, 5 female race walkers; ages 27.7 ± 3.4 yr, BMI 20.6 ± 1.7 kg/m^2) were recruited over three separate training camps during preparation for the 2016 Summer Olympic Games and the 2017 World Championships, and provided written informed consent in accordance with the Human Ethics Committee of the Australian Institute of Sport (ethics approval no. 20150802 and 20161201). Six male participants undertook two camps, however two of these data sets were incomplete due to insufficient tissue samples, resulting in 4 participants who had completed two camps being included in the final analysis. In addition, two additional (male) data sets were excluded from the final analysis due to their inability to complete one of the experimental trials due to injury (unrelated to bone). Therefore, our final data set provided a total of 32 trials (n=28 participants, 23 males, 5 females) with data for pre- (*Baseline*) and post-treatment (*Adaptation*), of which 18 trials (13 males, 5 females) also contributed to data from acute restoration to a HCHO diet (*Restoration*). Participants and elite coaches contributed to the concept and implementation of the research camps, helping to prioritize the themes of interest and contributing to the design of the training program and test protocols.

6.3.2 Study overview

Participants completed a 3.5-week block of intensified training and laboratory and field testing, supported by either a high-CHO (HCHO) or an isoenergetic LCHF diet (Figure 6.1, Table 6.1), consumed under strict dietary control (Mirtschin et al., 2018). Upon completion of the 3.5-week dietary intervention, a subset of participants (n=18) completed a further testing block under conditions of acute high CHO availability. Markers of bone metabolism were measured after an overnight fast, in response to an energy-matched meal of nutrient composition matching the intervention diet, and in response to a bout of strenuous exercise (Burke et al., 2017), at Baseline, Adaptation, and Restoration (Figure 6.1).

6.3.3 Dietary control

Details of dietary control are described briefly here; more details are described in prior work (Mirtschin et al., 2018). Participants were allocated into HCHO and LCHF groups based on preference. Both diets were isocaloric (Table 6.1), however dietary CHO and fat intakes differed between groups during intervention. Study diets were designed and individualized for each athlete by trained members of the research team including registered sports dietitians, a professional chef, and exercise physiologists. All meals were weighed (food scales accurate to 2 g) and provided for athletes at set meal times. In addition, a collection of snacks per individual meal plans were provided to the athletes each day. Any unconsumed items or changes made to menu plans were weighed and recorded for final analysis of dietary intakes. Compliance to the meal plans was assessed daily. Meal plans were designed and final dietary analysis of actual intakes was conducted using FoodWorks 8 Professional Program (Xyris Software Australia Pty Ltd, Australia). Further analysis of intakes was completed using Microsoft Excel.

6.3.4 Experimental design

Testing at Baseline, Adaptation, and Restoration involved a hybrid laboratory/field test of 25 km (males) or 19 km (females) at around 50 km race pace (75% of maximal oxygen uptake [VO_2max]) (Figure 6.1). Upon entering the laboratory in an overnight fasted and rested state between 0600 and 0800 in the morning (times were kept consistent within-participant), a cannula was inserted into an antecubital vein for collection of blood samples at rest (Fasting), immediately before exercise (2 h post-meal), immediately after exercise (Post-ex) and 3 h post-exercise (3 h post-ex). Blood was analyzed for concentrations of cross-linked C-terminal telopeptide of type I collagen (CTX), P1NP and total OC to determine the effects of dietary interventions and exercise on bone metabolism. The cannulas were flushed with 3 ml of saline every 30 min throughout the trials. A standardized breakfast (2 $g \cdot kg^{-1}$ CHO for both groups during Baseline and Restoration, or an isocaloric low CHO option for LCHF during Adaptation) was consumed 30 min after the first blood sample, after which the participants rested for 120 min before beginning the session. During the Baseline and Restoration exercise test, both groups ingested glucose (60 $g \cdot h^{-1}$) throughout the test, while during Adaptation, isocaloric high fat snacks were provided for the LCHF group. Upon completion of the exercise test, the participants rested in the laboratory for a further 3 h, and received a standardized recovery shake (1.5 $g \cdot kg^{-1}$ CHO for both groups during Baseline and Restoration, or an isocaloric low CHO option for LCHF during Adaptation; both shakes included 0.3 $g \cdot kg^{-1}$ protein) at 30 min post-exercise to improve satiety.

Analysis of serum bone modeling/remodeling biomarkers Blood samples were collected into a 3.5 mL EDTA BD Vacutainer Plus SST II tube, and allowed to clot by standing at room temperature for 2 h before centrifuging at 1,000G for 10 min for subsequent analysis of serum markers of bone resorption (CTX), bone formation (P1NP) and overall bone metabolism (OC). Analysis was undertaken by chemiluminescence on IDS-iSYS (Immunodiagnostic Systems Limited; Boldon, Tyne and Wear, UK). Inter-assay coefficient of variation as reported by the manufacturer was 6.2%, 4.6% and 6.1%, respectively. CVs were determined as follows: OC: 6 serum controls were run, using 3 reagents lots, in duplicate twice per day for 20 days, on 2 analyzers; P1NP: 3 serum controls were run, using 3 reagent lots, in quadruplicates once per day for 20 days, on 2 analyzers; CTX: 5 serum controls were run, using 3 reagent lots, in duplicate twice per day for 20 days, on 3 analyzers. In addition to these tests, the laboratory ran quality control samples throughout testing and the results were within the established acceptable manufacturer ranges.

6.3.5 Statistical analyses

Statistical analyses were conducted using SPSS Statistics 22 software (INM, New York, USA) and R (R Core Team, 2018) with a significance level set at $p \leq 0.05$. Normality of data was checked with a Shapiro-Wilk test and visual inspection of residual plots. General Linear Mixed models were fitted using the R package lme4 (Bates et al., 2015) and included random intercepts for Subjects and Camps to account for baseline inter individual heterogeneity and the partial cross-over design. Because the estimated Camp effect variance was 0, this random intercept was subsequently removed to resolve boundary issues in the Restricted Maximum Likelihood estimation. P-values were obtained using Type II Wald F tests with Kenward-Roger degrees of freedom. Initial models included all possible interactions but non-significant interaction terms were dropped for ease of interpretation. Fasting values and exercise-related area under curve (AUC; Pre-exercise to 3 h post-exercise (Matthews et al., 1990)) for all markers were compared with a two-way mixed analysis of variance (ANOVA), with *post hoc* tests of Student's t-tests for independent samples (between-groups) and for paired samples (within-groups); where normality was violated, Wilcoxon's test and Mann-Whitney U-test were used. Where a data point was missing, AUC was not calculated; this resulted in exclusion of 1 participant in the CTX AUC calculations, and 2 participants from both P1NP and OC calculations. Effect sizes were calculated based on the Classical Cohen's *d* while accounting for the study design by using the square root of the sum of all the variance components (specified random effects and residual error) in the denominator. Data are presented as means (95% confidence intervals [CI]).

6.4 Results

Bone modeling/remodeling biomarkers during fasting Compared to Baseline, fasting concentrations of CTX were increased after the LCHF diet (+22% [9, 35]: p=0.008, *d*=0.69), with a decrease in P1NP (-14% [-19, -9; p=0.001, *d*=0.99] and OC (-25% [-35, -14]; p<0.001, *d*=1.39) levels (Figure 6.2). In addition, the change in fasting P1NP (p<0.001, *d*=1.64) and OC (p<0.001, *d*=1.78) after the 3.5 week intervention was significantly different between the diets (Figure 6.2).

6.4.1 Exercise bone markers

CTX decreased post-meal independent of dietary intervention (Figure 6.3A, Figure 6.4A, p<0.001, *d*=1.63). At Adaptation, post-exercise CTX concentrations in LCHF increased above Baseline (p=0.001, *d*=1.67) and HCHO (p<0.001, *d*=0.62) (Figure 6.3A). LCHF decreased P1NP (Figure 6.3B, p<0.001, *d*=0.85) and OC across exercise (Figure 6.3C, p<0.001, *d*=0.99) compared to Baseline. At Restoration, post-exercise CTX returned to Baseline levels for LCHF (Figure 6.4A, p>0.05, *d*=0.20 compared to Baseline), while concentrations of P1NP (Figure 6.4B, p<0.001, *d*=0.23) and OC (Figure 6.4C, p<0.001, *d*=0.21) remained suppressed across exercise.

6.4.2 Bone marker exercise area under curve

At Adaptation, LCHF exercise-related AUC for CTX was greater [+81% (54, 109); p<0.001, *d*=1.52] than Baseline, and higher than HCHO (p=0.035, *d*=0.81) (Figure 6.3D). Exercise-related AUC for P1NP decreased at Adaptation for LCHF [-19% (-25, -12); p=0.003, *d*=1.27] compared with Baseline and was lower than HCHO (p=0.009, *d*=1.03) (Figure 6.3E), with similar outcomes for OC [-29% (-35, -23); p<0.001, *d*=2.0 and p<0.001, *d*=1.64, Figure 6.3F]. At Restoration, LCHF experienced a return of exercise-related AUC for CTX back to Baseline values [-43% (-21, 31); p=0.003, *d*=1.08 compared to Adaptation and no difference compared to HCHO; Figure 6.4D], meanwhile AUC for P1NP [+3% (-17, 48), p=1.000 compared to Adaptation and p=0.009, *d*=1.50 compared to HCHO; Figure 6.4E], OC [-3% (-19, 14), p=1.000 compared to Adaptation and p=0.010, *d*=1.47 compared to HCHO; Figure 6.4F] remained suppressed.

6.5 Discussion

Our data reveal novel and robust evidence of acute and likely negative effects on the bone modeling/remodeling process in elite athletes after a short-term ketogenic LCHF diet, including increased marker of resorption (at rest and post-exercise) and decreased formation (at rest and across exercise), with only partial recovery of these effects following acute restoration of CHO availability. Long-term effects of such alterations remain unknown, but may be detrimental to bone mineral density (BMD) and bone strength, with major consequences to health and performance. While ketogenic diets are of interest to athletes due to their ability to induce substantial shifts in substrate metabolism, increasing the contribution of fat-based fuels during exercise (Volek et al., 2015), we have previously reported the downside of a concomitantly greater oxygen cost and reduced performance of sustained high-intensity endurance exercise (Burke et al., 2017). The current study identifies further complexity in the interaction between the ketogenic diet and exercise with respect to markers of bone modeling/remodeling, in which catabolic processes are augmented and anabolic processes are reduced.

The LCHF diet is also popular within the general community for its purported health benefits, including rapid weight loss and improved glycemic control (Athinarayanan et al., 2019). However, data from animal studies (Bielohuby et al., 2010; Scheller et al., 2016) demonstrate that chronic LCHF diets are associated with impaired bone growth, reduced bone mineral content, compromised mechanical properties, and slower fracture healing. Furthermore, increased bone loss has been reported in children with intractable epilepsy placed on a medically supervised LCHF diet for 6 months (Simm et al., 2017; Bergqvist et al., 2008). In contrast, adults with type 2 diabetes mellitus who self-selected to consume a LCHF diet for 2 years experienced no changes in spinal BMD in comparison to a ‘usual care’ group (Athinarayanan et al., 2019). One explanation for these divergent outcomes involves interactions of the LCHF diet with the level of habitual contractile activity. Indeed in mice, a LCHF diet negated the positive benefits of exercise on BMD in trabecular bone (Scott et al., 2019), while in children with epilepsy, the rate of bone loss was greater in the more active patients (Simm et al., 2017). Therefore, the hormonal response to exercise undertaken with low CHO availability was of particular interest in our study.

Previous studies involving acute strategies of low CHO availability around exercise have identified effects on bone resorption, as measured by increased blood CTX concentrations. For example, males who undertook 60 min of treadmill running at 65% VO$_2$max following a CHO-rich breakfast (~1 $g\cdot kg^{1}$) showed small variations in CTX responses, but only around the exercise period, while dietary effects on parathyroid hormone, OC and P1NP were not detected (Scott et al., 2012). Meanwhile, a more strenuous protocol (120 min at 70% VO$_2$max) was associated with an attenuation of acute (pre-exercise to 2 h post-exercise) concentrations of IL-6, CTX, and P1NP when CHO was consumed (0.7 $g\cdot kg\cdot h^{1}$) during exercise (Sale et al., 2015). However, OC was unchanged by diet and no differences in markers of bone metabolism were detected over the subsequent three days, suggesting that these effects are transient and quickly reversed (Sale et al., 2015). Short-term effects were also reported when 24 elite male runners with energy-matched intake over an 8 d period were divided into a group who consumed CHO before, during, and immediately after each of their 13 training sessions (additional total CHO) while the others consumed an artificially sweetened placebo (de Sousa et al., 2014). Here, CTX concentrations were suppressed at 80 min of recovery following an interval training sessions in the CHO group with no dietary effects on P1NP or OC; furthermore, fasting concentrations of all markers were similar at baseline and on the ninth morning (de Sousa et al., 2014). Finally, Hammond and colleagues (2019) investigated the independent effects of low CHO availability and acute energy restriction during the recovery from one session of high-intensity interval running and the completion of a subsequent session (3.5 h into recovery). They reported lower CTX concentrations in the high CHO (control) diet compared with both of the other conditions across the various acute responses to exercise-related feeding, while there were no differences between the energy and CHO restricted trials. Meanwhile, only energy restriction produced an increase in IL-6 responses to exercise, and there were no differences in P1NP concentrations between dietary treatments (Hammond et al., 2019). Furthermore, 5 d of low vs optimal energy availability, which also resulted in a 2-fold difference in CHO availability, was shown to result in a significant difference in the AUC of fasting CTX (+85 vs +15%, respectively) and P1NP (-60 vs -25%, respectively) (Papageorgiou et al., 2017). To date, the only study to report an effect of acute manipulations of CHO around exercise on bone formation markers was that of Townsend et al. (2017), in which the immediate consumption of a protein-CHO feeding after a run to exhaustion at 75% VO_2max was associated with a suppression of the post-exercise rise in CTX levels and a higher concentration of P1NP. These authors concluded that immediate post-exercise meal ingestion may benefit bone health compared to delayed feeding, although the effects on CTX concentrations were reversed at 4 h post-exercise and a similar time course of P1NP changes was not provided; therefore, it appears that the overall effect on bone modeling/remodeling processes appears to follow meal ingestion patterns.

The novelty of the current study was the interrogation of the effects of ***prolonged*** adaptation to CHO restriction on bone metabolism. Unlike the previous investigations, we identified clear and consistent effects on bone metabolism at rest and in response to exercise following 3.5 weeks of a ketogenic LCHF diet (Figures 6.2-6.4), with increases in a marker of bone resorption (CTX) and decreases in markers of bone formation (P1NP) and metabolism (OC). Although some might argue that a complete adaptation to a LCHF diet requires much longer than the 3.5-week period utilized in the current study, it should be noted that adaptations in substrate metabolism and exercise economy have been reported across this (Burke et al., 2017; Shaw et al., 2019), and much shorter (Burke et al., 2019), time periods. Nevertheless, the current study is reflective of a shorter-term adaptation to a LCHF diet and our findings warrant further investigation across longer time periods.

Acute restoration of high CHO availability was only partially effective in reversing these outcomes. Here, marker of bone resorption returned to baseline with high CHO pre-exercise meal and CHO ingestion throughout exercise, while the other markers of bone metabolism remained suppressed, indicating impaired overall balance of bone metabolism. This supports the concept proposed by Hammond et al. (2019) that CTX is responsive to acute intake of CHO, possibly mediated through enteric hormone secretion. Meanwhile, differences in muscle glycogen concentration, which are not addressed by studies of acute feedings, may have a greater effect on OC and P1NP concentrations. Given the serious nature of injury risks and long-term outcomes of poor bone health in later life in endurance athletes, further consideration of the potential effects of the LCHF diet in exacerbating existing risk factors for poor bone health is warranted. In particular, we note that the impairment of bone metabolism around exercise and recovery would involve a significant portion of the day in athletes who undertake multiple training sessions, as well as being superimposed on the changes identified at rest.

The interaction of diet and exercise on bone metabolism is complex and requires more sophisticated investigation including replication of the current findings. Furthermore, evolving knowledge of inter-organ crosstalk suggests that outcomes of altered bone metabolism may be more far-reaching than the fate of the structural integrity of bone. Indeed, we note the recognition of muscle and bone as endocrine organs, with evidence that IL-6 released from contracting muscle has autocrine, paracrine and endocrine effects (Karsenty et al., 2017). This includes a purported feed-forward loop in which contraction-induced stimulation of osteocalcin in myofibers promotes the release of IL-6 and enhances muscle adaptation to exercise (Karsenty et al., 2017). Results of the current study challenge this synergistic relationship between osteocalcin signaling and IL-6, and remind us of the pleiotropic nature of the molecules stimulated by diet-exercise interactions.

Limitations: The data analysis undertaken in this study was a secondary outcome of our investigations of the ketogenic LCHF diet; these were not specifically powered to optimally address the potential effects on markers of bone modeling/remodeling. However, the detection of changes in the IL-6 response to prolonged exercise in our initial study (McKay et al., 2019) provided motivation to examine possible downstream effects. Because an identical protocol was undertaken in two separate studies of the LCHF diet, we were able to pool data from these investigations to double the sample size previously known to allow detection of changes in metabolism and performance. Indeed, changes in markers of bone metabolism in the response to the interaction of exercise and the dietary treatments were clearly detected with the pooled data, but were also identifiable in the case of the smaller sample size of the carbohydrate restoration arm of the current dataset. Therefore, we feel confident that our data are robust and warrant further investigation of this theme.

6.6 Conclusions

Despite recent interest in the potential benefits of LCHF diets on endurance performance or metabolic adaptation, the long-term health effects of this dietary intervention are largely unknown. We are the first to show that a 3.5-week ketogenic LCHF diet in elite endurance athletes has negative effects on the markers of bone modeling/remodeling at rest and during a prolonged high intensity exercise session. We also show only partial recovery of these adaptations with acute restoration of CHO availability. Given the injury risks and long-term outcomes underpinned by poor bone health in later life, in athletes as well as individuals who undertake exercise for health benefits, additional investigations of the ketogenic diet and its role in perturbing bone metabolism are warranted.

Acknowledgements

The study was funded by a Program Grant from the Australian Catholic University Research Funds to Professor Louise Burke (ACURF, 2017000034). The authors and funding agents do not have any conflicts of interests. Data were harvested from 2 trials registered at Australian New Zealand Clinical Trial Registry (ACTRN12619001015134 and ACTRN12619000794101), found at http://www.ANZCTR.org.au/ACTRN12619001015134.aspx and http://www.ANZCTR.org.au/ACTRN12619000794101.aspx

Conception and design of the experiments was undertaken by IAH, LMB, MLR, LG-L, APS, AKAM, JJL, MW, LM and KEA; Collection, assembly, analysis and interpretation of data was undertaken by IAH, LMB, MLR, LG-L, APS, AKAM, JJL, MW, LM and KEA; Manuscript was prepared by IAH, LMB, KEA and JAH. All authors approved the final version of the manuscript. IAH and LMB had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. We thank our research colleagues and supporters of the Supernova research series and acknowledge the commitment of the elite race-walking community

Table 6.1. Dietary intakes in the HCHO and LCHF groups.

	Intervention		**Restoration**	
	HCHO (n=14)	**LCHF (n=18)**	**HCHO (n=8)**	**LCHF (n=10)**
Energy (kJ·d^{-1})	14518 ± 2142	15138 ± 2104	13705 ± 1948	15706 ± 1774
Energy (kJ·kg·d^{-1})	229 ± 13	227 ± 23	219 ± 16	239 ± 27
Protein (g·d^{-1})	133 ± 22	143 ± 19	132 ± 24	151 ± 18
Protein (g·kg·d^{-1})	2.1 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.3 ± 0.2
Fat (g·d^{-1})	74 ± 14	318 ± 45***	77 ± 14	95 ± 12 **$$$
Fat (g·kg·d^{-1})	1.2 ± 0.1	4.8 ± 0.5***	1.2 ± 0.1	1.4 ± 0.2 **$$$
CHO (g·d^{-1})	549 ± 75	35 ± 5***	492 ± 60 $	552 ± 62 $$$
CHO (g·kg·d^{-1})	8.7 ± 0.4	0.5 ± 0.1***	7.9 ± 0.6 $$	8.4 ± 1.0 $$$

HCHO, high carbohydrate diet; LCHF, low carbohydrate high fat diet; CHO, carbohydrate.

**p<0.01, ***p<0.001 significant difference between diets

$p<0.05, $$$p<0.001 significantly different compared to Intervention

Figure Legends

Figure 6.1. Study flowchart and overview. Thirty-two data sets were gathered from 30 participants who participated in one or more training camps. After Baseline testing on a carbohydrate-rich (HCHO) diet, they elected to follow a 3.5-week energy-matched dietary intervention of either HCHO or ketogenic low carbohydrate-high fat (LCHF) principles. After Adaptation, the participants underwent an acute period of Restoration of high carbohydrate availability. At Baseline and at the end (Adaptation) of this intervention, as well as after acute carbohydrate reintroduction (Restoration) they undertook a test block including a 25 km (2 hr) hybrid laboratory/field race walking protocol at ~75% VO_2max. Venous blood samples were collected after an overnight fast, 2 hr after an energy-matched breakfast based on their diet (immediately pre-exercise), immediately post exercise and after 3 hr of passive recovery during which an intervention-matched recovery shake was consumed at 30 min. Blood samples were analyzed for serum concentrations of C-terminal telopeptide of type I collagen (CTX), procollagen 1 N-terminal propeptide (P1NP) and osteocalcin (OC).

Figure 6.2. Percentage change in fasting serum C-terminal telopeptide of type I collagen (CTX), procollagen 1 N-terminal propeptide (P1NP) and osteocalcin (OC) for high carbohydrate (HCHO; solid bars) and low CHO high fat (LCHF; striped bars) after the 3.5-week dietary intervention. Data are means ± standard deviations. ***p<0.001 Significant between-group difference; ##p<0.01, ###p<0.001 Significant change from Baseline within-group.

Figure 6.3. Time course of changes in bone marker concentrations across exercise (left panel) and exercise area under curve (right panel) for serum C-terminal telopeptide of type I collagen (CTX) (**A, D**), procollagen 1 N-terminal propeptide (P1NP) (**B, E**) and osteocalcin (OC) (**C, F**) after the 3.5-week dietary intervention. Black bars/symbols represent Baseline, gray bars/symbols represent Adaptation. Squares and circles represent high carbohydrate (HCHO) and low carbohydrate high fat (LCHF), respectively. Grey bars represent a hybrid laboratory/field 19-25 km walk test at ~75% VO_2max. Data are means ± standard deviations. ## p<0.01, ###p<0.001 denotes significant differences at time points or tests within diet groups; *p<0.05, **p<0.01, ***p<0.001 denotes significant differences between diet groups at a specific time point.

Figure 6.4. Time course of changes in bone marker concentrations across exercise (left panel) and exercise area under curve (right panel) for serum C-terminal telopeptide of type I collagen (CTX) (**A, D**), procollagen 1 N-terminal propeptide (P1NP) (**B, E**) and osteocalcin (OC) (**C, F**)

after acute reintroduction of carbohydrate (right panel). Gray bars/symbols represent Adaptation, and white bars/symbols represent Restoration. Squares and circles represent high carbohydrate (HCHO) and low carbohydrate high fat (LCHF), respectively. Grey bars represent a hybrid laboratory/field 19-25 km walk test at ~75% VO_2max. Data are means ± standard deviations. $$p<0.01, $$$p>0.001 denotes significant within-group difference compared to Restoration; *p<0.05, **p<0.01, ***p<0.001 denotes significant differences between diet groups at a specific time point.

Figure 6.1

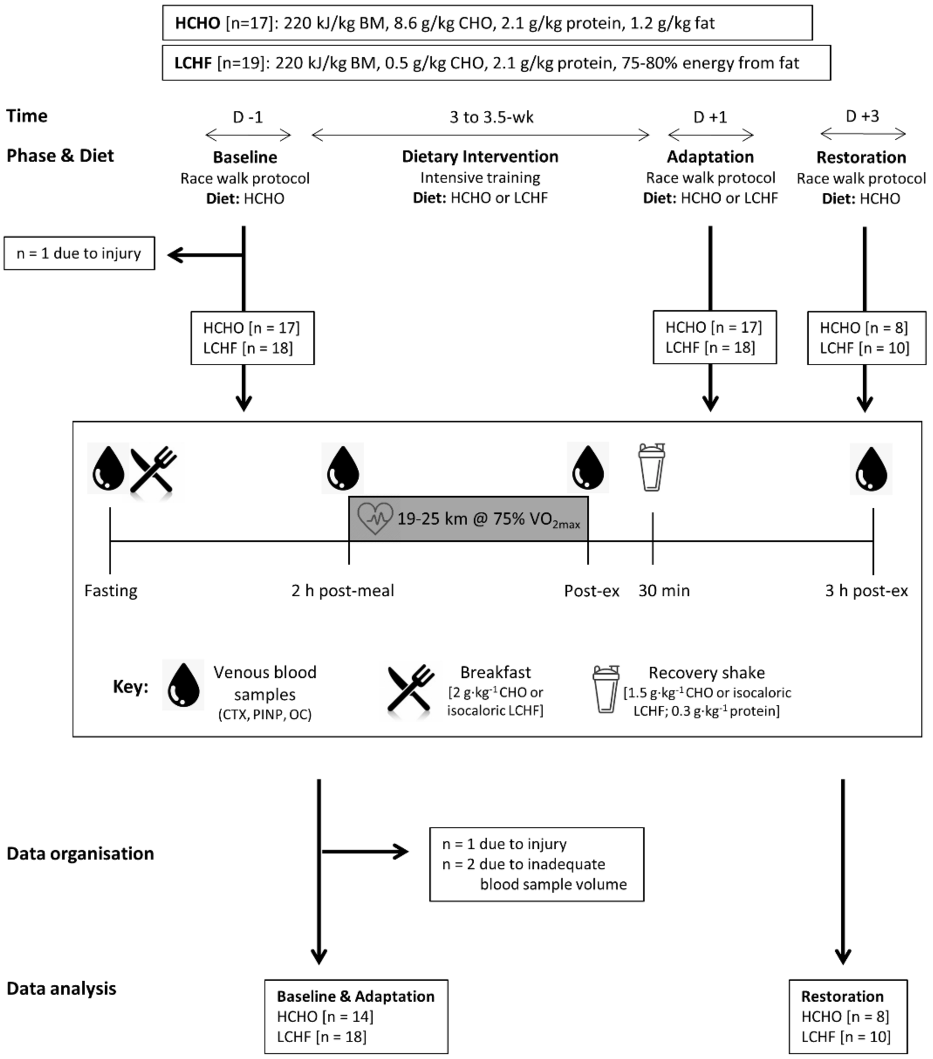

HCHO [n=17]: 220 kJ/kg BM, 8.6 g/kg CHO, 2.1 g/kg protein, 1.2 g/kg fat
LCHF [n=19]: 220 kJ/kg BM, 0.5 g/kg CHO, 2.1 g/kg protein, 75-80% energy from fat
Time
Phase & Diet
D -1
Baseline
Race walk protocol
Diet: HCHO
3 to 3.5-wk
Dietary Intervention
Intensive training
Diet: HCHO or LCHF
D +1
Adaptation
Race walk protocol
Diet: HCHO or LCHF
D +3
Restoration
Race walk protocol
Diet: HCHO
n = 1 due to injury
HCHO [n = 17]
LCHF [n = 18]
HCHO [n = 17]
LCHF [n = 18]
HCHO [n = 8]
LCHF [n = 10]
19-25 km @ 75% VO_{2max}
Fasting
2 h post-meal
Post-ex
30 min
3 h post-ex
Key:
Venous blood samples (CTX, PINP, OC)
Breakfast [2 $g \cdot kg^{-1}$ CHO or isocaloric LCHF]
Recovery shake [1.5 $g \cdot kg^{-1}$ CHO or isocaloric LCHF; 0.3 $g \cdot kg^{-1}$ protein]
Data organisation
n = 1 due to injury
n = 2 due to inadequate blood sample volume
Data analysis
Baseline & Adaptation
HCHO [n = 14]
LCHF [n = 18]
Restoration
HCHO [n = 8]
LCHF [n = 10]

Figure 6.2

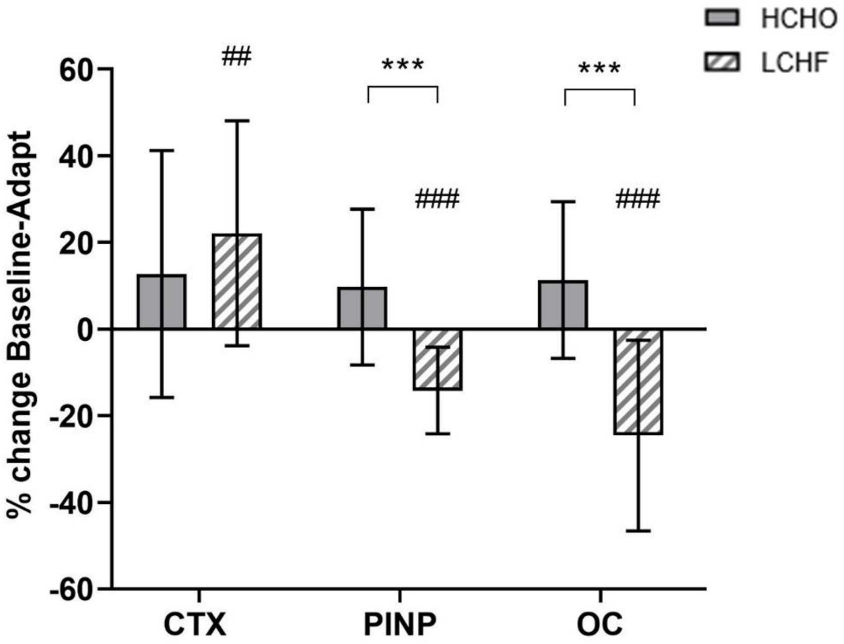
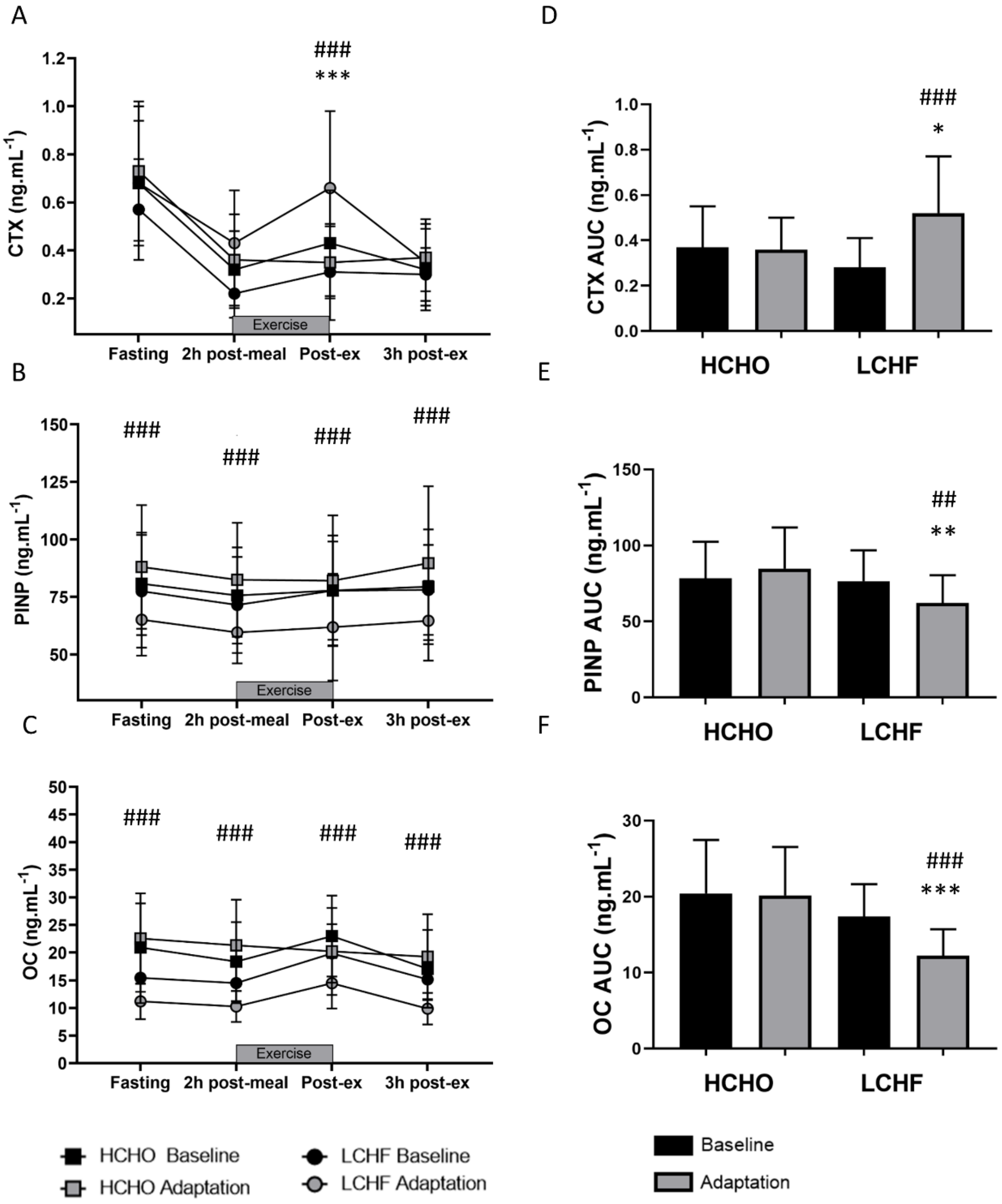


Figure 6.3


A
CTX (ng.mL^{-1})
1.2
1.0
0.8
0.6
0.4
0.2
###
***
Exercise
Fasting
2h post-meal
Post-ex
3h post-ex
D
CTX AUC (ng.mL^{-1})
1.0
0.8
0.6
0.4
0.2
0.0
###
*
HCHO
LCHF
B
PINP (ng.mL^{-1})
150
125
100
75
50
###
###
###
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Exercise
Fasting
2h post-meal
Post-ex
3h post-ex
E
PINP AUC (ng.mL^{-1})
150
100
50
0
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HCHO
LCHF
C
OC (ng.mL^{-1})
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45
40
35
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25
20
15
10
5
0
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Exercise
Fasting
2h post-meal
Post-ex
3h post-ex
F
OC AUC (ng.mL^{-1})
30
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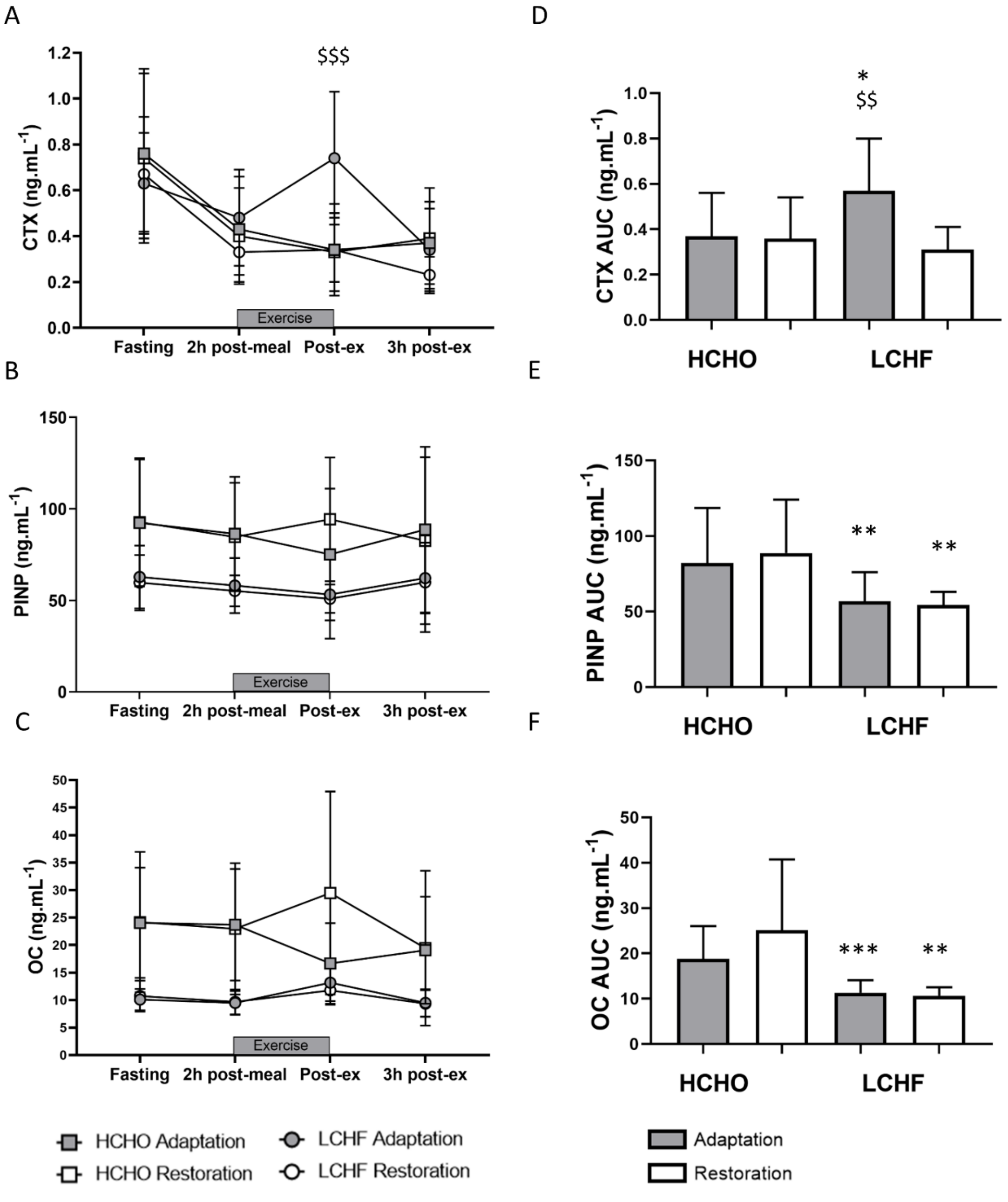
***
HCHO
LCHF
HCHO Baseline
HCHO Adaptation
LCHF Baseline
LCHF Adaptation
Baseline
Adaptation

Figure 6.4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CTX (ng.mL^{-1})
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1.0
0.8
0.6
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0.2
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Exercise
Fasting
2h post-meal
Post-ex
3h post-ex
CTX AUC (ng.mL^{-1})
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HCHO
LCHF
B
E
PINP (ng.mL^{-1})
150
100
50
0
PINP AUC (ng.mL^{-1})
**
**
C
F
OC (ng.mL^{-1})
50
45
40
35
30
25
20
15
10
5
0
OC AUC (ng.mL^{-1})
***
**
HCHO Adaptation
HCHO Restoration
LCHF Adaptation
LCHF Restoration
Adaptation
Restoration

6.7 Interlinking chapter

The third study of this thesis showed that a 3.5-week adherence to a ketogenic LCHF in elite race walkers increased marker of bone resorption and decreased markers of bone formation at rest and during a prolonged, intense exercise bout. These effects were only partially recovered with acute CHO feeding, where of note and perhaps concern is that markers of bone formation remained suppressed despite CHO intake around exercise. Therefore, this study suggests that long-term LCHF diets that are adequate in energy availability may pose a risk to the athlete's bone health.

The fourth and final study of the thesis examined the effects of 5 d of low energy and moderate CHO availability vs low CHO but high energy availability vs high energy and high CHO availability on markers of bone turnover in elite male race walkers.

7 STUDY 4: EFFECTS OF LOW ENERGY VS LOW CHO AVAILABILITY ON MARKERS OF BONE MODELLING AT REST AND DURING EXERCISE IN ELITE RACE WALKERS

Publication statement:

This chapter is comprised of the following paper in preparation for Bone.

Heikura, IA., Ross, ML., Tee, N., McKay, AKA., Sharma, AP., & Burke, LM. (2020). Effects of low energy vs low CHO availability on markers of bone modelling at rest and during exercise in elite race walkers *In preparation for Bone*

7.1 Abstract

Aims: To compare the effects of 5 days of high energy and high CHO availability (HCHO), high energy but low CHO availability (LCHF), or low energy but moderate CHO availability (LEA) on markers of bone modelling during prolonged exercise. **Methods:** World-class male race walkers (n=20) completed 5 d of HCHO (40 kcal·kg FFM·d^{-1}; ~9.6 g·kg·d^{-1} CHO), LCHF (40 kcal·kg FFM·d^{-1}; <0.5 g·kg·d^{-1} CHO) or LEA (15 kcal·kg FFM·d^{-1}; ~5.1 g·kg·d^{-1} CHO). Markers of bone breakdown (cross-linked C-terminal telopeptide of type I collagen, CTX), formation (procollagen 1 N-terminal propeptide , P1NP) and metabolism (osteocalcin, OC; undercarboxylated-OC, Glu-OC) were assessed at rest (fasted, pre-exercise) and 0, 1 and 3 h post-exercise at Baseline, and after the intervention (Adaptation). **Results:** LCHF showed a decrease from Baseline to Adaptation for Glu-OC at fasted (p=0.028), pre-exercise (p=0.004), post-exercise (p=0.001) and 3 h post-exercise (p=0.050), as well as for OC at fasted (p=0.030), pre-exercise (p=0.014), 1 h post-exercise (p=0.007) and 3 h post-exercise (p=0.006). LEA showed a decrease in Glu-OC at fasted state (p=0.026) and pre-exercise (p=0.016). LCHF decreased exercise area under curve for P1NP [-20% (-31, -9), p=0.013], OC [-26% (-32, -20), p=0.002] and Glu-OC [-31% (-50, -11), p=0.011]. CTX did not change significantly for any of the treatment groups. **Conclusion:** Markers of bone formation showed impairments after 5 d of LCHF diet. Meanwhile, markers of bone resorption showed no change. Dietary CHO availability may play a role independent of energy availability in bone formation. Future investigations on the effects of longer-term LEA in males are warranted.

7.2 Introduction

Energy availability (EA) is the cornerstone for optimal functioning of several body systems, including immune, endocrine and bone health (Mountjoy et al., 2018). Meanwhile, adequate CHO availability supports training quality (i.e. intensity) and race performance (Burke et al., 2017). More recently, CHO have also been linked to bone health, where reports show impaired bone modelling around endurance exercise following both acute (Sale et al., 2015; de Sousa et al., 2014) and long-term severe (Heikura et al., 2019) CHO restriction. Bone health is a key consideration for endurance athletes, who are at a high risk for impaired bone mineral density and stress fractures (Schofield & Hecht, 2012).

Although careful matching of energy and fuel intakes to the fuel requirements of daily training is important and should be emphasised for the majority of the year, occasionally the athlete may be forced to decrease EA within a short-term (i.e. several weeks) period of training/racing. One explanation for this behaviour is the need to periodise body composition to allow optimal race performance via higher power-to-weight ratio (Stellingwerff, 2018). A reduction in body mass or fat mass requires a caloric deficit, where moderate caloric deficits have been proven more beneficial than more extreme approaches in terms of health and performance (Garthe et al., 2011). A recent case study by Stellingwerff (2018) reported 9 years worth of body composition data of an Olympic level female middle-distance runner, where it was shown that body fat percentage fluctuated within each year, reaching a low point (9.2% compared to 10.9%) around peak racing period. This ”race physique” was reached via a 6-8 week dietary intervention in which energy and CHO availability were decreased to induce a small (~300 kcal) daily caloric deficit. This strategic and periodised approach maintained athlete health throughout her career (only ~2.8 missed menstrual cycles per year and two major injuries across 9 year career). While minor or brief periods of low EA are unlikely to cause problems, more severe or prolonged low EA poses a threat to athlete health in both females and males (Mountjoy et al., 2018; Ihle & Loucks; Zanker & Swaine, 2000).

Another dietary means to alter physique is manipulation of dietary CHO availability. Indeed, low-CHO, high fat (LCHF) diets (Volek et al., 2014; Burke, 2015), which provide adequate EA but limited CHO availability ($<50\ g \cdot d^{-1}$) to develop and maintain ketosis, have been promoted to endurance athletes for decades in an effort to enhance endurance performance via increased reliance on fat-based fuels during exercise (Volek et al., 2014). Recent research from our lab contradicts these claims by showing impaired exercise economy and lack of performance gains in elite race walkers following a 3-week LCHF diet (Burke et al., 2017), suggesting that this diet may play only a small and targeted role in sports nutrition (Burke, 2015). A final, important consideration of LCHF diets is their effect on athlete health, and especially bone health. Here, reports in rodents (Bielohuby et al., 2010) and children (Simm et al., 2017) suggest that long-term LCHF diets impair bone modelling and bone density, meanwhile studies in sedentary and/or overweight adults show no effect of LCHF on bone (Athinarayanan et al., 2019). In athletes and around exercise, acute CHO restriction has acutely impaired bone modelling (Sale et al., 2015); however these CHO restrictions have also simultaneously been a state of energy restriction in the absence of isocaloric provision of other macronutrients. Indeed, a recent report by Hammond et al. (2019) addressed this topic by comparing the acute effects of either isocaloric (EA of ~60 kcal·kg FFM·d^{-1}) high CHO (12 g·kg·d^{-1}) or non-ketogenic LCHF diet (3 g·kg·d^{-1}), or an energy restricted diet (EA of ~20 kcal·kg FFM·d^{-1}) matched for CHO content of the non-ketogenic LCHF diet, on bone modelling around a high-intensity exercise session. The study showed increased bone resorption in both CHO restricted groups regardless of EA, thus concluding that acute low CHO availability might be a more potent stimulus for bone resorption (Hammond et al., 2019). The only study to investigate the effects of longer-term severe CHO restriction in athletes showed impaired bone modelling (increased resorption and decreased formation) at rest and during exercise in a group of elite race walkers following a 3.5-week LCHF diet (<50 g·d^{-1} CHO) in comparison to an isocaloric high CHO (~8.5 g·kg·d^{-1}) diet (Heikura et al., 2019). In line with the work by Hammond et al., this study confirms the hypothesis that CHO availability might have an EA-independent effect on bone modelling at rest and around exercise. Therefore, collectively, available research suggests that CHO availability is a powerful signal (possibly independent of EA) affecting bone modelling. However, as these effects have mostly been studied separately, or at best in the acute setting around exercise, it is difficult to draw conclusions in terms of whether it is the lack of available energy in general, or CHO-based fuels specifically, that is driving the negative adaptations in bone modelling over a longer-term period.

Therefore, we aimed to compare the effects of 5 days of either (1) High energy and high CHO availability, (2) High energy but low CHO availability, and (3) Low energy but moderate-to-high CHO availability diets on markers of bone modelling in elite race walkers during a prolonged bout of exercise. We hypothesised that impairments to bone markers (shown by increased concentrations of marker of resorption, decreased concentrations of markers of formation, or both) are seen in the LCHF and LEA diets, as opposed to no change in the high energy and high CHO diet.

7.3 Methods

7.3.1 Participants

We recruited 20 world-class male race walkers to take part in this study/camp (Table 1). The participants were expected to be trained at the start of the camp, possess optimal EA [including normal resting metabolic rate (>29 kcal·kg FFM·d^{-1}), blood pressure (>90 mmHg / 60 mmHg), testosterone (low = within lowest quartile of reference range) and triiodothyronine (low = within lowest quartile of reference range)], and be free of injuries prior to the study. The study protocol was approved by the Ethics Committee of the Australian Institute of Sport (AIS; ethics approval number 20181203). The participants were fully informed about the study protocol prior to signing the informed consent.

7.3.2 Study design

We compared the effects 5 days of (1) High energy and CHO availability (HCHO), (2) Low energy but moderate-to-high CHO availability (LEA), or (3) High energy but low CHO availability (LCHF) diets on markers of bone modelling around a standardised, prolonged exercise bout (Figure 1). The study consisted of two phases: Dietary harmonisation (12 days) and Dietary intervention (5 days). During dietary harmonisation, all participants followed an identical HCHO diet. Thereafter, participants were allocated into one of the three dietary conditions [HCHO (n=6), LEA (n=7) or LCHF (n=7)] based on individual preference for the next 5 day period (Dietary intervention). We chose to allow the athletes decide their preferred diets partly to enhance compliance, but more importantly, to minimise the potential placebo effect; we felt that it was important to have each athlete follow a diet they believed would help their training and competition goals in the best possible way. Dietary intevention was preceded and followed by a 25 km race walk test, where markers of bone modelling were measured to determine the effects of dietary interventions on bone modelling.

7.3.3 Dietary standardisation

Dietary interventions. The diets were individualised by experienced sports dietitians using FoodWorks Professional Edition 9 (Xyris Software, Brisbane, Australia). During *harmonisation*, all participants followed a HCHO diet with a target EA of 40 (training days), 35 [high volume (>35 km·d^{-1}) days] or 45 (rest days) kcal·kg FFM·d^{-1}, where macronutrient targets were calculated as a percentage (%) of target energy intake (65% of energy from CHO, 15% of energy from protein, 20% of energy from fat). During *intervention*, HCHO continued to follow targets from the harmonisation phase, with the exception that a high-volume day (and subsequent reductions in target EA) was now defined as >30 km·d^{-1} training (this applied to all three dietary treatments). LCHF followed the same target EA as HCHO, however with low CHO (0.5 g·kg^{-1}), moderate protein (2.2 g·kg^{-1}) and high fat [remainder (~80%) of target energy] intakes. With the LCHF intervention, dietary fat was the only macronutrient that was adjusted based on target energy requirements. LEA had a target EA of 15 (training days), 10 (high volume days) or 20 (rest days) kcal·kg FFM·d^{-1}, where macronutrient targets were calculated as 60% of energy from CHO, 25% of energy from protein, 15% of energy from fat.

Effect of brief (5 d) dietary interventions on BM. It is important to note that acute or short-term (a couple days) changes in BM are challenging to interpret and while a linear trend may reflect actual changes in BM or body composition due to altered (increased or decreased) EA, there are a variety of other confounding factors including nutritional (changes in fibre intake or hydration status) and non-nutritional (einvironmental temperature, changes in training load) factors that may have a significant, short-term effect on BM and that may potentially mask or exacerbate true changes in BM. Therefore, although changes in BM after *harmonisation* and *intervention* blocks in the three intervention groups will be briefly described below, the reader should keep in mind the aforementioned challenges in interpreting this kind of short-term measurement data. No change in BM was detected across *harmonisation* in any diet group, which may show that we managed to maintain the athletes within optimal EA. Meanwhile, during the 5 d *intervention*, all diet groups lost some BM (HEA: -0.8 ± 0.3 kg; LEA: -1.6 ± 0.6 kg; LCHF: -2.0 ± 0.3 kg); here, the BM loss in the LCHF group was mainly caused by reductions in FFM (-2.7 ± 1.3 kg) likely reflecting depletion of muscle glycogen stores and associated water. Referring back to the claim at the beginning of this paragraph, it is noteworthy that during the 5 d *refeeding* period (all groups back to the harmonisation diet) following *intervention*, LCHF increased BM (due to a rebound of FFM back to the glycogen repleted levels) back to baseline values (0.0 ± 0.6 kg compared to baseline).

Energy availability calculations and estimation of daily energy targets. The methodology for determining individual and day-by-day specific energy and macronutrient targets for the current study has been described in full detail in Heikura et al. (2020). Briefly, we utilised a novel approach where we prospectively defined day-by-day dietary targets for each athlete based on information acquired from preliminary laboratory assessment [fat-free mass (FFM), exercise energy expenditure (EEE) and resting metabolic rate RMR)] and prospective weekly training plans. To estimate EEE, the participants completed an incremental test to exhaustion on a motorised treadmill during screening. The test began at a speed of 11 or 12 km·h^{-1} and included a total of four stages. Each stage lasted 4 min and increased in speed by 1 km·h^{-1} per stage, with a 1 min rest between each stage. Respiratory gases were collected for 60 sec at the end of each stage, and used to estimated EEE (kcal·min^{-1}) for each speed using the Weir equation (Weir, 1990); notably, sedentary energy expenditure [measured RMR as kcal/d divided by 1440 (24 hours equals 1440 min) to yield RMR as kcal·min^{-1}] was deducted from these values. Thereafter, EEE (kcal·min^{-1}) was converted into EEE (kcal·km^{-1}) and averaged across the four speeds for a simplified, time-efficient use in subsequent calculations and real-time within-day adjustments. As our participants were elite athletes, exercise training accounted for the majority of daily activity-induced energy expenditure. Therefore, no calculations were made for non-exercise activity thermogenesis. In case of cross-training, a metabolic equivalent of 4 (gym sessions) or 8 (swimming, bicycling) was used (Ainsworth et al., 2000). For running, EEE was estimated as body mass * distance (km) (Margaria et al., 1963). For all of these calculations, the resting component of the EEE was deducted from the calculations to reflect additional energy expended during exercise only. Daily EEE was estimated from planned training distance [planned km * EEE (kcal·km^{-1})]. Individual daily target energy intake was calculated as follows: (FFM * Target EA) + planned EEE.

To ensure daily EA was maintained within target values, the participants were interviewed at lunch and dinner times each day throughout the study to cross-reference actual training to prospective training plans. Where changes to training were made that exceeded the energy cost equivalent to 2 km of race walking [i.e. a change greater than ~112-169 kcal depending on individual (~2.4 kcal deviation from planned EA)], energy intakes were adjusted within-day to maintain actual EA within acceptable limits. All meals and snacks were weighed and provided for the athletes on a daily basis, and recorded by the members of the staff. Actual dietary intakes were analysed using the same software as during menu planning. Actual dietary intakes have been reported in Table 2.

7.3.4 Experimental design

Testing involved a 25 km long walk at around 50K race pace (Figure 1). Upon entering the laboratory in an overnight fasted and rested state, a cannula was inserted into an antecubital vein for collection of blood samples at rest (Fasted), immediately before exercise (Pre-exercise), immediately after exercise (0 min) and 1 h and 3 h post-exercise. Blood was analysed for concentrations of markers of bone resorption [cross-linked C-terminal telopeptide of type I collagen (CTX)], bone formation [procollagen 1 N-terminal propeptide (P1NP)], and bone modelling-related hormones [osteocalcin (OC) and undercarboxylated OC (Glu-OC)] to determine the effects of dietary interventions and exercise on markers of bone modelling. Here, increases in CTX and/or decreases in other markers would be expected to reflect impaired bone remodelling process. The cannulas were flushed with 3 ml of saline every 30 min throughout the trials. A standardised breakfast [2 $g \cdot kg^{-1}$ CHO for all groups at Baseline, or a diet-specific option for LCHF (isocaloric LCHF option) and LEA (2 $g \cdot kg^{-1}$ CHO) at Adaptation] was consumed 30 min after the first blood sample, after which the participants rested for 120 min before beginning the session. The session was a 25 km combined laboratory and field protocol with regular glucose ingestion (60 $g \cdot h^{-1}$) throughout the test for all groups at Baseline and for HCHO at Adaptation, while LCHF ingested isocaloric high fat snacks and LEA received 30 $g \cdot h^{-1}$ glucose at Adaptation. Upon completion of the exercise test, the participants rested in the laboratory for a further 3 hours, and received a standardised recovery shake (1.5 $g \cdot kg^{-1}$ CHO and 0.3 $g \cdot kg^{-1}$ protein for all groups at Baseline, or a diet-specific option for LCHF and LEA at Adaptation) at 30 min post-exercise to improve satiety.

7.3.5 Blood analysis

Blood was collected into a 3.5 ml EDTA BD Vacutainer Plus SST II tube. Blood was allowed to clot by standing at room temperature for 2 hours before centrifuging at 1000 G for 10 min. Serum was aliquoted into four 0.75 ml polypropylene tubes. Blood clotting, centrifuge and freeze time were kept consistent for all samples. Once aliquoted, the samples were immediately stored at -80°C and later used for analysis of CTX, P1NP, OC and Glu-OC. Analysis for CTX, P1NP, OC and Glu-OC was undertaken by enzyme-linked immunosorbent assays (ELISAs) supplied by Cloud-Clone Corp. (CTX: Katy, TX, USA), NovateinBio (P1NP: Woburn, MA, USA) and TaKaRa (OC and Glu-OC: Shiga, Japan). Inter-assay coefficient of variation was <12%, <10.6%, <2.4%, and <9.9% for CTX, P1NP, OC and Glu-OC, respectively.

7.3.6 Statistical analysis

Statistical analyses were conducted using SPSS Statistics 22 software (INM, New York, USA) with a significance level set at $p \leq 0.05$. Normality of data was checked with a Shapiro-Wilk test and visual inspection of residual plots. A three-way mixed ANOVA was used to compare differences in bone markers across exercise, between diets and between tests. Exercise-AUC (Pre-exercise to 3 h post-exercise) for all markers were compared with a two-way mixed ANOVA, with post hoc tests of Student's t-tests for independent (between-groups) and paired (within-groups) samples; where normality was violated, Wilcoxon's test and Mann-Whitney U-test were used. Data are presented as means [95% confidence intervals (CI)].

7.4 Results

7.4.1 Bone markers across exercise

No statistically significant interaction of diet*test*time was seen for Glu-OC, OC, CTX or P1NP ($p>0.05$). There were statistically significant interactions for test*time for Glu-OC [F(4, 68)=3.275, p=0.0196], for time*diet for P1NP [F(6.586, 55.985)=3.091, p=0.009], and for test*diet for Glu-OC [F(2, 17)=5.014, p=0.019] and OC [F(2,17)=8.453, p=0.003].

LCHF showed a significant decrease from Baseline to Adaptation for Glu-OC (Figure 4B) at fasted state (p=0.028), pre-exercise (p=0.004), post-exercise (p=0.001) and 3 h post-exercise (p=0.050). There was also a decrease in OC for LCHF (Figure 4A) at fasted state (p=0.030), pre-exercise (p=0.014), 1 h post-exercise (p=0.007) and 3 h post-exercise (p=0.006). LEA showed a significant decrease in Glu-OC (Figure 3B) at fasted state (p=0.026) and pre-exercise (p=0.016). No change was seen for HCHO (Figure 2 A-D) or LEA (Figure 3) or for LCHF (Figure 4) for any other marker or time point.

7.4.2 Bone marker area under curve across exercise

There was a significant 2-way interaction (test*diet) for OC [F(14.618, 17)=6.287, p=0.009) and Glu-OC [F(1.206, 17)=3.663, p=0.048). Exercise-related AUC decreased significantly for LCHF for P1NP [-20% (-31, -9), p=0.013], OC [-26% (-32, -20), p=0.002] and Glu-OC [-31% (-50, -11), p=0.011] (Figure 4 E-G) with no change for HCHO (Figure 2 E-G) or LEA (Figure 3 E-G). Meanwhile, CTX (Figures 2H, 3H and 4H) did not change significantly for any of the treatment groups.

7.5 Discussion

We investigated the effects of 5 d of either high energy and high CHO availability (HCHO), high energy but low CHO availability (LCHF), and low energy but moderate-to-high CHO availability (LEA) diets on markers of bone modelling during a prolonged bout of exercise. Our main findings indicate that, in comparison with a bout of prolonged strenuous exercise undertaken with high energy and CHO availability, 5 d of LCHF and exercise decreased a marker of bone formation but had no change on the markers of bone resorption, while 5 d of exposure to severe LEA was not associated with any diet-related changes. We conclude that dietary CHO availability may play a key role in optimal bone modelling, independently of energy availability, at least within a short-term period of 5 d and in the case of markers of bone formation.

Although no absolute threshold exists, low EA has generally been defined as <30 kcal·kg FFM·d^{-1} based on research in healthy sedentary females, where direct and negative effects on markers of bone modelling have been reported within 4 to 5 d (Ihle & Loucks 2004). In a step-wise manner, studies show a linear decline in the concentrations of P1CP (marker of bone formation) with decreasing EA (below 45 kcal·kg FFM·d^{-1}), meanwhile the changes in OC have been shown below 30 kcal·kg FFM·d^{-1}, and the concentrations of NTX (marker of bone resorption) appear to respond to decreased EA until after EA has been decreased to 10 kcal·kg FFM·d^{-1} (Loucks & Thuma, 2003; Ihle & Loucks, 2004). While this preliminary research has been carried out with females, emerging evidence suggests that male athletes can also suffer from low EA (Tenforde et al., 2016). Interestingly, the effects seem to be different between sexes. Papageorgiou et al. (2017) reported that 5 d of low EA (15 kcal·kg FFM·d^{-1}) decreased bone turnover ratio (P1NP:CTX) in females but not in males when compared to high EA (45 kcal·kg FFM·d^{-1}). In male athletes, 3 d of restricted energy intake (-50% of energy requirements) decreased P1NP concentrations compared to 3 d of balanced energy intake (100% of energy requirements) (Zanker & Swaine, 2000). Meanwhile, in active females, 3 d of low EA (15 kcal·kg FFM·d^{-1}) achieved by diet (reduced energy intake) but not by exercise (increased energy expenditure) reduced P1NP concentrations (Papageorgiou et al., 2018).

Studies have also shown that acute and long-term CHO restriction impairs bone modelling at rest and during exercise. For example, Sale et al. (2015) reported increased CTX and reduced P1NP concentrations across a 120 min endurance exercise session when no CHO was ingested during exercise, compared to a fed condition. Meanwhile, de Sousa et al. (2014) showed similar outcomes over a high intensity session with no CHO compared to regular high CHO feeding during the session. More recently, we showed that a 3.5-week adherence to a ketogenic LCHF diet in elite race walkers increased CTX and reduced P1NP and OC concentrations at rest and during exercise, and that markers of bone formation remained suppressed in the keto-adapted athletes despite acute CHO refeeding (Heikura et al., 2019).

Most studies on EA and bone have implemented matched macronutrient ratios (i.e. % of total energy intake; usually ~50-55% from CHO, 10-20% from protein and 30-35% from fats) for low and optimal EA treatments. Therefore, low EA treatments have also been low CHO availability treatments. For example, in Papageorgiou et al. (2017) CHO intakes in females and males were 154-206 g·d^{-1} (or ~2.6-2.8 g·kg·d^{-1}) and 302-398 g·d^{-1} (or ~5.0-5.5 g·kg·d^{-1}) for low and optimal EA treatments (15 and 45 kcal·kg FFM·d^{-1}, respectively). As the effects of low energy and low CHO availability become superimposed, it is impossible to determine whether the impairments seen to markers of bone modelling with low EA are linked to a decrease in energy or CHO availability, or a combination of both.

A recent study by Hammond et al. (2019) is the first to address this topic. In this investigation, the effects of 24 h of either high energy and high CHO availability (HCHO: ~12 g·kg·d^{-1} CHO and ~60 kcal·kg FFM·d^{-1}), low energy and low CHO (LCAL: ~3 g·kg·d^{-1} CHO and ~20 kcal·kg FFM·d^{-1}) or high energy and low CHO (LCHF: ~3 g·kg·d^{-1} CHO and ~60 kcal·kg FFM·d^{-1}) diets were investigated around an acute bout of twice-a-day endurance exercise. This study protocol provides a unique opportunity to investigate the independent effects of energy and CHO on bone modelling markers. The authors reported a lack of suppression in the concentrations of CTX around the second bout of high-intensity exercise with both LCHF and LCAL diets, as opposed to the HCHO diet. The study concluded that acute changes in markers of bone modelling might be more reflective of alterations in dietary CHO, as opposed to energy, availability (Hammond et al., 2019). While these findings are certainly interesting, it is noteworthy that the study implemented an acute 24 h dietary intervention, therefore it does not allow investigation of longer-term effects of energy and CHO on bone. Furthermore, the LCHF diet implemented in Hammond et al. provided ~3 g·kg·d^{-1} CHO, and therefore, was not a strict ketogenic LCHF diet. In contrast, in the current study, we implemented a 5 d period of either high energy and high CHO availability (HCHO: ~10 g·kg·d^{-1} CHO and ~40 kcal·kg FFM·d^{-1}), high energy but low CHO availability (LCHF: ~0.5 g·kg·d^{-1} CHO and ~40 kcal·kg FFM·d^{-1}), and low energy but moderate-to-high CHO availability (LEA: ~5 g·kg·d^{-1} CHO and ~15 kcal·kg FFM·d^{-1}) diets. Notably, our dietary interventions provided a step-wise reduction in dietary CHO content to enable comparison of three main-stream dietary approaches on bone modelling markers (Table 2). In contrast to the findings of Hammond et al. (2019), we showed decreased concentrations of bone formation markers with the LCHF diet (Figure 4), while no clear effects were seen with LEA (Figure 3) or HCHO (Figure 2). In line with Hammond et al., our results suggest that lack of dietary CHO may be a more potent stimulus for changes in markers of bone modelling (indicated as reduced P1NP which leads to overall impaired BT ratio) compared to overall EA. Our results confirm the findings of our previous study, where 3.5 weeks of LCHF diet (~0.5 g·kg·d^{-1} CHO and ~40 kcal·kg FFM·d^{-1}) led to decreased markers of bone formation during exercise, compared to a high CHO diet (~8.5 g·kg·d^{-1} CHO and ~40 kcal·kg FFM·d^{-1}). The current study shows that these outcomes can be seen in as little as 5 d of LCHF diet. Contrary to our previous findings (Heikura et al., 2019) and those by Hammond et al. (2019) of increased marker of bone resorption (CTX) after acute and long-term LCHF, respectively, we failed to show change in CTX after our 5 d dietary intervention. It is important to emphasise the short-term nature of the current study. Indeed, it is possible that effects of low EA on bone take longer and thus were not seen within this 5 d period. Whether longer-term LEA has a similar magnitude of effects on bone modelling as a LCHF diet does, remains to be seen.

7.6 Conclusions

Our findings of decreased concentrations of markers of bone formation around endurance exercise after 5 d of LCHF diet, in comparison to no change after a similar period of low energy but moderate CHO availability, suggest that CHO availability may have a significant, energy-independent, role in markers of bone modelling over a short-term period. Future investigations should focus on the underlying mechanisms and the time-course of these changes as a result of such diets.

Acknowledgements

The study was funded by a Program Grant from the Australian Catholic University Research Funds to Professor Louise Burke. The authors and funding agents do not have any conflicts of interests.

Conception and design of the experiments was undertaken by IAH, MLR, NT, AKAM and LMB; Collection, assembly, analysis and interpretation of data was undertaken by IAH, MLR, NT, AKAM and LMB; Manuscript was prepared by IAH and LMB. All authors approved the final version of the manuscript.

IAH and LMB had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. We thank our research colleagues and supporters of the Supernova research series and acknowledge the commitment of the elite race-walking community.

TABLES

Table 7.1. Participant characteristics at baseline.

	HCHO (n=6)			LEA (n=7)			LCHF (n=7)		
Age (yr)	30.7	±	2.9	31.3	±	2.1	30.9	±	4.7
BM (kg)	66.2	±	8.2	68.1	±	6.1	65.9	±	8.0
VO_2peak (ml·kg·min^{-1})	63.85	±	3.63	61.87	±	5.03	67.70	±	6.11
20 km PB (hh:min:s)	1:23:30	±	0:02:09	1:20:58	±	0:01:51	1:21:55	±	0:02:51

HCHO, high energy availability; LEA, low EA; LCHF, low carbohydrate high fat; BM, body mass; VO2peak, peak oxygen consumption; 20 km PB, 20 km race walking personal best.

Table 7.2. Dietary energy availability and macronutrient breakdown during harmonisation and intervention.

	Harmonisation			Intervention		
	HCHO (n=6)	**LEA (n=7)**	**LCHF (n=7)**	**HCHO (n=6)**	**LEA (n=7)**	**LCHF (n=7)**
Energy intake (kJ·kg·d^{-1})	246 ± 23	230 ± 5	239 ± 12	245 ± 17 ^{aaa}	144 ± 10	224 ± 12 ^{aaa}
CHO (g·kg·d^{-1})	9.6 ± 0.9	9.0 ± 0.2	9.3 ± 0.5	9.6 ± 0.7 $^{aaa, bbb}$	5.1 ± 0.4	0.5 ± 0.0 ^{aaa}
Protein (g·kg·d^{-1})	2.2 ± 0.2	2.1 ± 0.1	2.2 ± 0.1	2.2 ± 0.2	2.1 ± 0.2	2.2 ± 0.0
Fat (g·kg·d^{-1})	1.3 ± 0.1	1.2 ± 0.0	1.3 ± 1.1	1.3 ± 0.1 $^{aaa, bbb}$	0.6 ± 0.0	4.8 ± 0.3 ^{aaa}
EA (kcal·kg FFM·d^{-1})	39 ± 4	41 ± 2	41 ± 1	39 ± 1 ^{aaa}	14 ± 2	40 ± 1 ^{aaa}

HCHO, high energy availability; LEA, low EA; LCHF, low carbohydrate high fat; EA, energy availability; CHO, carbohydrate.; FFM, fat-free mass.

a $p<0.05$, aa $p<0.01$, aaa $p<0.001$ significant difference to LEA

b $p<0.05$, bb $p<0.01$, bbb $p<0.001$ significant difference to LCHF

FIGURES

Figure 7.1. Study overview. After an initial controlled high energy availability (HCHO) period (Dietary harmonisation), the participants were assigned to 5 days of either HCHO (high energy and carbohydrate availability), LEA (low energy but high carbohydrate availability) or LCHF (high energy but ketogenic low carbohydrate high fat diet). A standardised 25 km (~2 h) hybrid laboratory/field race walking protocol at ~75% VO2max was completed at Baseline and after dietary interventions (Adaptation). On the morning of each test, the participants consumed a standardised diet-specific breakfast and rested for 2 h before commencing the exercise test. After exercise, the participants rested in the laboratory for 3 h, where a recovery shake was consumed at 30 min post-exercise. During baseline testing, all participants followed the same (HCHO) diet and received identical meals around the test. During Adaptation, diet-specific meals were consumed. Venous blood samples were collected in the fasted state and immediately before (Pre-exercise), and immediately (0 min) and 1 and 3 h post-exercise for analysis of concentrations of C-terminal telopeptide of type I collagen (CTX), procollagen 1 N-terminal propeptide (P1NP), osteocalcin (OC) and undercarboxylated OC (Glu-OC).

Figure 7.2. Time course of changes in bone marker concentrations across exercise (left panel) and exercise area under curve (right panel) for blood osteocalcin (OC: A, E), undercarboxylated OC (Glu-OC: B, F), procollagen 1 N-terminal propeptide (P1NP: C, G), and C-terminal telopeptide of type I collagen (CTX: D, H) after 5 d of high energy availability (HCHO, n=6) diet. White symbols/bars represent Baseline; Black symbols/bars represent Adaptation. EX0, immediately post-exercise; EX1, 1 h post-exercise; EX3, 3 h post-exercise.

Figure 7.3. Time course of changes in bone marker concentrations across exercise (left panel) and exercise area under curve (right panel) for blood osteocalcin (OC: A, E), undercarboxylated OC (Glu-OC: B, F), procollagen 1 N-terminal propeptide (P1NP: C, G), and C-terminal telopeptide of type I collagen (CTX: D, H) after 5 d of low energy availability (LEA, n=7) diet. White symbols/bars represent Baseline; Black symbols/bars represent Adaptation. EX0, immediately post-exercise; EX1, 1 h post-exercise; EX3, 3 h post-exercise. *$p<0.05$ significant difference between tests.

Figure 7.4. Time course of changes in bone marker concentrations across exercise (left panel) and exercise area under curve (right panel) for blood osteocalcin (OC: A, E), undercarboxylated OC (Glu-OC: B, F), procollagen 1 N-terminal propeptide (P1NP: C, G), and C-terminal telopeptide of type I collagen (CTX: D, H) after 5 d of low carbohydrate-high fat (LCHF, n=7)

diet. EX0, immediately post-exercise; EX1, 1 h post-exercise; EX3, 3 h post-exercise. *$p<0.05$, **$p<0.01$, ***$p<0.001$ significant difference between tests.

Figure 7.1.

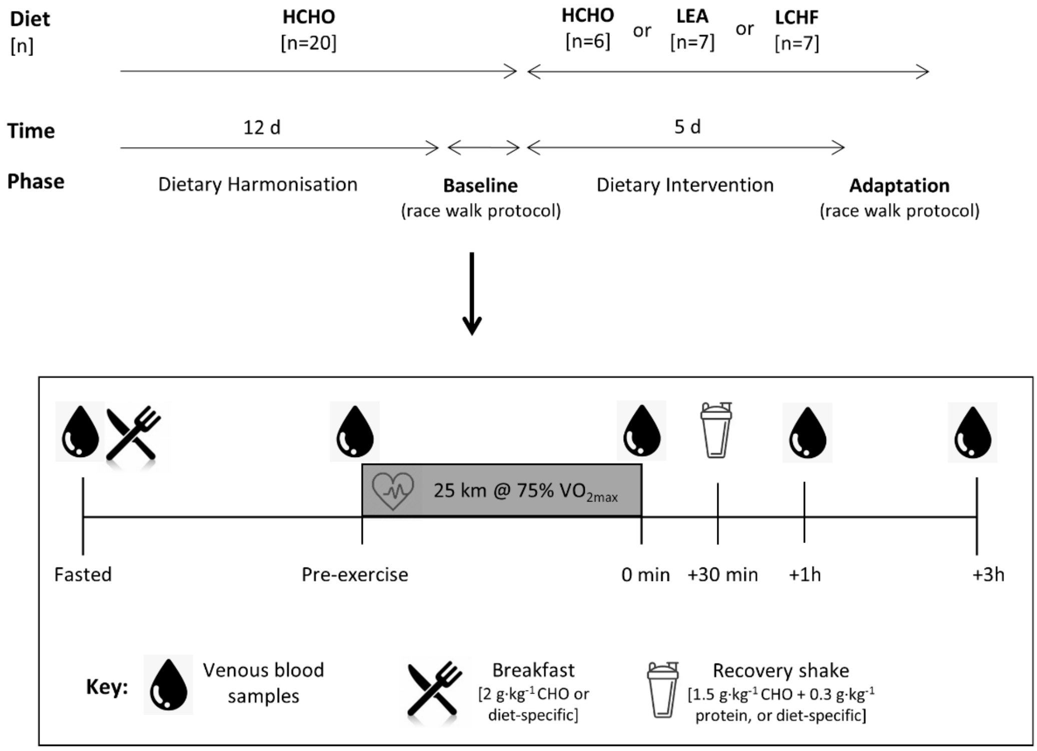
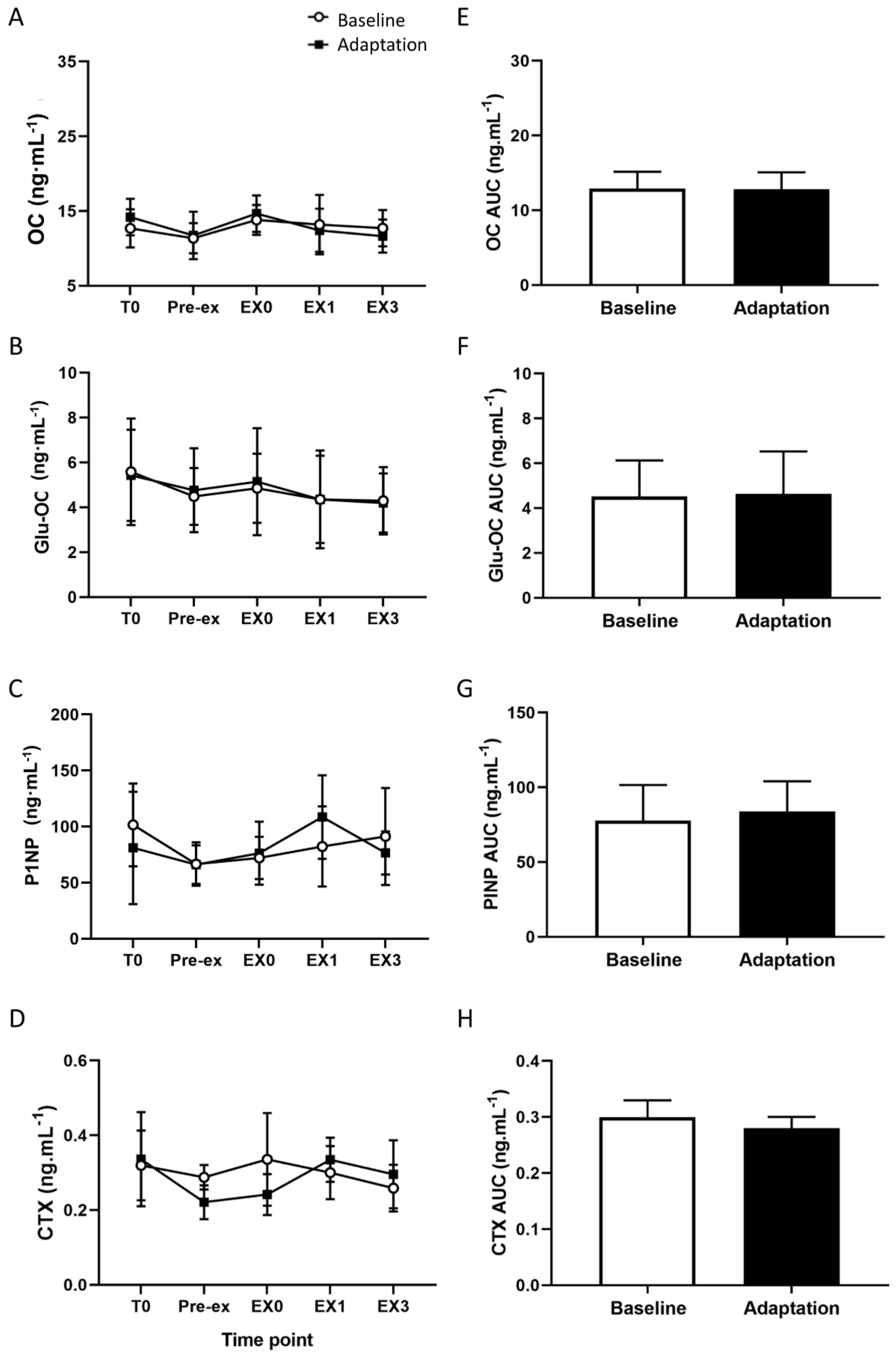

Diet
[n]
HCHO
[n=20]
HCHO
[n=6]
or
LEA
[n=7]
or
LCHF
[n=7]
Time
12 d
5 d
Phase
Dietary Harmonisation
Baseline
(race walk protocol)
Dietary Intervention
Adaptation
(race walk protocol)
25 km @ 75% VO_{2max}
Fasted
Pre-exercise
0 min
+30 min
+1h
+3h
Key:
Venous blood samples
Breakfast
[2 $g·kg^{-1}$ CHO or diet-specific]
Recovery shake
[1.5 $g·kg^{-1}$ CHO + 0.3 $g·kg^{-1}$ protein, or diet-specific]

Figure 7.2.


A
Baseline
Adaptation
OC (ng·mL^{-1})
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5
T0
Pre-ex
EX0
EX1
EX3
E
OC AUC (ng.mL^{-1})
30
20
10
0
Baseline
Adaptation
B
Glu-OC (ng·mL^{-1})
10
8
6
4
2
0
T0
Pre-ex
EX0
EX1
EX3
F
Glu-OC AUC (ng.mL^{-1})
10
8
6
4
2
0
Baseline
Adaptation
C
P1NP (ng·mL^{-1})
200
150
100
50
0
T0
Pre-ex
EX0
EX1
EX3
G
PINP AUC (ng.mL^{-1})
150
100
50
0
Baseline
Adaptation
D
CTX (ng.mL^{-1})
0.6
0.4
0.2
0.0
T0
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EX0
EX1
EX3
Time point
H
CTX AUC (ng.mL^{-1})
0.4
0.3
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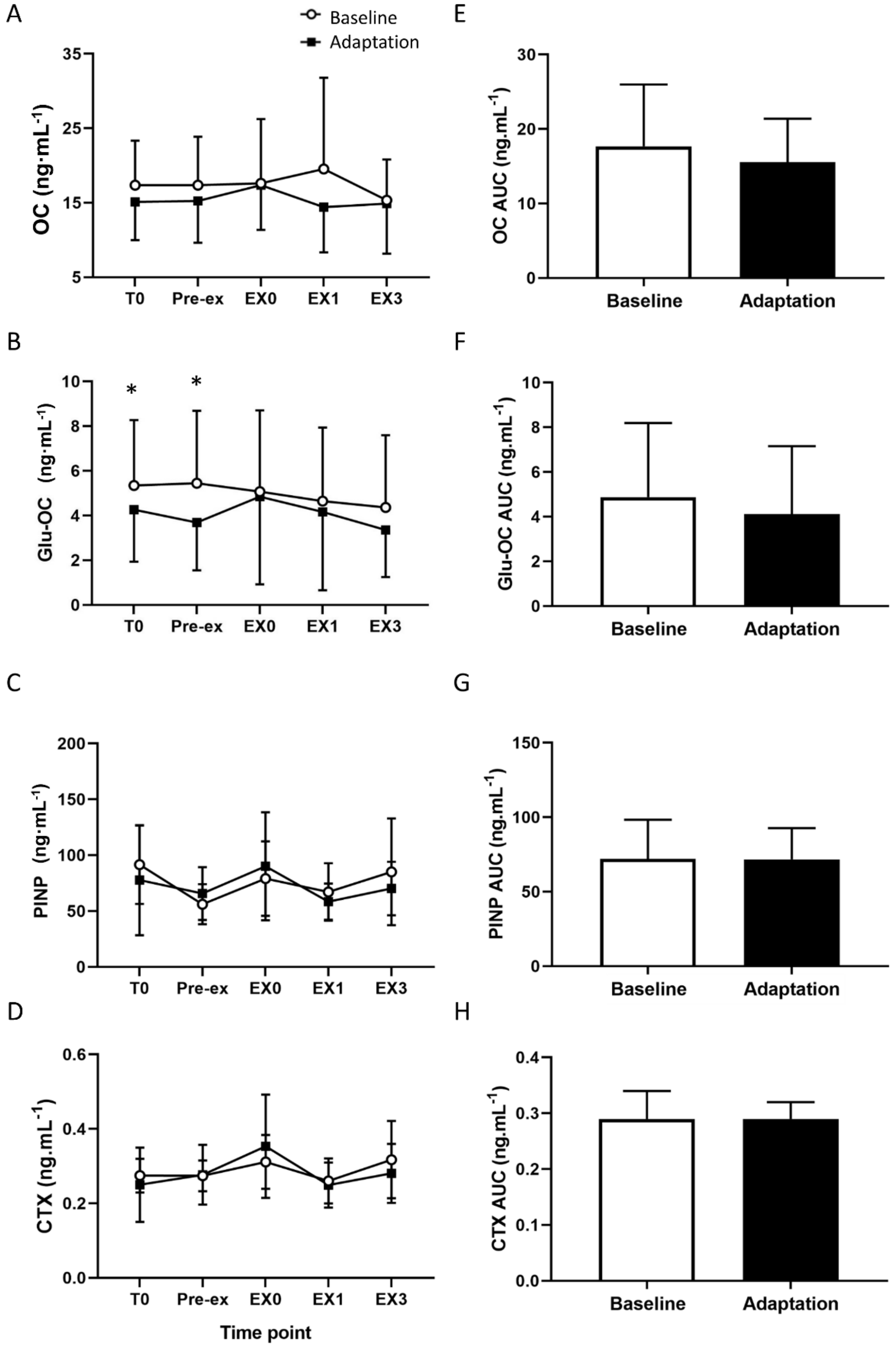
Baseline
Adaptation

Figure 7.3.


A
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Adaptation
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OC (ng·mL^{-1})
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Glu-OC (ng·mL^{-1})
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Glu-OC AUC (ng.mL^{-1})
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PINP (ng·mL^{-1})
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PINP AUC (ng.mL^{-1})
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Adaptation
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CTX (ng.mL^{-1})
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Pre-ex
EX0
EX1
EX3
Time point
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0.1
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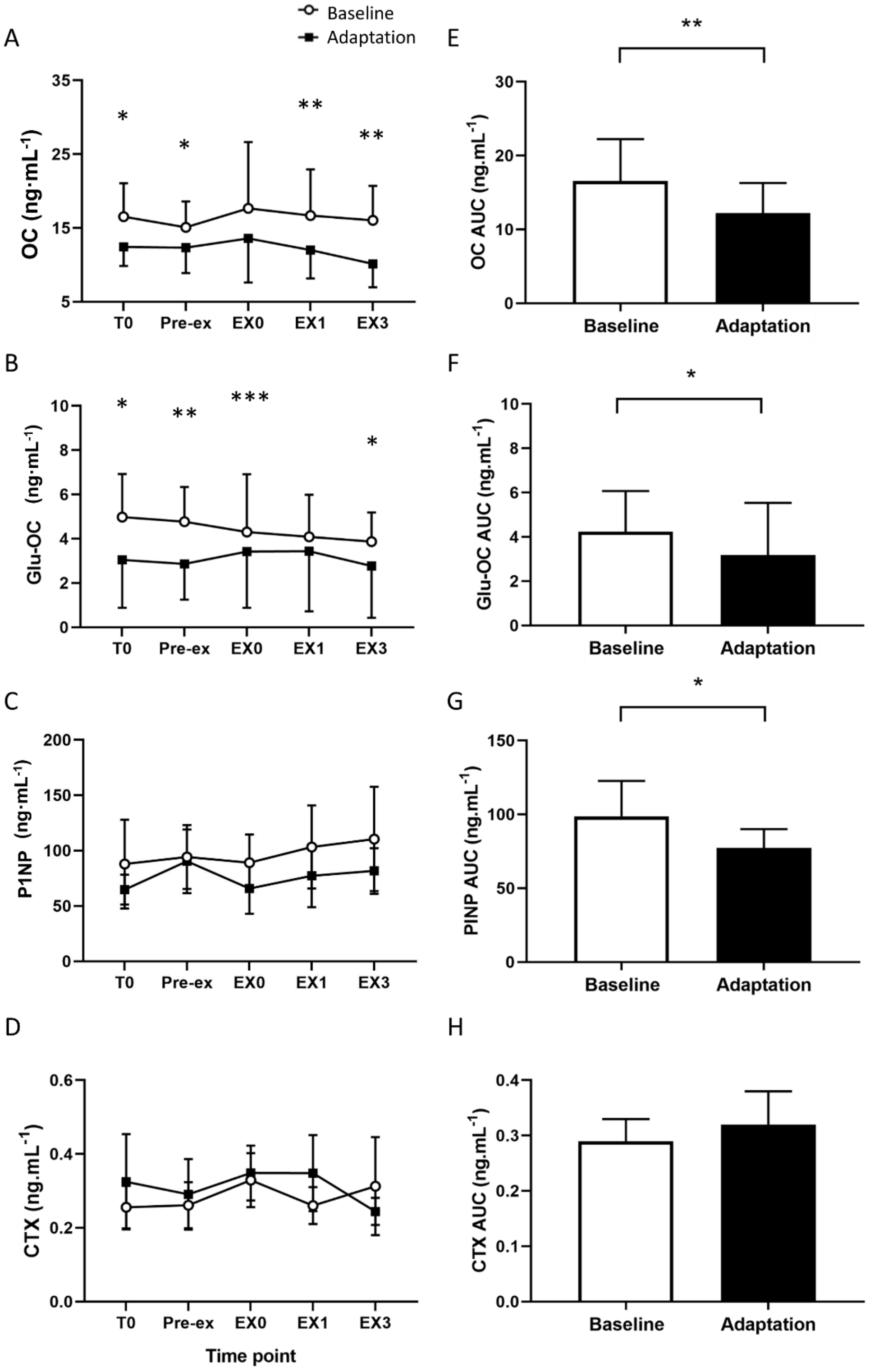
CTX AUC (ng.mL^{-1})
Baseline
Adaptation

Figure 7.4.



Baseline
Adaptation
A
OC (ng·mL-1)
35
25
15
5
*
*
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T0
Pre-ex
EX0
EX1
EX3
E
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OC AUC (ng.mL-1)
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Baseline
Adaptation
B
Glu-OC (ng·mL-1)
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Glu-OC AUC (ng.mL-1)
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Adaptation
C
P1NP (ng·mL-1)
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Pre-ex
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EX1
EX3
G
*
PINP AUC (ng.mL-1)
150
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Baseline
Adaptation
D
CTX (ng.mL-1)
0.6
0.4
0.2
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T0
Pre-ex
EX0
EX1
EX3
Time point
H
CTX AUC (ng.mL-1)
0.4
0.3
0.2
0.1
0.0
Baseline
Adaptation

8 DISCUSSION AND CONCLUSIONS

Current sports nutrition guidelines emphasise the need for a periodised and individualised nutrition plan to support the specific training and performance goals of the athlete while maintaining good health (Thomas et al., 2016). Therefore, some endurance athletes are likely to benefit from implementing a range of nutrition approaches including low to high energy and CHO availability to address specific goals such as training adaptation (occasional training or recovering with low CHO availability; Impey et al., 2018b; Marquet et al., 2016a,b), athletic performance (optimised energy and CHO availability based on event-specific demands; Stellingwerff et al., 2019) and optimal physique (integration of periods of reduced EA; Stellingwerff, 2018; Melin et al., 2019). Despite the well-established recommendations, evidence of whether elite endurance athletes actually implement current guidelines has been lacking. An alternative dietary strategy that has become popular among some endurance athletes in the pursuit of enhanced performance and physique manipulation is the LCHF diet (Volek et al., 2015). However, a frequently ignored component in discussions on a specific nutrition strategy is the effect of such a strategy on athlete health, which is directly linked to performance (Raysmith & Drew, 2016). Endurance athletes are especially prone to poor bone health and subsequent stress fractures (Schofield & Hecht, 2012). Therefore, this thesis aimed to address these gaps in the literature by investigating:

1. Self-reported dietary periodisation practices in world-class endurance athletes across macro-, meso- and micro-cycles of training and competition.
2. Day-by-day periodisation of energy and CHO availability in professional cyclists across a series of single-day races and concomitant effects of these strategies on physique and endocrine system.
3. The effects of a 3.5-week LCHF diet and acute CHO feeding on markers of bone modelling in world-class race walkers.
4. The effects of 5 d of low energy and moderate CHO availability vs high energy and high CHO availability (optimal EA diet) vs high energy and low CHO availability (LCHF diet) on markers of bone modelling in world-class race walkers.

The main findings from these independent but related studies are discussed in detail below. Collectively, these studies show evidence of periodised energy and CHO availability on macro (months to years), meso (weeks to months) and micro (between to within-days) level, implemented by elite endurance (middle- and long-distance running, race walking, road cycling) athletes mainly as a means to support performance and physique goals, and less frequently, as a means to enhance cellular adaptations. We also show changes in blood concentrations of markers of bone modelling (increased concentrations of markers of resorption and decreased concentrations of markers of formation) after short- and long-term LCHF diets (isocaloric to their high CHO control interventions), which suggests that in addition to EA, CHO availability may have an independent role in the bone modelling, and possibly, long-term bone health, of endurance athletes.

8.1 Novel findings of the current thesis

Dietary periodization has become a central theme in sports nutrition in the last decade (Thomas et al., 2016; Burke et al., 2018b). Despite emerging research and guidelines on how elite athletes should manage their nutrition at various levels of a periodised training program, literature with regards to actual knowledge and practices of periodised nutrition among elite athletes has been almost non-existent (Heikura et al., 2017a,b). Therefore, ***study 1*** aimed to collect qualitative data on a large cohort of elite track and field endurance athletes to address this knowledge gap. Specifically, based on our previous pilot study (Heikura et al., 2017a,b), we developed an online survey tool for distribution across the world, ending up with a final sample size of 104 elite athletes [of which 50% were major championship (Worlds or Olympics) qualifiers]. We detected a number of key repeated themes across various levels of training periodisation. Firstly, road athletes reported different nutritional practices to middle- and track-distance athletes, where the former were more likely to implement strategies of training with both low and high CHO availability within the annual training plan. Another distinction between distance groups was around manipulation of physique. Here, middle-distance athletes were the most conscious about the effects of nutrition strategies on outcomes such as body composition or muscle mass maintenance. We also reported a difference between sexes in terms of energy and CHO availability, where females were more likely to report to restrict intake of extra energy/CHO compared to males. Overall, this athlete cohort reported to focus mainly on training and race performance when making decisions on nutrition. Meanwhile, themes such as body composition manipulation, health, and practicality were less important. Finally, a relatively large proportion of athletes within this cohort were unaware of the use of nutrition to manipulate training adaptations, or felt that there were side-effects or challenges that prevented their use.

Of course, our study measured self-reported and self-assessed behaviours and some athletes may not have been willing to share their actual practices and may not have had the insight to distinguish between intended/deliberate practice and actual/accidental practice. Indeed, our pilot study identified some disconcordance between actual and reported behaviours around dietary periodisation elements in the studied cohort, which we suggested could be explained by the failure of the recording period (during an altitude training camp) to reflect habitual practices as well as the possibility of a lack of awareness by athletes of the dietary behaviours needed to achieve their intended practices (Heikura et al., 2017a, b). A more recent insight gained from the literature review in this thesis, which included data from the performance studies (Burke et al., 2017a) accompanying Study 3 in the current body of work, is that the stark dietary manipulations needed to create low CHO availability in lower calibre athletes may not be necessary in elite athletes. Indeed, the intensive training load and the sequencing of training sessions may in itself achieve the opportunity to train with low muscle glycogen stores and reduce the relative efficacy or value of additional dietary changes. To conclude, we demonstrate that elite track and field endurance athletes self-report periodised nutrition practices where the implementation of a specific strategy depends on the annual training/competition phase, and is guided by event-specific demands and influenced by the sex of the athlete. Further work should be done to bridge the gap between the researchers and the athletes in terms of the most recent strategies that emphasise low CHO availability training (more effective implementation of this strategy as well as investigation of current practices in other sports), as well as continue to probe its value for elite athletes. Finally, it is important to note that the survey tool was created for use among the track and field endurance events and in its current form, will not be suitable for use in other endurance sports due to specificity of the language and the description of the training and racing scenarios that are found in track & field but not in other sports (e.g. road cycling or cross-country skiing). Therefore, future research should aim to modify the survey tool for use in other sports (endurance and team sports) as well as take steps towards its validation, including a larger sample of athletes.

Study 2 continued with the theme of periodised nutrition. Here, we aimed to assess nutrition on a micro level of training and racing, that is, day-by-day dietary intakes of 6 professional male cyclists during the Classics. The current road cycling literature has heavily focused on stage racing and mean intakes are often reported (Table 5.1), while research on single-day racing and day-by-day analysis of intakes as well as overall assessment of EA have been lacking. Considering the recent, emerging evidence of the negative consequences of low EA in both female and male athletes (Mountjoy et al., 2018), and the possible significance of maintaining adequate within-day energy balance (for example, Torstveit et al., 2018), this topic is important yet has remained largely understudied to-date. Based on our analysis, we concluded that professional cyclists periodise energy and carbohydrate intakes day-by-day, where race days were characterised by low EA (14 vs 57 kcal·kg FFM·d^{-1}) but accompanied by high carbohydrate intakes (10.7 vs 6.4 g·kg·d^{-1}), meanwhile the opposite was true for rest days. These findings appear to be very distinct from stage racing where considerable effort is focused on increasing energy intake and EA on every day of racing. We also reported that low EA (<10 kcal·kg FFM·d^{-1}) every second day (i.e. on race days) led 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. These hormones are important for several reasons, and one key target tissue of their action is the bone (Lombardi et al., 2016). However, a further analysis of the data reveals important insights into these findings and necessitates further discussion around this topic. Indeed, during exit interviews, one of the cyclists (cyclist 4) admitted trying to limit food intake over the study period in an effort to lose weight, which included the rest days. This cyclist did indeed ingest considerably less energy and CHO compared to other cyclists on race and rest days as well as limited intake of CHO-rich foods in the 24 h recovery period post-race (Table 5.4). Importantly, cyclist 4 experienced a significant 27 % drop in testosterone concentrations across the 8 d period (786 to 571 ng·dL^{-1}; lowest quartile of reference range 280–485 ng·dL^{-1}), while the concentrations of testosterone for other cyclists remained stable (Figure 5.1). Therefore, the results of this one cyclist have likely skewed the data and affected our interpretations of findings. In fact, retrospectivecalculation of the average EA across the 8 d period (i.e. race and rest days pooled) showed that the mean EA for this period was 36 kcal·kg FFM·d^{-1} (range 26 to 47 kcal·kg FFM·d^{-1}). Therefore, it is likely that the absence of major shifts in hormone concentrations (apart from cyclist 4 and testosterone concentrations) may have been due to an overall sufficient EA across this study period. This finding, albeit observational and in a small number of athletes, challenges the idea that athletes should aim to meet energy demands on a daily basis and suggests that perhaps, where overall EA (across several days) falls within acceptable limits, these short-term (especially single-day) periods of extreme low EA are harmless in terms of athlete health. It should be emphasised though that this is a mere speculation based on observations in 6 male cyclists across a relatively brief 8 d period (which is still longer than many strictly controlled intervention studies on low EA; for example Ihle & Loucks, 2004; Papageorgiou et al., 2017; Zanker & Swaine, 2000). Therefore, the concept of multi-day rolling averages to assess chronic EI and EEE warrants further research. Also, future (controlled intervention) studies are needed in a larger sample size and across a longer time period.

In terms of health, the bone is an important consideration for endurance athletes. In fact, we recently reported that 60% of female and 50% of male elite distance runners had suffered from a stress fracture during their career (Heikura et al., 2018). The cause of these stress fractures are multi-factorial and could include: a) inappropriate training (especially sudden changes in weight-dependent sports); b) genetic disposition; c) poor EA, resulting in chronic RED-S and poor BMD (Tenforde et al., 2016). Bone is a dynamic tissue and exercise has a key role in maintaining and enhancing the strength and density of bone tissue (Kini & Nandeesh, 2012). However, nutrition and especially energy and CHO availability are also important. For example, there is evidence that low EA even in the presence of an exercise stimulus results in impaired bone modelling (Papageorgiou et al., 2017) and has likely long-term negative effects on BMD (Ackerman et al., 2015). More recently, studies have shown that restricting CHO intake in the acute time period around endurance exercise leads to impaired bone modelling (Scott et al., 2012; Sale et al., 2015; Townsend et al., 2017; Hammond et al., 2019). Longer-term CHO restriction in the form of the LCHF diet, on the other hand, has been shown that impair bone modelling and lead to increased prevalence of osteoporosis and risk of fractures, at least in rodents (Bielohuby et al., 2010; Scheller et al., 2016) and children with epilepsy (Bergqvist et al., 2008; Simm et al., 2017). Cumulative effects of a combination of LCHF and exercise have also been reported in rodents and children (Bielohuby et al., 2010; Simm et al., 2017).

The potential link between low CHO availability and bone health is an important consideration for endurance athletes, as many athletes may be tempted to implement LCHF diets in an effort to enhance the body’s capacity to oxidise fats as a fuel and consequently, prolong time to fatigue during endurance exercise (Volek et al., 2015; Burke, 2015). Therefore, ***study 3*** investigated the effects of a 3.5-week LCHF diet and acute CHO feeding on markers of bone modelling (CTX, P1NP, OC) in elite race walkers. We applied a strict dietary control (Mirtschin et al., 2018) over the study period, where athletes were divided into isocaloric treatments of either high CHO or LCHF diet. Markers of bone modelling were measured at rest, following a treatment-specific meal, and 0 and 3 h upon completion of a 2 h intense race walk protocol. We found clear and significant changes in bone modelling in the LCHF group, where markers of bone formation decreased (P1NP AUC -19%, OC AUC -29%) and resorption increased (CTX +81%) at rest and during exercise. When the exercise test was repeated after acute CHO feeding, markers of bone formation remained suppressed, while the marker of bone resorption returned to baseline values. These findings are in line with and of similar magnitude as reported in previous studies (Hammond et al., 2019; Sale et al., 2015) showing increased concentrations of bone resorption marker CTX following acute CHO restriction around endurance exercise. On the contrary, while the majority of this previous body of work has not been able to show changes in markers of bone formation, our study showed clear and significant decreases to both markers of bone formation (P1NP and OC) after the 3.5-week LCHF diet; these markers also remained suppressed despite acute CHO feeding. Whether these effects and differences compared to previous literature are due to the prolonged nature of CHO restriction applied in the current study, remains to be seen. Regardless, our findings demonstrate negative changes to bone modelling markers as a consequence of the LCHF diet and suggest tha endurance athletes might need to carefully consider the overall risk and reward of these diets for their long-term health. Indeed, given the injury risks and long-term outcomes underpinned by poor bone health in later life in athletes as well as individuals who undertake exercise for health benefits, additional investigations of the LCHF diet and its role in perturbing the bone modelling process are warranted.

Success in most endurance sports relies partly on the achievement of a high power-to-weight ratio (this topic has been discussed in detail in section 2.2 of this thesis), where a light and lean physique is likely to be beneficial due to enhanced economy of movement (i.e. reduced energy cost per distance covered). Therefore, endurance athletes are challenged by the need to achieve and maintain a “race weight” for optimal performance outcomes (Stellingwerff, 2018; Armstrong, 2000; McMahon, 2016). The manipulation of body weight and composition requires a change (decrease) in energy and macronutrient intakes or alternatively, the characteristics of training (duration, intensity, and type of sessions; i.e. increase in EEE): this often means that EA is reduced, usually via reduced EI as athletes may be more reluctant or unable to alter training stimulus. In females, even brief periods (less than one week) of low EA (less than 30 kcal·kg FFM·d^{-1}) have been shown to cause impairments to the endocrine system and bone modelling (for review, see Loucks et al., 2011). Emerging evidence suggests male athletes may suffer from low EA and associated impairments to health and performance (Tenforde et al., 2016; Heikura et al., 2018), albeit most of the evidence is speculative due to lack of intervention studies in this population. Another means to manipulate body composition is via altered macronutrient composition of the diet, where LCHF diet has been a popular means to reduce body weight, at least in clinical populations (Manninen, 2004; Petterson et al., 2013). Notably, most studies on the relationships between low EA and bone have implemented matched macronutrient ratios (i.e. % of total energy intake; usually ~ 50-55% from CHO, 10-20% from protein and 30-35% from fats) for low and optimal EA treatments (Papagergiou et al., 2017; Zanker & Swaine, 2000; Ihle & Loucks, 2004). Therefore, low EA treatments have also been reduced CHO availability treatments. Similarly, most available research on the effects of acute CHO restriction on the concentrations of markers of bone modelling has implemented a CHO restricted condition without controlling for the energy intake between treatments (Sale et al., 2015; Scott et al., 2012); again, these CHO restricted conditions have also been low or non-existent in energy content. Therefore, as the effects of low energy and low CHO availability become superimposed, it is impossible to determine whether the impairments seen to bone markers with low EA are linked to a decrease in energy or CHO availability, or a combination of both.

Accordingly, ***Study 4*** addressed this topic by implementing a 5 d study protocol of either 1) high energy and CHO availability (HCHO), 2) high energy but extremely low CHO availability (LCHF), or 3) low energy and moderate CHO availability (LEA). The purpose was to implement three common diets of endurance athletes, where a HCHO diet might be the most commonly followed or recommended, whereas the LCHF diet has gained interested among some athletes, and the LEA represents the "ideal" weight-loss diet for the athlete (i.e. reduced energy and CHO intake with adequate protein intake). The effects of these diets were examined in terms of bone markers (CTX, P1NP, OC, Glu-OC) during a prolonged, intense exercise bout in elite male race walkers. Our findings indicate significant decreases in the AUC around exercise for P1NP [-20% (-31, -9)], OC [-26% (-32, -20)], and Glu-OC [-31% (-50, -11)] in the LCHF group, with no change in the other dietary conditions. Meanwhile, despite a non-significant trend towards increased AUC for LCHF [+9% (-4, 22)], CTX concentrations showed no statistically significant change for any of the treatment groups. A recent study by Hammond et al. (2019) was the first to address the topic energy vs CHO availability and bone marker concentrations. In their study, the acute effects of 24 h of either high energy and high CHO availability, low energy and low CHO, or high energy and low CHO availability were investigated around an acute bout of twice-a-day endurance exercise. The authors reported a lack of suppression in the concentrations of CTX around the second bout of high-intensity exercise with both CHO restricted diets, and concluded that acute changes in bone markers might be more reflective of alterations in dietary CHO, as opposed to energy, availability. While these findings are certainly interesting, it is noteworthy that the study implemented an acute 24 h dietary intervention, therefore it does not allow investigation of longer-term effects of energy and CHO on bone. Furthermore, the LCHF diet implemented in the study provided ~3 $g \cdot kg \cdot d^{-1}$ CHO, and therefore, was not a ketogenic LCHF diet. In contrast, in study 4 of this thesis, our dietary interventions provided a step-wise reduction in dietary CHO content to enable comparison of three main-stream dietary approaches on bone marker concentrations. In contrast to the findings of Hammond et al. (2019), we showed decreases in markers of bone formation with the LCHF diet (Figure 7.4), while no clear effects were seen with LEA (Figure 7.3) or HCHO (Figure 7.2). In line with Hammond et al. (2019), our results suggest that lack of dietary CHO may be a more potent stimulus for impaired bone modelling process (indicated as reduced markers of bone formation) compared to overall EA.

8.2 Reflections on research with world-class athletes

A unique characteristic of the current thesis is that all four studies focus solely on ***elite*** endurance athletes (middle/distance runners, race walkers, and road cyclists). Within this context, an elite athlete is defined as an athlete competing at an international (Major Championship) level in his/her sport; this may be further defined according to their event/sport (e.g. membership of a UCI pro-cycling team, threshold of points awarded by IAAF). It is rare to get access to this athlete population and indeed, the majority of the sports nutrition literature has been conducted in sub-elite athletes (i.e. athletes competing in their sport but not fulfilling the criteria for an elite status as described above). This is an important consideration as many findings in trained, or worse yet, untrained, populations cannot be directly translated or even extrapolated into the real-life practice of working with elite athletes (Myburgh, 2003; Close et al., 2019) – yet this is often done in the absence of available research on the latter. Another valuable outcome from including elite athletes within a research study is their ability to influence the study goals (i.e. research will be more meaningful for this athlete population and outcomes are more likely to be applied into practice) and immediate access to study outcomes upon completion of data collection (in contrast to delays in advancing scientific knowledge due to the lengthy peer review process). Therefore, these athletes will benefit from participation by having the ability to suggest measures that are of interest or benefit specifically to them, and by having first access to novel data to aid their own athletic preparation (Sandbakk, 2018).

In studies 1 and 2, we were able to assess dietary periodization practices of elite endurance track and field athletes and professional road cyclists using self-report and direct measures, respectively, of energy and CHO availability. The strength of ***study 1*** was the ability of our online survey tool to assess self-reported dietary periodisation practices at all levels of the annual preparation period. In addition, we were able to reach a large cohort (n=104) of world-class athletes (50% major championship qualifiers) and assess not only practices but also reasons behind these nutrition choices. One limitation with this approach was the descriptive nature of the study, where, for example, terms such as “high” or “low” energy or CHO availability are merely descriptive, thus what might be considered as low CHO availability by one, might be interpreted as moderate to high CHO availability by another.

In ***study 2***, we were able to access professional male cyclists during a specialised racing period – The Classics (i.e. single-day racing, with a race usually every few days). This collaboration allowed us to describe and monitor in detail the training/racing characteristics as well as timing and intake of nutrition throughout the 8-day period, featuring 4 races. This study is the first to-date in the literature to describe day-by-day approach to nutrition and EA in professional cyclists, and the first overall to address the challenges of single-day racing. While we were challenged by last-minute drops in rider availability, which left us with smaller than anticipated sample size (n=6), and by limited access to more sophisticated laboratory testing (such as DXA for BMD, or RMR testing), we were nonetheless able to collect novel data around energy and macronutrient availability, hormone concentrations and physique outcomes. This study also gave us the opportunity to retrospectively discuss some of the challenges of working in the environment of professional cycling, which resulted in a comprehensive analysis and guidelines for further research (Table 5.2).

The opportunity to implement a nutrition intervention 1) with elite athletes and 2) using a real life training setting (such as a research camp that combines training and testing) is extremely rare but very useful, as it provides high ecological validity for the interpretation of study outcomes into the real-life athlete practice (Close et al., 2019). Indeed, this type of approach is highly applied in nature and possibly the only way to recruit elite athletes for a prolonged intervention study. The Supernova race walking research series (***studies 3 and 4*** of the current thesis) have managed to do exactly this. Here, we have had the honour to recruit and accommodate a large number (total n=52 across four research camps) of elite race walkers who contribute their physical (study outcomes) and intellectual (study design) output to research. The use of a hybrid laboratory/field exercise test has allowed us to test the effects of an intervention during a session that reflects actual, real-life training of these athletes. Also, and as mentioned above, building a dietary intervention study around the concept of a training camp has allowed us to maintain high ecological validity and offer invaluable group training opportunities for the athletes. Another beneficial outcome from this experience has been direct engagement with some of the best endurance athletes in the world, which will hopefully help us close the gap between research and practice (Sandbakk, 2018). Finally, and as mentioned earlier, in the end, each athlete is an individual (Archer et al., 2018), and by participating in research they will acquire individual data on how intervention x worked specifically for them.

Despite the benefits of research with truly elite athletes, working with this population also comes with its challenges. One of the challenges is the lack of control and standardisation over the athlete's training program. Indeed, for an elite athlete, every training day is likely to be different, and what is planned on paper might not be executed on the day due to factors such as fatigue, insufficient recovery from the previous day's training, or feeling better than usual. This might not be an entirely bad thing, as it allows for a high ecological validity (as opposed to studies that repeat the same exercise session 5 d a week). It is also (nearly) impossible to isolate the athlete or their training program for a more mechanistic study. Neither is it possible to collect data that causes disruptions to the athlete's training (including muscle biopsies or testing that would require change in habitual training). On the contrary, data on cellular events might not be directly translatable into the field (Close et al., 2019). In the big scheme of things, elite athletes are a small, rare, unique population and hence, research on elite athletes is likely to be characterised by small sample sizes to begin with, and also as a consequence of last minute dropouts due to reasons such as injuries, illness, visa complications, and so on. As these athletes are a rare breed, it will be more difficult to save the sample size by recruiting a new participant, compared to a study where untrained or moderately trained was the only prerequisite.

8.3 Reflections on the methodology of dietary assessment and standardisation in the field and in the laboratory

Another characteristic of the current thesis is the utilisation of several methods to collect data on dietary habits or intakes. Indeed, methodology used ranges from self-reported practices (not quantified intakes per se) (*study 1*), to researcher-assisted weighed dietary records (*study 2*), to researcher-led strict dietary standardisation and control (*studies 3 and 4*). Considering the several issues and challenges associated with assessment of dietary intake (Capling et al., 2017; Archer et al., 2018) and the importance of proper and careful dietary control around interventions (Close et al., 2019), this section will discuss some of the benefits and challenges of the dietary assessment or standardisation methodologies utilised in the current thesis. As detailed methodology for each study has been outlined in Chapter 3, this section will discuss these aspects specifically from the point of view of the learning outcomes and skills development of the PhD student. The discussion will focus on studies 2, 3 and 4; study 1 will be omitted here as it aimed to assess and characterise dietary periodization *practices*, not quantify intakes per se.

Study 2 gave us an opportunity to collect dietary intake data from a group of professional cyclists. Professional cycling is a unique sport and world where teams have their own chefs and rely almost solely on the chef in terms of nutrition support. We were able to collaborate with the team chef/nutritionist, which enabled us access to exact recipes used for meals, as well as exact brand names for snacks and sports foods. We were also able to assist riders in the weighing and recording of meal items. These factors led to a high-quality dietary intake data set that depicts actual intakes in professional cyclists with likely the highest precision possible.

The experience from strict dietary control required to implement a 3 to 4 week dietary treatment has been unique. In studies 3 and 4, dietary intakes were determined for each athlete according to not only their dietary treatment but also personal preferences. In ***study 3***, energy and macronutrient targets were assigned per athlete BM but remained the same throughout the study, regardless of training volume. Meanwhile, in ***study 4***, where the diets were designed around the concept of EA, the methodology was the most advanced in nature. This methodology is unique and in contrast to previous intervention studies on EA, where a strict control of energy intakes and EEE has been possible due to a short duration of the intervention (often 3 to 5 d) and the inclusion of sedentary or recreationally active participants (where the researchers have full control of the training program of the participants). For example, preliminary research by Prof Anne Loucks and colleagues implemented step-wise reductions in EA to study the effects decreasing EA on various body systems (for example, Loucks & Thuma, 2003; Ihle & Loucks, 2004). In these studies, sedentary female participants were provided a predetermined amount of food (in the form of liquid dietary products) while completing a set amount of steady state exercise in the laboratory environment (until target EEE for the day was met). While these studies were clearly sophisticated in design, their application to real life is not ideal. Indeed, athletes rarely complete the same type and volume of training each day; moreover, in the case of reduced EA, athletes are more likely to achieve a caloric deficit by reductions in CHO and fat, while protein intake is likely to remain unchanged. More recent studies by Koehler et al. (2016) and Papageorgiou et al. (2017, 2018) followed similar methodology to the investigations of Loucks et al. with the exception of participant type (active but non-elite female and male participants) and type of food provision (meal plans including “real food” instead of liquid dietary products). While the benefits of these more recent studies include inclusion of both female and male participants and the provision of real foods, the challenges noted with regards to earlier literature (i.e. a set amount of training per day and fixed macronutrient ratios in the low EA treatment) remain. Indeed, overall, the approach utilised in the previous literature on the effects of low EA on health outcomes poorly reflects the real-world requirements of dietary support of elite athletes, where training is different every day and may sometimes change with short notice. Therefore, upon embarking on our 23 d intervention in study 4, we realised that the method used in previous EA research would not be practical in the current study where we were likely to face a challenge of manipulating individual dietary EI targets and subsequently, meal plans, acutely in the face of changes to training volume within- and between-days. Accordingly, we modelled a pilot spreadsheet designed to create prospective, individualised targets day-by-day for each athlete (based on projected and actual training volumes and target EA values) and to assist us in real-time adjustments to diet plans (in case of sudden changes to daily training load) (Heikura et al., 2020). Due to changes in planned training, considerable amount of time was spent adjusting intakes within-day and subsequently, food was added or taken away from that day's meal plan depending on whether training-induced EEE had increased or decreased, respectively, below or above a predetermined threshold. These actions required continuous monitoring and interactions between the research team and the athletes. Although study 4 was undoubtedly the most challenging of all four studies in terms of both researcher work load and athlete burden, it was also a unique experience and provided important insights into the usefulness and practicality (or lack of) within-day adjustments to dietary intakes based on acute changes in training load.

8.4 Reflections on the PhD experience

I arrived in Australia in October 2015 for Supernova 1 race walking research study/camp to volunteer and learn from the best in the field. At the time I considered to have established a quite solid background in exercise and sports science (BSc and MSc in Exercise Physiology from the University of Jyväskylä, Finland). While this was true, I had no idea I had barely scratched the surface. Fast forward four years and I can easily say that my time in Australia and specifically, at the Australian Institute of Sport, working with Prof Louise Burke and her team, along with several collaboration opportunities with overseas colleagues including, for example, Dr Trent Stellingwerff (Canadian Sport Institute Pacific) and Mr Mark Quod (UCI cycling team Mitchelton-Scott) has exponentially expanded my theoretical and practical knowledge and skill set in the area of sports nutrition and exercise physiology. I have learned that sports nutrition can be a super exciting field and is in fact so much more than the "five servings of vegetables a day" kind of thing. Looking back, I feel incredibly lucky to have had the opportunity to develop my skill set in the physiology (RMR and DXA measurements, venepuncture and cannulation, hybrid field/lab testing, rectal probes for core temperature measurement and breath hydrogen measures to assess gut adaptations) and biochem (ELISA kits) labs during various research studies (6 separate Supernova camps, 3 marathon camps and a triathlon camp as well as a collagen research study run by a fellow PhD student Bek Alcock). My sports nutrition knowledge and skills have benefited from the unique experience of being deeply immersed in the world of sports nutrition in the research setting, which has meant careful planning, adjusting and measuring of meals (to the gram, per each individual athlete requirements), day after day, preparing hundreds of bottles of individualised (to volume and flavour) sports drinks and snacks for training support, while simultaneously aiming to maintain a balance between scientific rigor and keeping the athletes happy throughout a month of dietary control. Further experience has come from outside of the PhD, where I have been fortunate to assist in Athlete Availability Program testing (several sports across a 2-year period) – a huge thanks to Dr Mick Drew for trusting me with this work. I have also been able to participate in casual work in the Altitude House – thanks to AIS Physiology for this cool opportunity. At the end of the day, it has been the experience of a lifetime, something I doubt I would have been able to get anywhere else. And while I have learned a lot, I realise there is so much more left to learn – this is a profession where you will never be finished. I love the fact and can't wait to se what the future holds; whatever it is, I am confident that my time in Australia has prepared me for it.

8.5 Future directions – where to from here?

The studies within this thesis have addressed several gaps in the literature of dietary periodisation and effects of low energy and CHO availability on markers of bone modelling, as outlined in the previous sections. However, several topics warrant further investigation. One of the biggest gaps in the literature remains ***the long-term (several weeks to months) effects of continuous, altered (often reduced) energy and CHO availability on bone health*** of athletes. While deleterious effects on markers of bone modelling have been shown in as little as 5 d of low EA (Ihle & Loucks, 2004; Papagergiou et al., 2017, 2018; Zanker & Swaine, 2000) or acute CHO restriction around exercise (Scott et al., 2012; Sale et al., 2015; de Sousa et al., 2014; Townsend et al., 2017; Hammond et al., 2019), it is very likely that these effects need to be repeated and accumulated over time to induce negative functional outcomes (e.g. decreased BMD and increased fracture incidence). The bone tissue has a relatively slow turnover (as opposed to other body systems such as the endocrine system or muscle protein synthesis), and studies in amenorrheic athletes often show minimal or no imporvements in BMD across 6 to 12 month periods of increased EA (Cialdella-Kam et al., 2014; Singhal et al., 2019). We showed in study 3 of the thesis that markers of bone modelling were negatively altered after 3.5 weeks of LCHF diet; however, it is unclear whether these changes were prolonged enough to transfer into, eventually, decreased BMD. Thus, future investigations should focus on determining the time course of changes in bone metabolic (blood markers of bone remodelling) outcomes to transfer into structural changes of the bone tissue. In other words, how well do acute changes reflect the likelihood of longer-term outcomes, and how long periods of low energy or CHO are safe in terms of bone health? Indeed, future ***research should address the safety of brief exposures to low EA***, which may be inevitable in elite sports where BM and physique play an important role in exercise economy and ultimately, performance.

Another consideration is ***the depth of exposure to low EA or low CHO availability***. For example, could the negative effects of energy or CHO restriction on markers of bone breakdown and formation be avoided by utilising a stepwise reduction in energy and/or CHO availability (i.e. gradual drops from baseline to target EA, as opposed to a single, sudden, massive drop as is usually seen in the intervention studies)? It can be hypothesised that a more modest approach might lead to a lower stress response as the body would have more time to gradually get used to the new, lower level of energy and CHO availability. Research around weight loss has indeed shown that slower weight loss rates lead to a more beneficial outcome in terms of fat loss and maintenance of lean mass (Garthe et al., 2011). Whether the same is true for endocrine and bone systems, is currently not known.

In terms of length of exposure, another point to consider ***would be whether exposure is continuous or not***: here, the addition of refeed/recovery days in between a low EA intervention might be an effective strategy to maintain better overall health of the athlete (Peos et al., 2019). For example, athletes could restrict energy intake every other day, on easy training days and/or every other week, compared to continuous energy restriction. Research in sedentary individuals suggests that longer diet breaks might be useful (Byrne et al., 2018) but whether short (one or two days) periods are helpful in athletes remains to be seen. It is likely that ***length and depth of exposure have combined effects***. Therefore, whether there is an AUC for low EA (length of exposure multiplied by depth of energy deficit) and a threshold below which harmful effects are seen, remains to be investigated.

The ***composition of macronutrients within a diet*** is not irrelevant. Endurance athletes require CHO based fuels for successful completion of high-intensity training and racing (Hawley & Leckey, 2015) and a 3.5 week extreme CHO restriction in the form of a ketogenic LCHF diet impaired economy and performance gains in elite race walkers (Burke et al., 2017). In addition, CHO appear to have a role in maintaining optimal endocrine function especially via thyroid (T3; Spaulding et al., 1976), metabolic (leptin; Jenkins et al., 1997) and reproductive (LH; Loucks & Verdun, 1998) systems. These systems have effects on bone health and therefore, extreme CHO restriction may not be a suitable dietary approach to endurance athletes. In support of this, studies 3 and 4 of this thesis showed impaired markers of bone modelling after 5 d and 3.5 weeks of LCHF diet despite adequate EA. A better approach to physique management (e.g. weight loss) might be to reduce dietary fat intake, followed by modest reductions in dietary CHO (Helms et al., 2014). However, further research on optimal macronutrient ratios during a weight loss diet in athletes are needed.

Timing of meals within-day is another tempting component around low EA research. It is well known that physique athletes carefully time meals around training, thus maintaining optimal fuel availability around the times the body needs the fuels, and these athletes have been shown to reach extremely low levels of body fat while maintaining lean mass levels and usually without massive sacrifices to health within a medium term (several months) period (Mitchell et al., 2017). Additionally, research in athletes has shown that maintenance of energy balance within day (i.e. incorporating meals in close proximity to exercise) is associated with better health and physique parameters in both female and male athletes (Torstveit et al., 2018; Fahrenholtz et al., 2018; Deutz et al., 2000). Timing of meals adds to the complexity of the topic of periodising energy and CHO availability across the day and between days. Finally, ***whether some individuals may be more safe to implement low EA / weight-loss strategies than others, and which factors might play a role*** (e.g. age, gender, injury/illness history and susceptibility, baseline endocrine status) would be important to investigate so as to make sure interventions are only undertaken with athletes that can handle the added stress of energy and/or CHO restriction.

8.6 Conclusions

This series of research studies has addressed several gaps in the literature and contributed novel insights into the nutrition periodisation practices of elite endurance athletes and into the interactions between acute and prolonged dietary energy and CHO restriction and markers of bone modelling in world-class endurance athletes. The key findings can be summarised as: 1) World-class track and field endurance athletes periodise nutrition across all levels of training and racing, where key incentive appears to be performance optimisation, 2) Professional cyclists micro-periodise energy and CHO availability to match fuel intakes to the day-by-day demands of training and racing, 3) Prolonged, extreme CHO restriction (the LCHF diet) changes markers of bone formation (decrease) and resorption (increase) during a prolonged intense exercise bout, and these effects are not fully recovered with acute CHO feeding, and 4) Short-term CHO restriction in the form of a LCHF diet decreases cocentrations of markers of bone formation during a prolonged exercise bout, while no effects are seen with a diet providing low energy but moderate CHO availability. Athletes should continue to aim to match fuel intake to the goals and demands of training and racing across all levels of a periodised program. Care should be taken with extreme CHO restriction as this appears to lead to impairments in bone modelling markers at rest and during exercise.

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10 RESEARCH PORTFOLIO APPENDIX

10.1 Publication statements of contribution of others

1. **Heikura, IA.**, Stellingwerff, T., & Burke, LM. (2018). Self-reported periodization of nutrition in elite female and male runners and race walkers. *Front Physiol*. Dec 3;9:1732. doi: 10.3389/fphys.2018.01732. eCollection 2018

Contribution statement: IH was primarily responsible for the conception and design, collection and assembly of data, data analysis and interpretation, drafting, revising and approval of final manuscript. TS was involved in the conception and design, data analysis and interpretation, drafting, revising and approval of final manuscript. LB was involved in the conception and design, data analysis and interpretation, drafting, revising and approval of final manuscript.

Approximate percentage contributions: I.A. Heikura 70%; T. Stellingwerff 15%; L.M. Burke 15%

I acknowledge that my contribution to the above paper is 70%.

[redacted]

I.A. Heikura Date: 1/11/2019

As principal supervisor of this project, I certify that the above contributions are true and correct:

[redacted]

L.M. Burke Date: 1/11/2019

Coauthor signatures:

[redacted]

T. Stellingwerff Date: 1/11/2019

2. **Heikura, IA.**, Quod, M., Strobel, N., Palfreeman, R., Civil, R., & Burke, LM. (2019). Alternate-D Low Energy Availability During Spring Classics in Professional Cyclists. *Int J Sports Physiol Perform*. Mar 12:1-35. doi: 10.1123/ijspp.2018-0842. [Epub ahead of print]

Contribution statement: IH was primarily responsible for the conception and design, collection and assembly of data, data analysis and interpretation, drafting, revising and approval of final manuscript. MQ was involved in the conception and design, collection and assembly of data, data analysis and interpretation, and approval of final manuscript. NS was involved in the conception and design, collection and assembly of data, data analysis and interpretation, and approval of final manuscript. RP was involved in the conception and design, data analysis and interpretation, and approval of final manuscript. RC was involved in the conception and design, collection and assembly of data, data analysis and interpretation, and approval of final manuscript. LB was involved in the conception and design, data analysis and interpretation, drafting, revising and approval of final manuscript.

Approximate percentage contributions: I.A. Heikura 70%; M. Quod 7.5%; N. Strobel 7.5%; R. Palfreeman 2.5%; R. Civil 2.5%; L.M. Burke 10%

I acknowledge that my contribution to the above paper is 70%.

[redacted]

I.A. Heikura Date: 1/11/2019

As principal supervisor of this project, I certify that the above contributions are true and correct:

[redacted]

L.M. Burke Date: 1/11/2019

Coauthor signatures:

[redacted]

M. Quod Date: 1/11/2019

[redacted]

N. Strobel Date: 1/11/2019

[redacted]

R. Palfreeman Date: 1/11/2019

[redacted]

R. Civil Date: 1/11/2019

3. **Heikura, IA.,** Burke, LM., Hawley, JH., Ross, ML., Garvican-Lewis, L., Sharma, AP., McKay, AKA., Leckey, JJ., Welvaert, M., McCall, L., & Ackerman, KE. (2019). A short-term ketogenic diet impairs markers of bone health in response to exercise. *Accepted for publication in Frontiers in Endocrinology*

Contribution statement: IH was primarily responsible for the conception and design, collection and assembly of data, data analysis and interpretation, drafting, revising and approval of final manuscript. LB was involved in the conception and design, collection and assembly of data, data analysis and interpretation, drafting, revising and approval of final manuscript. JH was involved in the conception and design, data analysis and interpretation, drafting, revising and approval of final manuscript. MR was involved in the conception and design, collection and assembly of data, and approval of final manuscript. LGL was involved in the conception and design, collection and assembly of data, and approval of final manuscript. AS was involved in the conception and design, collection and assembly of data, and approval of final manuscript. AM was involved in the conception and design, collection and assembly of data, and approval of final manuscript. JL was involved in the conception and design, collection and assembly of data, and approval of final manuscript. MW was involved in the conception and design, data analysis and interpretation, and approval of final manuscript. LM was involved in the data analysis and interpretation, and approval of final manuscript. KA was involved in the data analysis and interpretation, drafting, revising and approval of final manuscript.

Approximate percentage contributions: I.A. Heikura 60%; L.M. Burke 15%; J.A. Hawley 5%; M.L. Ross 3%; L. Garvican-Lewis 2%; A. Sharma 2%; A.K.A. McKay 2%; J.J. Leckey 2%; M. Welvaert 2%; L. McCall 2%; K.A. Ackerman 5%.

I acknowledge that my contribution to the above paper is 60%.

[redacted]

I.A. Heikura Date: 1/11/2019

As principal supervisor of this project, I certify that the above contributions are true and correct:

[redacted]

L.M. Burke Date: 1/11/2019

Coauthor signatures:

[redacted]

J.A. Hawley Date: 1/11/2019

[redacted]

M.L. Ross Date: 1/11/2019

[redacted]

L. Garvican-Lewis Date: 1/11/2019

[redacted]

A.P. Sharma Date: 1/11/2019

[redacted]

A.K.A. McKay Date: 1/11/2019

[redacted]

J.J. Leckey Date: 1/11/2019

[redacted]

M. Welvaert Date: 1/11/2019

[redacted]

L. McCall Date: 1/11/2019

[redacted]

K.A. Ackerman Date: 1/11/2019

4. **Heikura, IA.,** Ross, ML., Tee, N., McKay, AKA., Sharma, AP., & Burke, LM. (2020). Effects of low energy vs low CHO availability on markers of bone modelling at rest and during exercise in elite race walkers *In preparation for Bone*

Contribution statement: IH was primarily responsible for the conception and design, collection and assembly of data, data analysis and interpretation, drafting, revising and approval of final manuscript. MR was involved in the conception and design, collection and assembly of data, and approval of final manuscript. NT was involved in the conception and design, collection and assembly of data, data analysis and interpretation, and approval of final manuscript. AM was involved in the conception and design, collection and assembly of data, and approval of final manuscript. AS was involved in the conception and design, collection and assembly of data, and approval of final manuscript. LB was involved in the conception and design, collection and assembly of data, data analysis and interpretation, drafting, revising and approval of final manuscript.

Approximate percentage contributions: I.A. Heikura 70%; M.L. Ross 5%; N. Tee 5%; A.K.A. McKay 2.5%; A. Sharma 2.5%; L.M. Burke 15%;

I acknowledge that my contribution to the above paper is 70%.

[redacted]

I.A. Heikura Date: 1/11/2019

As principal supervisor of this project, I certify that the above contributions are true and correct:

[redacted]

L.M. Burke Date: 1/11/2019

Coauthor signatures:

[redacted]

M.L. Ross Date: 1/11/2019

[redacted]

N. Tee Date: 1/11/2019

[redacted]

A.K.A. McKay Date: 1/11/2019

[redacted]

A.P. Sharma Date: 1/11/2019

10.2 Conference statements of contribution of others

1. **Heikura, IA.**, Stellingwerff, T. & Burke, LM. Self-reported periodisation of nutrition in elite female and male runners and race walkers.

   Poster presentation at International Sports and exercise Nutrition Conference, Newcastle Upon Tyne, UK. December 2018.

   Contribution statement: This presentation was based on the work from study 1 (author contributions listed in section 10.1) and was created and delivered by IH with assistance from TS and LB and reviewed by all authors.

2. **Heikura, IA.**, Cox, GC, McKay, AKA., Burke, LM. Acute Implementation of train/recovery with low carbohydrate availability does not compromise bone metabolism in world-class triathletes.

   Mini-oral presentation at European College of Sport Sciences conference, Dublin, Ireland. July 2018.

   Contribution statement: This presentation was based on the work from a study not part of this thesis and was created and delivered by IH.

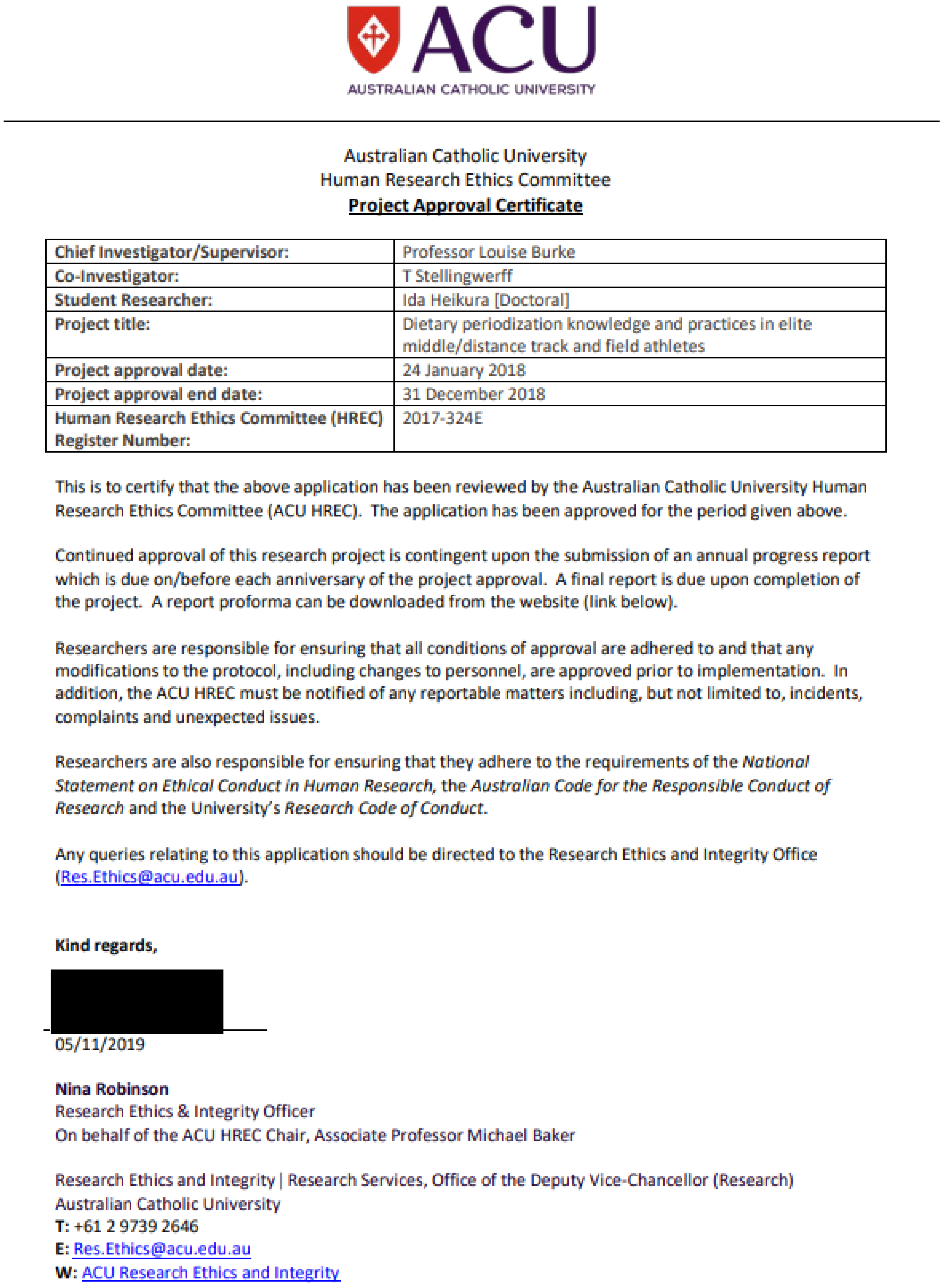
3. **Heikura, IA.**, Burke, LM., Bergland, D., Uusitalo, ALT., Mero, AA. & Stellingwerff, T. Impact of elite runner's energy availability on bone mineral density, health, injuries and haemoglobin responses at altitude.

   Poster presentation, SPIN Summit 2017, Vancouver, Canada. October 2017.

   Contribution statement: This presentation was based on the work from a study not part of this thesis and was created by IH with the assistance from TS and LB and delivered at the conference by TS.

10.3 Ethics approvals for studies 1-4

Study 1



AUSTRALIAN CATHOLIC UNIVERSITY

Australian Catholic University
Human Research Ethics Committee
Project Approval Certificate

Chief Investigator/Supervisor:	Professor Louise Burke
Co-Investigator:	T Stellingwerff
Student Researcher:	Ida Heikura [Doctoral]
Project title:	Dietary periodization knowledge and practices in elite middle/distance track and field athletes
Project approval date:	24 January 2018
Project approval end date:	31 December 2018
Human Research Ethics Committee (HREC) Register Number:	2017-324E

This is to certify that the above application has been reviewed by the Australian Catholic University Human Research Ethics Committee (ACU HREC). The application has been approved for the period given above.

Continued approval of this research project is contingent upon the submission of an annual progress report which is due on/before each anniversary of the project approval. A final report is due upon completion of the project. A report proforma can be downloaded from the website (link below).

Researchers are responsible for ensuring that all conditions of approval are adhered to and that any modifications to the protocol, including changes to personnel, are approved prior to implementation. In addition, the ACU HREC must be notified of any reportable matters including, but not limited to, incidents, complaints and unexpected issues.

Researchers are also responsible for ensuring that they adhere to the requirements of the *National Statement on Ethical Conduct in Human Research*, the *Australian Code for the Responsible Conduct of Research* and the University's *Research Code of Conduct*.

Any queries relating to this application should be directed to the Research Ethics and Integrity Office (Res.Ethics@acu.edu.au).

Kind regards,

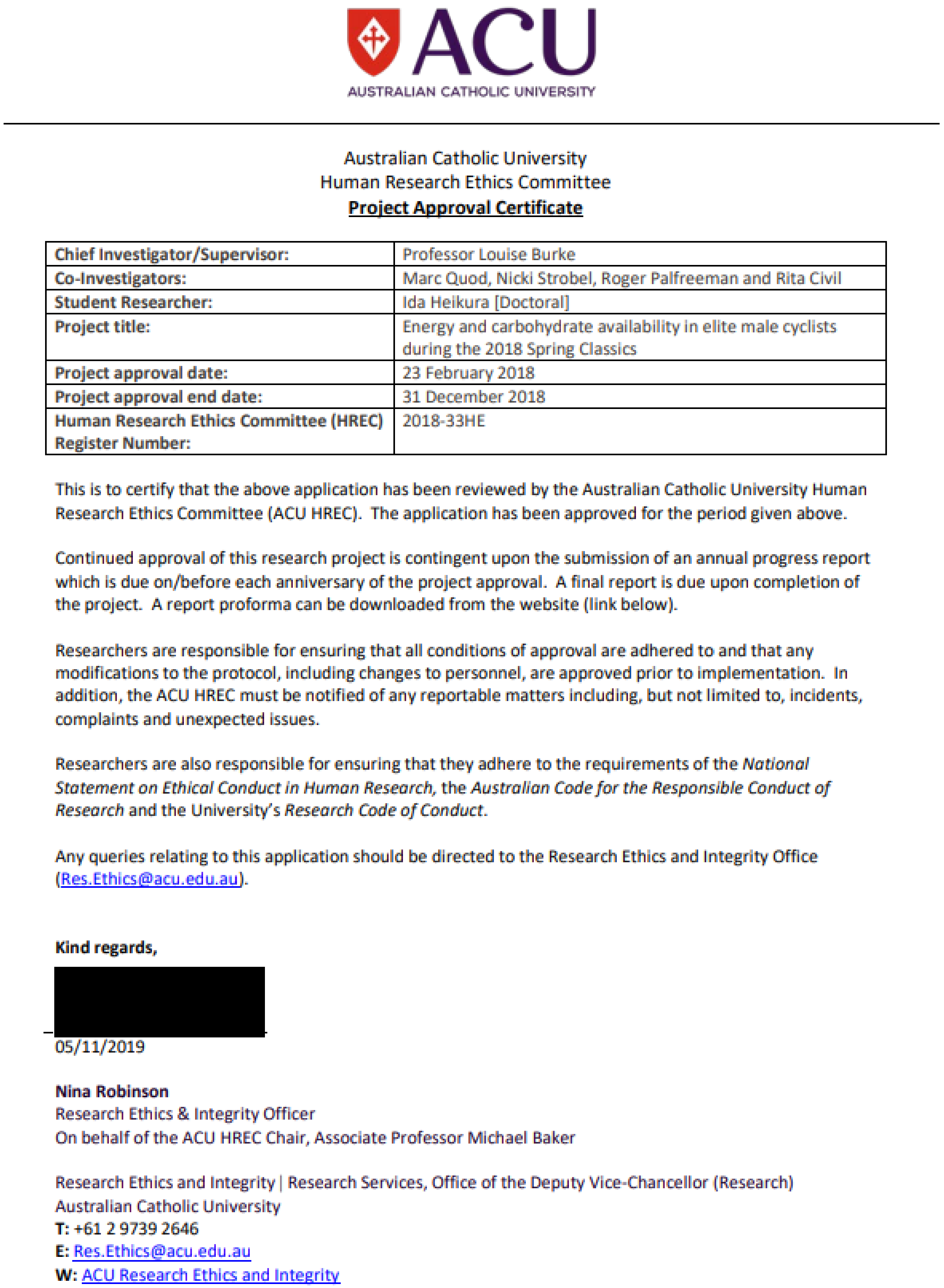
[redacted]

05/11/2019

Nina Robinson
Research Ethics & Integrity Officer
On behalf of the ACU HREC Chair, Associate Professor Michael Baker

Research Ethics and Integrity | Research Services, Office of the Deputy Vice-Chancellor (Research)
Australian Catholic University
T: +61 2 9739 2646
E: Res.Ethics@acu.edu.au
W: ACU Research Ethics and Integrity

Study 2



Australian Catholic University
Human Research Ethics Committee
Project Approval Certificate

Chief Investigator/Supervisor:	Professor Louise Burke
Co-Investigators:	Marc Quod, Nicki Strobel, Roger Palfreeman and Rita Civil
Student Researcher:	Ida Heikura [Doctoral]
Project title:	Energy and carbohydrate availability in elite male cyclists during the 2018 Spring Classics
Project approval date:	23 February 2018
Project approval end date:	31 December 2018
Human Research Ethics Committee (HREC) Register Number:	2018-33HE

This is to certify that the above application has been reviewed by the Australian Catholic University Human Research Ethics Committee (ACU HREC). The application has been approved for the period given above.

Continued approval of this research project is contingent upon the submission of an annual progress report which is due on/before each anniversary of the project approval. A final report is due upon completion of the project. A report proforma can be downloaded from the website (link below).

Researchers are responsible for ensuring that all conditions of approval are adhered to and that any modifications to the protocol, including changes to personnel, are approved prior to implementation. In addition, the ACU HREC must be notified of any reportable matters including, but not limited to, incidents, complaints and unexpected issues.

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Any queries relating to this application should be directed to the Research Ethics and Integrity Office (Res.Ethics@acu.edu.au).

Kind regards,

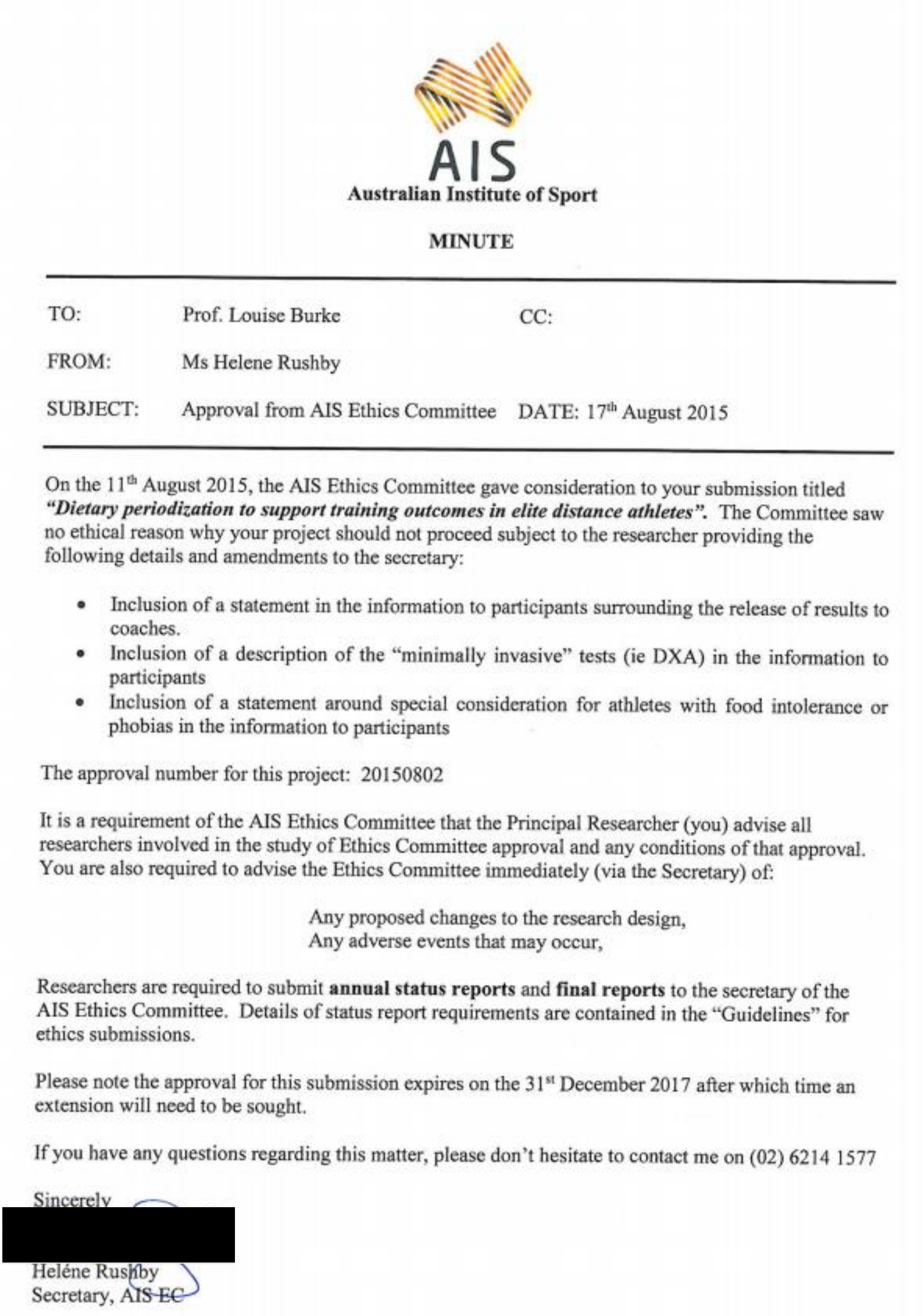
[redacted]

05/11/2019

Nina Robinson
Research Ethics & Integrity Officer
On behalf of the ACU HREC Chair, Associate Professor Michael Baker

Research Ethics and Integrity | Research Services, Office of the Deputy Vice-Chancellor (Research)
Australian Catholic University
T: +61 2 9739 2646
E: Res.Ethics@acu.edu.au
W: ACU Research Ethics and Integrity

Study 3



AIS
Australian Institute of Sport

MINUTE

TO:	Prof. Louise Burke	CC:	
FROM:	Ms Helene Rushby		
SUBJECT:	Approval from AIS Ethics Committee	DATE: 17th August 2015	

On the 11th August 2015, the AIS Ethics Committee gave consideration to your submission titled ***"Dietary periodization to support training outcomes in elite distance athletes".*** The Committee saw no ethical reason why your project should not proceed subject to the researcher providing the following details and amendments to the secretary:

- Inclusion of a statement in the information to participants surrounding the release of results to coaches.
- Inclusion of a description of the "minimally invasive" tests (ie DXA) in the information to participants
- Inclusion of a statement around special consideration for athletes with food intolerance or phobias in the information to participants

The approval number for this project: 20150802

It is a requirement of the AIS Ethics Committee that the Principal Researcher (you) advise all researchers involved in the study of Ethics Committee approval and any conditions of that approval. You are also required to advise the Ethics Committee immediately (via the Secretary) of:

Any proposed changes to the research design,
Any adverse events that may occur,

Researchers are required to submit **annual status reports** and **final reports** to the secretary of the AIS Ethics Committee. Details of status report requirements are contained in the "Guidelines" for ethics submissions.

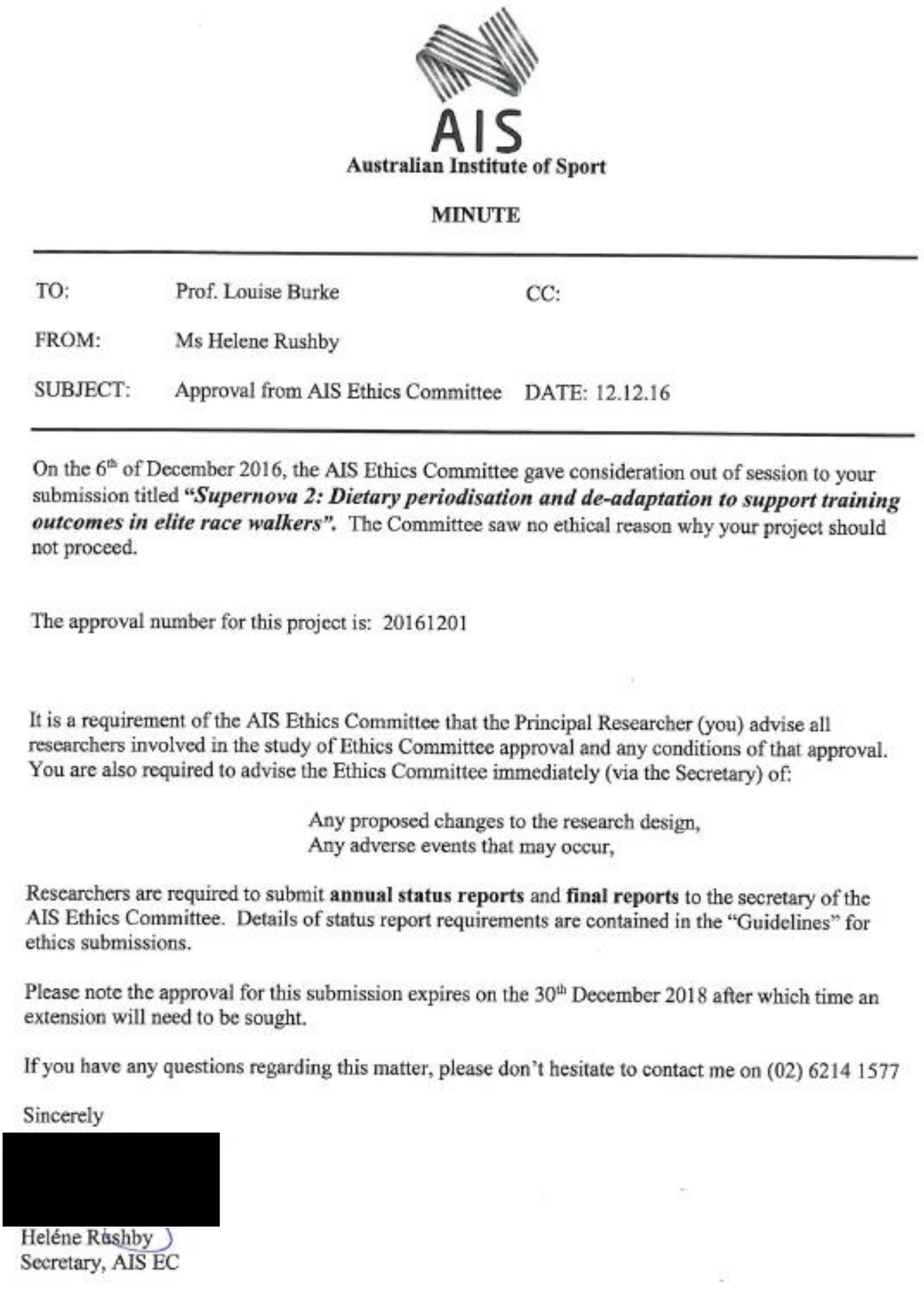
Please note the approval for this submission expires on the 31st December 2017 after which time an extension will need to be sought.

If you have any questions regarding this matter, please don't hesitate to contact me on (02) 6214 1577

Sincerely

[redacted]

Heléne Rushby
Secretary, AIS EC



Australian Institute of Sport

MINUTE

TO:	Prof. Louise Burke	CC:	
FROM:	Ms Helene Rushby		
SUBJECT:	Approval from AIS Ethics Committee	DATE: 12.12.16	

On the 6th of December 2016, the AIS Ethics Committee gave consideration out of session to your submission titled ***"Supernova 2: Dietary periodisation and de-adaptation to support training outcomes in elite race walkers".*** The Committee saw no ethical reason why your project should not proceed.

The approval number for this project is: 20161201

It is a requirement of the AIS Ethics Committee that the Principal Researcher (you) advise all researchers involved in the study of Ethics Committee approval and any conditions of that approval. You are also required to advise the Ethics Committee immediately (via the Secretary) of:

Any proposed changes to the research design,
Any adverse events that may occur,

Researchers are required to submit **annual status reports** and **final reports** to the secretary of the AIS Ethics Committee. Details of status report requirements are contained in the "Guidelines" for ethics submissions.

Please note the approval for this submission expires on the 30th December 2018 after which time an extension will need to be sought.

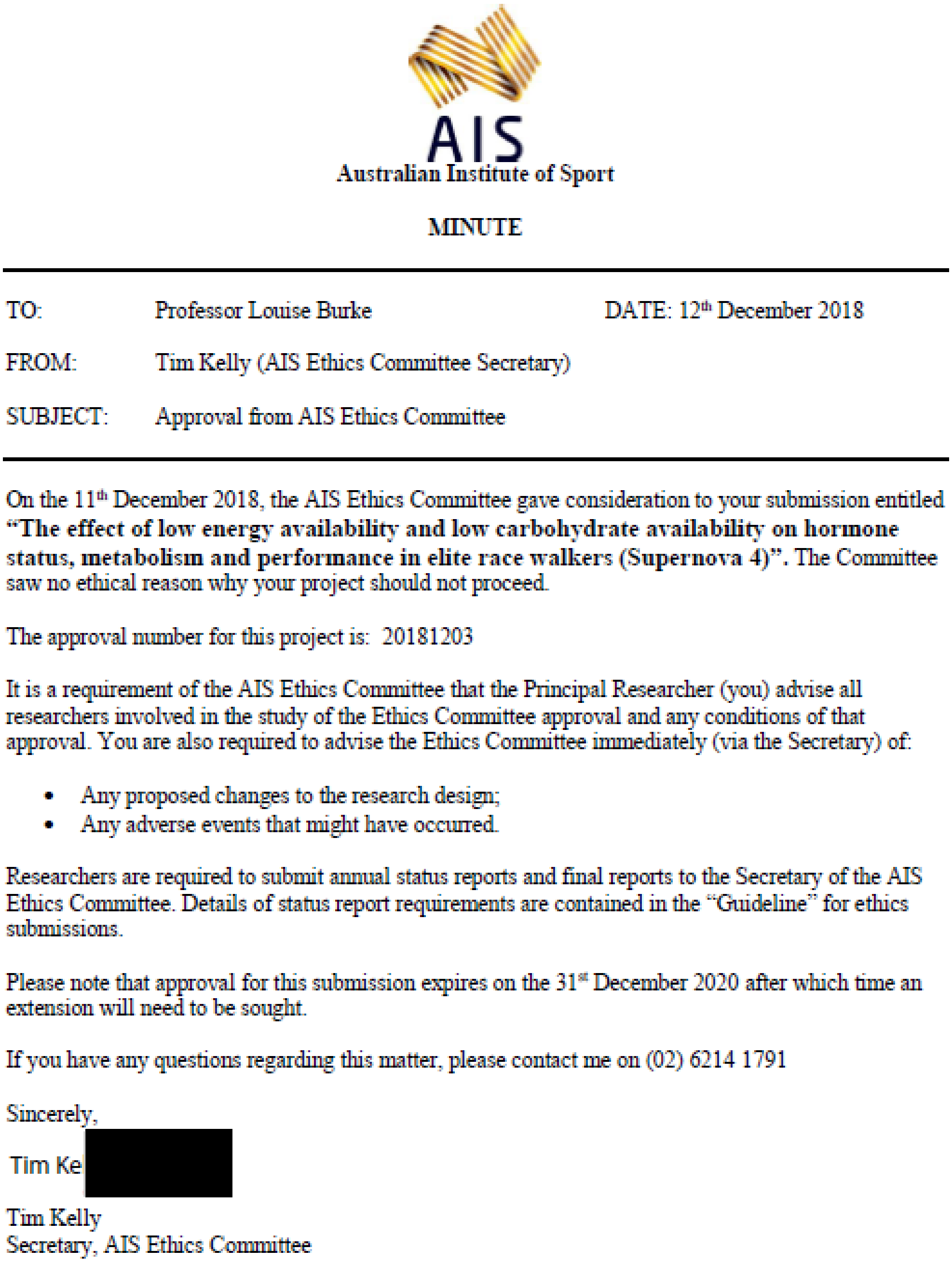
If you have any questions regarding this matter, please don't hesitate to contact me on (02) 6214 1577

Sincerely

[redacted]

Heléne Rushby
Secretary, AIS EC

Study 4



AIS
Australian Institute of Sport

MINUTE

TO:	Professor Louise Burke	DATE: 12th December 2018
FROM:	Tim Kelly (AIS Ethics Committee Secretary)	
SUBJECT:	Approval from AIS Ethics Committee	

On the 11th December 2018, the AIS Ethics Committee gave consideration to your submission entitled **"The effect of low energy availability and low carbohydrate availability on hormone status, metabolism and performance in elite race walkers (Supernova 4)".** The Committee saw no ethical reason why your project should not proceed.

The approval number for this project is: 20181203

It is a requirement of the AIS Ethics Committee that the Principal Researcher (you) advise all researchers involved in the study of the Ethics Committee approval and any conditions of that approval. You are also required to advise the Ethics Committee immediately (via the Secretary) of:

- Any proposed changes to the research design;
- Any adverse events that might have occurred.

Researchers are required to submit annual status reports and final reports to the Secretary of the AIS Ethics Committee. Details of status report requirements are contained in the "Guideline" for ethics submissions.

Please note that approval for this submission expires on the 31st December 2020 after which time an extension will need to be sought.

If you have any questions regarding this matter, please contact me on (02) 6214 1791

Sincerely,

Tim Ke [redacted]

Tim Kelly
Secretary, AIS Ethics Committee

End of document