Periodization of carbohydrate availability for endurance training with a focus on pre-training intake

Submitted by

Jeffrey A Rothschild

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Sport Performance Research Institute New Zealand Faculty of Health and Environmental Sciences Auckland University of Technology, Auckland, New Zealand

Primary Supervisor: Dr. Daniel Plews

Secondary Supervisor: Professor Andrew Kilding

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Abstract

Endurance athletes of all levels face the same question — what should I eat before exercise? A surge in sport nutrition research and practitioners over the past 25 years has led to increased awareness around the role of nutrition in sports performance. However, limited data are available on the day-to-day practices of endurance athletes, how well they reflect evidence-based recommendations, or what dictates those choices. Furthermore, important questions relating to the effects of pre-exercise nutrition intake on performance, fat oxidation, hunger, and molecular signaling remain unanswered. Therefore, the purpose of this thesis was to better understand the pre-exercise nutrition beliefs and practices of endurance athletes by investigating: (i) stated beliefs and practices of athletes, (ii) scientific validity of common beliefs reported by athletes, and (iii) actual pre-exercise nutrition practices of athletes.

The first chapter of the thesis was an international survey of ~2,000 endurance athletes aimed at understanding the beliefs and self-reported pre-exercise nutrition practices of endurance athletes. Nearly two-thirds of athletes reported the use of fasted-state training, and it was observed that many athletes may not be following best-practice recommendations of varying dietary intake in relation to training sessions to optimize endurance training adaptations. Conflicting beliefs relating to the effects of fasted training on metabolism and exercise capacity were also found among athletes, often aligned with differences in competitive level, sex, and/or habitual dietary pattern.

In response to the discordant beliefs observed among athletes, the next three studies aimed to elucidate the metabolic, molecular, and performance effects of pre-exercise nutrition choices. In an acute cross-over study of 17 trained male cyclists (Chapter 5), a carbohydrate-rich breakfast reduced fat oxidation compared to exercising both in the overnight-fasted state and following pre-exercise protein ingestion, but there were no differences between the three trials for average power during high-intensity intervals, perceived exertion, oxidative stress, or hunger. These findings suggest that a low-carbohydrate breakfast can be a viable alternative to fasted-state

training for athletes who wish to optimize fat oxidation during exercise, and that athletes can complete ~1-hr of exercise in the overnight-fasted state without compromising high-intensity training capacity or experiencing additional hunger. The next two chapters utilized multivariable regression techniques on data pooled from published studies to investigate factors influencing skeletal muscle AMP-activated protein kinase (AMPK) signaling (Chapter 6) and substrate oxidation during cycling exercise (Chapter 7). Pre- and peri-exercise carbohydrate intake had negligible influence on AMPK activation during exercise, whereas disrupting cellular energy charge had a large influence, suggesting high-intensity exercise may drive an adaptative response irrespective of nutritional factors. Chapter 7 reports factors commonly known to influence substrate oxidation during exercise such as exercise duration and intensity, age, sex, fitness level, muscle glycogen, and daily dietary intake explained only ~59% of the variation in respiratory exchange ratio (RER) during exercise. In addition, factors that could be easily modified by athletes such as exercise duration and intensity, daily macronutrient intake, and pre- and peri-exercise carbohydrate intake, only explained 36% of the variation in RER during exercise suggesting most of what dictates RER during exercise cannot be easily controlled on a daily basis.

The final study of the thesis monitored the daily diet, training, and sleep habits of 55 endurance athletes throughout 12 weeks of training with focus on the relationship between carbohydrate intake and training (Chapter 8), and the influence of prior-day carbohydrate intake on subjective recovery status (Chapter 9). It was found that many endurance athletes do not follow recommended practices of adjusting daily carbohydrate intake, or if they do, the magnitude of adjustment is small relative to changes in training volume and/or intensity (Chapter 8). Chapter 9 demonstrated total daily carbohydrate intake has minimal influence on subjective recovery scores the following day, after accounting for other factors related to training load and sleep. The data also displayed nonergodicity, an important phenomenon where group-level findings cannot be generalized to individuals, and a novel method was used to predict how an individual athlete would respond to carbohydrate intake.

Collectively, the studies in this thesis demonstrate that endurance athletes differ greatly in their beliefs and practices related to pre-exercise nutrition, and in many cases do not follow contemporary evidence-based recommendations. Novel findings include: 1) 63% endurance athletes report the use of fasted-state training, 2) beliefs and practices relating to pre-exercise nutrition differ based on sex, competitive level, and habitual dietary pattern, 3) the pre-exercise meal has minimal influence on work capacity, perceived exertion, oxidative stress, hunger, or AMPK activation during exercise, 4) although pre-exercise carbohydrate ingestion increases carbohydrate oxidation during exercise, most of what dictates substrate oxidation during exercise cannot be easily controlled on a daily basis, and 5) for most individuals, daily carbohydrate intake does not influence recovery status the following morning. These findings contribute to the sport nutrition literature and can be used by nutritionists, coaches, and athletes to make better-informed pre-exercise fueling choices.

Attestation of Authorship

"I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning."

Jeffrey Rothschild

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List of Common Abbreviations and Nomenclature

ACC - acetyl coenzyme A carboxylase
AIC - Akaike's information criterion
AMP – Adenosine monophosphate
ADP – Adenosine diphosphate
ATP – Adenosine triphosphate
AMPK – AMP-activated protein kinase
ARIMA – Autoregressive Integrated Moving Average
AU – Arbitrary units
BCAA/EAA – Branched-chain amino acids/ essential amino acids
CHO - Carbohydrate
CI – Confidence interval
Cr - Creatinine
CS – Citrate Synthase
CTI – Carbohydrate Training Index
FFA – Free fatty acid
GE – Gross efficiency
HIIE – High-intensity interval exercise
HIIT – High-intensity interval training
HR – Heart rate
HRV – Heart rate variability
IMTG – Intramuscular triglyceride
LCHF – Low carbohydrate, high fat
MAPK – Mitogen-activated protein kinase
MCT – Medium chain triglyceride
mRNA - Messenger RNA
p-ACC – Phosphorylated ACC
p-AMPK – Phosphorylated AMPK
PCr - Phosphocreatine
PCR – Polymerase Chain Reaction
PDH – Pyruvate dehydrogenase
PPAR - Peroxisome proliferator-activated receptor
PRS – Perceived recovery status
RER – Respiratory exchange ratio
RMSE – Root mean squared error
RONS – Reactive oxygen and nitrogen species
RPE – Rating of perceived exertion
RQ – Respiratory quotient
SD – Standard deviation
SIT – Sprint interval training
sRPE – Session RPE
TCA - Tricarboxylic acid cycle

TSS – Training stress score
VAS – Visual analog scale
$\dot{V}E.\dot{V}CO2^{-1}$ - ventilatory equivalent for carbon dioxide
$\dot{V}E.\dot{V}O2^{-1}$ - ventilatory equivalent for oxygen
VIF – Variance inflation factor
VO_{2max} – Maximal oxygen consumption
VO_{2peak} – Peak oxygen consumption
VT – Ventilatory threshold
VTΔ20 – 20% of the difference between VT and peak power
W_{max} – Maximal power
W_{peak} – Peak power
β-HAD – β-hydroxyacyl coenzyme A dehydrogenase

Ethical Approval

All original research studies conducted as part of this thesis were approved by the Auckland University of Technology Ethics Committee and in accordance with the Declaration of Helsinki. The ethical approval codes for these studies were 19/415 (19/11/2019), 19/420 (19/3/2020), and 22/7 (10/2/2022).

Publications and Presented Abstracts

Peer-reviewed journal articles

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Rothschild J, Islam H, Bishop D, Stewart T, Kilding A, Plews D. 2022. Factors Influencing AMPK Activation During Cycling Exercise: A Pooled Analysis and Meta-Regression. Sports Med. 52:1273–1294.

Rothschild J, Kilding A, Broome S, Stewart T, Cronin J, Plews D. 2021. Pre-exercise carbohydrate or protein ingestion influences substrate oxidation but not performance or hunger compared with cycling in the fasted state. Nutrients 13(4), 1291.

Rothschild J, Kilding A, Plews D. 2021. Pre-exercise nutrition habits and beliefs of endurance athletes vary by sex, competitive level, and diet. J Am Coll Nutr 40(6), 517–528.

Rothschild J, Kilding A, Plews D. 2020. What should I eat before exercise? Pre-exercise nutrition and the response to endurance exercise: Current prospective and future directions. Nutrients 12(11), 3473.

Rothschild J, Kilding A, Plews D. 2020. Prevalence and determinants of fasted training in endurance athletes: a survey analysis. Int J Sport Nutr Exerc Metab 30(5), 345–356.

Manuscripts under review

Rothschild J, Stewart T, Kilding A, Plews D. The influence of dietary carbohydrate on perceived recovery status differs at the group and individual level – evidence of nonergodicity among endurance athletes.

Rothschild J, Stewart T, Kilding A, Plews D. A Predicting daily recovery during long-term endurance training using machine learning analysis.

Conference presentations

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Statement of Contribution

Chapter 3: Prevalence and determinants of fasted training in endurance athletes: a survey analysis
Jeffrey Rothschild 85%, Prof. Andrew Kilding 7.5%, Dr. Daniel Plews 7.5%.

Chapter 4: Pre-exercise nutrition habits and beliefs of endurance athletes vary by sex, competitive level, and diet.
Jeffrey Rothschild 85%, Prof. Andrew Kilding 7.5%, Dr. Daniel Plews 7.5%.

Chapter 5: Pre-exercise carbohydrate or protein ingestion influences substrate oxidation but not performance or hunger compared with cycling in the fasted state
Jeffrey Rothschild 80%, Dr. Sophie Broome 2.5%, Prof. John Cronin 2.5%, Dr. Tom Stewart 5%, Prof. Andrew Kilding 5%, Dr. Daniel Plews 5%.

Chapter 6: Factors Influencing AMPK Activation During Cycling Exercise: A Pooled Analysis and Meta-Regression
Jeffrey Rothschild 80%, Dr. Hashim Islam 4%, Prof. David Bishop 4%, Dr. Tom Stewart 4%, Prof. Andrew Kilding 4%, Dr. Daniel Plews 4%.

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Chapter 8: Self-reported dietary intake of endurance athletes during 12 weeks of training
Jeffrey Rothschild 80%, Prof. James Morton 5%, Dr. Tom Stewart 5%, Prof. Andrew Kilding 5%, Dr. Daniel Plews 5%.

Chapter 9: The influence of dietary carbohydrate on perceived recovery status differs at the group and individual level – evidence of nonergodicity among endurance athletes
Jeffrey Rothschild 85%, Dr. Tom Stewart 5%, Prof. Andrew Kilding 5%, Dr. Daniel Plews 5%.

Jeffrey Rothschild ________________________

Prof. Andrew Kilding ________________________

Dr. Daniel Plews ________________________

Dr. Tom Stewart ________________________

Prof. David Bishop

Dr. Sophie Broome

Prof. John Cronin ________________________

Dr. Hashim Islam ________________________

Prof. James Morton

1. Introduction

This Chapter provides a brief introduction to the relationship between pre-exercise nutrition intake and exercise, leading to the rationale for the aims and objectives of this thesis.

1.1 Background and Rationale

Endurance athletes of all levels face the same question — what should I eat before exercise? A surge in the number of sports nutrition publications and dedicated sports nutrition professionals over the past 25 years has led to increased awareness around the role of nutrition in sports performance. However, the day-to-day nutrition practices of endurance athletes are not well characterized, and important questions relating to the effects of pre-exercise nutrition intake on performance, fat oxidation, hunger, and molecular signaling remain unanswered. For example, there are limited data describing what athletes eat before training, what dictates those choices, and whether their beliefs and practices are supported by scientific evidence.

Strategies that alter nutrient availability before and during exercise can influence training adaptations by increasing the exercise stimulus and/or enhancing or blunting cellular responses to exercise-induced perturbations [1]. These adaptations can include increased mitochondrial size and function, as well as increased rates of fat oxidation, indicating a pivotal role for carbohydrate as both a fuel source and a metabolic signal to elicit a desired adaptation [2]. Specific strategies to alter nutrient availability can include carbohydrate ingestion before or during exercise, restricting carbohydrate between training sessions, and exercising in the overnight-fasted state. An obvious but important distinction is that fasted training reduces overall energy and nutrient availability whereas low- and high- carbohydrate meals provide energy intake before exercise. This can trigger different cellular signaling cascades that are responsive to the availability of carbohydrate [3], fatty acids [4, 5], and/or amino acids [6].

It is also noteworthy that overnight fasting reduces liver glycogen [7], but does not directly affect muscle glycogen concentration [8] (although it may contribute to a lack of muscle refueling from a training session undertaken on the previous evening). In contrast, restricting carbohydrate between training sessions allows exercise to be commenced with low muscle glycogen concentrations [9]. Therefore, it is important for the athlete, coach, and/or practitioner to differentiate strategies that reduce muscle and/or liver glycogen content when planning nutrition intake around specific training sessions. For example, in the overnight-fasted state (i.e., low liver glycogen and normal muscle glycogen) performance during short duration (< 60 min) exercise is unlikely to be impaired [10] and fat oxidation is generally increased [11]. In contrast, training that is commenced with low muscle glycogen can increase fat oxidation and markers of training adaptations but may compromise high-intensity exercise capacity [12, 13].

Despite the rationale for strategically reducing carbohydrate availability around specific workouts, there may also be negative implications. For athletes doing a high volume of training, performing exercise in the overnight-fasted state could more likely lead to low energy availability, which can be associated with hormonal and immune dysfunction [14]. Exercising in the overnight-fasted state may also increase intestinal permeability compared with exercise following a carbohydrate -containing breakfast [15]. An alternative to performing training sessions in the overnight-fasted state is to consume a breakfast that includes protein but not carbohydrate. This could potentially reduce feelings of hunger while reducing muscle protein breakdown, and still allow high levels of fat oxidation during exercise without affecting key signaling molecules [16-18]. However, few studies have investigated the effects of pre-exercise protein ingestion.

Another emerging area of research involves investigating how the day-to-day nutrition practices of athletes align with evidence-based recommendations to modulate carbohydrate intake according to changes in training and the goals of each training session. Prior to this thesis, knowledge of athlete practices has largely been limited to surveys [19-22], case studies [23, 24], or short-duration (3–7 d) observations [25-27]. Nutrition-related knowledge among athletes and coaches has been reported to be poor [28-31], and many athletes fail to meet the current best-practice recommendations for dietary carbohydrate intake [25, 32-34]. Data on the daily pre-exercise nutrition intake patterns and beliefs are limited in athletes competing in sports other than running, as well as for non-elite endurance athletes. It is also of interest to understand the intake of athletes in relation to their daily training load across longer (> 1 wk) periods of observation. Gaining a better understanding of the current practices of athletes regarding pre-training fuel intake can allow for relevant lab-based studies to be designed to optimize nutrition and training recommendations.

In light of the above, the purpose of this thesis was to better understand the pre-exercise nutrition beliefs and practices of endurance athletes by investigating: (i) stated beliefs and practices of athletes, (ii) scientific validity of common beliefs reported by athletes, and (iii) actual pre-exercise and daily nutrition practices of athletes.

Key research questions investigated in this thesis are:

1. How many athletes train in the overnight-fasted state?
2. What dictates if people perform or avoid training in the overnight-fasted state?
3. How do athletes adjust dietary intake based on training?
4. How does pre-training dietary intake influence subjective effort and wellbeing during exercise?
5. How do pre-exercise nutrition choices influence molecular signaling markers?
6. Does nutrition intake influence daily recovery status during longer-term training?

1.2 Thesis Organization

This thesis is comprised of 10 chapters presented in five main sections (Figure 1.1). Chapters 3-10 are written as stand-alone chapters formatted specifically for publication in peer-reviewed journals (abstract, introduction, methodology, results, discussion). Citations are presented in a standard referencing format as a single bibliography at the end of the thesis.

The first section consists of an introduction and literature review, focusing on the metabolic and physiological effects of pre-exercise nutrition.

The second section focuses on the stated beliefs and practices relating to pre-exercise nutrition, analyzing a survey completed by ~2000 endurance athletes. Chapter 3 focuses on fasted-state training, and Chapter 4 examines what is consumed when athletes do include a pre-training meal/snack along with differences in beliefs and practices based on sex, competitive level, and habitual dietary pattern.

The third section focuses on the scientific validity of common beliefs reported by athletes by looking at the metabolic, molecular, and performance effects of pre-exercise nutrition choices. This includes an acute cross-over study of 17 trained male cyclists (Chapter 5), a modeling analysis investigating the role of factors influencing skeletal muscle AMPK activation (Chapter 6), and a modeling analysis investigating factors influencing substrate oxidation during cycling exercise (Chapter 7).

The fourth section focuses on the pre-exercise nutrition practices of athletes, beginning with descriptive data from a 12-week study that monitored the daily diet, training, and sleep habits of 55 endurance athletes with an emphasis on carbohydrate intake in relation to training load (Chapter 8). Chapter 9 examines the influence of prior-day carbohydrate intake on subjective recovery status, along with an investigation into group-level vs. individual analysis.

The final section (Chapter 10) provides an overall discussion and summary of the main findings, followed by practical applications, limitations, directions for future research, and concluding remarks.

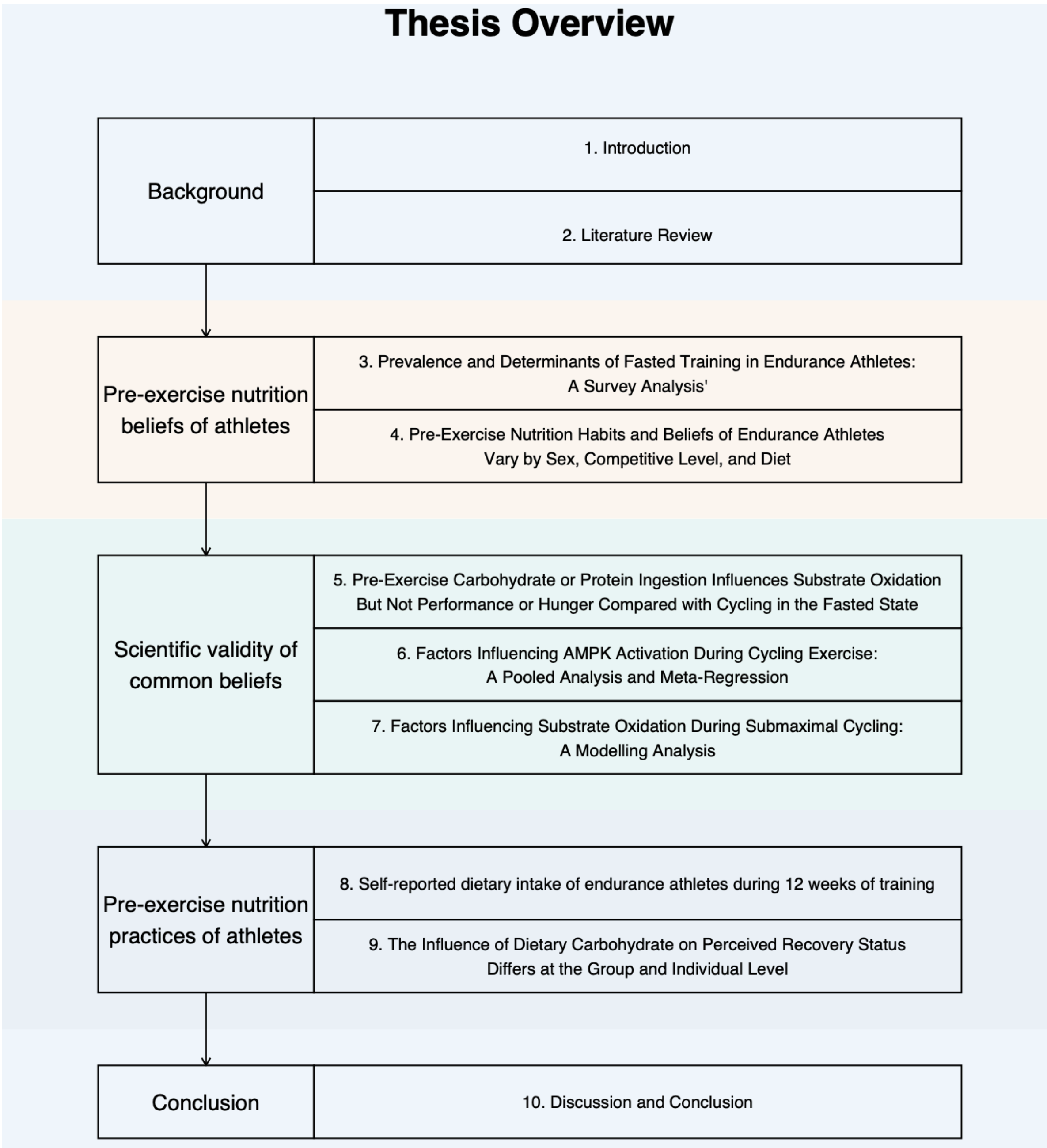

Figure 1.1 Overview of thesis structure.

2. Literature Review: Pre-exercise Nutrition and the Response to Endurance Exercise

This chapter provides an overview of research findings that examine the influence of pre-exercise nutrition ingestion on the metabolic, physiological, and performance responses to endurance training, along with the pre-exercise nutrition practices of athletes. Studies using morning training sessions will be considered in the context of fasted-state training, because it is otherwise difficult to be sure that sufficient time has occurred between the previous meal and an afternoon/evening training session to ensure that baseline metabolic conditions have been re-established.

Elements of this chapter are taken from the following publication:

Rothschild, J. A., Kilding, A. E., & Plews, D. J. (2020). What should I eat before exercise? Pre-exercise nutrition and the response to endurance exercise: Current prospective and future directions. *Nutrients*, *12*(11), 3473.

2.1. Introduction

Exercise duration and intensity are the two most important factors influencing the adaptive response to endurance training [35]. However, strategies altering nutrient availability before and during exercise can also impact training adaptations by modulating the exercise stimulus and/or cellular responses to the exercise-induced perturbations [2]. Specific strategies to alter nutrient availability can include exercising in the overnight-fasted state, restricting carbohydrate (CHO) ingestion between training sessions, and increasing CHO ingestion before or during exercise [36]. Although performance may be improved following pre-exercise CHO ingestion [37, 38], exercise undertaken with reduced availability of CHO can increase the activation of key signaling proteins compared with exercise performed with high CHO availability [2], potentially influencing longer-term training adaptations.

Among the primary intracellular signals comprising the endurance training response are mechanical stretch, reactive oxygen and nitrogen species (RONS), calcium flux, the ratio of adenosine monophosphate and adenosine triphosphate (AMP:ATP), and the availability of endogenous CHO and free fatty acids (FFA) [39, 40]. Nutritional intake (i.e., the size, type, and timing of the pre-exercise meal) has the potential to modify signaling across several of these pathways, primarily related to energy sensing and nutrient availability. This can occur by affecting training intensity (including total work done, and muscle contractile stress), substrate metabolism, and/or oxidative stress (Figure 2.1).

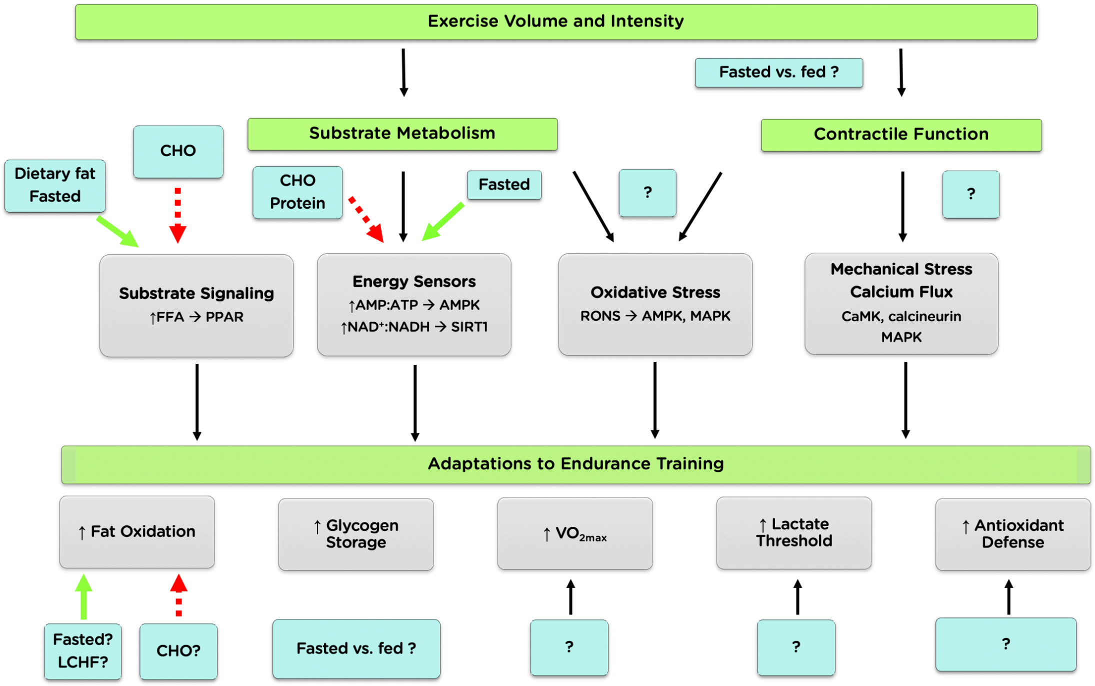

Figure 2.1. Schematic of potential impact of pre-exercise nutrition on the training response. Green arrows suggest the potential to increase or augment specific signalling, and red dashed arrows suggest the potential to decrease or impair specific signalling. Abbreviations: AMP, Adenosine monophosphate; ATP, Adenosine triphosphate; AMPK, AMP-activated protein kinase; CaMK, calcium/calmodulin-stimulated protein kinase; CHO, carbohydrate; FFA, free fatty acids; LCHF, low-CHO high-fat; MAPK, mitogen-activated protein kinase; NAD, Nicotinamide adenine dinucleotide; NADH, reduced NAD; PPAR, peroxisome proliferator-activated receptor; RONS, Reactive Oxygen and Nitrogen Species; VO_{2max}, maximal oxygen consumption.

Although some lines of evidence suggest ingesting CHO before exercise can attenuate favorable endurance training adaptations, contrasting findings have been reported. For example, ingesting CHO has decreased [41], increased [15], or had no effect [42] on the activity of the 5′ AMP-activated protein kinase (AMPK) following exercise. Similarly, training-induced improvements in maximal oxygen consumption (VO_{2max}) have been reported to increase [43], decrease [44], or remain unchanged [45] following 4–6 weeks of CHO-fed compared with fasted-state training. These contrasting findings can be a source of confusion for athletes, coaches, and practitioners, and highlight the need to better understand the influence of pre-exercise nutrition intake, as well as the beliefs and practices of athletes trying to apply science-based recommendations to their daily training. The following sections will review the influence of pre-exercise nutrition ingestion on acute responses and longer-term adaptations to endurance exercise.

2.2 Acute Responses to Pre-Exercise Nutrition Intake

Most pre-exercise nutrition interventions have been conducted in an acute context. However, acute responses to training do not always correspond with long-term adaptations [46, 47]. For example, increased fat oxidation observed during a "sleep-low" training protocol did not lead to a sustained increase in whole-body fat oxidation following the intervention [48]. Likewise, blunting key mitochondrial signaling proteins with CHO ingestion during acute exercise did not impair training-induced improvements in performance or mitochondrial biogenesis [49, 50]. Despite this, the accumulation over time of transient, exercise-induced changes in gene expression are thought to be the driving factor behind many adaptations to training [51]. Therefore, it is relevant to consider the acute effects of pre-exercise nutrition in addition to longer-term adaptations.

2.2.1. Metabolism and Substrate Oxidation

The liver plays a key role in metabolic regulation during extended exercise [52]. Despite the ~40% reduction in liver glycogen following an overnight fast [7], blood glucose concentration can be maintained at normal levels during exercise due to increased gluconeogenesis and/or decreased utilization of glucose in skeletal muscle [53, 54]. However, fatigue during extended exercise is often associated with reduced blood glucose concentrations [55], supporting a critical role for liver glycogen in achieving optimal performance during extended exercise. Based on this observation, it could be expected that the duration of exercise is an important factor in determining performance differences between fed and fasted-state exercise (discussed in section 2.2.3). This also helps explain why the addition of fructose, which enhances liver glycogen repletion compared with glucose-based CHO ingestion [56], to a CHO-rich breakfast can enhance extended endurance performance [57].

Exercising in the fasted state generally allows higher levels of fat oxidation than exercise performed in the CHO-fed state during low-to-moderate intensity exercise [11] and can increase the relative intensity where maximal fat oxidation occurs [58]. Ingesting CHO before exercise increases plasma glucose and insulin levels, leading to a reduction in hepatic glucose output and an increase in skeletal muscle glucose uptake during exercise [59]. This can lower fat oxidation by decreasing plasma FFA availability via insulin-mediated inhibition of lipolysis [60], and also by inhibiting fat oxidation within the muscle due to an increased glycolytic flux [61]. Intramuscular triglycerides (IMTG) provide a key substrate for fat oxidation, primarily during exercise in the fasted state [62, 63], although their use declines and the oxidation of plasma FFA increases as the duration of exercise extends [64].

Up to 6 h may be required following a CHO-rich meal for substrate oxidation and glucose homeostasis to return to levels observed during fasted-state exercise [65]. However, consumption of protein before and during steady-state exercise does not affect FFA availability or whole body fat oxidation compared with fasted-state exercise commenced with normal [16] or lowered [17] muscle glycogen concentration, despite elevated insulin levels. This may be related to the increases in catecholamine levels during exercise, which are an important determinant of the adipose tissue lipolytic rate and can override the inhibition by insulin [66]. Protein ingestion before exercising in a low-glycogen state has no effect on rates of muscle protein synthesis but could plausibly reduce muscle protein breakdown during exercise [67]. It also appears possible that pre-exercise protein ingestion increases amino acid oxidation during exercise [67], but further quantification of its influence is needed. Although, most research investigating the effect of pre-exercise nutrient intake on substrate utilization during subsequent exercise has compared CHO intake to a (non-nutritive) placebo, the use of pre-exercise protein ingestion is a noteworthy and under-researched area.

In contrast with exercise performed in the overnight-fasted state, which is typically undertaken with reduced hepatic but not muscle glycogen stores [8], restricting CHO between training sessions allows exercise to be undertaken with reduced muscle glycogen concentrations [9]. During exercise with low muscle glycogen there is an increase in the oxidation of fat [68] and amino acids [18, 69], and a reduction in muscle glycogen breakdown [68, 70, 71]. When exercise is undertaken with normal muscle glycogen levels, muscle glycogen breakdown is generally similar between CHO-fed and fasted-state exercise [63, 72-74]. When muscle glycogen breakdown has been found to increase in the CHO-fed vs. fasted state it has been in the context of a high-CHO meal fed 4 h before starting exercise, suggesting the increased glycogen utilization may have been derived from the glycogen synthesized following the meal [75].

The respiratory exchange ratio (RER), represents an indirect measure of the skeletal muscle respiratory quotient (RQ) — the quantity of CO_2 produced in relation to O_2 consumed [76]. The RER can be used under certain conditions (e.g., steady-state exercise below the second ventilatory threshold) to estimate the relative contributions of fat and carbohydrate to energy production with higher values equating to increased carbohydrate reliance and lower values representing increased fat reliance [77]. Exercise performed in the fasted state typically results in a lower RER compared with exercising in the CHO-fed state [11]. This apparent contradiction with the lack of difference in muscle glycogen breakdown can be explained by differences in plasma glucose and FFA in the CHO-fed vs. fasted state [62]. It is also well established that the RER increases with exercise intensity [78], and decreases with exercise duration [79-81]. Other factors known to influence the RER during exercise include sex [82], habitual dietary intake [83-85], CHO ingestion during exercise [80, 86], CHO ingestion before exercise [38, 87-90], and length of time before exercise food is consumed [91, 92], among others. Training status is also relevant when considering the metabolic effects of a pre-exercise meal, as trained athletes typically show a greater capacity for fat oxidation compared with untrained or recreationally active populations [81, 93].

2.2.2. Cell Signaling

Among the key intracellular signals influencing skeletal muscle adaptations to endurance training are changes in the AMP:ATP ratio, contraction-induced changes in mechanical strain, increased calcium flux, an increase in RONS, and the availability of endogenous CHO and FFA [39, 40, 94]. Nutritional intake has the potential to modify signaling across several of these pathways, primarily related to energy sensing and nutrient availability (Figure 2.1)

2.2.2.1. Energy Sensing and the AMP-Activated Protein Kinase

The 5′ AMP-activated protein kinase (AMPK) is a cellular energy sensor that regulates cellular and whole-body energy balance by inhibiting ATP-consuming pathways and activating ATP-producing pathways [95]. Repeated AMPK activation leads to a range of beneficial metabolic adaptations that include increases in glucose uptake, glycolytic flux, fat oxidation, and mitochondrial biogenesis [96], thereby contributing to training-induced improvements in endurance capacity. The degree of AMPK activation during exercise may be influenced by several factors including exercise intensity [97], duration [98], training status [99], muscle glycogen [100], and nutrient availability [1], however the relative importance of each has yet to be determined.

In the case of studies in which exercise was commenced with normal muscle glycogen stores, observations of a blunting of AMPK-α2 activity associated with pre-exercise CHO intake [41, 50] have been associated with exercise at lower intensities than those reporting no differences in AMPK-α2 activity between CHO-fed and fasted-state exercise [42, 101]. Following exercise that is undertaken with low, compared with normal muscle glycogen levels, greater increases in the activity of AMPK-α2 have been observed following steady-state endurance exercise at 65–70% VO_{2max} [102-104], yet similar increases in AMPK activity and/or phosphorylation were seen following both exhaustive and non-exhaustive high-intensity exercise undertaken with high and low muscle glycogen levels [13, 105, 106]. Therefore, ingesting CHO before exercise may dampen AMPK activity during low but not high-intensity exercise. Future research could seek to better understand the interplay between exercise intensity and CHO ingestion before and/or during exercise, bearing in mind that interactions between CHO ingestion and exercise intensity may be different during continuous and intermittent exercise [107].

2.2.2.2. Contraction-Induced Signaling

Another key intramuscular signal comes from increased calcium released during muscle contraction. Calcium-dependent transcriptional pathways play important roles in regulating fat oxidation, mitochondrial biogenesis, and muscle fiber-type changes via myocyte enhancer factor 2 and p38 mitogen-activated protein kinase (MAPK) [108-111]. Few studies have compared the effects of nutrition interventions on calcium-dependent, contraction-induced signaling pathways. There appear to be minimal effects of exercise performed in the fed vs. fasted-state or with varying levels of muscle glycogen [68, 105, 112, 113], but some evidence suggests p38 may be sensitive to nutrient status [114, 115]. Although more research is needed, the independence of these pathways from nutritional influence could help to explain why similar longer-term changes can be observed when training under differing nutritional conditions.

2.2.2.3. Substrate Signaling

Exercise performed in the overnight-fasted state generally results in higher levels of FFA compared with CHO-fed exercise, and an inverse relationship is seen between FFA concentration and CHO oxidation during exercise [65]. In addition to acting as substrate for β-oxidation in the mitochondria, FFA also play a role in molecular signaling cascades that regulate fatty acid metabolism and mitochondrial biogenesis, via activation of peroxisome proliferator-activated receptors (PPAR), MAPKs, and sirtuin 1 [39, 116-118]. Some studies have found differences in FFA between the fed and fasted state throughout an entire bout of exercise [79, 119], while others have shown differences appearing from 20 [75], 30 [120], 45 [37], or 60 min [121] into exercise. These differences do not exhibit any clear patterns relating to meal size, time of ingestion, or exercise intensity, warranting further examination into reasons for observed discrepancies. Similar levels of FFA are found during exercise in the fasted-state and following ingestion of a high-fat meal [122, 123] or following pre-exercise protein ingestion with normal [16] and low [17] muscle glycogen levels. However, FFA may be increased during exercise in trained, compared with untrained participants [124], and in females, compared with males [125]. Although studies directly testing this are currently lacking, differences in FFA-related signaling could be hypothesized to account for a significant portion of differences observed in the training response between fasted-state and CHO-fed training. Despite an ability to increase FFA and fat oxidation capacity, there is currently no strong evidence supporting improvements in mitochondrial volume or respiratory function after a high fat diet when measured in skeletal muscle biopsy samples obtained in the resting state [118].

2.2.2.4. Reactive Oxygen and Nitrogen Species

Rather than simply a byproduct of oxidative stress, RONS play a direct role in regulating the response to both acute exercise (e.g., muscle contractile function, glucose uptake, blood flow, and cell bioenergetics) and longer-term exercise training (e.g., mitochondrial biogenesis, muscle hypertrophy, angiogenesis, and redox homeostasis) [126]. Considering their importance, few studies have examined the influence of a pre-exercise meal on the oxidative stress response to a bout of exercise. At rest, a high-CHO meal can evoke a greater postprandial oxidative stress response compared with a high-fat meal [127], while the addition of olive oil to a meal reduced post-meal increases in oxidative stress markers, such as NADPH oxidase and 8-isoprostane, both of which have been associated with endurance training adaptations [128-130]. Acute and chronic fruit ingestion can dampen lipid oxidation during exercise [131], and fruit-derived phenolic compounds may promote muscle fiber-type transformation [132]. Whey protein can also impact the antioxidant defense system by enhancing activity of the endogenous antioxidant enzymes [133]. It is currently unknown how various pre-exercise meals affect oxidative stress in response to exercise and if there are any longer-term training implications.

2.2.3. Performance

Compared with exercising in the fasted state, fed-state exercise has been found to generally enhance prolonged (>60 min), but not shorter duration aerobic exercise performance [10]. However, ingesting CHO during exercise minimizes the differences between consuming CHO or a placebo prior to exercise [134-137]. Although most studies have compared pre-exercise CHO ingestion to a placebo/fasted condition, minimal performance differences have been observed between high-fat and high-CHO pre-exercise meals [90, 122, 136, 138]. The vast majority of studies comparing performance in the fed or fasted state have used steady-state endurance exercise [10], but similar effects of exercise duration are found with interval training, as performance was improved in the fed state for 90 min of high-intensity intermittent running [139, 140], but not short-duration interval training [141-143]. However, some studies have shown a benefit of pre-exercise CHO ingestion on exercise capacity tests lasting ~5–10 min [144, 145]..

The amount, type, and timing of the pre-exercise meal also have the potential to influence performance outcomes. The amount of CHO (25–312 g) consumed prior to exercise does not have a meaningful effect on time trial performance [38, 87, 88, 146], and no differences in performance have been observed following pre-exercise ingestion of solid vs. liquid CHO [73], solid vs. gel-based CHO [147, 148], or fast-food vs. sport supplements [149]. Timing of the pre-exercise meal also has minimal effects when consumed 15, 45, or 75 min [91], 15 or 60 min [142], or 5 or 35 min [150] before exercise. However, improved performance was observed when consuming 32 g of CHO 30-min, but not 120-min, before exercise [144], and when consuming 250 g, but not 42 g of CHO, 3.5 h before exercise [145]. However, in the latter case it is likely that glycogen stores were not matched at the start of exercise [145]. It also may be an oversimplification to group all CHO-containing meals into the same category and ignore the CHO composition, as the addition of fructose to a pre-exercise meal enhanced endurance capacity compared with an isocaloric glucose-based meal [57]. There is some fear of hypoglycemia from consuming CHO between 30–60 min prior to exercise; however, despite occurring in a small number of cases, there does not appear to be any detrimental performance effects or any relationship between low blood glucose concentrations and performance [151].

The perception of breakfast is also a consideration when comparing the acute performance effects of pre-exercise CHO and fasted exercise. Trained cyclists completed a ~20 min cycling time-trial more quickly when they perceived they had consumed breakfast (CHO or placebo) prior to the start of the exercise, compared with a fasted exercise session [152]. However, when a time-trial was preceded by 2 h of steady-state cycling, there were no placebo effects observed [153], suggesting placebo effects may be minimized with longer exercise durations. When undertaking exercise with reduced muscle glycogen levels, the perception of CHO availability also augmented high-intensity interval training (HIIT) capacity, although performance was not restored to that of CHO consumption [154].

Overall, the importance of consuming CHO before exercise increases as the exercise duration increases, and exercising in the fed vs. fasted state appears to have a far greater effect on performance than the size or timing of the meals, unless the difference in meal timing is at least 90 min. To differentiate pre-exercise energy vs. CHO intake, future studies should compare fed vs. fasted exercise, along with pre-exercise protein ingestion, in the absence of CHO, prior to both interval training and steady-state performance tests.

2.3. Training Adaptations

The following section will focus on the influence of pre-exercise nutrition intake on longer-term adaptations to training.

2.3.1. Skeletal Muscle Adaptation

A challenge when comparing skeletal muscle adaptations across available studies is the variety of methods that have been used to compare high vs. low CHO availability around training sessions (Figure 2.2). Of the studies examining the effects of longer-term (≥4 weeks) training in the fasted state on endurance adaptations [43-45, 155-157], only one [156] has used endurance-trained subjects. Furthermore, almost all studies using moderate-intensity continuous endurance training in the fasted state also provided the fed groups with CHO during exercise, which can independently influence both acute [158] and chronic [159] responses to exercise. Other studies have examined pre-exercise CHO supplementation, though not necessarily in the overnight-fasted state and using untrained subjects [160, 161]. Additionally, fasted state training has been used as part of studies comparing low vs. high muscle glycogen [162] and once vs. twice daily training [163].

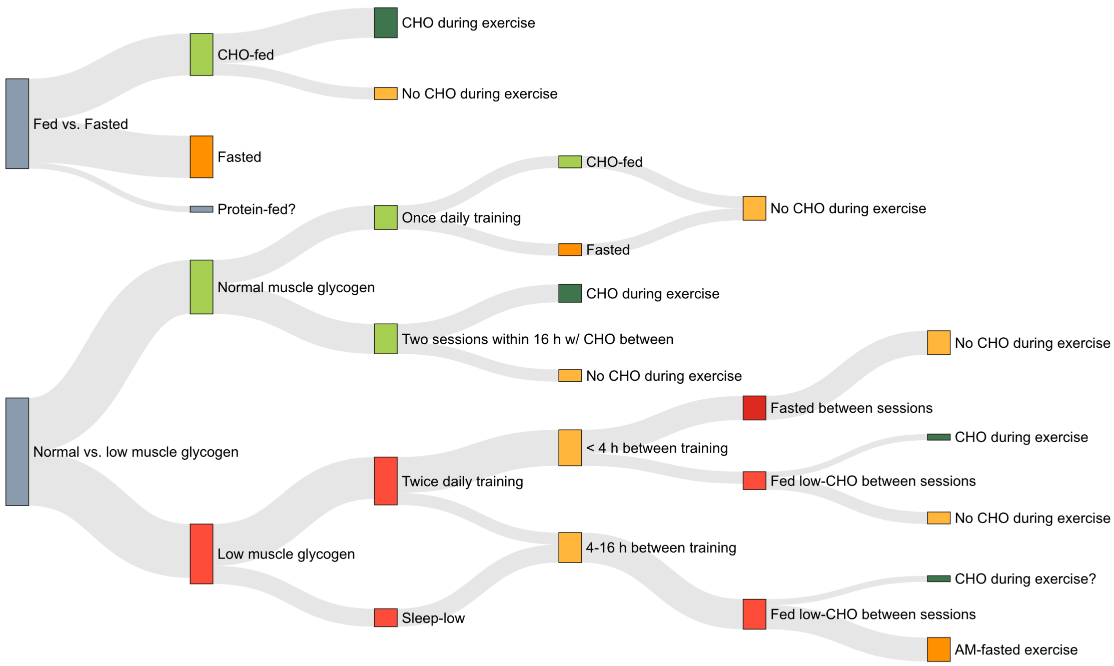

Figure 2.2. Comparison of the various methods of altering CHO availability used in training studies. Protocols used to commence training with a reduced availability of endogenous carbohydrate include overnight fasting, and training twice within a 24 h period consuming low-CHO nutrition between sessions or remaining in the fasted state. Some studies have fed carbohydrate during exercise, while others have not. Thickness of the line is related to the number of studies using a given approach. Question marks represent areas yet to be studied. Created from [9, 12, 43-45, 48, 63, 155-157, 159, 162-167], [1]which included 307 participants (10.7% female), 26.3 ± 4.2 years, VO_{2max} 53.2 ± 11.0 mL kg^{-1} min^{-1}.

2.3.1.1. Substrate Usage

Fat oxidation is higher during an acute bout of exercise performed in the overnight-fasted, compared with the CHO-fed state, and with low compared with high muscle glycogen. Despite these acute differences, most studies have found no longer-term differences in fat oxidation following 4–6 weeks of fed or fasted-state training when testing participants in the fed [44, 45, 157] or fasted [63, 160] state. Similar findings have been reported in the "sleep-low" context, where fat oxidation is increased during fasted training sessions performed with low muscle glycogen compared with exercising in the fed-state, but no differences in fat oxidation were observed following one [162], three [165], or four [48] weeks of training when tested in the fed state. However, it is possible that longer time periods of fasted training may be needed before relevant differences in fat oxidation would be observed, as proteins involved in fat oxidation have been increased following fasted, but not fed-state training [43, 45]. Studies that have reported improvements in fat oxidation following training with low compared with normal muscle glycogen tested subjects in the fasted state and trained twice daily with only water ingested between the sessions [12, 163]. Though speculative, these differences could be related to FFA signaling, which are increased during exercise and increased even further if no food is ingested in the hours following exercise [118]. This would imply the second session of each twice-daily training day would have been commenced with elevated FFA. Finally, IMTG usage during exercise was increased after 6 weeks of fasted (but not fed) training when tested in the fasted state [157], but there were no differences when tested in a fed state, while also providing additional CHO [45]. Taken together, it appears that increases in fat oxidation following fasted-state or low-glycogen training may not be relevant during typical racing conditions when consuming CHO before and during exercise, but more studies in endurance-trained athletes are needed to compare acute and chronic changes, with a focus on FFA-related signaling pathways.

2.3.1.2. Mitochondrial Markers

A key feature of the adaptive response to endurance training are changes in the activity of enzymes involved in the tricarboxylic acid (TCA) cycle and the β-oxidative pathway [168]. Activity of citrate synthase (CS) is the most widely used biomarker of mitochondrial content in skeletal muscle because of the strong correlation between resting CS activity and resting mitochondrial content when measured using the "gold standard" transmission electron microscopy [169]. Similar changes in CS activity have been observed between fasted and fed-state training following 3–6 weeks of moderate-intensity training [43, 44], HIIT [155, 160], and sprint interval training [170, 171]. A key enzyme of the β-oxidative pathway, β-hydroxyacyl coenzyme A dehydrogenase (β-HAD), is also generally not impacted by pre-exercise nutrition [43, 44, 155, 170, 171]. However, one study has shown an increase in both CS and β-HAD only with fasted, but not CHO-fed training [157]. It is possible this difference may be related to the very large amount of CHO ingested in the fed-training group (~2 g kg^{-1} 90 min prior and 1 kg^{-1} $hour^{-1}$ during exercise), as studies showing similar adaptations between fed and fasted training used smaller (e.g., 1–1.5 g kg^{-1} CHO) pre-training meals [44, 155]. Increases in succinate dehydrogenase activity following twice-daily training were blunted when ingesting CHO before and during the second workout, which was commenced with lowered muscle glycogen [159], suggesting a strong, and potentially underappreciated influence of ingesting CHO during exercise that adds complexity when interpreting the current literature. The negative influence of CHO ingestion during exercise on the adaptive response could be related to reduced FFA [86], and is unlikely related changes in muscle glycogen, but further investigation is needed.

Greater increases in CS have been reported in two studies that had subjects train twice-daily every other day, inducing low muscle glycogen during the second bout of exercise, compared with once-daily training with normal muscle glycogen [9, 12]. In these studies, the two sessions were 1–2 h apart and subjects received only water between sessions. In contrast, other studies using twice-daily training but feeding low- or high-CHO meals between sessions found similar training-induced increases in CS activity between groups [164, 167]. When comparing two different “train-low” protocols (2 h vs. 15 h between low-glycogen training sessions), greater elevations in acute signaling and mitochondrial adaptations were observed when training with 2 h between sessions without ingesting any food [166, 172]. Thus, it appears that remaining in the fasted state following the first bout of exercise may be an important factor in the augmented adaptations observed following twice-daily training. Overall, the exercise training itself seems to be the primary driver of changes in mitochondrial content, though very large pre-exercise meals (>1.5 g/kg CHO) and CHO ingestion during exercise may have blunting effects on some signaling pathways.

2.3.1.3. VO_{2max} and Peak Aerobic Power

Studies comparing fasted and fed training have reported no differences in VO_{2max} following 4 weeks of sprint interval training (SIT) [156], 6 weeks of aerobic training [45, 157], and 3 weeks of mixed intensity training [173]. However, greater training-induced increases in VO_{2max} have also been reported following both fasted vs. fed-state training [44] and fed vs. fasted-state training [43]. Reasons for these divergent findings are unclear, as both studies used untrained participants performing 4–6 weeks of steady-state aerobic training. Similar improvements in VO_{2max} and peak power were seen in untrained men following 8 weeks of HIIT with or without prior CHO [160], and following exercise undertaken with low or high muscle glycogen levels in trained and untrained athletes [9, 159, 167, 174, 175].

2.3.1.4. Summary and Future Directions

Pre-exercise nutrition intake would not be expected to have an effect on VO_{2max} (which is largely affected by central adaptations [176]), but may affect peripheral adaptations that are influenced by fuel availability such as the substrate usage and mitochondrial size, particularly in untrained participants. Although there is potential for pre-exercise nutrition intake to influence adaptations to endurance training, the lack of research in endurance-trained subjects, the very large amounts of CHO ingested before exercise in some studies, and the provision of CHO both before and during exercise in other studies makes extrapolating results to trained athletes challenging. Additionally, some of the strongest evidence suggesting low-glycogen training can magnify signaling responses to exercise is based on studies performing the experimental exercise session a few hours after a glycogen-lowering exercise bout [12, 163, 164], and some of these effects might be largely attributable to performing two exercise sessions in close proximity [172].

Future training studies should compare fasted-state training against low-CHO and moderate-CHO pre-exercise meals, in the context of both HIIT and steady-state continuous endurance training to determine if there are differential effects on fat oxidation and/or mitochondrial biogenesis. It would also be of interest to investigate if there is a threshold for the amount of pre-exercise CHO ingested, independent of muscle glycogen levels [2], above which adaptations may be negatively impacted but below which adaptations are not impaired. Additionally, sex-based differences in the response to training programs should be investigated, as females accounted for just ~10% of participants in the training studies discussed.

2.3.2. Performance Changes

Studies comparing fed vs. fasted training have reported similar improvements in time-to-exhaustion during a maximal incremental test [157, 177] and 1-hour time-trial performance [157] following 6 weeks of endurance training, and similar improvements in 20-min time-trial performance following 3 weeks of SIT performed in the fasted state or with pre-exercise whey protein ingestion [171]. In contrast, time-to-fatigue at 85% VO_{2max} improved more in trained cyclists performing SIT in the fasted state compared to those that consumed CHO (>2.5 $g.kg^{-1}$ CHO prior and CHO drink during exercise), despite performing less work during training sessions [156].

Some studies comparing high vs. low glycogen training have reported similar performance improvements between groups [12, 48, 159, 163, 167], however greater improvements were seen following one and three weeks of sleep-low training [162, 165, 178], twice-daily training with low-CHO vs. high-CHO consumption between sessions [164], and twice- vs. once-daily training [9]. Two studies using a combination of tactics to vary CHO availability around training sessions (i.e., periodized-CHO) found similar improvements between chronic high-CHO and periodized-CHO diets, both of which resulted in greater improvements than a chronic low-CHO diet [174, 175]. Future training studies should compare pre-exercise protein ingestion against CHO-fed and fasted-state training in the context of both HIIT and steady-state continuous endurance training. Additionally, it would be of interest to study whether a delayed CHO ingestion strategy [179] in the context of low glycogen or fasted-state training has any influence on the adaptive response and whether it might be training specific (e.g., high- vs. low-intensity training).

2.4 Pre-exercise Nutrition Practices of Athletes

A limited number of studies have characterized the pre-training nutrition practices of athletes and have most commonly been in populations of elite runners and race walkers. Training in the overnight-fasted state is used as a tool for reducing CHO availability by some but not all athletes. Surveys have reported 21–48% of elite runners and race walkers perform some training sessions in an overnight-fasted state . A case study of elite marathon runners reported they trained with low CHO-availability 1-3 times per week, with 90% of sessions being after an overnight fast and 10% being reduced glycogen training from twice-daily training without CHO restoration between sessions [24], and a 7-d study of elite Kenyan runners reported daily morning runs at moderate to high-intensity in the overnight-fasted state [27].

The concept of adjusting daily carbohydrate intake in relation to changes in exercise volume and intensity has been referred to as "fueling for the work required" [2], and is in line with current sport nutrition guidelines [180]. This practice has the potential to influence signaling pathways regulating training-induced skeletal muscle adaptations [2, 181], manage body composition, and reduce the risk of inadequate energy availability [180]. However, there is limited evidence of how athletes practice it during real-world, day-to-day training. This could be related to several challenges, including athlete adherence to longer-term data collection, difficulty quantifying training loads (particularly when athletes train in multiple exercise modalities), and the lack of any objective method to quantify the relationship between training and dietary CHO intake.

Surveys have shown roughly half of athletes report increasing CHO intake within the 4 h prior to key training sessions (i.e., high intensity or >90 min) [19, 20], yet only 17% report reducing CHO intake before easy sessions [20] and 20% report eating less food on easy training days or the day after easy training days [19]. However, at the group level an observational study of elite runners and race walkers found no differences in pre-exercise CHO intake before key workouts compared with easy workouts [25]. Laboratory-based research in non-athletes has shown an 11% increase in energy intake before a planned session of aerobic exercise [182], but it is possible that many athletes who train regularly may not make the same planned adjustments. It is also possible that some athletes may alter their pre-exercise nutrition without realizing it.

A number of other studies have analyzed the dietary intake and practices of endurance athletes, but have focused primarily on either macronutrient intake and meal frequency [183-186], or nutrition before a single training session or race [34], rather than on the dietary habits, beliefs, and practices related to pre-exercise nutritional intake. Furthermore, data on the pre-exercise nutrition intake patterns and beliefs are lacking in athletes competing in sports other than running. Particularly for non-elite endurance athletes, it is currently unclear how many athletes regularly perform fasted-state training, what percentage of training sessions are done fasted, and what dictates if someone trains in the fasted state. It is also unknown how many athletes consume protein, in the absence of carbohydrate, prior to training.

2.5. Summary

The availability of endogenous and exogenous CHO, fat, and protein before and during exercise can influence the acute and longer-term responses to endurance exercise. Acutely, CHO ingestion inhibits fat oxidation, however evidence of a sustained increase in capacity for fat oxidation following long-term training in the fasted state is lacking. Contrasting findings related to the influence of CHO ingestion on mitochondrial signaling may be related to the amount of carbohydrate consumed and the intensity of exercise. Performance is improved following pre-exercise CHO ingestion for longer but not shorter duration exercise, while training-induced performance improvements following various pre-exercise nutrition strategies vary based on the type of nutrition protocol used.

In addition to wider participant demographics, more research is needed looking at the acute and longer-term effects of pre-exercise protein ingestion, using endurance-trained subjects, examining fasted-state training compared with ingesting moderate and low-CHO meals before exercise, and fasted vs. fed-state training without CHO ingestion during exercise. A better understanding of the prevalence and rationale for (or against) training in the fasted state would also help practitioners and researchers to better serve the needs of athletes. In addition, there is a need to characterize the nutrition practices of endurance athletes in their day-to-day training environments, particularly in relation to daily changes in training load. Although it is recommended that CHO intake be modulated according to changes in training load, there have yet to be any longer-term (> 7 d) studies of athlete practices. This is likely due in part to challenges relating to longer term data collection, and the current lack of objective method for relating training volume and dietary intake. However, the use of technology such as smartphone apps and online fitness platforms can open new possibilities for field-based research.

3. Prevalence and Determinants of Fasted Training in Endurance Athletes: A Survey Analysis

This chapter describes the prevalence and determinants of exercise performed in the overnight-fasted state among endurance athletes of varying backgrounds and competitive levels.

This chapter contains the following publication:

Rothschild, J. A., Kilding, A. E., & Plews, D. J. (2020). Prevalence and determinants of fasted training in endurance athletes: A survey analysis. International journal of sport nutrition and exercise metabolism, 30(5), 345-356.

3.1 Abstract

Athletes may choose to perform exercise in the overnight-fasted state for a variety of reasons related to convenience, gut comfort, or augmenting the training response, but it is unclear how many endurance athletes use this strategy. We investigated the prevalence and determinants of exercise performed in the overnight-fasted state among endurance athletes using an online survey, and examined differences based on sex, competitive level, and habitual dietary pattern. The survey was completed by 1,950 endurance athletes (51.0% female, mean age 40.9 ±11.1 years). The use of fasted training was reported by 62.9% of athletes, with significant effects of sex ($p < 0.001$, Cramer's V (φ_c) = 0.18 [90% CI 0.14,0.22]), competitive level ($p < 0.001$, $\varphi_c = 0.09$ [90% CI 0.5,0.13]), and habitual dietary pattern ($p < 0.001$, $\varphi_c = 0.26$ [90% CI 0.22,0.29]). Males, non-professional athletes, and athletes following a low-carbohydrate, high-fat diet were most likely to perform fasted training. The most common reasons for doing so were related to utilising fat as a fuel source (42.9%), gut comfort (35.5%), and time constraints/convenience (31.4%), while the most common reasons athletes avoided fasted training were that it doesn't help their training (47.0%), performance was worse during fasted training (34.7%), or greater hunger (34.6%). Overall, some athletes perform fasted training because they think it helps their training while others avoid it because they think it is detrimental to their training goals, highlighting a need for future research. These findings offer insights into the beliefs and practices related to fasted-state endurance training.

3.2 Introduction

A key contributor for achieving optimal endurance performance is providing adequate carbohydrate (CHO) to working muscles, at a rate dependent upon the intensity and duration of exercise [187]. However, it has become evident that altering nutrient availability before, during, and after endurance exercise can enhance cellular responses to exercise-induced perturbations [188]. For example, exercise performed with reduced CHO-availability can increase the activation of key mitochondrial signalling proteins compared with exercise performed with higher CHO-availability [189]. In an attempt to optimize both adaptations and performance, a periodised approach to CHO ingestion has emerged, whereby CHO ingestion is varied according to the type and goals of the training session [36].

Strategies to modulate CHO availability include low-CHO, high-fat (LCHF) diets, restricting CHO-ingestion between training sessions, increasing CHO-ingestion before or during exercise, and exercising in the overnight-fasted state. Overnight fasting reduces liver glycogen [7], but does not affect muscle glycogen concentration [8], while restricting CHO between training sessions allows exercise to be performed starting with low muscle glycogen concentrations [9]. A case study of elite marathon runners reported they trained with low CHO-availability 1-3 times per week, with 90% of sessions being after an overnight fast and 10% being reduced glycogen training from twice-daily training without CHO restoration between sessions [24]. Elite Kenyan runners perform daily morning runs at moderate to high-intensity in the overnight-fasted state [27], and a recent survey found 48% of elite endurance athletes report performing at least some training sessions in an overnight-fasted state [20].

Athletes may choose to perform training sessions in the overnight-fasted state for a variety of reasons, including convenience, gut comfort [190], or in an attempt to augment the training response. Greater training-induced improvements in maximal fat oxidation and markers of mitochondrial biogenesis have been reported with fasted, compared with fed-state training in some [157], but not all [155] studies, highlighting a need for further research.

Despite being acknowledged as a strategic method to lower CHO availability for a specific training session via reduced liver glycogen [36], data on the use of training in the overnight-fasted state is lacking in athletes competing in sports other than running, and in non-elite endurance athletes. Therefore, the aim of this study is to describe the prevalence and determinants of exercise performed in the overnight-fasted state among endurance athletes of varying backgrounds and competitive levels. Secondary outcomes are to determine if differences in nutrition practices or beliefs related to sex, competitive level, or habitual dietary pattern exist.

3.3 Methods

Participants. Active endurance athletes ≥ 18 years old were invited to participate. Eligibility criteria insisted that athletes: 1) had been training for at least one year; 2) regularly completed at least 4 exercise sessions per week; and 3) had participated in at least one organised endurance event of any distance at any time. Study protocols and materials were approved by the Auckland University of Technology Ethics Committee (19/415).

Survey. The anonymous online survey was developed collaboratively by the authors, and conducted using Qualtrics software (Provo, UT, USA). Our survey expanded on the survey tool used by Heikura et al. [20], in the investigation of the larger dietary practices of energy and CHO periodization used by free-living track and field endurance athletes. The survey was circulated internationally in English via social media and email, with data collected between December 2019 and March 2020. Informed consent was provided by participants prior to beginning the survey. Participants were asked 40-47 questions, dependent upon logic functions that skipped irrelevant questions. The median response time to complete the survey was 10.4 minutes. Results presented are part of a larger survey on beliefs and practices related to pre-exercise nutrition intake in endurance athletes. The major themes explored in the survey included general background (demographics, competitive level, training history), fasted training (is it used? why/why not?), determinants of breakfast composition (does it vary based on the duration, intensity, or mode of workout?), pre-exercise intake (how often are CHO-containing or low-CHO foods consumed before training?), wake-up and eating times before training that is shorter and longer than 90 min, pre-exercise nutrition beliefs, and dietary supplements consumed within the 1 h before exercise. The full survey is available at https://bit.ly/3dNJEVv. Data relating to breakfast composition and the use of dietary supplements are not reported here. Several questions related to habitual dietary pattern and the use of fasted training were adopted from a previously published questionnaire [20], but the majority of questions were designed for this study and have not been previously used. The survey was reviewed for face validity by three sport nutrition experts, piloted among 15 athletes, and updated based on feedback received.

The options for self-reported competitive level included professional, elite non-professional (qualify and compete at the International level), high-level amateur (qualify and compete at National Championship-level events), amateur (enter races but don't expect to win), and recreational (train but do not participate in competition). For habitual dietary pattern, respondents could choose among no dietary plan, gluten-free, high-CHO (>50% dietary intake), high-protein, low-CHO (>2.2 g protein per kg body mass, <130 g CHO), LCHF (<130 g CHO), paleo (avoid grains, legumes, and dairy products), paleo for athletes (paleo with more carbs around training), periodised carb (habitually lower CHO and then increase before key training, OR habitually high-CHO and then restrict before key training), pescatarian (vegetarian that also consume fish), vegan (avoid all animal products), and vegetarian (include some dairy or eggs). For fasted-state training, participants were asked *"Thinking of your training over the past 12 months, do you ever intentionally train in the fasted state (e.g. train first thing in the morning without having eaten any food – if you drink coffee-only, consider that as fasted-state training)?".*

Statistical analysis. Only responses from completed surveys were analysed. Descriptive data are presented as number (n) and percentage (%) of responses. Participant age is presented as mean ±SD and was analysed using the Kruskal-Wallis test with Dwass, Steel, Critchlow-Fligner pairwise post-hoc comparisons. Categorical data were assessed by chi-square analysis and Cramer's V (φ_c) effect size with 90% confidence intervals (CI), using R and jamovi software [191, 192]. Effects are interpreted as small (0.1-0.3), medium (0.3-0.5), large (>0.5), however this categorisation varied based on the degrees of freedom [193] and differences in interpretation are noted where applicable. Where significant differences were found, chi-square post hoc testing using Fisher's exact test was performed using the Fifer package in R software with Bonferroni corrections.

Participants could select multiple answers for several questions, such as which sports they trained for and which dietary patterns they followed. Therefore, the percentage of responses to some questions added up to over 100%. Answers that were filled in for "other" were grouped into common sub-groups for reporting, with those totalling ≥ 4.0% of responses included in the figures but not analysed. Three participants chose "prefer not to say" for sex and were omitted from sex-based analyses. For analyses based on event duration athletes were grouped into four categories: those training for events <60 min, 1-3 h, 3-12 h and >12 h. For dietary analyses, paleo and paleo for athletes were combined into a single group. For analyses based on competitive level, recreational athletes were combined into the amateur group, and a series of questions related to pre-exercise nutrition beliefs that used a 5-pt Likert scale was folded down into a 3-pt scale (disagree/neutral/agree) for analysis.

3.4 Results

Participants

The survey was completed by 1,950 participants (48.8% male, 51.0% female, 0.2% declined to give sex; mean age: 40.9 ±11.1 years [range 18-78], Fig. 3.1a). The primary sports represented were running (57.2%), triathlon (50.3%), cycling (36.5%), and swimming (18.3%, Fig. 3.1b). The typical duration of the competitive events the respondents trained for was < 60 min (n = 183, 9.4%), 1-3 h (n = 592, 30.4%), 3-12 h (n = 1026, 52.6%), >12 h (n = 149, 7.6%, Fig 3.1c). Additional background information is shown in figures 3.1d-f.

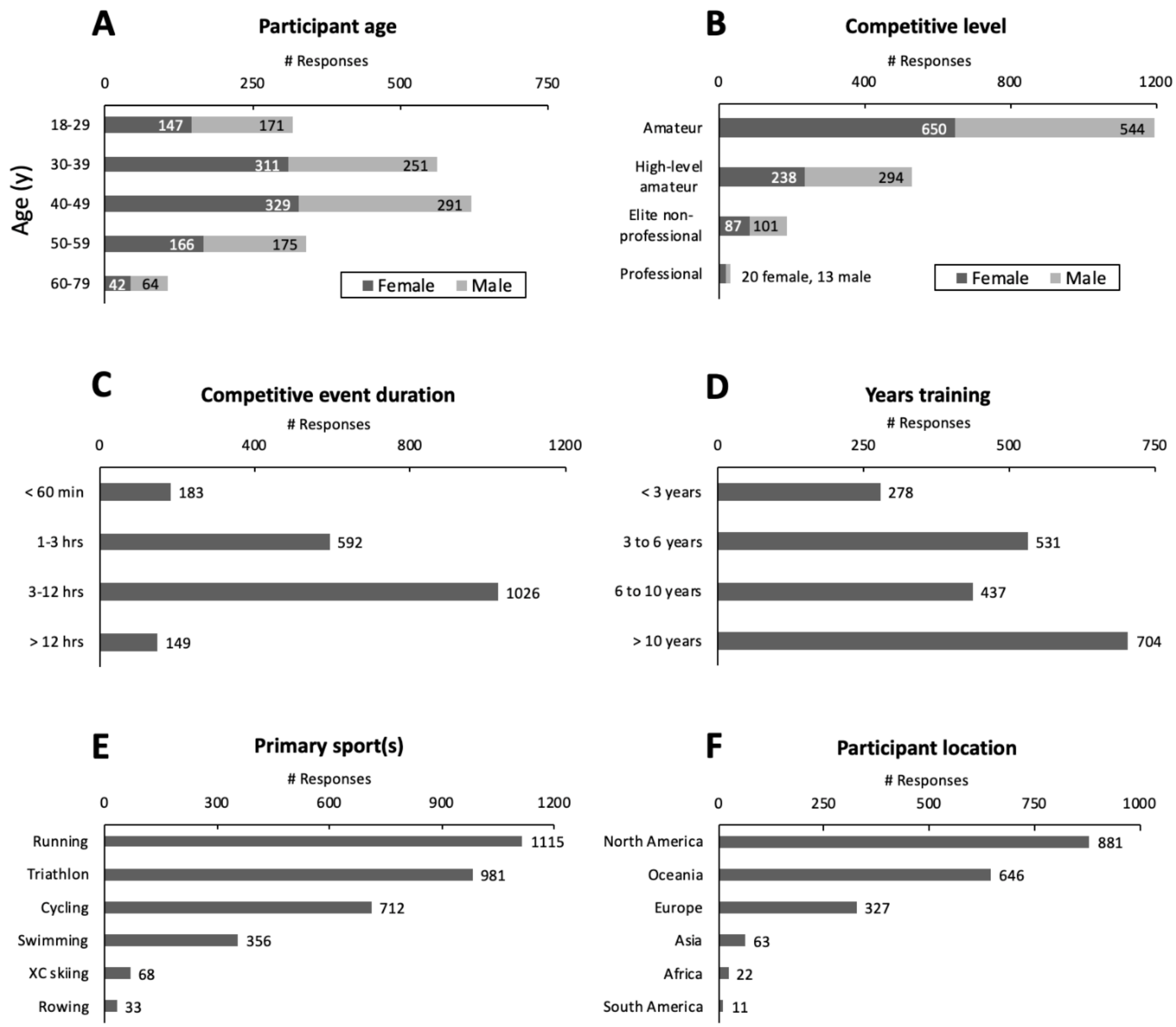

Figure 3.1. Participant demographics. Age (A), competitive level (B), typical event duration (C), number of years training (D), sports participated in (E), and location grouped by continent (D) of survey respondents. N = 1,950, except (A, B), where three subjects ages 21 (high-level amateur), 23 (professional), and 33 years (amateur) declined to give their sex. For (E), respondents could select more than one primary sport. Other sports written into the survey included duathlon, biathlon, adventure racing, kayaking, race walking, and stand-up paddling (all < 1.0% of respondents). XC skiing: Cross-country skiing. For (F) participants were from 57 different countries and grouped by continent.

Weekly training

The most common training volume was 10-15 h per week (n = 856, 43.9%), followed by 7-9 h per week (n = 617, 31.6%), 16-20 h per week (n = 246, 12.6%), < 7 h per week (n = 185, 9.5%), 21-30 h per week (n = 43, 2.2%), and >30 h per week (n = 3, 0.2%). When asked specifically about exercise sessions that started before 9 am, 33.0% of respondents (n = 643) reported 5-7 sessions per week, 29.6% (n = 578) reported 1-2 sessions per week, 26.7% (n = 520) reported 3-4 sessions per week, and 10.7% (n = 209) reported not exercising before 9 am.

Fasted training

Overall, 62.9% (n = 1,227) of respondents reported performing at least some training sessions in the overnight-fasted state (Fig. 3.2). There were significant effects of sex ($p < 0.001$, $\varphi_c = 0.18$ [90% CI 0.14,0.22]), competitive level ($p < 0.001$, $\varphi_c = 0.09$ [90% CI 0.5,0.13]), and habitual dietary pattern ($p < 0.001$, $\varphi_c = 0.26$ [90% CI 0.22, 0.29]). Males were more likely than females and professional athletes were less likely than all other levels to perform fasted training (Tables 3.1, 3.2). A significantly greater number of athletes following a LCHF dietary regimen performed fasted training compared with any other dietary group, while athletes that do not follow any specific dietary plan perform less fasted training compared with those following a periodised-CHO or high-protein diet, and athletes following a periodised-CHO diet perform more fasted training compared with vegetarian and high-CHO diets (Table 3.3). There were significant interactions between habitual dietary patterns and frequency of fasted training ($p < 0.001$, $\varphi_c = 0.14$ [90% CI 0.11, 0.16]), with LCHF performing more fasted-state training than nearly every other group (Table 3), but no effects of sex or competitive level. Additionally, there was no relationship between reported training volume (hours per week) and the use of fasted training ($p = 0.940$, $\varphi_c = 0.03$ [90% CI 0.00, 0.06]).

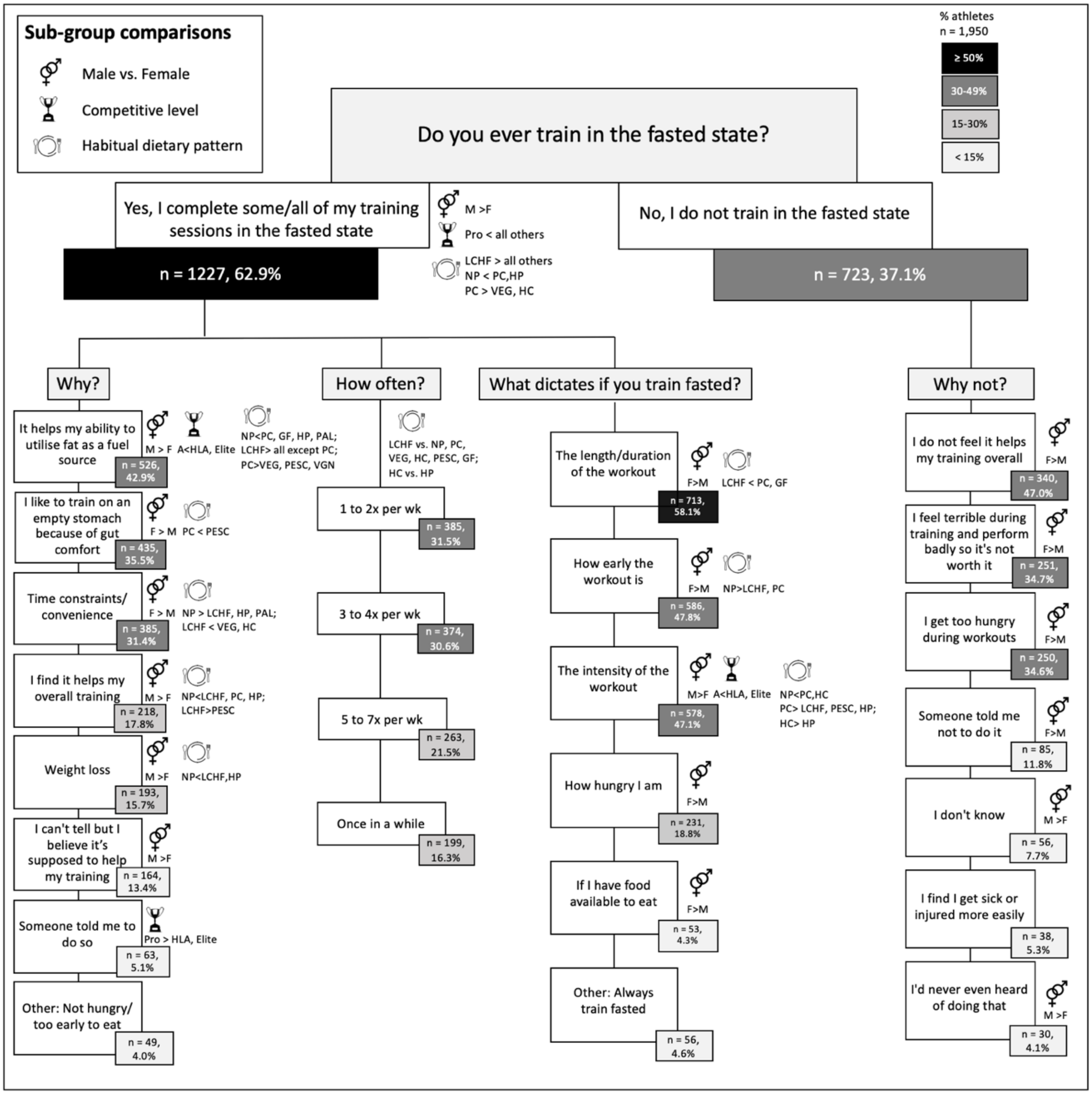

Figure 3.2. The prevalence, frequency, and determinants of training in the fasted state, and reasons why it is/isn't used. Percentages (%) reflect the % of athletes that chose a specific answer in relation to all athletes that were presented with a given question. Significant differences between sub-groups ($p < 0.05$) are depicted with symbols and a brief description of the direction of the differences. Sex abbreviations - F: Female; M: Male. Competitive level abbreviations – A: Amateur; HLA: high-level amateur; Elite: elite non-professional; Pro: professional. Dietary pattern abbreviations – LCHF: low-carb, high-fat; GF: gluten-free; HC: high-carbohydrate; HP: high-protein, low-carb; NP: no dietary plan; PAL: Paleo; PC: periodised carbohydrate; PESC: pescatarian; VEG: vegetarian; VGN: vegan.

The most common reasons for performing fasted training were related to utilising fat as a fuel source (n = 526, 42.9%), gut comfort (n = 435, 35.5%), and time constraints/convenience (n = 385, 31.4%; Fig. 3.2, Tables 3.1–3.3). More males cited reasons such as increasing the ability to utilise fat, helping their training overall, and weight loss, while females more often cited gut comfort and time constrains/convenience as their reasons for using fasted training. Amateurs were less likely than high-level amateur and elite athletes to use fasted training with the goal of increasing their fat-burning capacity, while professionals were more likely than high-level amateur and elite athletes to use fasted training because someone had told them to (Table 3.2). Reasons for fasting also varied based on habitual dietary patterns (Table 3.3).

The decision to perform fasted training was most-often based on the length/duration of the training session (n = 713, 58.1%), how early the session is (n = 586, 47.8%), and the intensity of the session (n = 578, 47.1%; Fig. 3.2). Additionally, a number of athletes reported that they always train without eating (n = 56, 4.6%). Sex differences were found between all factors that influence the decision to train in the fasted state (Table 3.1). High-level amateur and elite athletes based their decision on the intensity of the workout more than amateur athletes ($p < 0.001$, $\varphi_c = 0.19$ [90%CI 0.15, 0.24], Table 3.2), and differences were evident among dietary habits for workout duration ($p = 0.002$, $\varphi_c = 0.14$ [90%CI 0.10, 0.18]), time ($p < 0.001$, $\varphi_c = 0.18$ [90%CI 0.14, 0.23]), and intensity ($p < 0.001$, $\varphi_c = 0.21$ [90%CI 0.17, 0.26]) to dictate whether or not to perform fasted training (Table 3.3). The most common length of time performing fasted training was more than three years (n = 583, 47.7%), followed by 1–3 years (n = 453, 37.1%), and less than one year (n = 186, 15.2%).

Among those who do not perform exercise in the fasted state, the most common reasons for avoiding it were they do not feel that it helps their training (n = 340, 47.0%), perform worse during training (n = 251, 34.7%), and get too hungry (n = 250, 34.6%; Fig. 3.2). Sex differences were seen for all but one of the reasons for not using fasted-state training (Table 3.1), while no sub-group differences were found for competitive level or habitual dietary pattern (data not shown). Additional reasons for avoiding fasted-state training included a belief that females should not train fasted (n = 15, 2.1%), and not training in the morning (n = 15, 2.1%). Regardless of whether they perform exercise in the fasted state, athletes were asked if/where they had ever heard that it was a good thing to do. Responses included social media/online (n = 851, 43.6%), another athlete (n = 454, 23.3%), coach (n = 343, 17.6%), nutritionist (n = 297, 15.2%), physiologist (n = 138, 7.1%), or other sources such as personal reading (n = 83, 4.3%), while 29.2% (n = 569) reported they had never been told that fasted training was good for them.

Pre-exercise nutrition beliefs

Significant differences were seen in all questions relating to pre-exercise nutrition beliefs when analysed by sex and habitual dietary pattern (Table 3.4). The largest effects were seen for “skipping breakfast will allow me to get a better workout” which varied by sex ($p < 0.001$, φ_c = 0.16 [90% CI 0.13,0.20], small effect), competitive level ($p < 0.001$, φ_c = 0.08 [90% CI 0.04,0.11], small effect) and diet ($p < 0.001$, φ_c = 0.23 [90% CI 0.21,0.26], medium effect), and “skipping breakfast allows me to burn more fat during my workout” which also varied by sex ($p < 0.001$, φ_c = 0.25 [90% CI 0.22,0.29], small effect), competitive level ($p = 0.006$, φ_c = 0.07 [90% CI 0.03,0.11], small effect) and diet ($p < 0.001$, φ_c = 0.23 [90% CI 0.21,0.26]).

Table 3.1. Sex differences in fasting practices of endurance athletes

	Male	**Female**	**Total**	**p-value, Cramer's V [90% Confidence Interval]**
Completed responses	n = 952 (48.9%)	n = 995 (51.1%)	n = 1,947	
Athletes that perform some or all training in the overnight-fasted state	72.0%	54.4%	63.0%	**$p < 0.001$, $\varphi_c = 0.18$ [90% CI 0.14, 0.22]**
Why do you train in the AM-fasted state? [1]				
It helps my ability to utilise fat as a fuel source	53.9%	29.0%	42.9%	**$p < 0.001$, $\varphi_c = 0.25$ [90% CI 0.2, 0.3]**
I like to train on an empty stomach because of gut comfort	29.6%	42.9%	35.5%	**$p < 0.001$, $\varphi_c = 0.14$ [90% CI 0.09, 0.19]**
Time constraints/convenience	26.3%	37.7%	31.3%	**$p < 0.001$, $\varphi_c = 0.12$ [90% CI 0.08, 0.17]**
I find it helps my overall training	21.8%	12.8%	17.8%	**$p < 0.001$, $\varphi_c = 0.12$ [90% CI 0.08, 0.17]**
Weight loss	18.7%	12.0%	15.7%	**$p = 0.001$, $\varphi_c = 0.09$ [90% CI 0.05, 0.14]**
I can't actually tell, but I believe it's supposed to help my overall training	15.3%	10.9%	13.4%	**$p = 0.024$, $\varphi_c = 0.06$ [90% CI 0.03, 0.12]**
Someone told me to do so	5.1%	5.2%	5.1%	$p = 0.958$, $\varphi_c = 0.00$ [90% CI 0.0, 0.0]
For a morning workout, what dictates if you train in the fasted or fed state? [1]				
The length/duration of the workout	52.6%	65.2%	58.2%	**$p < 0.001$, $\varphi_c = 0.13$ [90% CI 0.09, 0.18]**
How early the workout is	40.3%	57.1%	47.7%	**$p < 0.001$, $\varphi_c = 0.17$ [90% CI 0.12, 0.22]**
The intensity of the workout	50.2%	43.3%	47.1%	**$p = 0.015$, $\varphi_c = 0.07$ [90% CI 0.04, 0.12]**
How hungry I am	13.7%	25.1%	18.8%	**$p < 0.001$, $\varphi_c = 0.15$ [90% CI 0.10, 0.19]**
Whether or not I have food available to eat	3.2%	5.7%	4.3%	**$p = 0.031$, $\varphi_c = 0.06$ [90% CI 0.03, 0.11]**
Why do you not perform fasted training? [2]				
I do not feel it helps my training overall	39.3%	51.5%	47.0%	**$p = 0.002$, $\varphi_c = 0.12$ [90% CI 0.07, 0.18]**
I feel terrible during the session and perform badly so it's not worth it	28.1%	38.5%	34.7%	**$p = 0.004$, $\varphi_c = 0.11$ [90% CI 0.06, 0.17]**
I get too hungry during workouts	21.7%	42.3%	34.7%	**$p < 0.001$, $\varphi_c = 0.21$ [90% CI 0.15, 0.27]**
Someone told me not to do it	4.1%	16.3%	11.8%	**$p < 0.001$, $\varphi_c = 0.18$ [90% CI 0.13, 0.25]**
I don't know	15.4%	3.3%	7.8%	**$p < 0.001$, $\varphi_c = 0.22$ [90% CI 0.16, 0.28]**
I find I get sick or injured more easily	3.4%	6.4%	5.3%	$p = 0.080$, $\varphi_c = 0.07$ [90% CI 0.04, 0.13]
I'd never even heard of doing that	7.9%	2.0%	4.2%	**$p < 0.001$, $\varphi_c = 0.14$ [90% CI 0.09, 0.21]**

[1] Percentages are based on the number of people in each column who perform fasted training. [2] Percentages are based on the number of people in each column who do not perform fasted training. Significant interactions ($p < 0.05$) in bold.

Table 3.2. Differences in fasting practices of endurance athletes based on competitive level

	Amateur	High-level amateur	Elite non-professional	Professional	Total	Post hoc comparisons	p-value, Cramer's V [90% Confidence Interval]
Completed responses	n = 1,195 (61.3%)	n = 533 (27.3%)	n = 188 (9.6%)	n = 34 (1.7%)	n = 1,950		
Age (mean, SD) [1]	42.6 (10.6) †	39.2 (11.6)	36.9 (11.3)	31.1 (6.0) ^	40.9 (11.1)	†, ^	**p < 0.001**
Athletes that perform some or all training in the fasted state	63.0%	64.2%	64.9%	29.4%	62.9%	Pro < all others	**p < 0.001, φc = 0.09 [90% CI 0.05, 0.13]**
Why do you train in the AM-fasted state? [2]							
It helps my ability to utilise fat as a fuel source	38.2%	47.4%	56.6%	70.0%	42.9%	Amateur < HLA, Elite	**p < 0.001, φc = 0.13 [90% CI 0.09, 0.18]**
I like to train on an empty stomach because of gut comfort	36.0%	33.9%	36.1%	40.0%	35.5%		p = 0.906, φc = 0.02 [90% CI 0.0, 0.07]
Time constraints/convenience	32.7%	30.4%	27.9%	10.0%	31.4%		p = 0.314, φc = 0.05 [90% CI 0.01, 0.10]
I find it helps my overall training	16.1%	18.4%	24.6%	20.0%	17.8%		p = 0.166, φc = 0.06 [90% CI 0.02, 0.11]
Weight loss	14.6%	18.4%	13.9%	30.0%	15.7%		p = 0.220, φc = 0.06 [90% CI 0.0, 0.11]
I can't actually tell, but I believe it's supposed to help my overall training	14.3%	11.4%	13.1%	10.0%	13.4%		p = 0.601, φc = 0.04 [90% CI 0.0, 0.09]
Someone told me to do so	5.7%	3.8%	3.3%	30.0%	5.1%	Pro > HLA, Elite	**p = 0.002, φc = 0.11 [90% CI 0.07, 0.16]**
For a morning workout, what dictates if you train in the fasted or fed state? [2]							
The length/duration of the workout	55.6%	62.6%	59.8%	70.0%	58.1%		p = 0.144, φc = 0.07 [90% CI 0.05, 0.11]
How early the workout is	48.5%	46.8%	44.3%	70.0%	47.8%		p = 0.413, φc = 0.05 [90% CI 0.0, 0.10]
The intensity of the workout	39.8%	55.6%	66.4%	70.0%	47.1%	Amateur < HLA, Elite	**p < 0.001, φc = 0.19 [90% CI 0.15, 0.24]**
How hungry I am	18.9%	18.1%	20.5%	20.0%	18.8%		p = 0.952, φc = 0.02 [90% CI 0.0, 0.06]
Whether or not I have food available to eat	4.4%	4.1%	4.9%	0.0%	4.3%		p = 0.895, φc = 0.02 [90% CI 0.0, 0.07]

Significant interactions (p < 0.05) in bold. HLA: high-level amateur, NS: not statistically significant; Pro: professional.
[1] Age vs. level analysed using the Kruskal-Wallis- test. [2] Percentages are based on the number of people in each column who perform fasted training. † = different from all other levels (p< 0.001), ^ = different from all other levels (p < 0.02)

Table 3.3. Differences in fasting practices of endurance athletes based on habitual dietary pattern

	No dietary plan	LCHF	Periodised carb	Vegetarian	High-carbohydrate	Pescatarian	Gluten-free	High-protein, low-carb	Paleo	Vegan	Post hoc differences	p-value, Cramer's V [90% Confidence Interval]
Total responses (% of respondents, n = 1,950)	n = 957 (49.1%)	n = 191 (9.8%)	n = 191 (9.8%)	n = 137 (7.0%)	n = 137 (7.0%)	n = 122 (6.3%)	n = 117 (6.0%)	n = 111 (5.7%)	n = 83 (4.3%)	n = 82 (4.2%)		**p < 0.001**
Athletes that perform some or all training in the fasted state	56.7%	96.3%	79.1%	56.9%	54.7%	68.0%	64.1%	74.8%	67.5%	65.9%	NP < PC, HP; LCHF > all others; PC > VEG, HC	**p <0.001, φc =0.26 [90% CI 0.22, 0.29]**
How often do you train in the AM-fasted state? [1]												
1-2x per week	32.5%	21.9%	39.3%	28.2%	41.3%	36.6%	36.5%	24.4%	30.9%	35.2%	[2] LCHF vs. NP, PC, VEG, HC, PESC, GF; HC vs. HP	**p <0.001, φc =0.14 [90% CI 0.11, 0.16]**
3-4x per week	30.1%	38.8%	24.7%	25.6%	22.7%	26.8%	29.7%	24.4%	32.7%	29.6%		
5-7x per week	19.9%	34.4%	17.3%	23.1%	10.7%	18.3%	18.9%	37.8%	23.6%	18.5%		
Once in a while, not weekly	17.5%	4.9%	18.7%	23.1%	25.3%	18.3%	14.9%	13.4%	12.7%	16.7%		
Why do you train in the AM-fasted state? [1]												
It helps my ability to utilise fat as a fuel source	27.1%	79.3%	66.9%	41.0%	45.3%	28.9%	50.7%	54.2%	53.6%	38.9%	NP<PC, GF, HP, PAL; LCHF> all except PC; PC>VEG, PESC, VGN	**p <0.001, φc = 0.38 [90% CI 0.35, 0.42]**
I like to train on an empty stomach because of gut comfort	35.7%	35.3%	21.9%	42.3%	36.0%	47.0%	40.0%	37.3%	33.9%	46.3%	PC < PESC	**p = 0.009, φc = 0.13 [90% CI 0.08, 0.17]**
Time constraints/convenience	39.8%	17.4%	21.9%	38.5%	38.7%	33.7%	24.0%	18.1%	16.1%	22.2%	NP > LCHF, HP, PAL; LCHF < VEG, HC	**p <0.001, φc = 0.21 [90% CI 0.17, 0.26]**
I find it helps my overall training	10.7%	35.9%	25.8%	16.7%	20.0%	14.5%	17.3%	30.1%	19.6%	24.1%	NP<LCHF, PC, HP; LCHF>PESC	**p <0.001, φc = 0.23 [90% CI 0.19, 0.27]**
Weight loss	11.6%	21.7%	17.2%	17.9%	21.3%	10.8%	16.0%	27.7%	19.6%	16.7%	NP < LCHF, HP	**p = 0.003, φc = 0.13 [90% CI 0.10, 0.18]**
I can't actually tell, but I believe it's supposed to help my overall training	14.7%	8.2%	13.9%	16.7%	16.0%	12.0%	9.3%	12.0%	14.3%	14.8%		p = 0.546, φc = 0.08 [90% CI 0.03, 0.12]
Someone told me to do so	4.4%	5.4%	9.3%	6.4%	2.7%	3.6%	8.0%	4.8%	3.6%	5.6%		p = 0.458, φc = 0.08 [90% CI 0.04, 0.12]
For a morning workout, what dictates if you train in the fasted or fed state? [1]												
The length/duration of the workout	57.3%	47.3%	67.5%	62.8%	61.3%	63.9%	73.3%	54.2%	55.4%	66.7%	LCHF < PC, GF	**p = 0.002, φc =0.14 [90% CI 0.10, 0.18]**
How early the workout is	56.9%	34.8%	37.1%	48.7%	41.3%	54.2%	38.7%	44.6%	35.7%	42.6%	NP >LCHF, PC	**p <0.001, φc = 0.18 [90% CI 0.14, 0.23]**
The intensity of the workout	42.0%	44.6%	72.8%	52.6%	64.0%	49.4%	53.3%	36.1%	58.9%	50.0%	NP< PC, HC; PC>	**p <0.001, φc = 0.21 [90% CI 0.17, 0.26]**

											LCHF, PESC, HP; HC>HP	
How hungry I am	21.4%	13.6%	19.2%	16.7%	16.0%	14.5%	29.3%	19.3%	7.1%	25.9%	Post hoc NS	**p = 0.023, φc =0.12 [90% CI 0.09, 0.16]**
Whether or not I have food available to eat	4.2%	2.2%	4.6%	6.4%	8.0%	6.0%	2.7%	4.8%	3.6%	7.4%		p = 0.589, φc =0.07 [90% CI 0.08, 0.11]

[1] Percentages are based on the number of people in each column who perform fasted training. [2] Comparisons are between diets across all frequencies of fasted training. Significant interactions ($p < 0.05$) in bold. Dietary pattern abbreviations - LCHF: low-carb, high-fat; GF: gluten-free; HC: high-carbohydrate; HP: high-protein, low-carb; NP: no dietary plan; PAL: paleo; PC: periodised carbohydrate; PESC: pescatarian; VEG: vegetarian; VGN: vegan; NS: not statistically significant.

Table 3.4. Differences in nutrition-exercise beliefs by sex, competitive level, and habitual dietary pattern.

	If I have an early morning workout, I would rather not have a full stomach so I don't eat anything			If I have an early morning workout, I always eat something small beforehand like a banana, piece of toast, or a bar			The quality of my workout is the same whether I eat or do not eat beforehand			Skipping breakfast will allow me to get a better workout			Skipping breakfast will allow me to burn more fat during my workout		
	Agree	**Neutral**	**Disagree**	**Agree**	**Neutral**	**Disagree**	**Agree**	**Neutral**	**Disagree**	**Agree**	**Neutral**	**Disagree**	**Agree**	**Neutral**	**Disagree**
Total (n = 1,950)	48.9%	15.0%	36.1%	49.4%	10.3%	40.3%	25.9%	22.7%	51.4%	13.9%	30.7%	55.4%	43.0%	34.4%	22.6%
Sex															
Male (n = 952)	51.2%	18.3%	30.6%	41.8%	11.4%	46.7%	29.8%	22.7%	47.5%	18.5%	33.6%	47.9%	55.3%	30.1%	14.6%
Female (n = 995)	46.7%	12.0%	41.3%	56.8%	8.9%	34.3%	22.3%	22.6%	55.1%	9.5%	27.9%	62.5%	31.4%	38.5%	30.2%
	p < 0.001, φc = 0.13 [90% CI 0.09, 0.16]			**p < 0.001, φc = 0.15 [90% CI 0.12, 0.19]**			**p < 0.001, φc = 0.09 [90% CI 0.06, 0.13]**			**p < 0.001, φc = 0.16 [90% CI 0.13, 0.20]**			**p < 0.001, φc = 0.25 [90% CI 0.22, 0.29]**		
Competitive level															
Amateur (n = 1,195)	50.8%	15.0%	34.2%	49.5%	9.6%	40.9%	26.4%	24.4%	49.3%	14.6%	33.6%	51.7%	40.7%	37.4%	21.9%
High-level amateur (n = 533)	47.7%	14.4%	37.9%	49.5%	9.8%	40.7%	25.7%	19.5%	54.8%	13.9%	25.9%	60.2%	46.9%	31.7%	21.4%
Elite non-professional (n = 188)	43.1%	16.5%	40.4%	46.8%	15.4%	37.8%	26.6%	21.8%	51.6%	11.2%	28.2%	60.6%	46.3%	25.5%	28.2%
Professional (n = 34)	32.4%	17.6%	50.0%	61.8%	11.8%	26.5%	11.8%	17.6%	70.6%	2.9%	17.6%	79.4%	44.1%	23.5%	32.4%
	p = 0.161, φc = 0.05 [90% CI 0.01, 0.09]			p = 0.178, φc = 0.05 [90% CI 0.01, 0.09]			p = 0.069, φc = 0.05 [90% CI 0.02, 0.09]			**p <0.001, φc = 0.08 [90% CI 0.04, 0.11]**			**p = 0.006, φc = 0.07 [90% CI 0.03, 0.11]**		
Habitual dietary pattern															
No dietary plan (n = 957)	44.0%	15.7%	40.3%	54.5%	10.3%	35.1%	26.3%	23.6%	50.1%	9.3%	29.5%	61.2%	34.1%	41.6%	24.3%
LCHF (n = 191)	75.9%	14.1%	9.9%	11.0%	8.4%	80.6%	40.3%	26.2%	33.5%	43.5%	39.3%	17.3%	85.3%	11.0%	3.7%
Periodised carb (n = 191)	50.8%	19.4%	29.8%	40.3%	14.7%	45.0%	24.1%	17.8%	58.1%	13.1%	36.1%	50.8%	61.8%	26.2%	12.0%
Vegetarian (n = 137)	48.2%	16.8%	35.0%	53.3%	10.9%	35.8%	17.5%	27.0%	55.5%	13.1%	32.1%	54.7%	36.5%	39.4%	24.1%
High-carbohydrate (n = 137)	40.9%	10.2%	48.9%	62.8%	8.8%	28.5%	19.7%	16.1%	64.2%	8.8%	27.7%	63.5%	44.5%	24.1%	31.4%
Pescatarian (n = 122)	54.1%	16.4%	29.5%	48.4%	9.8%	41.8%	23.8%	32.8%	43.4%	14.8%	32.8%	52.5%	45.1%	28.7%	26.2%
Gluten-free (n = 117)	51.3%	11.1%	37.6%	58.1%	10.3%	31.6%	22.2%	22.2%	55.6%	7.7%	32.5%	59.8%	44.4%	30.8%	24.8%
High-protein, low-carb (n = 111)	55.9%	15.3%	28.8%	40.5%	6.3%	53.2%	27.0%	27.0%	45.9%	29.7%	32.4%	37.8%	57.3%	30.9%	11.8%
Paleo (n = 83)	49.4%	19.3%	31.3%	47.0%	10.8%	42.2%	27.7%	20.5%	51.8%	16.9%	28.9%	54.2%	49.4%	25.3%	25.3%

Vegan (n = 82)	53.7%	9.8%	36.6%	46.3%	19.5%	34.1%	26.8%	19.5%	53.7%	11.0%	30.5%	58.5%	35.4%	40.2%	24.4%
	p <0.001, φc = 0.15 [90% CI 0.13, 0.18]			**p <0.001, φc = 0.21 [90% CI 0.18, 0.23]**			**p <0.001, φc = 0.12 [90% CI 0.09, 0.14]**			**p <0.001, φc = 0.23 [90% CI 0.21, 0.26]**			**p <0.001, φc = 0.23 [90% CI 0.21, 0.26]**		

Percentages are in relation to the given n for each row. Interactions shown as, p-value, Cramer's V [90% Confidence Interval (CI)]. Significant interactions ($p < 0.05$) in bold. Cramer's V effect sizes interpreted for sex as 0-0.1 (negligible) and 0.1-0.3 (small); for competitive level and diet as 0-0.07 (negligible), 0.07-0.21 (small), and 0.21-0.35 (medium)

3.5 Discussion

The purpose of this study was to describe the prevalence and determinants of exercise performed in the overnight-fasted state among endurance athletes. To our knowledge, this is the first large-scale study (n = 1,950) characterising these habits and beliefs among endurance athletes across a range of ages, competitive levels, and nationalities. Novel findings of this study include: 1) 62.9% of endurance athletes report performing some or all of their training in the overnight-fasted state, 2) males perform fasted training more than females, 3) people following low-CHO or periodised-CHO diets perform more fasted training than those following other dietary patterns, and 4) many people perform fasted training because they think it helps their training, while others avoid fasted training because they don't think it helps their training, highlighting the need for more research on the effects of fasted training across a range of populations and levels of athlete.

Of the participants in this study, 62.9% reported performing some or all of their training in the overnight-fasted state. While 63-65% of non-professional athletes reported the use of fasted training sessions, only 29.4% of professional athletes reported they perform fasted training. This finding is in line with previous surveys of professional endurance athletes reporting 21-48% perform some training sessions in an overnight-fasted state (Heikura, et al., 2017; Heikura et al., 2018). Although these differences might be attributed to the higher training volumes of professional endurance athletes, in our survey there was no relationship between reported training volume (hours per week) and the use of fasted training. However, it is possible that the smaller sample size of professional athletes in our survey (n = 34) may not be truly representative of this population.

Differences in fasting practices between levels were echoed in responses pertaining to nutrition-exercise beliefs. For example, ~71% of professional athletes disagreed with the statement "The quality of my workout is the same whether I eat or do not eat beforehand", while only ~50% of the non-professional athletes disagreed, and ~79% of professional athletes disagreed with the statement "Skipping breakfast will allow me to get a better workout", compared with only 52-

60% of non-professional athletes (Table 3.4). This discrepancy may be related to differences in typical workout duration. If professionals workout longer than non-professionals, they might be expected to feel more hindered by fasted-state exercise. In support, a recent meta-analysis reported that fed-state exercise enhanced prolonged, but not shorter duration aerobic exercise performance compared to exercising in the fasted state [10].

Sex differences were found across many of the survey responses. Males reported more fasted training than females, while females more often felt it does not help their overall training and were more often told they should not perform exercise in the fasted state (Table 3.1). Although very little research has compared exercise in the overnight-fasted state between sexes, a 4-wk study in untrained participants found training-induced changes in mitochondrial enzymes were not different between fed and fasted groups but women had greater increases with fed training while men had greater increases with fasted training [44]. However, the claim that women should avoid fasted-state training is refuted by a 6-wk study in untrained females that found similar training-induced improvements among fed and fasted groups [155]. In light of the overall lack of sport nutrition research using females subjects [194], further research on sex differences in the response to fasted-state endurance training is warranted.

Our data highlight the disparity between the large number of athletes reporting the use of fasted training and the available research in trained athletes. Despite being recognised as a method of modulating CHO-availability [36], there is limited research looking at the effects of training (≥4 wk) in the fasted state on endurance adaptations using trained subjects [156]. Furthermore, nearly all of the studies using moderate-intensity continuous endurance training in the fasted state provided the non-fasted groups with very large meals (~120-160 g CHO) prior to exercise as well as CHO during exercise [43, 45]. Practical application is limited, as athletes might typically consume smaller meals before exercise, and CHO ingestion during exercise minimises differences between exercise in the CHO-fed or fasted state [137]. Therefore, research is needed using trained athletes looking at the effects of training in the overnight-fasted state compared with a breakfast that contains small to moderate amounts of CHO.

It is well-established that fat oxidation during submaximal endurance exercise can be increased by exercising in the fasted state [11] and/or by consuming a low-CHO diet [195]. Indeed, the most common reason for performing exercise in the overnight-fasted state in the present study was a desire to increase fat utilisation, and the low-CHO and periodised-CHO groups reported both the greatest amount of fasted training and the highest percentage of respondents selecting increased fat utilisation as a reason for doing fasted training. However, it should be acknowledged that longer-term changes in fat utilisation with fasted-state training are less clear and may depend on the testing conditions. For example, six weeks of fasted-state training increased the intensity at which maximal fat oxidation occurred more than training in a CHO-fed state [157], while another 6-wk study found no differences in fat oxidation rates when tested in a fed state while also providing additional CHO [45]. Although high capacity for fat oxidation is important during extended-duration endurance events [196], it is unclear how much this can be increased through fasted-state training. This uncertainty is reflected in the large number of athletes who believe fasted-state training is not beneficial or are unsure of its merit. Studies in trained athletes are needed to determine if longer-term changes in fat oxidation, and performance, following fasted-state training are relevant during typical racing conditions when competing in the fed state and consuming CHO. It would also be of interest to study fasted-state training both compared with, and as a part of, low-CHO, high-CHO, and nutritionally periodised dietary patterns.

Despite our large sample size, we acknowledge some limitations of this study largely related to the non-randomised and non-controlled responses to our survey. Due to a self-selection bias [197], it is possible that the respondents who choose to participate may not represent all endurance athletes. While it has been commonly observed that people under-report dietary intake [198, 199], the accuracy of self-reported training patterns and habitual pre-exercise nutrition intake is unclear. There has not been any assessment of survey reliability, however, the survey was reviewed by three nutrition and exercise science experts for face validity. Additionally, when adjusting for multiple comparisons across a large number of groups (e.g. four competitive levels and ten dietary patterns), p-values that might be expected to attain statistical significance (i.e. $p < 0.05$) may be unusually inflated and obscure significant differences. The small number of professional athletes also limits the statistical power to detect differences between all sub-groups, as does the low cell count for some of the diet-related variables. However, by default, professional athletes will always be a minority compared to other groups.

3.6 Conclusion

To our knowledge, this is the first large-scale study looking at the habits and beliefs related to exercising in the overnight-fasted state in endurance athletes across a range of ages, competitive levels, and nationalities. Nearly two-thirds (62.9%) of endurance athletes perform training sessions in the overnight-fasted state, but the frequency and reasons for doing so differ based upon sex, competitive level, and habitual dietary pattern. This study offers insight for coaches, sports scientists, and nutritionists into the beliefs and practices related to fasted-state endurance training. Males, non-professionals, and those following lower-CHO or periodised-CHO diets are most likely to perform exercise in the overnight-fasted state. This study also highlights conflicts among athletic sub-groups around why fasted training is or isn’t beneficial, demonstrating the need for further research in the area.

4. Pre-Exercise Nutrition Habits and Beliefs of Endurance Athletes Vary by Sex, Competitive Level, and Diet

This chapter investigates the self-reported beliefs and practices relating to pre-exercise nutrition intake among endurance athletes of varying ages and competitive levels, focusing on differences based on sex, competitive level, and habitual dietary pattern.

This chapter contains the following publication:

Rothschild, J. A., Kilding, A. E., & Plews, D. J. (2021). Pre-exercise nutrition habits and beliefs of endurance athletes vary by sex, competitive level, and diet. Journal of the American College of Nutrition, 40(6), 517-528.

4.1. Abstract

Objective: The purpose of this study was to determine the self-reported beliefs and practices relating to pre-exercise nutrition intake among endurance athletes of varying ages and competitive levels, and examine differences based on sex, competitive level, and habitual dietary pattern.

Methods: An anonymous online survey was circulated internationally in English and completed by 1,950 athletes of varying competitive levels (51.0% female, mean age 40.9 years [range 18:78]). Survey questions included training background, determinants of pre-exercise nutrition intake and composition, and timing relative to exercise.

Results: Prior to morning exercise, 36.4, 36.0, and 27.6% of athletes consume carbohydrate-containing food/drinks before almost every workout, some of the time, and never/rarely, respectively, with significant effects of sex ($p < 0.001$, Cramer's V (φ_c) = 0.15) and competitive level ($p < 0.001$, $\varphi_c = 0.09$). Nutritional intake before exercise varied based on workout duration for 47.6% of athletes, with significant effects of sex ($\varphi_c = 0.15$) and habitual diet ($\varphi_c = 0.19$), and based on workout intensity for 39.1% of athletes, with significant effects of sex ($\varphi_c = 0.13$) and habitual diet ($\varphi_c = 0.17$, all $p < 0.001$). Additionally, 89.0% of athletes reported using at least some type of dietary supplement (including caffeine from coffee/tea) within 1 h before exercise.

Conclusions: Overall, nearly all factors measured relating to pre-exercise nutrition intake varied by sex, competitive level, habitual dietary pattern and/or intensity/duration of the training session and suggest a large number of athletes may not be following current recommendations for optimizing endurance training adaptations.

4.2. Introduction

The type and amount of nutritional intake before an exercise session can affect the physiological responses that occur in response to the exercise stress [188]. For example, ingestion of carbohydrate (CHO) prior to exercise leads to a reduction in hepatic glucose output and an increase in skeletal muscle glucose uptake during exercise [59], along with a reduction in fat oxidation during low-to-moderate intensity exercise [11]. Pre-exercise CHO ingestion can also enhance prolonged endurance performance compared with exercising in the fasted state [200]. Although performance may be improved from pre-exercise CHO ingestion, exercise performed with reduced availability of muscle glycogen can increase the activation of key mitochondrial signaling proteins compared with exercise performed with normal glycogen concentration [189], potentially influencing longer-term training adaptations. In an attempt to optimize both training adaptations and acute performance during key training sessions, current sport nutrition guidelines suggest adjusting energy and CHO intake in response to an athlete's training schedule [180]. This can allow some training to be performed with high CHO-availability in order to enhance glycolytic and CHO-oxidation pathways, and some training to be performed with low CHO-availability to increase the activation of acute cell signaling pathways related to mitochondrial biogenesis and lipid oxidation [201].

In light of the above rationale, it is unclear to what degree pre-exercise nutrition practices vary among athletes on a day-to-day basis. A survey of elite runners and race walkers found that just 15% of athletes reported increasing energy intake within the 4 h prior to training sessions on hard training days, and only 16% reported reducing CHO intake before easy sessions [20]. A number of studies have analyzed the dietary intake and practices of endurance athletes, but have focused primarily on either macronutrient intake and meal frequency [183-186], nutrition before a single training session or race [34], or specific dietary components such as caffeine [202] or sodium intake [203], rather than on the overall dietary intake, beliefs, and practices related to pre-exercise nutritional intake.

Gaining a better understanding of how athletes are fueling prior to training can allow for relevant lab-based studies to be designed in an effort to optimize nutrition and training recommendations, and also inform areas where education for coaches and athletes might be needed. Therefore, the aim of this study is two-fold. First, to determine the self-reported beliefs and practices relating to pre-exercise nutrition intake among endurance athletes of varying ages and competitive levels. Secondly, to determine if differences in nutrition practices or beliefs related to sex, competitive level, or habitual dietary pattern exist.

4.3. Methods

Survey. The survey was developed collaboratively by the authors. It was reviewed for face validity by three sport nutrition experts, piloted among 15 athletes, and updated based on feedback received. The survey was conducted anonymously online, using Qualtrics software (Provo, UT, USA), and circulated internationally in English via social media and email. Data was collected between December 2019 and March 2020. Participants were asked between 40 and 47 questions, dependent upon logic functions that skipped irrelevant questions. The median response time to complete the survey was 10.4 min, which was below our 15-minute target [204]. Results presented herein are part of a larger survey on beliefs and practices related to pre-exercise nutrition intake in endurance athletes. The major themes explored in the survey are shown in Table 4.1. For the full survey see the online supplemental material. Findings specifically related to training in the overnight-fasted state are reported elsewhere [21]. Study protocols and materials were approved by the Auckland University of Technology Ethics Committee (19/415). Informed consent was provided by participants prior to beginning the survey.

Table 4.1. Overview of key themes of the questionnaire

I. Background	Demographics, competitive level, training history
II. Fasted training	Do you perform fasted training? Why/why not?
III. Determinants of breakfast content	Does it vary based on the duration, intensity, or mode of the workout?
IV. Pre-exercise intake	How often do you consume CHO-containing or low-CHO foods before training?
V. Effect of workout duration	When do you wake up and when do you eat before training that is shorter and longer than 90 min?
VI. Pre-exercise nutrition beliefs	Questions answered using a 5-pt Likert scale
VII. Dietary supplements	Which supplements do you consume within the 1 h before exercise?

The options for participants to report their competitive level included professional, elite non-professional (qualify and compete at the International level), high-level amateur (qualify and compete at National Championship-level events), amateur (enter races but don't expect to win), and recreational (train but do not participate in competition). For habitual dietary pattern, respondents could choose among no dietary plan, gluten-free, high-CHO (>50% dietary intake), high-protein, low-CHO (>2.2 g protein per kg body weight, less than 130 g CHO), low-CHO, high-fat (LCHF; < 130 g CHO), paleo (avoid grains, legumes, and dairy products), paleo for athletes (paleo with more carbs around training), periodized CHO (habitually lower CHO and then increase before key training, OR habitually high-CHO and then restrict before key training), pescatarian (vegetarian that also consume fish), vegan (avoid all animal products), and vegetarian (include some dairy or eggs).

Participants. Active endurance athletes were invited to participate if they were ≥ 18 years, and had been consistently training for at least one year, were regularly completing at least 4 exercise sessions per week and had participated in at least one organized endurance event of any distance at any time. The survey was completed by 1,950 participants (48.8% male, 51.0% female, 0.2% declined to give sex, mean age 40.9 years [range 18:78]) from 57 different countries. The primary sports represented were running (57.2%), triathlon (50.3%), cycling (36.5%), and swimming (18.3%). The self-reported competitive level included professional (n = 34, 1.7%), elite non-professional (n = 188, 9.6%), high-level amateur (n = 533, 27.3%), and amateur (n = 1195, 61.3%) athletes. The typical duration of the competitive events the respondents trained for was < 60 min (n = 183, 9.4%), 1-3 h (n = 592, 30.4%), 3-12 h (n = 1026, 52.6%), >12 h (n = 149, 7.6%). Weekly training volume and daily training distribution are shown in Figure S1.

Statistical analysis. Only responses from completed surveys were analyzed. Descriptive data are presented as number (n) and percentage (%) of responses. Categorical data were assessed by chi-square analysis and the Cramer's V (φ_c) effect size statistic with 90% confidence intervals (CI), using R and jamovi software [191, 192]. Effects are interpreted as small (0.1-0.3), medium (0.3-0.5), large (>0.5), however this categorization varied based on the degrees of freedom [193] and differences in interpretation are noted where applicable. Where significant differences were found, chi-square post hoc testing using Fisher's exact test was performed with the Fifer package in R software with Bonferroni corrections.

Participants were allowed to select more than one answer for several questions, such as which sports they trained for and which dietary patterns they followed. Therefore, the percentage of responses to some questions added up to more than 100%. Three participants chose "prefer not to say" for sex and were left out of analyses performed based on sex. For analyses based on competitive level, recreational and amateur were combined into a single group. For dietary analysis, paleo and paleo for athletes were combined into a single group. For analyses based on event duration athletes were grouped into four categories; those training for events <60 min, 1-3 h, 3-12 h and >12 h. A series of questions that asked how often the participants consume various supplements prior to exercise could be answered as never, sometime, or often/always. Responses for "sometimes" and "often/always" were combined for analysis and reported as consuming these things "at least some of the time".

4.4. Results

Habitual diet. Approximately half of the respondents (n = 957, 49.1%) reported they did not follow any specific dietary pattern. Males were more likely to follow LCHF and high-CHO dietary patterns, while females were more likely to follow vegetarian, pescatarian, and gluten-free dietary patterns (Table 4.2). The percentage of athletes following a periodized-CHO pattern increased with competitive level, from 8.0% of amateur athletes to 26.5% of professional athletes (Table 4.3). Amateur athletes were also more likely than elite athletes to follow a high-protein, low-CHO diet, and less likely than high-level amateurs to follow a high-CHO diet (Table 4.3).

Pre-workout nutrition intake. Overall, 62.9% (n = 1,227) of athletes reported performing at least some training sessions in the overnight-fasted state. Regarding macronutrient intake prior to a morning exercise session, 36.4% (n = 709) consume CHO-containing food or drinks before almost every workout, 36.0% (n = 702) consume CHO some of the time, and 27.6% (n = 539) never or rarely consume CHO before a workout (Fig. 4.1a). Carbohydrate consumption before exercise differed by sex ($p < 0.001$, $\varphi_c = 0.15$ [90%CI 0.12,0.19]), competitive level ($p < 0.001$, $\varphi_c = 0.09$ [90%CI 0.07,0.12], small effect), and habitual diet ($p < 0.001$, $\varphi_c = 0.31$ [90%CI 0.29,0.34], large effect; Fig. 4.2, Tables 4.2–4.4). In contrast, only 3.9% of athletes (n = 76) reported consuming low-CHO foods (e.g. eggs or protein powder) before almost every morning exercise session, 23.6% (n = 460) consume low-CHO foods before some workouts, and 72.5% (n = 1,414) report never consuming low-CHO foods before a morning exercise session, with sub-group differences seen among multiple dietary patterns ($p < 0.001$, $\varphi_c = 0.17$ [90%CI 0.14,0.19]; Fig. 4.1a, 4.2, Table 4.4).

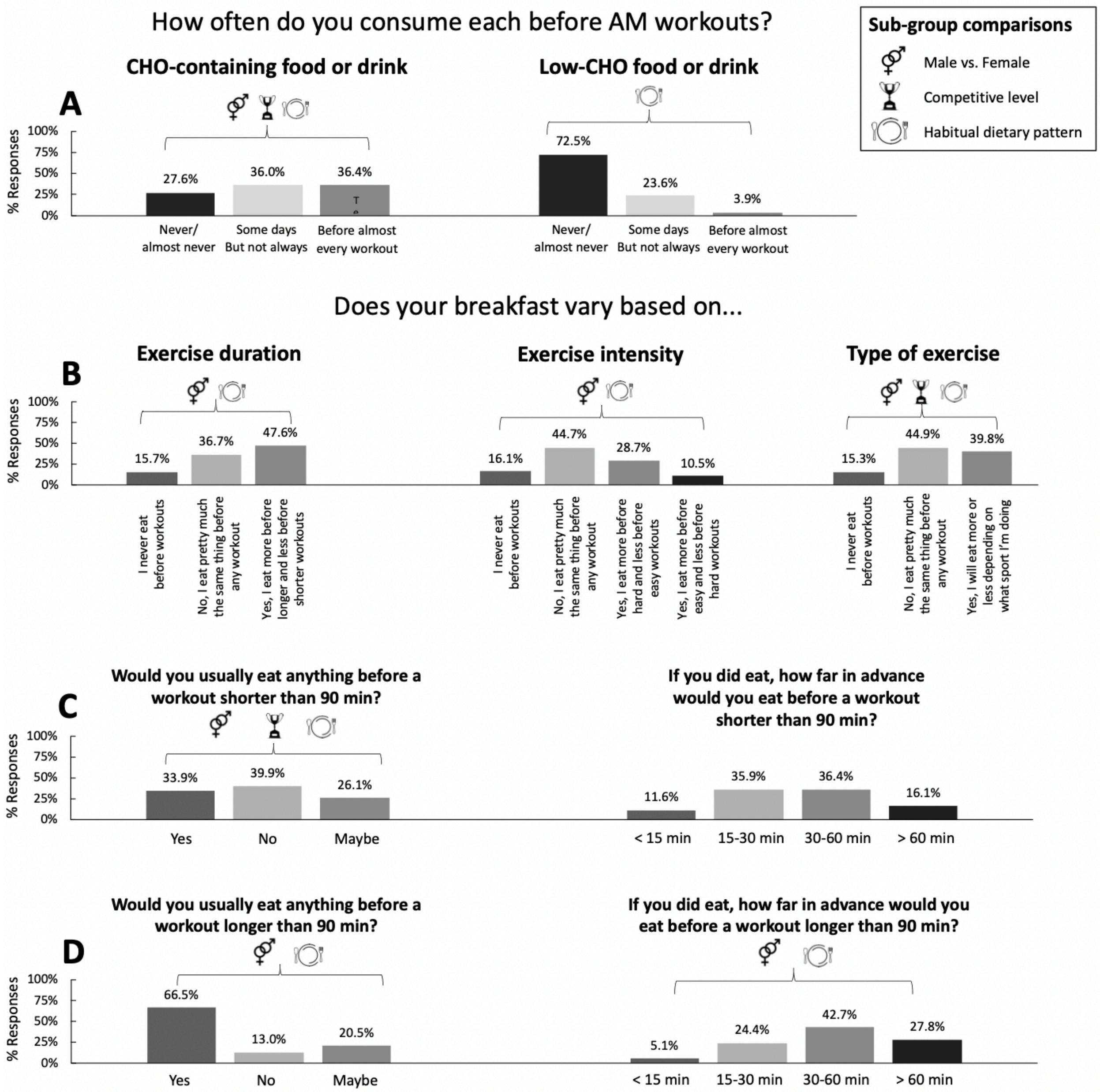

Figure 4.1. Frequency of various pre-exercise nutrition ingestion strategies (A), determinants of pre-exercise nutrition intake (B), and pre-exercise nutrition ingestion and food timing before workouts shorter than 90 min in duration (C) and longer than 90 min in duration (D) adopted by trained endurance athletes. Statistically significant ($p < 0.05$) sub-group interactions are depicted with symbols. N = 1,950. For (A), examples of low-CHO foods were provided in the survey question (e.g., eggs and avocado, or protein powder, without CHO such as bread, cereal, or juice).

In relation to the duration of a workout, 47.6% of athletes reported eating more before longer and less before shorter workouts, with significant effects of sex ($p < 0.001$, $\varphi_c = 0.15$ [90%CI 0.11,0.19]) and habitual diet ($p < 0.001$, $\varphi_c = 0.19$ [90%CI 0.17,0.22]; Fig. 4.1b, 4.2, Tables 4.2, 4.4).

Nutritional intake before exercise varied based on workout intensity for 39.1% of athletes, with significant effects of sex ($p < 0.001$, $\varphi_c = 0.13$ [90%CI 0.10,0.17]) and habitual diet ($p < 0.001$, $\varphi_c = 0.17$ [90%CI 0.15,0.19]; Fig. 4.1b, 4.2, Tables 4.2, 4.4). The modality of the workout (i.e. swim vs. bike, etc.) affected the pre-exercise intake for 39.8% of athletes with significant effects of sex ($p < 0.001$, $\varphi_c = 0.17$ [90%CI 0.14,0.21]), competitive level ($p = 0.002$, $\varphi_c = 0.07$ [90%CI 0.05,0.10], small effect), and diet ($p < 0.001$, $\varphi_c = 0.20$ [90%CI 0.17,0.22]; Fig. 4.1b, 4.2, Tables 4.2–4.4).

Table 4.2. Sex differences in pre-exercise nutrition practices of endurance athletes

	Male	Female	Total	p-value, Cramer's V [90% Confidence Interval]
Completed responses	48.9%	51.1%	n = 1,947	
How often do you consume carbohydrate-containing food or drinks prior to a morning exercise session?				**p < 0.001, φc = 0.15 [90%CI 0.12,0.19]**
Before almost every workout	29.8%	42.5%	36.3%	
Some days but not always	36.8%	35.3%	27.7%	
Never or almost never	33.4%	22.2%	36.0%	
How often do you consume a low-carbohydrate food or drinks prior to a morning exercise session?				p = 0.063, φc = 0.05 [90%CI 0.03,0.09]
Before almost every workout	4.0%	3.8%	3.9%	
Some days but not always	25.8%	21.4%	23.6%	
Never or almost never	74.8%	70.2%	72.5%	
Does your breakfast vary based on workout duration?				**p < 0.001, φc = 0.15 [90%CI 0.11,0.19]**
I never eat so I don't care	20.2%	11.5%	15.7%	
No, I eat the same thing	38.8%	34.6%	36.6%	
Yes, I eat more before longer workouts	41.1%	54.0%	47.7%	
Does your breakfast vary based on workout intensity?				**p < 0.001, φc = 0.13 [90%CI 0.10,0.17]**
I never eat so I don't care	20.5%	12.0%	16.1%	
No, I eat the same thing	44.9%	44.4%	44.6%	
Yes, I eat less before hard workouts and more before easy	9.7%	11.3%	10.5%	
Yes, I eat more before hard workouts and less before easy	25.0%	32.4%	28.8%	
Does your breakfast vary based on workout type/sport?				**p < 0.001, φc = 0.17 [90%CI 0.14,0.21]**
I never eat so I don't care	20.4%	10.5%	15.3%	
No, I eat the same thing	47.0%	42.8%	44.8%	
Yes, it varies	32.7%	46.7%	39.9%	
Habitual dietary pattern				
No dietary plan	47.8%	50.2%	49.0%	p = 0.298, φc = 0.02 [90%CI 0.0,0.06]
LCHF	13.6%	6.2%	9.8%	**p < 0.001, φc = 0.12 [90%CI 0.09,0.16]**
Periodised carb	11.0%	8.6%	9.8%	p = 0.077, φc = 0.04 [90%CI 0.02,0.08]
Vegetarian	5.8%	8.2%	7.0%	**p = 0.034, φc = 0.05 [90%CI 0.02,0.09]**
High-CHO	10.4%	3.8%	7.0%	**p < 0.001, φc = 0.13 [90%CI 0.09,0.17]**
Pescatarian	4.4%	8.0%	6.3%	**p < 0.001, φc = 0.07 [90%CI 0.04,0.11]**
Gluten-free	2.4%	9.4%	6.0%	**p < 0.001, φc = 0.15 [90%CI 0.11,0.19]**
High-protein, low-carb	5.3%	6.1%	5.7%	p = 0.403, φc = 0.02 [90%CI 0.0,0.06]
Paleo	3.5%	5.0%	4.3%	p = 0.089, φc = 0.04 [90%CI 0.0,0.08]
Vegan	3.9%	4.5%	4.2%	p = 0.485, φc = 0.02 [90%CI 0.0,0.05]

Significant interactions ($p < 0.05$) in bold. Percentages are based on the number of responses for each column. CHO: Carbohydrate; LCHF: Low-CHO, high-fat.

Table 4.3. Differences in pre-exercise nutrition practices of endurance athletes based on competitive level

	Amateur	High-level amateur	Elite non-professional	Professional	Total	p-value, Cramer's V [90% Confidence Interval]	Post hoc comparisons
Completed responses by sex							
Male	45.5%	55.2%	53.7%	38.2%	48.8%	**$p < 0.001$, $\varphi c = 0.09$ [90%CI 0.07,0.12]**	Amateur and HLA different
Female	54.4%	44.7%	46.3%	58.8%	51.0%		
Prefer not to say	0.1%	0.2%	0.0%	2.9%	0.2%		
Total[1]	n = 1,195 (61.3%)	n = 533 (27.3%)	n = 188 (9.6%)	n = 34 (1.7%)	n = 1950		
How often do you consume carbohydrate-containing food or drinks prior to a morning exercise session?							
Before almost every workout	33.7%	37.7%	42.6%	73.5%	36.4%	**$p < 0.001$, $\varphi c = 0.09$ [90%CI 0.07,0.12]**	Pro vs. all others
Some days but not always	36.0%	38.8%	31.4%	17.6%	36.0%		
Never or almost never	30.3%	23.5%	26.1%	8.8%	27.6%		
How often do you consume low-carbohydrate food or drinks prior to a morning exercise session?							
Before almost every workout	4.5%	3.0%	3.2%	0.0%	3.9%	**$p = 0.037$, $\varphi c = 0.06$ [90%CI 0.04,0.08]**	post hoc NS
Some days but not always	23.3%	21.8%	26.6%	44.1%	23.6%		
Never or almost never	72.1%	75.2%	70.2%	55.9%	72.5%		
Does your breakfast vary based on workout duration?							
I never eat so I don't care	17.1%	14.1%	14.4%	0.0%	15.7%	$p = 0.066$, $\varphi c = 0.05$ [90%CI 0.01,0.09]	
No, I eat the same thing	36.2%	36.8%	39.4%	38.2%	36.7%		
Yes, I eat more before longer workouts	46.7%	49.2%	46.3%	61.8%	47.6%		
Does your breakfast vary based on workout intensity?							
I never eat so I don't care	17.7%	14.4%	13.3%	2.9%	16.1%	**$p = 0.012$, $\varphi c = 0.06$ [90%CI 0.04,0.08]**	post hoc NS
No, I eat the same thing	45.5%	44.3%	41.0%	44.1%	44.7%		
Yes, I eat less before hard workouts and more before easy	9.3%	10.7%	17.0%	11.8%	10.5%		
Yes, I eat more before hard workouts and less before easy	27.5%	30.6%	28.7%	41.2%	28.7%		
Does your breakfast vary based on workout type/sport?							
I never eat so I don't care	16.7%	14.3%	12.2%	0.0%	15.3%	**$p = 0.002$, $\varphi c = 0.07$ [90%CI 0.05,0.10]**	Amateur vs HLA, Pro
No, I eat the same thing	46.7%	41.5%	42.0%	50.0%	44.9%		

Yes, it varies	36.7%	44.3%	45.7%	50.0%	39.8%		
Habitual dietary pattern							
No dietary plan	49.5%	49.2%	47.3%	41.2%	49.1%	p = 0.758, φc = 0.02 [90%CI 0.0,0.06]	
LCHF	10.4%	7.7%	13.8%	0.0%	9.8%	**p = 0.012, φc = 0.07 [90%CI 0.05,0.11]**	post hoc NS
Periodised carb	8.0%	10.1%	17.0%	26.5%	9.8%	**p < 0.001, φc = 0.12 [90%CI 0.08,0.15]**	Amateur < Elite and Pro; HLA < Pro
Vegetarian	6.3%	8.3%	8.0%	8.8%	7.0%	p = 0.370, φc = 0.04 [90%CI 0.0,0.07]	
High-CHO	5.4%	9.9%	8.5%	11.8%	7.0%	**p = 0.002, φc = 0.08 [90%CI 0.05,0.12]**	Amateur < HLA
Pescatarian	6.9%	6.6%	1.6%	2.9%	6.3%	**p = 0.017, φc = 0.07 [90%CI 0.04,0.11]**	Elite < Amateur and HLA
Gluten-free	6.0%	5.6%	5.3%	14.7%	6.0%	p = 0.215, φc = 0.05 [90%CI 0.01,0.09]	
High-protein, low-carb	6.4%	5.6%	1.6%	2.9%	5.7%	**p = 0.033, φc = 0.06 [90%CI 0.03,0.1]**	Amateur > Elite
Paleo	4.8%	3.6%	3.7%	0.0%	4.3%	p = 0.519, φc = 0.04 [90%CI 0.0,0.08]	
Vegan	3.7%	5.4%	4.8%	0.0%	4.2%	p = 0.247, φc = 0.05 [90%CI 0.01,0.09]	

Significant interactions (p < 0.05) in bold. Percentages are based on the number of responses for each column except [1], where percentages are based on the total across the row. CHO: Carbohydrate; HLA: high-level amateur; LCHF: Low-CHO, high-fat; NS: not statistically significant; Pro: professional.

Table 4.4. Differences in pre-exercise nutrition practices of endurance athletes based on habitual dietary pattern

	No dietary plan	LCHF	Periodised carb	Vegetarian	High-CHO	Pescatarian	Gluten-free	High-protein, low-carb	Paleo	Vegan	p-value, Cramer's V [90% Confidence Interval]	Post hoc differences
Total responses[1]	n = 957 (49.1%)	n = 191 (9.8%)	n = 191 (9.8%)	n = 137 (7.0%)	n = 137 (7.0%)	n = 122 (6.3%)	n = 117 (6.0%)	n = 111 (5.7%)	n = 83 (4.3%)	n = 82 (4.2%)		
How often do you consume carbohydrate-containing food or drinks prior to a morning exercise session?											**p <0.001, ϕc = 0.31 [90%CI 0.29,0.34]**	NP vs. PC; LCHF vs. all others; PC vs. Veg, HC; HC vs. GF, PAL; HP vs. all except PAL
Before almost every workout	42.3%	1.6%	21.5%	44.5%	54.7%	34.4%	29.9%	18.0%	28.9%	36.6%		
Some days but not always	36.3%	16.2%	54.5%	40.1%	29.9%	41.8%	42.7%	29.7%	36.1%	42.7%		
Never or almost never	21.4%	82.2%	24.1%	15.3%	15.3%	23.8%	27.4%	52.3%	34.9%	20.7%		
How often do you consume low-carbohydrate food or drinks prior to a morning exercise session?											**p <0.001, ϕc = 0.17 [90%CI 0.14,0.19]**	NP vs. LCHF, PC, GF, HP, paleo; LCHF vs. Veg, HC, Pesc; PC vs. Veg, HC, Pesc; Veg vs. GF, HP; HC vs. GF, HP, Paleo;
Before almost every workout	2.6%	7.9%	5.8%	0.7%	1.5%	1.6%	10.3%	12.6%	8.4%	2.4%		
Some days but not always	20.7%	36.1%	38.7%	20.4%	16.8%	20.5%	28.2%	26.1%	31.3%	19.5%		
Never or almost never	76.7%	56.0%	55.5%	78.8%	81.8%	77.9%	61.5%	61.3%	60.2%	78.0%		
Does your breakfast vary based on workout duration?											**p <0.001, ϕc = 0.19 [90%CI 0.17,0.22]**	HP vs. NP, PC, HC; LCHF vs. all others
I never eat so I don't care	12.9%	44.0%	10.5%	13.9%	9.5%	15.6%	11.1%	27.9%	18.1%	13.4%		
No, I eat the same thing	38.9%	33.5%	32.5%	36.5%	34.3%	29.5%	38.5%	27.9%	31.3%	41.5%		
Yes, I eat more before longer workouts	48.3%	22.5%	57.1%	49.6%	56.2%	54.9%	50.4%	44.1%	50.6%	45.1%		
Does your breakfast vary based on workout intensity?											**p <0.001, ϕc = 0.17 [90%CI 0.15,0.19]**	NP vs. PC, HP; LCHF vs. all except HP; PC vs. HP
I never eat so I don't care	13.5%	44.0%	10.5%	13.9%	10.2%	14.8%	8.5%	30.6%	15.7%	14.6%		
No, I eat the same thing	47.9%	32.5%	38.7%	40.9%	45.3%	41.0%	51.3%	36.0%	39.8%	45.1%		
Yes, I eat less before hard workouts and more before easy	12.2%	2.6%	5.8%)	10.9%	13.1%	13.1%	12.0%	6.3%	9.6%	9.8%		
Yes, I eat more before hard workouts and less before easy	26.4%	20.9%	45.0%	34.3%	31.4%	31.1%	28.2%	27.0%	34.9%	30.5%		
Does your breakfast vary based on workout type/sport?											**p <0.001, ϕc = 0.20 [90%CI 0.17,0.22]**	
I never eat so I don't care	12.7%	44.5%	9.4%	12.4%	8.8%	13.9%	7.7%	27.9%	13.3%	13.4%		

No, I eat the same thing	47.0%	34.0%	41.9%	46.7%	44.5%	38.5%	48.7%	38.7%	50.6%	47.6%		NP vs. HP; LCHF vs. all except HP; HP vs. PC, HC, GF
Yes, it varies	40.2%	21.5%	48.7%	40.9%	46.7%	47.5%	43.6%	33.3%	36.1%	39.0%		

Significant interactions ($p < 0.05$) in bold. Percentages are based on the number of responses for each column except [1], where percentages are based on the total across the row. Dietary pattern abbreviations – CHO: carbohydrate; LCHF: low-carb, high-fat; GF: gluten-free; HC: high-carbohydrate; HP: high-protein, low-carb; NP: no dietary plan; PAL: paleo; PC: periodised carbohydrate; PESC: pescatarian; VEG: vegetarian; VGN: vegan.

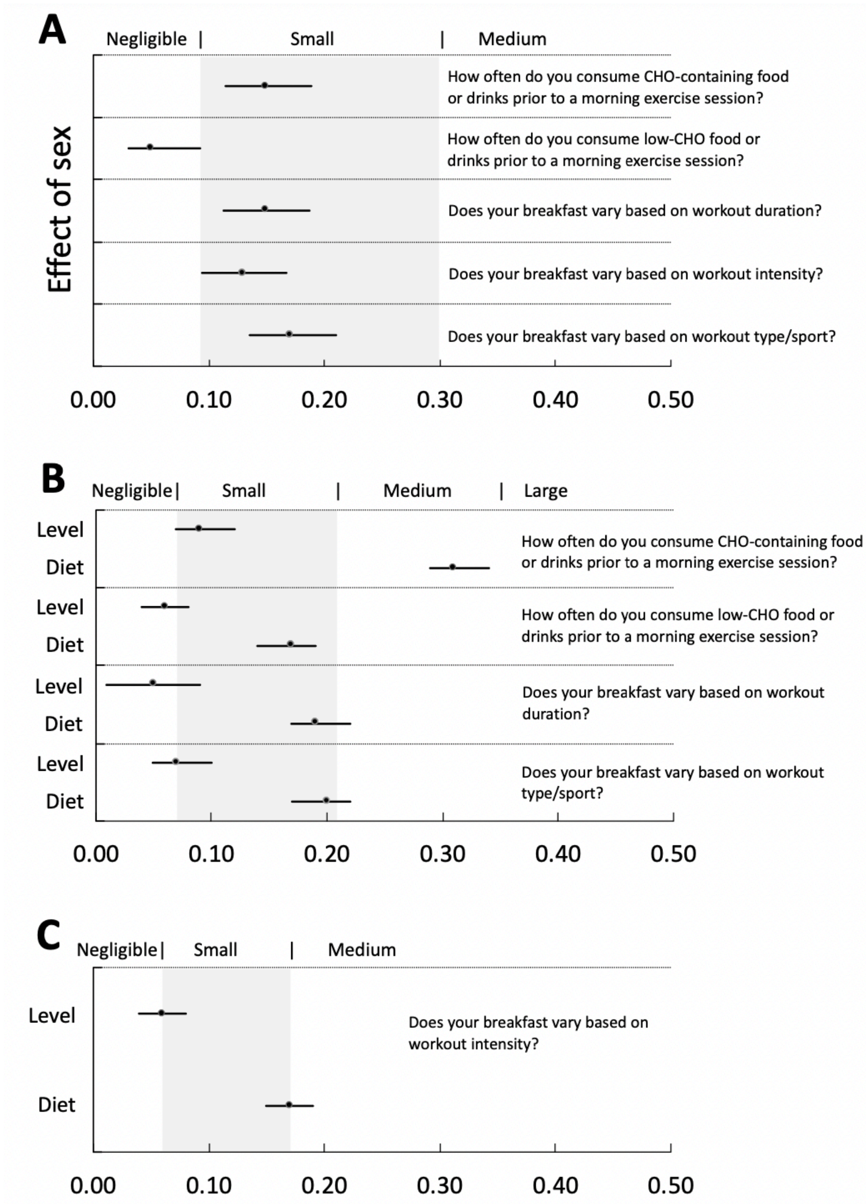

Figure 4.2. Sub-group interactions (as Cramer's V effect size with 90% Confidence Interval) for effects of sex (A), and for level and diet (B and C). Shaded areas represent a "small" effect, to the right of each shaded area represents a "medium" effect and to the left a negligible effect. Interpretations of magnitude are varied based on differing degrees of freedom [193].

For workouts *shorter* than 90 mins, responses for whether or not the athlete would usually eat were split between yes (33.9%), maybe (26.1%) and no (39.9%), with significant effects of sex (p <0.001, φ_c = 0.13 [90% CI 0.10,0.17]), competitive level (p = 0.027, φ_c = 0.06 [90%CI 0.04,0.09]), and habitual diet (p <0.001, φ_c = 0.21 [90% CI 0.18,0.23]; Fig. 4.1c, Table S4.1). The majority of athletes (72.0%) reported waking up and eating between 15-60 mins before training (Fig. 4.1c, Tables S4.2,S4.3). For workouts *longer* than 90 mins, 66.5% of athletes reported they would eat before training, 20.5% might eat, and 13.0% would not eat, with significant effects of sex ($p < 0.001$, $\varphi_c = 0.20$ [90% CI 0.16,0.23]) and habitual diet ($p < 0.001$, $\varphi_c = 0.24$ [90% CI 0.22,0.27]; Fig. 1d, Table S4.1). Sub-group interactions were also found between wake-up time and sex ($p < 0.001$, $\varphi_c = 0.10$ [90% CI 0.07,0.14]), level ($p = 0.005$, $\varphi_c = 0.06$ [90% CI 0.03,0.10]), and habitual diet ($p = 0.009$, $\varphi_c = 0.08$ [90% CI 0.05,0.12]), and between how far in advance the athlete would eat and both sex ($p = 0.009$, $\varphi_c = 0.08$ [90% CI 0.04,0.12]) and habitual diet ($p = 0.019$, $\varphi_c = 0.09$ [90% CI 0.05, 0.13]; Tables S4.2, S4.3). Additionally, significant sub-group effects of habitual diet were found when asked if they agree with the statement "If I have an early morning workout, I will always wake up early so that I have enough time to eat a full breakfast beforehand" ($p < 0.001$, $\varphi_c = 0.15$ [90% CI 0.11, 0.18]). Athletes on a high-CHO diet were most likely to agree (43.1%), while those following LCHF were least likely to agree (8.9%, Table S4.2).

Supplementation. Overall, 89.0% of athletes (n = 1,735) reported using at least some type of dietary supplement (including caffeine from coffee/tea) within 1 h before exercise (Fig. 4.3). Males more often use dietary nitrate, beta-alanine, vitamin E, vitamin C, creatine, sodium bicarbonate, and other caffeine sources (e.g. from pre-workout drink or caffeine supplement), while females reported greater use of protein powder and branched chain amino acid/essential amino acid (BCAA/EAA) supplements (all $p < 0.05$, Table S4.4, Fig. 4.3). For competitive level the largest effects were seen for beta-alanine and sodium bicarbonate, with usage increasing at each level from 4.8% and 2.7% of amateurs to 35.3% and 14.7% of professional athletes, respectively (Table S4.4). The largest effects of habitual diet were seen for protein powder and vitamin B12, with periodized-CHO using protein powder most often (48.2%) and vegans using the most vitamin B12 (34.1%; Table S4.4). Other supplements reported to be taken prior to exercise included vitamin D (2.5%), iron (1.3%), medium-chain triglycerides (0.8%), l-carnitine (0.7%), probiotics (0.5%), and curcumin (0.5%).

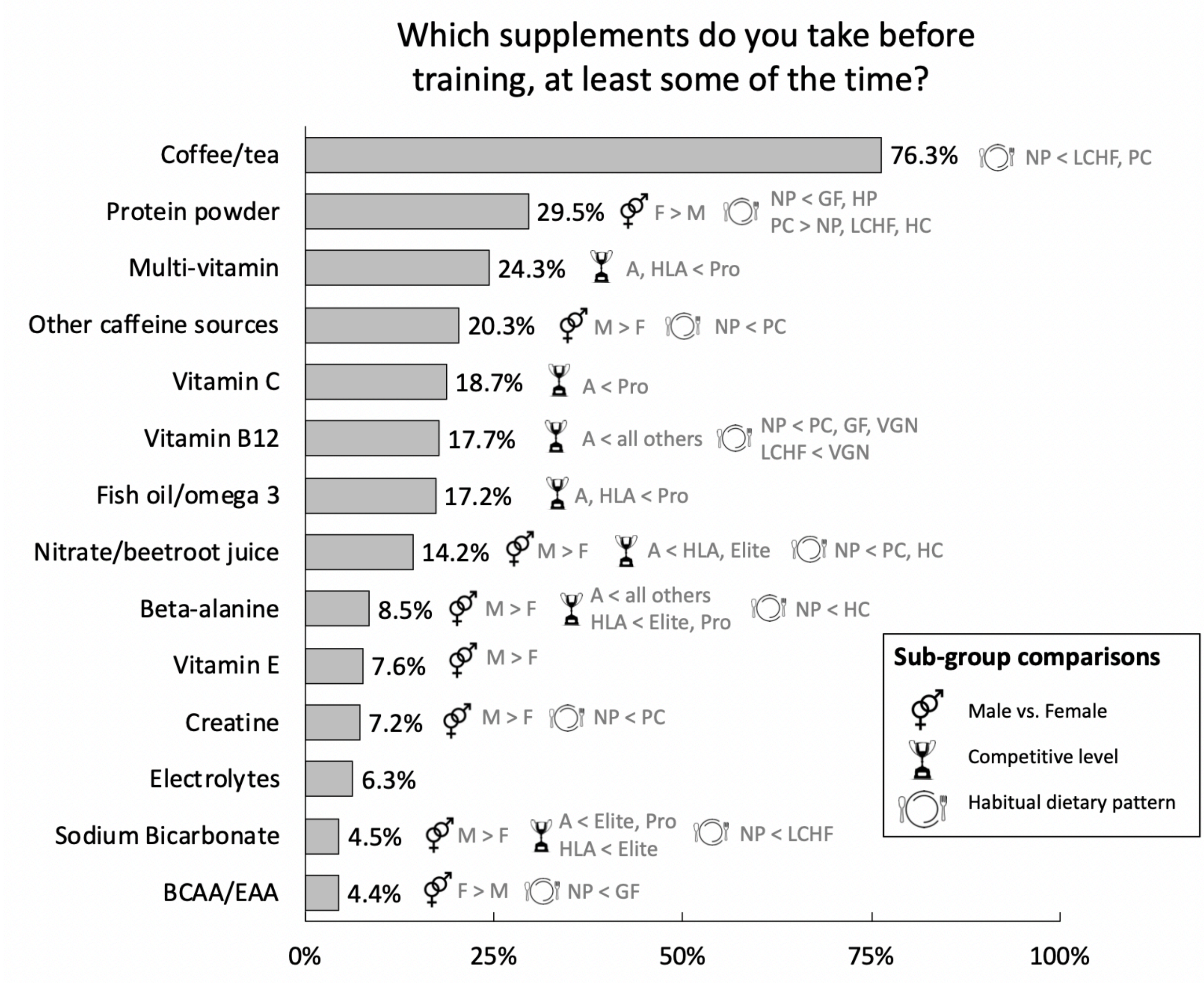

Figure 4.3. Supplements consumed by endurance athletes (N = 1,950) within 1 h before exercise, at least some of the time. Significant differences between sub-groups ($p < 0.05$) are depicted with symbols and a direction of the differences. Detailed analyses are shown in Table S4. BCAA/EAA: Branched chain amino acid/essential amino acids. Sex abbreviations - F: Female; M: Male. Competitive level abbreviations – A: Amateur; HLA: high-level amateur; Elite: elite non-professional; Pro: professional. Dietary pattern abbreviations – LCHF: low-carb, high-fat; GF: gluten-free; HC: high-carbohydrate; HP: high-protein, low-carb; NP: no dietary plan; PAL: Paleo; PC: periodised carbohydrate; PESC: pescatarian; VEG: vegetarian; VGN: vegan.

4.5. Discussion

To our knowledge, this is the first large-scale study describing the daily pre-exercise nutrition practices across a wide sample of endurance athletes. There are several novel findings of this study, including: 1) less than half of athletes vary their pre-exercise nutrition choices based on workout duration, workout intensity, or mode of workout, and 2) nearly all factors measured relating to pre-exercise nutrition intake vary by sex, competitive, level, and/or habitual dietary pattern.

Current sport nutrition guidelines suggest adjusting energy and CHO intake in response to an athlete's training schedule [180]. However, in our survey only 9.8% of athletes reported following a periodized-CHO dietary pattern (adjusting CHO intake based on their planned training sessions), and less than half of respondents reported eating differently depending on the duration (47.6%) or intensity (39.2%) of the upcoming morning exercise session. Similar to other surveys of elite runners and race walkers who are largely not following current recommendations of varying their pre-exercise nutrition [19, 20], it appears that athletes and coaches may benefit from additional education related to modifying pre-exercise nutrition choices before different types of workouts. Although laboratory-based research in non-athletes has shown an 11% increase in energy intake when provided an *ad libitum* meal before a planned session of aerobic exercise [182], it is possible that many athletes who train regularly may not make the same planned adjustments. At the same time, it is also possible that some athletes in this survey may indeed alter their pre-exercise nutrition without realizing it.

A large number of athletes (62.9%) reported performing at least some training sessions in the overnight-fasted state. For athletes doing a high volume of training, performing exercise in the fasted state could more likely lead to a negative energy balance, which can be associated with hormonal and immune dysfunction [14]. An alternative to performing training sessions in the fasted state is to consume a breakfast that includes protein but not CHO. This can reduce feelings of hunger while potentially reducing muscle protein breakdown and still allow high levels of fat oxidation during exercise [16, 18]. Recent research has shown that fat oxidation was not different during submaximal exercise following the ingestion of whey protein compared with a placebo [16], suggesting that exercise performed following a protein-rich breakfast may result in similar intra-muscular signaling compared with exercise performed in the fasted state. However, only 27.5% of respondents reported ever performing exercise following a low-CHO breakfast. Although the majority of research looking at pre-exercise nutrition has focused on CHO, the use of pre-exercise protein represents an interesting and under-researched opportunity. Future studies should examine the effects of a protein-rich breakfast compared with a CHO-rich breakfast as well as with exercise performed in the overnight-fasted state, consumed prior to both low and high-intensity endurance exercise.

We also found that 49.1% of athletes do not habitually follow any type of specific dietary pattern, while the most commonly consumed dietary patterns were LCHF (9.8%) and periodized-CHO (9.8%). This is similar to a survey of elite runners and race walkers reporting that 50% of athletes did not follow a unique dietary pattern, however in that study the most common dietary patterns were periodized-CHO (22%), high-CHO (13%), and gluten-free (10%) [20]. A study in recreational marathon runners found that 67% of participants did not follow any specific dietary pattern, while the most common patterns were vegetarian/vegan/ pescatarian (9%), Paleo (4%), gluten-free (4%), and low-CHO (3%) [205]. Dietary intake can impact adaptations to training by increasing the exercise stimuli and/or affecting the cellular responses to exercise-induced perturbations [206]. Indeed, intervention studies have shown more favorable effects of high-CHO and periodized-CHO diets, compared with a low-CHO diet in elite athletes [174], however no differences between low- and moderate-CHO diets in moderately-trained athletes has also been reported [207, 208]. Future studies should examine the effect of training status in the responses to diet-training interventions.

A secondary aim of this study was to determine if there are differences in nutrition practices related to sex, competitive level, or habitual dietary pattern. We found a number of significant sub-group interactions, which is in accordance with previous reports of differences in nutrition knowledge and habits based on the sex [183, 209, 210] and caliber [210-212] of athletes. In our survey males more-often followed LCHF and high-CHO dietary patterns, while females more-often followed vegetarian, pescatarian, and gluten-free dietary patterns (Table 2). However, females were more likely to consume CHO-containing foods before exercise, and also more likely than males to adjust their pre-exercise intake based on the duration, intensity, or mode of exercise. The finding that males were more likely to follow a higher-CHO diet has been previously reported among some [20, 183, 213] but not all [33, 34, 214] athletic populations. We found no sex differences among athletes who reported not following any specific dietary pattern, which is in contrast with research in recreational athletes that found females were more likely to follow a specific dietary pattern than males [205]. Analysis by competitive level revealed a number of significant effects, however with the exception of dietary supplement usage (discussed below), the effect sizes were negligible to small (Table 4.3, Fig. 4.2). Professional athletes were most likely to report consuming CHO before every training session and reported the greatest use of a periodized-CHO diet. As shown in Figure 4.2, sex and habitual dietary pattern appear to influence pre-exercise nutrition choices to a greater degree than competitive level.

A noteworthy observation is that the responses to nearly every question differed based on habitual dietary pattern. The largest differences were seen among athletes following a LCHF diet, who responded differently than all other groups when asked if they would eat prior to both short (< 90 min) and long (> 90 min) workouts, and if they varied their breakfast based on either the duration or intensity of the upcoming workout. In light of the large differences in habits and beliefs related to pre-exercise nutrition intake, researchers may wish to consider habitual dietary pattern, in addition to sex and competitive level, when planning and recruiting participants for nutrition-based exercise research.

Dietary supplements also have the potential to modify the adaptive response to endurance training (either positively or negatively), by affecting acid–base balance, oxidant signaling, or cumulative training load, all of which can impact the cellular signaling responses to training [215]. A number of sex differences were found with regard to supplementation, with males more often reporting the use of dietary nitrate, beta-alanine, vitamin E, vitamin C, creatine, sodium bicarbonate, and non-coffee caffeine sources, while females reported greater use of protein powder and BCAA/EAA supplements. Previous studies have shown vitamin E, protein, and creatine are more likely to be used by men [216], which is in line with our findings with the exception of females in our study reporting the use of protein powder more often than men.

Supplements with the greatest potential to augment the adaptative response include sodium bicarbonate, beta-alanine, and dietary nitrate/beetroot juice [215]. In our survey these supplements were used by only a small percentage of athletes, however higher-level athletes used them more than lower-level athletes (Fig. 4.3, Table S4.4). This is in accordance with previous findings that elite athletes use supplements more than non-elite athletes [216]. It is notable that 18.1% of athletes (including 41.2% of professionals) reported using vitamin C prior to training sessions, which has unclear evidence of benefit and some evidence that it may even blunt some favorable adaptations to endurance training [217].

Caffeine ingestion (from coffee or other caffeine sources) before at least some training sessions was reported by 81.2% of the respondents. Caffeine is well-established to improve endurance performance [218], and has been shown to attenuate reductions in power during training sessions performed with low CHO-availability [219]. Training studies looking at caffeine and longer-term endurance adaptations are lacking, though caffeine has been shown to augment adaptations to resistance training [220]. Research into the effects of caffeine ingestion on longer-term endurance training adaptations is warranted.

Interestingly, supplementation was often misaligned with the sub-groups who might benefit from them the most. For example, although females and vegetarians have reduced stores of muscle carnosine [221], in our survey they had among the lowest usage of beta-alanine; a supplement which can raise intramuscular carnosine levels (Stellingwerff et al., 2012; Table S4). Vegetarians may also respond better than omnivores to creatine supplementation [223], yet in our survey vegetarians and vegans had below-average supplementation rates. However, caution must be used when considering dietary supplement intake, as we were specifically asking about what people are taking during the 1 h pre-exercise window and are likely underestimating the true prevalence of each supplement.

Limitations of this study are largely related to the non-randomized and non-controlled responses. The use of qualitative questions (i.e. answering “some days” or “often/always” rather than a specific number of times per week or per month) may have been subject to the different interpretations by the individual respondents. While dietary intake is often mis-reported [198, 199], the accuracy of self-reported training patterns and habitual strategies relating to pre-exercise intake nutrition intake is unclear. Due to a self-selection bias [197], it is also possible that the respondents who chose to participate may not represent all endurance athletes. The wide range of participant ages may have also influenced the findings, as food preferences tend to change with age [224]. Additionally, when making comparisons across a large number of groups (e.g. four competitive levels and ten dietary patterns), significant between-group differences might have been obscured by inflated p-values due to the Bonferroni correction factors.

4.6. Conclusion

To our knowledge, this is the first large-scale study describing the pre-exercise nutrition practices across a wide sample of endurance athletes. Fewer than half of the athletes surveyed vary their pre-exercise nutrition choices based on workout duration, workout intensity, or mode of workout, and nearly all factors measured relating to pre-exercise nutrition intake varied by sex, competitive, level, and/or habitual dietary pattern. Furthermore, a large number of athletes may not be following optimal dietary or supplementation strategies for maximizing endurance training adaptations. This research offers insight for coaches, sports scientists, and nutritionists into the beliefs and practices related to the pre-exercise nutrition choices of endurance athletes. This study also highlights the need for a better understanding of how pre-exercise nutrition should alter for different training intensities and durations.

5. Pre-Exercise Carbohydrate or Protein Ingestion Influences Substrate Oxidation but Not Performance or Hunger Compared with Cycling in the Fasted State

The survey responses (Chapters 3–4) revealed several discordant beliefs among endurance athletes relating to the influence of pre-exercise nutrition choices on exercise capacity, fat utilization during exercise, and hunger. In addition, very few athletes reported ever consuming a low-carbohydrate (protein-rich) meal prior to training, despite emerging research suggesting potential utility for athletes who don't want to train in a fasted state. Accordingly, this chapter examines the effects of three pre-exercise nutrition strategies (carbohydrate-rich breakfast, protein-rich breakfast, or fasting) on metabolism, exercise capacity, hunger, and oxidative stress during cycling.

This chapter contains the following publication:

Rothschild, J. A., Kilding, A. E., Broome, S. C., Stewart, T., Cronin, J. B., & Plews, D. J. (2021). Pre-exercise carbohydrate or protein ingestion influences substrate oxidation but not performance or hunger compared with cycling in the fasted state. Nutrients, 13(4), 1291.

5.1 Abstract

Nutritional intake can influence exercise metabolism and performance, but there is a lack of research comparing protein-rich pre-exercise meals with endurance exercise performed both in the fasted state and following a carbohydrate-rich breakfast. The purpose of this study was to determine the effects of three pre-exercise nutrition strategies on metabolism and exercise capacity during cycling. On three occasions, seventeen trained male cyclists (VO_{2peak} 62.2 ± 5.8 mL·kg^{-1}·min-1, 31.2 ± 12.4 years, 74.8 ± 9.6 kg) performed twenty minutes of submaximal cycling (4 × 5 min stages at 60%, 80%, and 100% of ventilatory threshold (VT), and 20% of the difference between power at the VT and peak power), followed by 3 × 3 min intervals at 80% peak aerobic power and 3 × 3 min intervals at maximal effort, 30 min after consuming a carbohydrate-rich meal (CARB; 1 g/kg CHO), a protein-rich meal (PROTEIN; 0.45 g/kg protein + 0.24 g/kg fat), or water (FASTED), in a randomized and counter-balanced order. Fat oxidation was lower for CARB compared with FASTED at and below the VT, and compared with PROTEIN at 60% VT. There were no differences between trials for average power during high-intensity intervals (367 ± 51 W, p = 0.516). Oxidative stress (F_2-Isoprostanes), perceived exertion, and hunger were not different between trials. Overall, exercising in the overnight-fasted state increased fat oxidation during submaximal exercise compared with exercise following a CHO-rich breakfast, and pre-exercise protein ingestion allowed similarly high levels of fat oxidation. There were no differences in perceived exertion, hunger, or performance, and we provide novel data showing no influence of pre-exercise nutrition ingestion on exercise-induced oxidative stress.

5.1. Introduction

Nutritional intake before exercise can influence performance and the physiological responses to an exercise session [225]. Exercise performed with reduced carbohydrate (CHO) availability can increase fat oxidation, increase the activation of cell signaling pathways, and promote oxidative adaptations in skeletal muscle [2, 11]. At the same time, sufficient CHO ingestion before and/or during exercise is recommended for exercise sessions requiring a high quality, duration, and/or intensity [36]. It is therefore suggested that CHO ingestion be varied according to the goals and type of each exercise session to optimize both training adaptations and acute performance, yet there is wide variance among athletes regarding appropriate nutritional intake before exercise [22].

Strategies to vary CHO availability before exercise include ingesting high- or low-CHO meals, and exercising in the overnight-fasted state. We recently reported nearly two-thirds of endurance athletes (63%) train in the overnight-fasted state, while 72% consume CHO before some or all training sessions, and only 28% ever consume low-CHO meals before exercise [21, 22]. Athletes perform fasted-state training primarily to increase fat oxidation and improve gut comfort during exercise, while athletes that avoid fasted training do so because they feel their workout quality deteriorates and/or they will be too hungry during exercise [21]. It is well established that performing low-to-moderate intensity exercise in the overnight-fasted state can induce higher levels of fat oxidation compared with exercise performed following ingestion of CHO [11]. However, several studies have shown fat oxidation during exercise to be similar following protein ingestion compared with a placebo (fasted) condition [16, 17]. Therefore, pre-exercise protein ingestion may be an alternative to performing fasted-state training that could reduce hunger while maintaining high levels of fat oxidation. Additional research is needed to better understand differences in substrate oxidation between CHO-fed, protein-fed, and fasted-state training, as previous studies using pre-exercise protein ingestion have either not had a CHO control group [16, 17], performed extended exercise at a single intensity [16, 17, 226], or provided very large (>1000 kcal) pre-exercise meals [92, 136].

From a performance standpoint, fed-state exercise generally enhances prolonged (> 60 min), but not shorter duration (< 60 min) aerobic exercise compared with exercising in the fasted state [10], although few studies have used a high-intensity interval training (HIIT) model to measure performance despite HIIT being performed by virtually all endurance athletes [35]. Total work performed during HIIT has been reported to be increased in the fed, compared with the fasted state during some [140, 156] but not all [143] studies. To our knowledge, no studies have compared pre-exercise CHO, protein, and fasted-state training on HIIT work capacity.

More important for athletes and coaches than an acute training session are the longer-term training adaptations. Exercise-induced oxidative stress provides a key signal for the adaptative response to an exercise session with greater exercise-induced oxidative stress being associated with improved adaptations [126, 128], but it is unknown how this might be affected by various pre-exercise meals. At rest, a high-CHO meal can evoke a greater postprandial oxidative stress response compared with a high-fat meal [127], while whey protein can enhance endogenous antioxidant enzyme activity [133]. Furthermore, CHO ingestion before and during exercise can decrease exercise-induced oxidative stress during longer-duration moderate-intensity cycling [227]. Therefore, an understanding of how nutrition influences exercise-induced oxidative stress would be valuable as it could inform pre-exercise nutrition strategies.

Research comparing pre-exercise protein with pre-exercise CHO ingestion and fasted-state training across a range of exercise intensities is needed and could help endurance athletes and coaches make better pre-exercise nutrition choices. Accordingly, the aim of this crossover study was to determine the effects of three different pre-exercise nutrition strategies on substrate oxidation, performance during HIIT, and exercise-induced oxidative stress. We hypothesized that the fasted and protein conditions would have the highest fat oxidation, while cycling power during HIIT would be highest following CHO ingestion. Secondary outcomes were to determine the influence of the pre-exercise meal on cycling gross efficiency, heart rate (HR), and rating of perceived exertion (RPE) during moderate and high intensity cycling, along with hunger and gut comfort before and after exercise.

5.3. Materials and Methods

Participants: Seventeen trained cyclists and triathletes participated in this study (31.2 ± 12.4 years, 181.9 ± 6.4 cm, 74.8 ± 9.6 kg, VO_{2peak} 62.2 ± 5.8 mL·kg^{-1}·min^{-1}, peak aerobic power 425 ± 55 W/5.7 ± 0.6 W·kg^{-1}, average weekly training volume 13.6 ± 3.1 h). Participants were required to be 18–55 years old, with a training history of at least 8 h per week for the previous two years, and a VO_{2peak} >55 mL/kg/min. One participant completed only two of the three trials due to an injury unrelated to this study. Sample size was determined based on previously reported differences in respiratory exchange ratio (RER) of 0.06–0.08 and differences in fat oxidation of 0.13 g·min^{-1} between fed and fasted groups during submaximal steady-state cycling [66, 228]. All study protocols and materials were approved by the Auckland University of Technology Ethics Committee (19/420).

Participants reported to the laboratory on four occasions, seven days apart. Participants were asked to refrain from exercise, caffeine, and alcohol 24 h before each visit and kept a 24 h food log in order to replicate dietary intake prior to each testing day. Instructions on keeping a food log were provided.

Visit 1: After obtaining written informed consent and completing a health screening, a graded exercise test was performed to determine maximal oxygen consumption (VO_{2peak}). Participants cycled on an electronically braked cycle ergometer (Excalibur Sport, Lode BV, Groningen, The Netherlands) at 60 W for three minutes followed by a 30 W per minute increase until volitional fatigue. Expired gas was collected and analyzed continuously using a computerized metabolic system with mixing chamber (TrueOne2400, ParvoMedics, Sandy, UT, USA), with the VO_{2peak} recorded as the highest 15-s average. Peak power (W_{max}) was determined by the workload in the last completed stage plus the workload relative to the time spent in the last incomplete stage [power of completed stage + (30*(seconds at uncompleted stage/60)]. The ventilatory threshold (VT) was identified as the work rate where the ventilatory equivalent for oxygen ($\dot{V}E.\dot{V}O_2^{-1}$) began to increase in the absence of changes in the ventilatory equivalent for carbon dioxide ($\dot{V}E.\dot{V}CO_2^{-1}$), with 15 W deducted to account for the lag in VO_2 during the incremental test [229].

Following a 10-min rest, participants were familiarized with the HIIT protocol using 3 x 3-min intervals. The first interval was set at 80% W_{max}, performed in a cadence-independent manner, while subsequent intervals used the cadence-dependent linear mode set to produce a workload of 80% W_{max} at their preferred cadence. Three intervals were deemed appropriate to minimize the likelihood of training effects and because the participants were fatigued from the VO_{2peak} testing. Participants were asked at the start of the session about their weekly training volume, recorded as self-reported hours per week, and how often they perform exercise in the overnight-fasted state (i.e., without ingesting any calorie-containing foods or beverages). All trials were conducted under standard laboratory conditions (17–19 °C, 40–65% relative humidity), with participants fan cooled during exercise.

Visits 2–4: Participants reported to the laboratory in an overnight-fasted state (~10 h), with each visit at the same time of day. Upon arrival, participants completed a five-question survey that assessed fatigue, sleep quality, muscle soreness, stress, and mood on a five-point scale (scores 1 to 5), with overall well-being determined by summing the five scores [230]. Participants also rated their subjective sensations of hunger and gut discomfort upon arrival and again at the end of each session using paper-based visual analogue scales (VAS) with written anchors of "not hungry at all"/"no discomfort" and "extremely hungry"/"extreme discomfort" placed 0 and 100 mm, respectively [231]. A urine sample was obtained upon arrival (before meal consumption) and within five minutes of completing the exercise session.

In a randomized and counter-balanced order, participants received one of three meals to be consumed within a 5-min window. A CHO-rich meal (CARB; 1 g/kg CHO), a protein-rich meal (PROTEIN; 0.45 g/kg protein + 0.24 g/kg fat), or 500 mL water (FASTED). A 70-kg person received 51 g white bread (Tip Top, New Zealand) with 19 g raspberry jam (Barkers, New Zealand) and 500 mL of a 7% CHO-electrolyte drink [4:1 glucose-to-fructose ratio; Replace, Horleys, New Zealand] for CARB, and 25 g whey protein isolate (ICE, Horleys, New Zealand) with 33 g peanut butter (Forty Thieves, New Zealand) and 500 mL water for PROTEIN. A small amount of fat was included with PROTEIN to keep the two trials isocaloric and mimic real-world application. Total energy content of the CARB and PROTEIN meals was 4 kcal per kg body mass (299 ± 38 kcal). All groups consumed 500 mL fluid and could drink water ad libitum during the remainder of the session.

Thirty minutes after ingestion of the meal, participants began the sub-maximal cycling portion of the testing which included 4 × 5-min stages at a power equivalent to 60%, 80%, and 100% of VT (VT60, VT80, VT100, respectively), and 20% of the difference between VT and W_{max} (VTΔ20), to measure substrate oxidation, energy expenditure, heart rate (HR), and perceived exertion (RPE) (Figure 5.1). Expired gas was continuously measured using a metabolic cart (TrueOne2400, ParvoMedics, Sandy, UT, USA), with average values during the final two minutes of each stage analyzed. Intensity was normalized to the VT to reduce inter-subject variability in the physiological and perceived responses to exercise compared with using a percentage of VO_{2peak} [232].

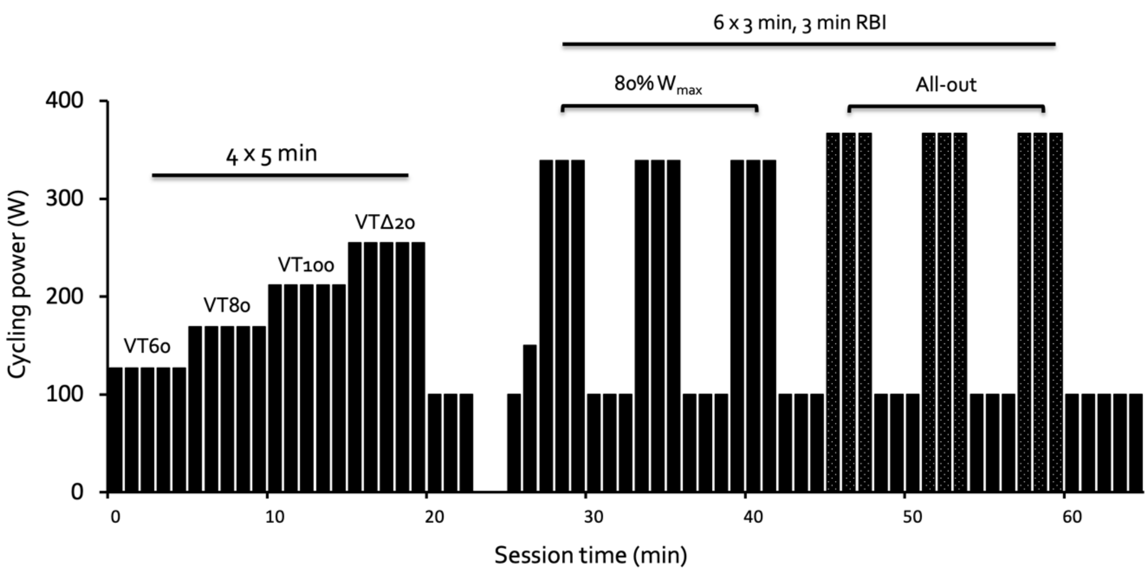

Figure 5.1. Schematic overview of cycle testing sessions using wattage from an example participant. The 5-min intervals were performed at intensities equivalent to 60%, 80%, and 100% of their ventilatory threshold (VT 60, VT80, VT100, respectively), and 20% of the difference between the ventilatory threshold and peak power (Wmax, VTΔ20), followed by a 3-min cool down at 100 W. The 3-min intervals included a lead-in of 1 min at 100 W and 1 min at 150 W, followed by intervals 1–3 at 80% of Wmax, and intervals 4–6 performed as maximal efforts.

Rate of energy expenditure (EE) was calculated using the formulas of Jeukendrup and Wallis [76], with cycling gross efficiency (GE) calculated as GE(%) = (mechanical work (kcal/min)/energy expenditure (kcal/min)) x 100. Whole-body rates of CHO and fat oxidation were calculated using standard equations, assuming 9.75 kcal/g fat and 4.07 kcal/g CHO [76].

Following a 3-min static rest, participants performed 6 × 3-min cycling intervals with 3 min of active recovery (100 W) between each interval (Figure 1). The first three intervals were performed at 80% of W_{max}, in a cadence-independent manner. Intervals 4–6 used the cadence-dependent linear mode set to produce a workload of 80% W_{max} at their preferred cadence, with participants instructed to produce their maximal power output across intervals 4–6 by increasing the cycling cadence. Immediately following intervals 3 and 6, a 0.3µL blood sample was collected from the left index fingertip and analyzed immediately using a portable blood lactate analyzer (Lactate Pro 2, Carlton, Australia). Power output (W) during HIIT was analyzed as mean power (W) for each interval. Heart rate was measured using a chest-strap (Polar T31, Polar, Inc., Kempele, Finland), with average values during the final 30 s of each interval analyzed. Rating of perceived exertion (RPE) was recorded following each submaximal stage and each high-intensity interval using Borg's 6–20 scale [233], and at the end of the session ($_s$RPE) using a 10-point scale [234]. We chose to have the work rate "clamped" for the first three intervals to compare HR, RPE, and lactate across conditions at a fixed cycling power, and have three intervals performed as maximal efforts to be used to determine work capacity during HIIT.

A competitive immunoassay was used for the quantitation of urinary F_2-isoprostanes (Kit #51635, Cayman Chemicals, Ann Arbor, USA) as previously described [235]. Samples were purified using solid-phase extraction cartridges. For standardizing urine dilution, creatinine levels were measured using a commercially available kit (Kit #500701, Cayman Chemicals, Ann Arbor, USA). Due to technical problems the number of samples analyzed was n = 12 for CARB, n = 12 for FASTED, and n = 11 for PROTEIN.

Statistical analysis: A series of linear mixed models were used to estimate differences in the exercise-induced changes between the three treatment conditions (CARB, PROTEIN, FASTED). These were fit using the lme4 R package. For the submaximal portion, intensity (four levels: VT60, VT80, VT100, VTΔ20) was added as a fixed effect, while interval (three levels) was considered a fixed effect for the high-intensity portion. When examining differences pre-post exercise (for hunger, gut comfort, and oxidative stress measures), time point (two levels; pre and post) was added as a fixed effect. For all models, treatment order was included as a fixed effect (given the crossover design) and participant ID was specified as a random intercept. Interactions between the treatment and other fixed effects were explored, and the optimal, best-fitting model for each outcome was decided based on the likelihood ratio test. The fit of each model was checked by visualizing the Q–Q and other residual plots to ensure approximate residual normality and heteroscedasticity, using the performance R package. Model-estimated means were calculated using the emmeans R package and presented as estimated means ± 95% confidence interval (CI). Contrasts between each treatment (within each intensity, interval, or time point) were estimated, with multiple comparisons adjusted using the Holm correction. A standardized effect size for each contrast (delta total variance; δt) was computed by dividing the mean difference by the population SD (calculated as the square root of the sum of the variance components of the random effects)[236]. Effect size are interpreted as small (0.2), medium (0.5) and large (0.8) [193]. One participant was excluded from the submaximal mixed models because their intensity was more than 3 SDs above the mean, due to an overestimation of the VT. The level of significance for all analyses was set at $p < 0.05$, and all analyses were carried out with R version 4.0.3.

5.4. Results

Submaximal Exercise

Relative exercise intensity for the four submaximal stages was 40.8 ± 4.4, 50.6 ± 5.9, 60.6 ± 7.2, and 71.8 ± 6.3%VO_{2peak}. For HR, RPE, RER, energy expenditure, VO_2, and CHO oxidation there were significant pairwise differences between each intensity level (all $p < 0.001$, Figure 5.2). Contrasts between treatments at each intensity revealed HR, measured as percentage of each individual's maximal HR, was lower for FASTED compared with both CARB and PROTEIN ($p < 0.05$, Figure 5.2A). Gross cycling efficiency was higher ($p < 0.01$) for FASTED compared with PROTEIN at each intensity (Figure 5.2B), while RPE was not different between treatments (Figure 2C). RER was higher ($p < 0.05$) for CARB compared with FASTED at VT60, VT80, and VT100, and compared with PROTEIN at VT60 (Figure 5.2D). Fat oxidation was lower ($p < 0.05$) for CARB compared with FASTED at VT60, VT80, and VT100, and compared with PROTEIN at VT60 (Figure 2E). Carbohydrate oxidation was different ($p < 0.05$) between CARB and FASTED at VT60, VT80, and VT100 (Figure 5.2F). Table 1 shows effect sizes with 95% confidence intervals for all contrasts at each submaximal intensity. Estimated means, confidence intervals, and *p*-values for all submaximal values are provided in Tables S5.1–S5.7.

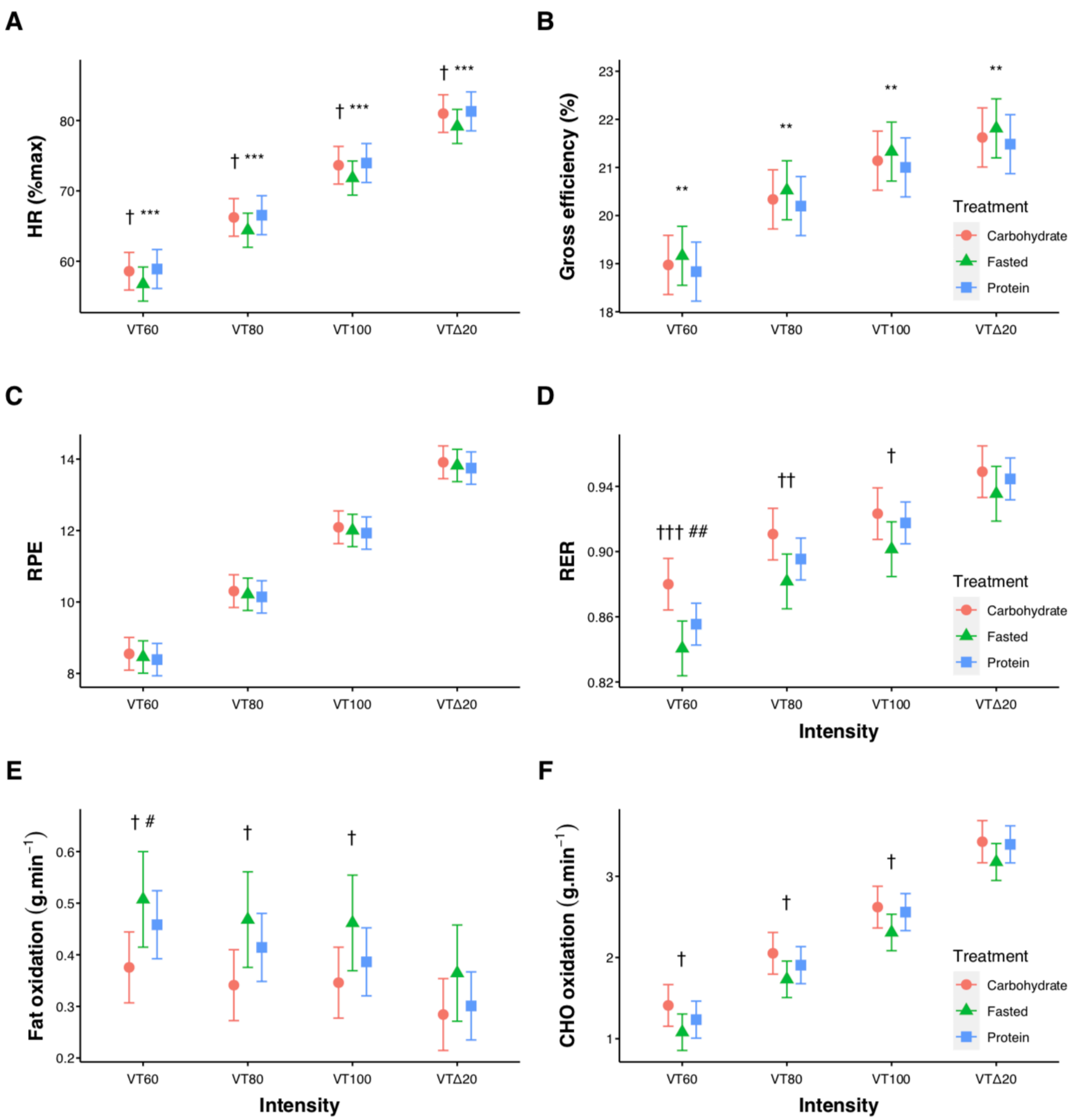

Figure 5.2. Influence of pre-exercise nutrition interventions on submaximal cycling heart rate (HR, (A)), cycling gross efficiency (B), rating of perceived exertion (RPE, (C)), respiratory exchange ratio (RER, (D)), fat oxidation (E), and carbohydrate (CHO) oxidation (F) during submaximal cycling. Significant differences CARB vs. FASTED († $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$), significant differences CARB vs. PROTEIN (# $p < 0.05$, ## $p < 0.01$), significant differences FASTED vs. PROTEIN (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Error bars represent 95% confidence intervals of the model-estimated mean.

Table 5.1. Effect sizes [95% confidence intervals] for all contrasts during submaximal exercise.

Contrast	Intensity	Heart Rate	Gross Efficiency	RPE	RER	Fat Oxidation (g/min)	Carbohydrate Oxidation (g/min)
CARB-FASTED	**VT60**	Small ES = 0.34 [0.07, 0.61]	Trivial ES = −0.15 [−0.32, 0.02]	Trivial ES = 0.08 [−0.23, 0.39]	Large ES = 1.29 [0.69, 1.89]	Large ES = −0.85 [−1.46, −0.25]	Medium ES = 0.69 [0.18, 1.21]
CARB-PROTEIN	**VT60**	Trivial ES = −0.06 [−0.37, 0.25]	Trivial ES = 0.11 [−0.06, 0.28]	Trivial ES = 0.15 [−0.16, 0.45]	Large ES = 0.80 [0.28, 1.33]	Medium ES = −0.54 [−1, −0.07]	Small ES = 0.37 [−0.1, 0.84]
FASTED-PROTEIN	**VT60**	Small ES = −0.4 [−0.55, −0.24]	Small ES = 0.26 [0.09, 0.42]	Trivial ES = 0.07 [−0.23, 0.37]	Small ES = −0.49 [−1.03, 0.06]	Small ES = 0.32 [−0.2, 0.84]	Small ES = −0.33 [−0.82, 0.17]
CARB-FASTED	**VT80**	Small ES = 0.34 [0.07, 0.61]	Trivial ES = −0.15 [−0.32, 0.02]	Trivial ES = 0.08 [−0.23, 0.39]	Large ES = 0.95 [0.36, 1.54]	Large ES = −0.82 [−1.43, −0.22]	Medium ES = 0.67 [0.16, 1.19]
CARB-PROTEIN	**VT80**	Trivial ES = −0.06 [−0.37, 0.25]	Trivial ES = 0.11 [−0.06, 0.28]	Trivial ES = 0.15 [−0.16, 0.45]	Medium ES = 0.50 [−0.02, 1.02]	Small ES = −0.47 [−0.94, −0.01]	Small ES = 0.31 [−0.16, 0.77]
FASTED-PROTEIN	**VT80**	Small ES = −0.4 [−0.55, −0.24]	Small ES = 0.26 [0.09, 0.42]	Trivial ES = 0.07 [−0.23, 0.37]	Small ES = −0.45 [−0.99, 0.09]	Small ES = 0.35 [−0.17, 0.87]	Small ES = −0.37 [−0.86, 0.13]
CARB-FASTED	**VT100**	Small ES = 0.34 [0.07, 0.61]	Trivial ES = −0.15 [−0.32, 0.02]	Trivial ES = 0.08 [−0.23, 0.39]	Medium ES = 0.71 [0.13, 1.3]	Medium ES = −0.75 [−1.35, −0.15]	Medium ES = 0.66 [0.14, 1.17]
CARB-PROTEIN	**VT100**	Trivial ES = −0.06 [−0.37, 0.25]	Trivial ES = 0.11 [−0.06, 0.28]	Trivial ES = 0.15 [−0.16, 0.45]	Trivial ES = 0.19 [−0.33, 0.7]	Small ES = −0.26 [−0.73, 0.2]	Trivial ES = 0.13 [−0.34, 0.59]
FASTED-PROTEIN	**VT100**	Small ES = −0.4 [−0.55, −0.24]	Small ES = 0.26 [0.09, 0.42]	Trivial ES = 0.07 [−0.23, 0.37]	Medium ES = −0.53 [−1.07, 0.01]	Small ES = 0.49 [−0.03, 1.01]	Medium ES = −0.53 [−1.02, −0.03]
CARB-FASTED	**VTΔ20**	Small ES = 0.34 [0.07, 0.61]	Trivial ES = −0.15 [−0.32, 0.02]	Trivial ES = 0.08 [−0.23, 0.39]	Small ES = 0.44 [−0.14, 1.03]	Medium ES = −0.52 [−1.13, 0.09]	Medium ES = 0.53 [0, 1.05]
CARB-PROTEIN	**VTΔ20**	Trivial ES = −0.06 [−0.37, 0.25]	Trivial ES = 0.11 [−0.06, 0.28]	Trivial ES = 0.15 [−0.16, 0.45]	Trivial ES = 0.14 [−0.37, 0.66]	Trivial ES = −0.11 [−0.58, 0.36]	Trivial ES = 0.07 [−0.4, 0.54]
FASTED-PROTEIN	**VTΔ20**	Small ES = −0.4 [−0.55, −0.24]	Small ES = 0.26 [0.09, 0.42]	Trivial ES = 0.07 [−0.23, 0.37]	Small ES = −0.30 [−0.84, 0.24]	Small ES = 0.41 [−0.11, 0.94]	Small ES = −0.46 [−0.96, 0.04]

Submaximal exercise consisted of 4 × 5-min stages at a power equivalent to 60%, 80%, and 100% of the first ventilatory threshold (VT) (VT60, VT80, VT100, respectively), and 20% of the difference between VT and Wmax (VTΔ20). [a] RER: Respiratory exchange ratio, RPE: Rating of perceived exertion.

Individual data points for RER across the submaximal exercise stages in relation to each individual's VO_{2peak} are shown in Figure 5.3. There was a significant effect of both treatment and intensity on RER ($p < 0.001$), but no significant interaction between treatment and intensity (p = 0.283).

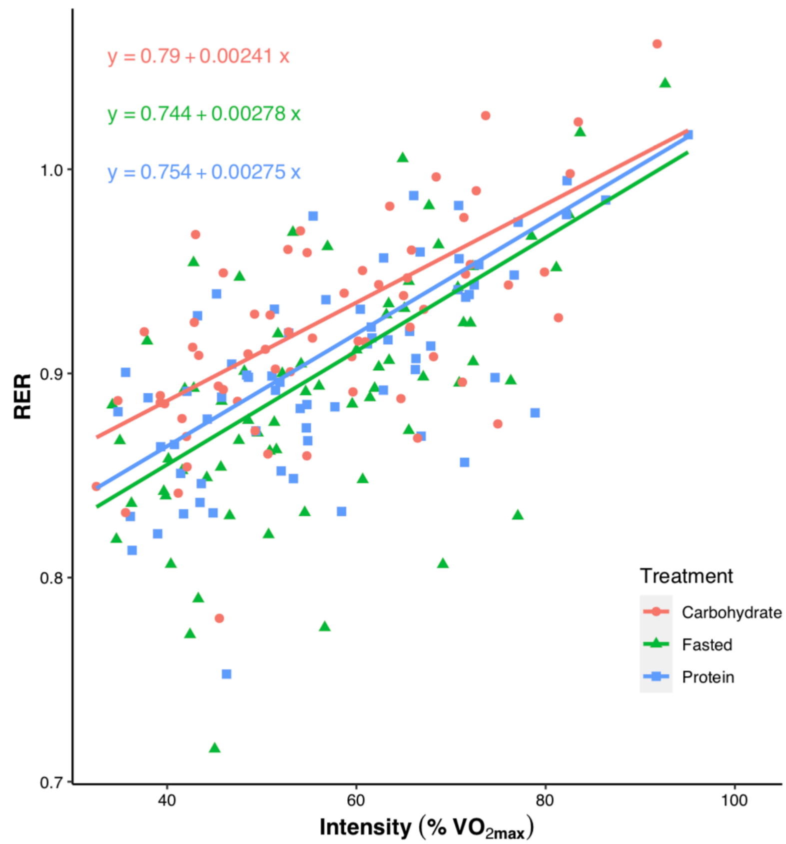

Figure 5.3. Correlations between each individual's relative exercise intensity as a percentage of VO2peak and respiratory exchange ratio (RER) during submaximal exercise, separated by treatment. Significant effects are found for treatment (p < 0.001) and intensity (p < 0.001). Trend line is based on model-estimated values.

High-Intensity Exercise

Intervals 1–3 were performed at 80%W_{max}, corresponding to 340 ± 44 W. The subsequent three intervals were performed as maximal efforts, with no differences between treatments for average power (p = 0.516, Figure 5.4A). Similarly, there were no differences in RPE or lactate (Figure 4B,C) between trials. However, contrasts between treatments showed HR was higher during PROTEIN compared with FASTED at each interval (all p = 0.004, Figure 5.4D).

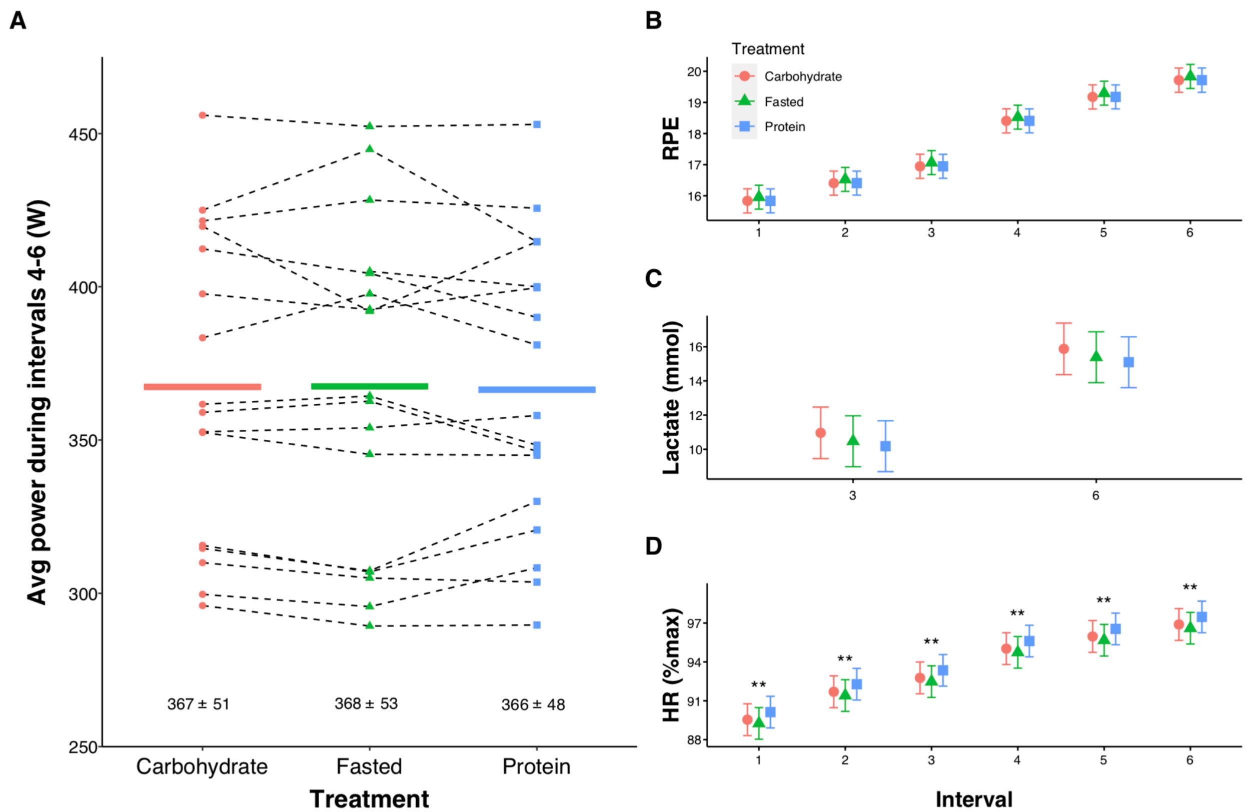

Figure 5.4. Influence on mean power during three all-out 3-min efforts (A), rating of perceived exertion (RPE, B), lactate (C), and heart rate (HR, D) during high-intensity interval training. In (A) individual data points are shown with solid lines representing means and numeric values reflecting mean ± SD. In (B–D), error bars represent 95% confidence intervals of the model-estimated mean. **Significant differences for FASTED vs. PROTEIN ($p < 0.01$).

Pre-Post Exercise

There was no effect of exercise (p = 0.510) or treatment (p = 0.595) on urinary F_2-Isoprostanes (Figure 5.5A). Hunger decreased from pre to post exercise (p < 0.001), with no effect of treatment, while gut discomfort following exercise was higher with protein compared with FASTED (p = 0.032) and CARB (p = 0.012, Figure 5.5B,C). Overall session RPE was not different between trials (p = 0.076), but there was a trend for CARB (7.9 (95%CI 7.5, 8.2) to be lower than FASTED (8.3 (95% CI 7.9, 8.5), p = 0.101, data not shown). When asked how often they typically perform exercise in the overnight-fasted state 59% of participants reported “rarely or never (less than 1x per week)”, 18% reported “sometimes (1–2x per week)”, and 24% reported “often or always (>2x per week)”.

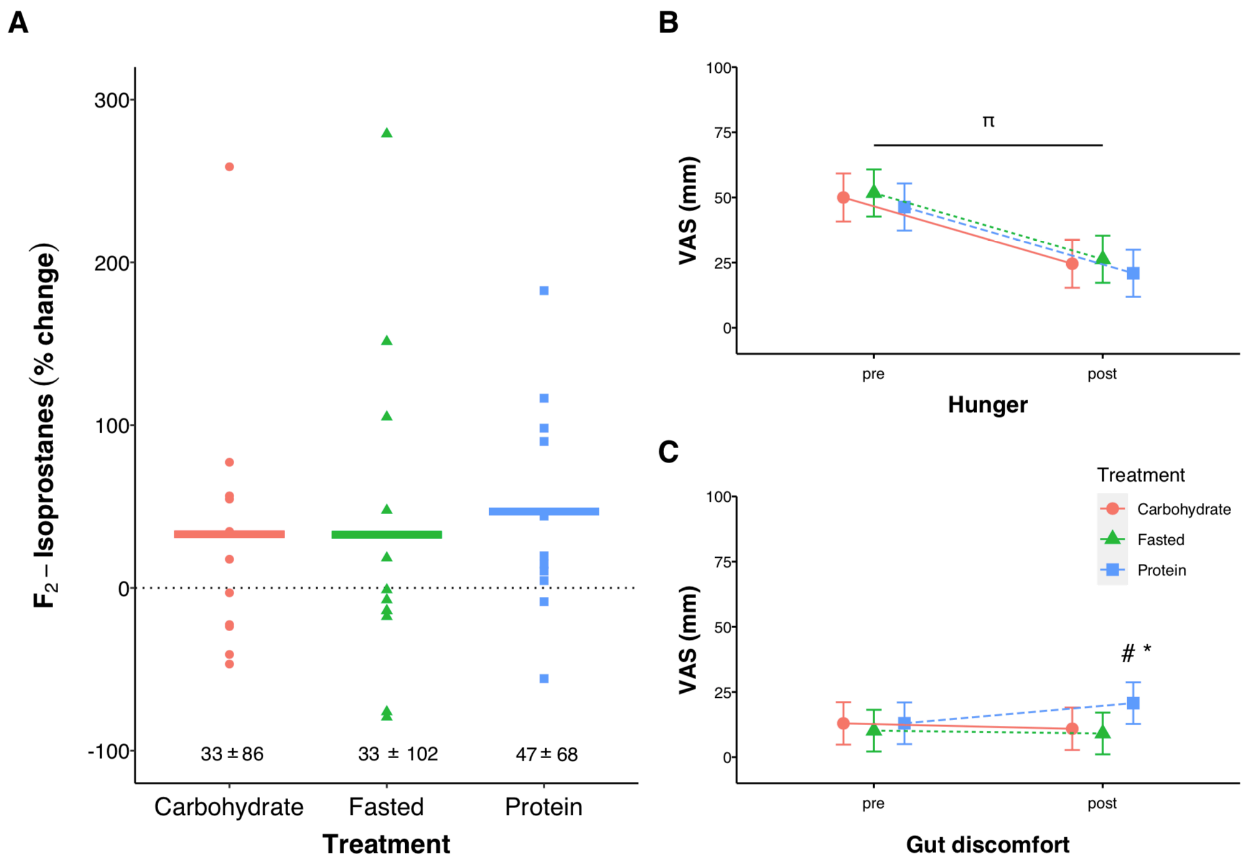

Figure 5.5. Urinary F2-Isprostane percent change (pre to post-exercise, (A)), and subjective levels of hunger (B) and gut discomfort (C) before and after exercise using a visual analog scale (VAS). All measurements at 'pre' were taken upon arrival to the lab in the overnight-fasted state, before being provided any nutrition treatment. In (A) individual data points are shown with solid lines representing means and the numeric values reflecting mean ± SD. In (B,**C**) error bars represent 95% confidence intervals of the model-estimated mean. $^{\pi}$ significant effect of time ($p < 0.001$), significant effects of treatment ($^{\#}$PROTEIN vs. CARB, $p = 0.012$, * PROTEIN vs. FASTED, $p = 0.032$) at the post time point. For (**A**), $n = 12$ for Carbohydrate, $n = 12$ for Fasted, and $n = 11$ for Protein.

5.5. Discussion

To our knowledge, this is the first study to compare continuous exercise and HIIT performed in the overnight-fasted state with both CHO-rich and protein-rich pre-exercise meals. These data reveal exercising in the overnight-fasted state can increase fat oxidation during submaximal exercise compared with exercising following a CHO-rich breakfast, and that pre-exercise protein ingestion allows similarly high levels of fat oxidation. Furthermore, there were no between-group differences in RPE, hunger, or performance during HIIT, and we provide novel data showing no influence of pre-exercise nutrition ingestion on F_2-Isoprostanes, a measure of exercise-induced oxidative stress.

It is well established that exercising in the fasted state allows higher levels of fat oxidation than exercise performed in the CHO-fed state during low-to-moderate intensity exercise, with these differences reduced as exercise intensity increases [11, 225]. Accordingly, we found the FASTED group had a lower RER (and thus higher levels of fat oxidation) compared with CARB at and below the VT, but not above (Figure 5.2D). Ingestion of PROTEIN resulted in a lower RER than CARB at the lowest intensity, but differences between groups were reduced as the intensity increased. Fat oxidation for PROTEIN was numerically lower, but not significantly different than FASTED (e.g., 0.46 vs. 0.51 $g.min^{-1}$ at VT60). The lack of statistical significance is likely due to the wide variation in RER observed between participants (Figure 5.3). In line with our findings, others have reported a protein-rich pre-exercise meal increased fat oxidation during moderate-intensity exercise compared with a CHO-rich meal [237], and there were no differences in fat oxidation when consuming whey protein before and during steady-state cycling compared with a placebo (fasted) trial [16]. Similar levels of fat oxidation between fasted and protein-fed exercise have also been reported during cycling between 58%–86% VO_{2max} [136], running at 55%–60% heart rate reserve [226], and cycling at ~50% VO_{2max} in a glycogen-depleted state [17]. A primary reason endurance athletes perform fasted-state training is a desire to increase fat oxidation during exercise [21], and our findings provide further evidence supporting the use of both pre-exercise protein ingestion and fasted-state training to increase fat oxidation during low-intensity exercise compared with a CHO-rich breakfast, which likely impairs fat oxidation.

Decreased cycling efficiency was observed in PROTEIN compared with FASTED, however the practical relevance of these differences is likely minimal. This may be accounted for by meal-induced thermogenesis, which is increased during exercise [238] and is nearly three-fold higher following protein compared with CHO ingestion [239, 240]. We also observed elevated HR in both fed conditions (Figure 5.3A), which could be related to the increased meal-induced thermogenesis, and/or increased sympathetic nervous system activity following glucose ingestion [241].

It is more common for athletes to perform lower-intensity exercise in the overnight-fasted state, as many feel their performance during higher-intensity exercise will be diminished [21]. Despite this belief, we found work capacity and RPE during HIIT were not different between trials. It is possible the assumption of diminished work capacity during fasted-state HIIT comes from observations of decreased power during HIIT performed with reduced muscle glycogen concentrations [163, 219]. However, exercise performed in the overnight-fasted state lowers hepatic but not muscle glycogen [8], and therefore fasted exercise with normal muscle glycogen levels would not be expected to have the performance decrement. Similar to our findings, no effect of a mixed macronutrient breakfast on HIIT performance or RPE has been reported compared with exercising in the overnight-fasted state [143]. However, others have shown benefit of pre-exercise CHO ingestion on an exercise capacity test lasting ~8–10 min [144]. Factors that influence central fatigue may be important during short-duration exercise, which is not limited by glycogen depletion. Central fatigue may be reduced by ingesting CHO [242] or branched-chain amino acids [243]. In our study work capacity was not influenced by pre-exercise CHO or protein ingestion, but it is possible that longer-duration exercise would have revealed differences between treatments.

Many athletes perform fasted-state training to improve gut comfort during exercise, while one of the most common reasons for avoiding training in the fasted state is related to hunger [21]. To this end, we obtained subjective ratings of hunger and gut comfort before and after exercise using a VAS. Hunger decreased following exercise, with no differences between trials. It is possible that post-exercise hunger scores would have been higher following lower-intensity exercise, as blood lactate accumulation is associated with the suppression of the hunger hormone ghrelin and subjective appetite [244]. Indeed, sprint interval exercise has induced a greater suppression of appetite during exercise compared with continuous endurance exercise [245]. Following moderate-intensity exercise there has been reduced hunger with a CHO-rich pre-exercise meal compared with fasted-state exercise [246], and with a protein-rich compared with CHO-rich pre-exercise meal [237]. Gut discomfort in our study was low before exercise (~12 out of 100) and increased following exercise only in PROTEIN. Protein is known to increase the risk of gut discomfort during exercise in some [247, 248], but not all [16], studies. However, in our study, the observed increase was modest (post-exercise gut discomfort ~21 out of 100), suggesting pre-exercise protein ingestion could still be an effective strategy for those who would prefer to eat before exercise while maintaining higher levels of fat oxidation.

Despite the increase in fat oxidation and maintenance of HIIT performance, exercising in the overnight-fasted state could more likely lead to a negative energy balance, which can be associated with hormonal and immune dysfunction [14]. For example, the average athlete in this study riding at the first ventilatory threshold will burn ~850 kcal per hour, creating a large calorie deficit when exercising in the fasted state. Even for the athlete who can achieve energy balance, body composition may be influenced by large or frequent within-day energy deficits [249]. The results of this study therefore highlight the utility of pre-exercise protein ingestion as a method of providing energy intake while still allowing higher levels of fat oxidation, particularly for those who want to increase fat burning without incurring a large caloric deficit.

Reactive oxygen species play a direct role in regulating the response to acute exercise and are critical for longer-term exercise training adaptations [128, 129, 215]. We found no effect of nutrition or exercise on F_2-Isoprostanes, one of the preferred markers for the detection of organism-wide oxidative stress [250]. Similar to others [251], we observed a large degree of inter-individual variability in exercise-induced oxidative stress (Figure 5A). It is potentially surprising that we didn't see an increase in F_2-Isoprostanes following exercise as most, but not all, studies have reported exercise-induced increases [250]. This result could be due to the well-trained status of the participants [252], the exercise protocol (HIIT lasting < 60 min), or the sample size and high interindividual variability in the response. It is important to further investigate the effects of pre-exercise nutrition on exercise-induced oxidative stress, potentially using different oxidative stress markers or different exercise protocols (e.g., sprint interval training, and/or longer-duration continuous exercise), as this could have implications for longer-term training adaptations.

The size and timing of the nutrient ingestion was chosen to maximize ecological validity. Ingesting a small amount of CHO (e.g., 1 g/kg) just prior to exercise (e.g., 30 min) is similar to the day-to-day practices of endurance athletes [22] and in contrast with previous studies using extremely large test meals [92] provided up to 4 h prior to exercise [75]. No differences in performance have been observed when CHO was consumed 15, 45, or 75 min [91], 15 or 60 min [142], or 5 or 35 min [150] before exercise. There also appears to be no effect of meal size on substrate oxidation during exercise, as similar values were found with 45 and 156 g of CHO consumed 4 h prior to exercise [87], and 25, 75, or 200 g of CHO consumed 45 min prior to exercise [88]. Therefore, our findings should be generalizable to a range of pre-exercise meal sizes and timings.

The potential for placebo effects must be acknowledged, as it is challenging to blind participants to their treatments when using solid and/or familiar foods (e.g., jam sandwich or peanut butter). Cycling time-trial performance has improved when subjects perceived they had consumed breakfast (either a viscous placebo or 2 g/kg CHO) before exercise, compared with a water-only trial [152]. A placebo effect is more likely during short-duration exercise when muscle glycogen use is not a limiting factor for performance [152, 153, 253]. The duration of exercise in our study was 60 min, with roughly 30% of that time spent at high intensity implying glycogen depletion was not a limiting factor for performance. Although only seven out of 17 study participants reported regularly performing training sessions in the overnight-fasted state, there were no performance differences when accounting for their habitual use of fasted training (data not shown). In a survey of endurance athletes, 26% agreed and 51% disagreed with the statement, "the quality of my workout is the same whether I eat or do not eat beforehand" [21]. Therefore, it is likely a large inter-individual variation exists with regard to the perception of breakfast and its influence on performance. However, is must also be acknowledged that the influence of the pre-exercise meal on performance will be related to duration as well as intensity of exercise [10, 225].

Future studies are needed to extend these findings to other populations including women and untrained individuals. There are known sex differences in substrate use and molecular signaling during exercise [254, 255], and trained athletes have a greater capacity for fat oxidation compared with untrained or recreationally active populations [93]. Additionally, calculations of energy expenditure assume negligible contribution of protein oxidation [76]. Although not quantified in this study, there is the possibility of increased protein oxidation during exercise in the PROTEIN group that may have been unaccounted for. This protein oxidation may to some extent account for the reduction in GE in the PROTEIN group. It has been reported that protein oxidation contributes up to 10% of total oxygen consumption [256], and can vary depending on training status [257], habitual diet [258], muscle glycogen levels [69], and pre-exercise protein ingestion [67], but further quantification of the influence of the pre-exercise meal is needed. Finally, training studies are needed to determine if longer-term adaptations to continuous and/or HIIT may be differentially influenced by pre-exercise nutrition choices.

5.6. Conclusion

In summary, fat oxidation during submaximal exercise was highest in the overnight-fasted state and following pre-exercise protein ingestion. There were no differences in work capacity, RPE, oxidative stress, or hunger between treatments. Consuming a low-CHO meal before submaximal exercise will not meaningfully impair fat oxidation and eating a high- or low-CHO meal does not confer additional performance benefit during HIIT compared with training in the overnight-fasted state. Therefore, athletes who wish to increase fat oxidation but promote energy balance can use pre-exercise protein ingestion as a viable alternative to fasted training sessions. Conversely, for shorter duration higher intensity sessions, fasted training and protein ingestion are also viable choices as performance was not compromised, suggesting athletes can choose whether to eat based on personal preference. However, longer-term training studies are needed as the net adaptive responses of chronic CHO or protein ingestion prior to exercise is unknown.

6. Factors Influencing AMPK Activation During Cycling Exercise: A Pooled Analysis and Meta-Regression

The 5’ AMP-activated protein kinase (AMPK) is a cellular energy sensor, providing a key signal in the adaptive response to endurance training. Many factors can influence AMPK activation during exercise including exercise intensity, fitness level, muscle glycogen content, and pre-exercise carbohydrate intake. Some athletes avoid carbohydrate ingestion before or during exercise out of fear of blunting some of the favorable training response. However, it is unclear if pre-exercise carbohydrate intake blunts mitochondrial signaling in response to exercise after accounting for other important variables such as intensity, duration, and muscle glycogen content, among others. This chapter examines nutrition and exercise-related factors influencing AMPK activation during exercise. Although multiple skeletal muscle proteins have established roles in the training response, AMPK was chosen because it is sensitive to cellular energy status and is among the most well-studied signaling proteins.

This chapter contains the following publication:

Rothschild, J. A., Islam, H., Bishop, D. J., Kilding, A. E., Stewart, T., & Plews, D. J. (2022). Factors influencing AMPK activation during cycling exercise: a pooled analysis and meta-regression. *Sports Medicine*, 52(6):1273-94.

6.1 Abstract

Background The 5' AMP-activated protein kinase (AMPK) is a cellular energy sensor that is activated by increases in the cellular AMP/ADP:ATP ratios and plays a key role in metabolic adaptations to endurance training. The degree of AMPK activation during exercise can be influenced by many factors that impact cellular energetics including exercise intensity, exercise duration, muscle glycogen, fitness level, and nutrient availability. However, the relative importance of these factors for inducing AMPK activation remains unclear and robust relationships between exercise-related variables and indices of AMPK activation have not been established.

Objectives The purpose of this analysis was to 1) investigate correlations between factors influencing AMPK activation and the magnitude of change in AMPK activity during cycling exercise, 2) investigate correlations between commonly reported measures of AMPK activation (AMPK-α2 activity, phosphorylated (p)-AMPK, and p-acetyl coenzyme A carboxylase (p-ACC), and 3) formulate linear regression models to determine the most important factors for AMPK activation during exercise.

Methods Data were pooled from 89 studies, including 982 participants (93.8% male, VO_{2max} 51.9 ± 7.8 ml kg^{-1} min^{-1}). Pearson's correlation analysis was performed to determine relationships between effect sizes for each of the primary outcome markers (AMPK-α2 activity, p-AMPK, p-ACC) and factors purported to influence AMPK signaling (muscle glycogen, carbohydrate ingestion, exercise duration and intensity, fitness level, and muscle metabolites). General linear mixed-effect models were used to examine which factors influenced AMPK activation.

Results Significant correlations ($r = .19$–$.55$, $p < .05$) with AMPK activity were found between end-exercise muscle glycogen, exercise intensity, and muscle metabolites phosphocreatine, creatine, and free ADP. All markers of AMPK activation were significantly correlated, with the strongest relationship between AMPK-α2 activity and p-AMPK ($r = .56$, $p < .001$). The most important predictors of AMPK activation were the muscle metabolites and exercise intensity.

Conclusion Muscle glycogen, fitness level, exercise intensity, and exercise duration each influence AMPK activity during exercise when all other factors are held constant. However, disrupting cellular energy charge is the most influential factor for AMPK activation during endurance exercise.

6.2 Introduction

The 5' AMP-activated protein kinase (AMPK) is a cellular energy sensor that is activated by increases in the AMP:ATP and ADP:ATP ratios [259]. AMPK activation switches on ATP-producing pathways (acutely, by phosphorylating downstream metabolic enzymes such as acetyl coenzyme A carboxylase [ACC], and chronically, by affecting gene expression), while concurrently switching off ATP-consuming processes [260]. Repeated AMPK activation leads to a range of beneficial metabolic adaptations that include increases in glucose uptake, glycolytic flux, fat oxidation, and mitochondrial biogenesis [96], thereby contributing to training-induced improvements in endurance capacity.

Since the first reports of exercise-induced AMPK activation in human skeletal muscle were published 20 years ago [97, 261, 262], several key factors influencing its activity have emerged including exercise intensity [97], exercise duration [98], muscle glycogen content [100], fitness level [99], and nutrient availability [1] (Fig. 6.1). However, contrasting findings related to the relative influence of these factors have been reported in the literature. For example, AMPK activation is most commonly seen at exercise intensities above 60% VO_{2max} [97, 261, 263], but low-intensity exercise performed for a long duration can also increase AMPK activity [264]. AMPK activity has increased with exercise duration in most [98, 99, 261], but not all [104, 265], studies, and exercise undertaken with low, compared with normal muscle glycogen levels, has sometimes [102-104], but not always [13, 105, 106, 266], increased AMPK activity during exercise. Concentrations of free AMP (AMP_{free}) and ADP (ADP_{free}) are also proposed to be a key determinant of AMPK activity during exercise [262, 263, 266-268], although findings have again been inconsistent [42, 269]. Thus, a better understanding of the interaction and relative impact of factors that influence AMPK activation during endurance exercise is needed.

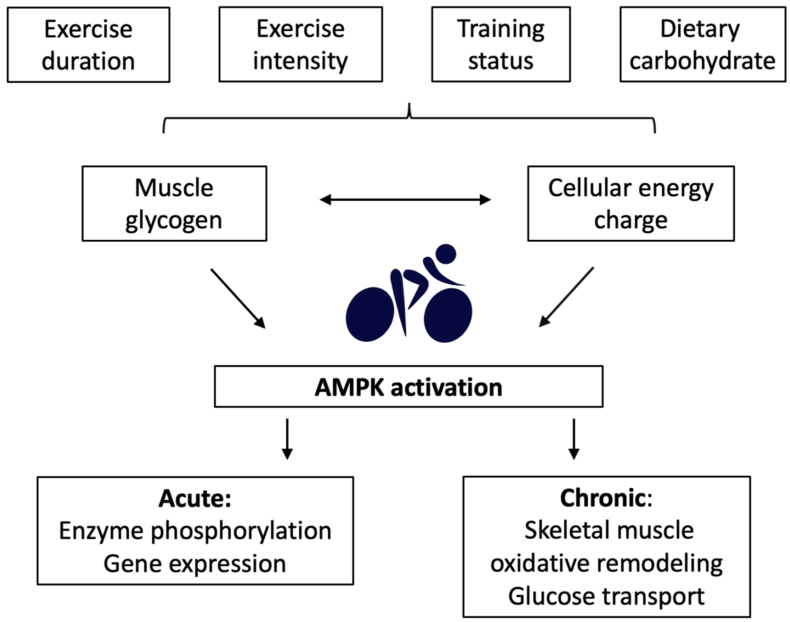

Figure 6.1. Schematic of AMPK activation. Activation of 5' AMP-activated protein kinase (AMPK) during exercise is influenced by changes in cellular energy charge (e.g., AMP/ADP:ATP ratio) and muscle glycogen concentrations, which are affected by exercise duration, exercise intensity, training status of an individual, and dietary carbohydrate intake. Acutely, AMPK induces phosphorylation of metabolic enzymes and the transcription of metabolic genes, while repeated activation contributes to chronic adaptations to endurance exercise by enhanced expression of metabolic enzymes/proteins involved in glucose transport, fat oxidation and mitochondrial biogenesis.

AMPK is a heterotrimeric protein complex that consists of three subunits (α, β, γ), which exist in skeletal muscle in several isoforms (α1, α2, β1, β2, γ1, γ2, and γ3) [260]. Early studies measuring skeletal muscle AMPK activation in response to exercise reported activity of the two catalytic α isoforms (α1 and α2), with the α2 subunit generally found to be more responsive to exercise [97, 261]. The activity of AMPK (α1 and α2) is stimulated more than 100-fold by phosphorylation of threonine-172, which accounts for the vast majority, though not all, of its activation [270-272]. Based on this, phospho-specific antibodies recognizing AMPK phosphorylated at Thr-172 (p-AMPK), and antibodies recognizing the downstream target ACC phosphorylated at the AMPK target site Ser-80 of ACC isoform 1 (analogous to Ser-79 in rodent ACC1) or Ser-221 of ACC isoform 2 (analogous to Ser-212 on rodent ACC2) are widely used as surrogate markers of AMPK activation (collectively referred to as p-ACC herein) [273]. AMPK phosphorylates both ACC isoforms, but ACC2 is the dominant isoform expressed in human skeletal muscle and sensitive to changes in AMPK activity in response to exercise [263, 274, 275]. In addition to phosphorylation, AMPK can be further activated allosterically by increases in the AMP/ADP:ATP ratios [95].

Due to strong correlations between AMPK-α2 activity and both p-AMPK$^{Thr-172}$ [276] and p-ACC^{Ser-79} [269], studies frequently use p-AMPK or p-ACC as an indirect marker of AMPK activity [107, 277, 278]. However, there are several reasons why p-AMPK and p-ACC may not be fully representative markers. First, allosteric activation by AMP can increase p-ACC without a change in Thr-172 phosphorylation [279]. Furthermore, p-AMPK at Thr-172 reflects phosphorylation of both AMPK-α1 and -α2 subunits and may be less sensitive for detecting changes in AMPK activity that are only occurring in the α2/β2/γ3 heterotrimer – the complex primarily activated in skeletal muscle during exercise [274]. A disassociation between AMPK activity and p-ACC has also been observed during prolonged low-intensity exercise [264]. Therefore, it would be valuable to better understand the relationship between the various markers of AMPK signaling.

Given the aforementioned contradictory findings on the relative importance of various factors for AMPK activation, and the complexities surrounding the use of surrogate markers of AMPK activity, the purpose of this analysis was to: 1) investigate correlations between factors influencing AMPK activation and the magnitude of change in AMPK activity during cycling exercise, 2) investigate correlations between commonly reported measures of AMPK activation during exercise (AMPK-α2 activity, p-AMPK, and p-ACC), and 3) formulate linear regression models to determine which factors best explain AMPK activation during exercise. To this end, we performed the largest pooled analyses of studies examining exercise-induced AMPK activation to date (~1000 participants) and provide novel insight into the factors influencing AMPK activation and relationships between markers of AMPK activity.

6.3 Methods

6.3.1 Literature Search

A literature search was performed using PubMed/MEDLINE using the terms ("AMPK"[Text Word] OR "acetyl coa carboxylase"[Text Word] OR "ACC"[Text Word] OR "AMP activated protein kinase"[Text Word]) AND "cycling"[Text Word]. A secondary search was performed through the Google Scholar database using the reference lists of all included publications and the studies that cited them.

6.3.2 Inclusion Criteria

All studies included in this analysis met the following inclusion criteria: (1) the study used human participants; (2) the study reported at least one of the markers of interest (AMPK-α2 activity, p-$AMPK^{Thr\text{-}172}$, or p- $ACC^{Ser\text{-}79/80 \text{ or } Ser\text{-}212/221}$) along with at least one of the following markers: maximal oxygen consumption (VO_{2max}), pre- and post-exercise muscle glycogen concentration, exercise intensity (as a percent of VO_{2max}), exercise intensity (as a percent of maximal power determined from an incremental exercise test), or exercise duration (min); (3) skeletal muscle biopsies were performed at rest and immediately post-exercise, as differences in p-AMPK have been reported between 0 and 30 min following exercise [280-282]; (4) two-legged cycling exercise was used, due to differences in substrate utilization between cycling and running and the variation in muscle sampling for biopsies in running studies (i.e., gastrocnemius vs. vastus lateralis) [283, 284]; (5) subjects had a VO_{2max} > 30 mL kg^{-1} min^{-1}, which includes ~90% of men under the age of 60 [285]; (6) analysis was performed on whole muscle and was not fiber-type specific.

6.3.3 Data Extraction

For studies reporting primary outcomes at multiple time points during exercise, each point was treated separately; for example, pre-exercise to 60 min and pre-exercise to 120 min [42, 99, 264]. For studies that included multiple groups, data were used if the interventions included factors that were accounted for in the analysis (e.g., differences in muscle glycogen levels, duration, or intensity) but only the control groups were used if the intervention arm included a variable not analyzed, such as hypoxia [267, 286], beetroot juice ingestion [287], antioxidant supplementation [288], or arginine infusion [289]. When AMPK activity was analyzed both with and without the presence of AMP [97, 262, 263, 269], the activity analyzed in the presence of AMP was used to be in accordance with the other studies. If insufficient data were reported, corresponding authors were contacted by-email. DigitizeIt software (Version 2.3, DigitizeIt, Germany) was used for the extraction of data from figures when raw data were not available.

To compare across studies using continuous exercise, which is often prescribed as a percentage of VO_{2max}, and high-intensity intermittent exercise (HIIE), which is often prescribed based on Watts (W), relative exercise intensity was standardized using both a percentage of VO_{2max} and percentage of the maximal power derived from an incremental exercise test (%W_{max}). Training load was calculated by multiplying the exercise intensity relative to W_{max} by the effective duration of the exercise session (in minutes). The training load value did not include recovery periods for which the exercise intensity was either not reported or when recovery was performed at a relative exercise intensity < 50% W_{max}. Because the recommended test duration to determine W_{max} (and VO_{2max}) is 8–12 min [290], a W_{peak} value determined from a longer-duration test will be underestimated [291, 292]. Therefore, to enable the most accurate comparisons between studies, a correction factor was used (Eq. 1) for studies that reported W_{peak} values from testing protocols > 12 min in duration to estimate the W_{max} value that would be expected from a 12-min incremental exercise test. This correction is based on the bioenergetic model proposed by Morton [293], confirmed by Adami et al. [291], and used in a previous meta-analysis [294].

(Eq. 1) $y = 1.8569x^{-0.242}$

A correction factor (y) was calculated based on the duration of the longer testing protocol expressed in minutes (x) using Eq. 1. The correction factor was then used to convert the reported peak power from the longer testing protocol (W_{peak}) to the estimated value of maximal power for a standard 12-min testing protocol ($W_{max'}$, both values expressed in Watts), using the following equation:

(Eq. 2) $W_{max'} = W_{peak} / y$

When $\dot{V}O_{2max}$ was reported but not W_{max} we used the equation by Hawley and Noakes [295] to estimate W_{max} (Eq. 3), which is based on the strong correlation between $\dot{V}O_{2max}$ and maximal cycling power output (r = .97, standard error of estimate 0.28 L min^{-1}).

(Eq. 3) VO_{2max} (L min^{-1}) = 0.01141 * W_{max} (W) + 0.435

6.3.4 Statistical Analysis

Standardized effect sizes were calculated as Hedges' g using pre- and post-exercise measurements. Pearson's correlation analysis was performed to determine relationships between the effect sizes for each of the primary outcome markers (AMPK-α2 activity, p-AMPK, p-ACC) and factors purported to influence AMPK signaling (muscle glycogen, carbohydrate ingestion, exercise duration and intensity, fitness level, and muscle metabolites). Using the *lme4* R package [296], general linear mixed-effect models were used to examine which factors were related to AMPK activation, with study ID specified as a random intercept. The following fixed effects were tested: muscle glycogen (starting level, end-exercise level, absolute reduction during exercise, and rate of reduction), exercise duration (min), exercise intensity (%VO_{2max}), exercise intensity (%W_{max}), training load (AU; duration * average intensity as a percentage of W_{max}), carbohydrate intake within 4 h of exercise (g), fitness level (VO_{2max}), and the Hedges' g effect sizes for the muscle metabolites creatine (Cr), phosphocreatine (PCr), PCr:total Cr (PCr+Cr), AMP_{free}, ADP_{free}, and lactate. Extreme outliers (>3 IQRs above the median) were removed from analyses for rate of glycogen reduction (> 17 mmol kg^{-1} dry mass min^{-1}) and exercise intensity (> 110 %W_{max}). Data for p-ACC measured at different phosphorylation sites were combined, as the likelihood ratio test showed no significant influence of the specific site (p = .738). To determine the best set of variables for predicting AMPK activation, the *glmulti* package [297] was used to generate all possible permutations of the fixed effects, selecting the best-fit models using Akaike's information criterion (AIC) scores [298]. Multicollinearity was assessed using the variance inflation factor (VIF), with > 5 as a cutoff to indicate excessive collinearity [299]. To focus on the influence of the modifiable variables, we also created models without the muscle metabolite markers. Estimated means for each of the three AMPK markers were calculated using the *emmeans* package [300]. Model fit is reported as marginal R^2, which describes the proportion of variance explained by the fixed effects alone, and conditional R^2, which describes the proportion of variance explained by both the fixed and random effects [301]. To separate the influence of ADP_{free}, exercise intensity, and muscle glycogen, partial correlations between these factors were calculated using the *ppcor* package [302]. The level of significance was set at $p < 0.05$. Descriptive statistics are provided as mean ± SD. Statistical analysis was carried out with R version 4.0.3 (The R foundation for Statistical Computing, Vienna, Austria).

6.4 Results

Eighty-nine studies were analyzed, which included 982 participants (6.2% female, mean age 26.7 ± 8.6 years, BMI 24.4 ± 1.9, VO_{2max} 51.9 ± 7.9 mL kg^{-1} min^{-1}). There were 77 data points for AMPK-α2 activity [13, 42, 97-99, 102-104, 261-267, 269, 289, 303-316], 157 for p-AMPK [5, 15, 17, 42, 62, 86, 99, 102, 105, 107, 112-114, 157, 172, 177, 264, 267, 274, 280-282, 286, 288, 289, 304-306, 308, 310, 311, 313, 317-349], and 157 for p-ACC [5, 15, 42, 47, 99, 102-105, 112, 114, 177, 262-264, 266, 267, 269, 274, 277, 278, 280-282, 286-289, 304-306, 308-315, 318, 319, 323-326, 328-334, 338-340, 342-346, 348, 350-353].

6.4.1 Correlations

Muscle glycogen concentration at the start of exercise showed no relationship with any marker of post-exercise AMPK activity (Fig. 6.2a-c). However, significant correlations were found between AMPK-α2 activity and both end-exercise muscle glycogen levels and the reduction in glycogen during exercise (absolute reduction and the rate of reduction), while p-AMPK correlated only with end-exercise glycogen levels and p-ACC correlated only with the rate of muscle glycogen breakdown (mmol kg^{-1} dry mass min^{-1}, Fig. 6.2d-l). Starting and end-exercise muscle glycogen levels ($r = .76$, $p < .001$) and starting muscle glycogen and exercise-induced glycogen reduction were also correlated (both absolute reduction, $r = .61$, $p < .001$, and reduction per min, $r = .39$, $p < .001$, supplemental Fig. S6.1). There was no relationship between the amount of carbohydrate consumed before or during exercise and AMPK activation (supplemental Fig. S6.2).

Exercise duration and training load (the product of exercise duration and average intensity as $\%W_{max}$) were not correlated with any marker of AMPK activity with the exception of a negative correlation between training load and p-ACC, while exercise intensity was positively correlated with p-AMPK and p-ACC when prescribed relative to VO_{2max} and with p-ACC when prescribed relative to W_{max} (Fig. 6.3). Fitness level (as determined by VO_{2max}) was not correlated with any marker of AMPK activity (Fig. 6.4a-c), but people with a higher VO_{2max} showed smaller disruptions in muscle metabolites such as PCr, Cr, and PCr: total Cr during exercise (Fig. 6.4d-f), as well as

AMP_{free} (r = -.32, p = .049, data not shown). Among the muscle metabolites, the exercise-induced increase in ADP_{free} was correlated only with AMPK-α2 activity, while no correlations were found for AMP_{free} (Fig. 6.5). Changes in PCr and Cr were correlated with AMPK-α2 activity only, while PCr:total Cr and lactate were correlated with p-ACC (Fig. 6.6, supplemental Fig. S6.3). Overall, none of the variables were significantly correlated with all three measures of AMPK activation, while ending glycogen, glycogen reduction rate, and exercise intensity (%VO_{2max}) were correlated with two out of three markers of AMPK activation (Fig. 6.7).

Partial correlations were calculated to determine if ADP_{free} is an independent predictor of AMPK activation or simply reflects a greater exercise intensity or greater glycogen depletion. The increase in ADP_{free} remained correlated with AMPK-α2 activity when removing glycogen reduction (r = .49, p = .017) and exercise intensity (r = .52, p = .011), but had no influence on p-AMPK or p-ACC (supplemental Table 6.1). Similarly, ending muscle glycogen remained correlated with AMPK-α2 activity when removing ADP_{free} (r = -.44, p = .033) and exercise intensity (r = -.33, p = .021), but had no influence on p-AMPK or p-ACC when removing these factors (supplemental Table 6.1).

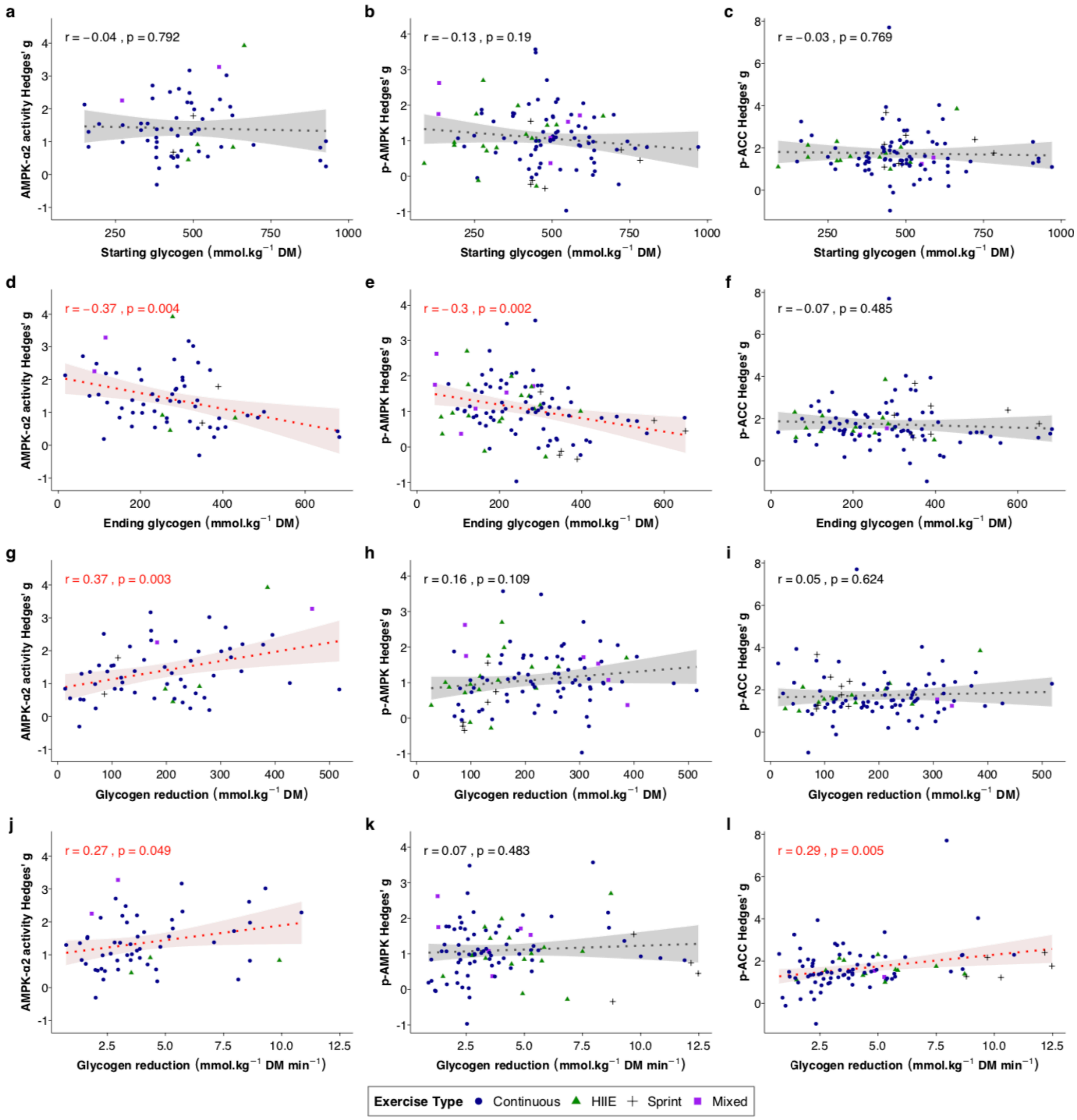

Figure 6.2. Linear correlations between Hedges' g effect size for change in markers of 5' AMP-activated protein kinase (AMPK) activity (pre to post exercise) and muscle glycogen levels at the start of exercise (a-c) and the end of exercise (d-f), absolute glycogen reduction during exercise (g-i), and the calculated rate of glycogen reduction per minute (j-l). CHO: carbohydrate, DM: dry mass. Exercise type is depicted separately as continuous exercise (circle), high-intensity interval exercise (HIIE, intermittent bouts > 30 s in duration, triangle), sprint exercise (single or repeated efforts < 30 s, cross), and mixed intensity exercise sessions (square). Shaded areas represent 95% confidence intervals, with significant correlations (p < 0.05) highlighted in red.

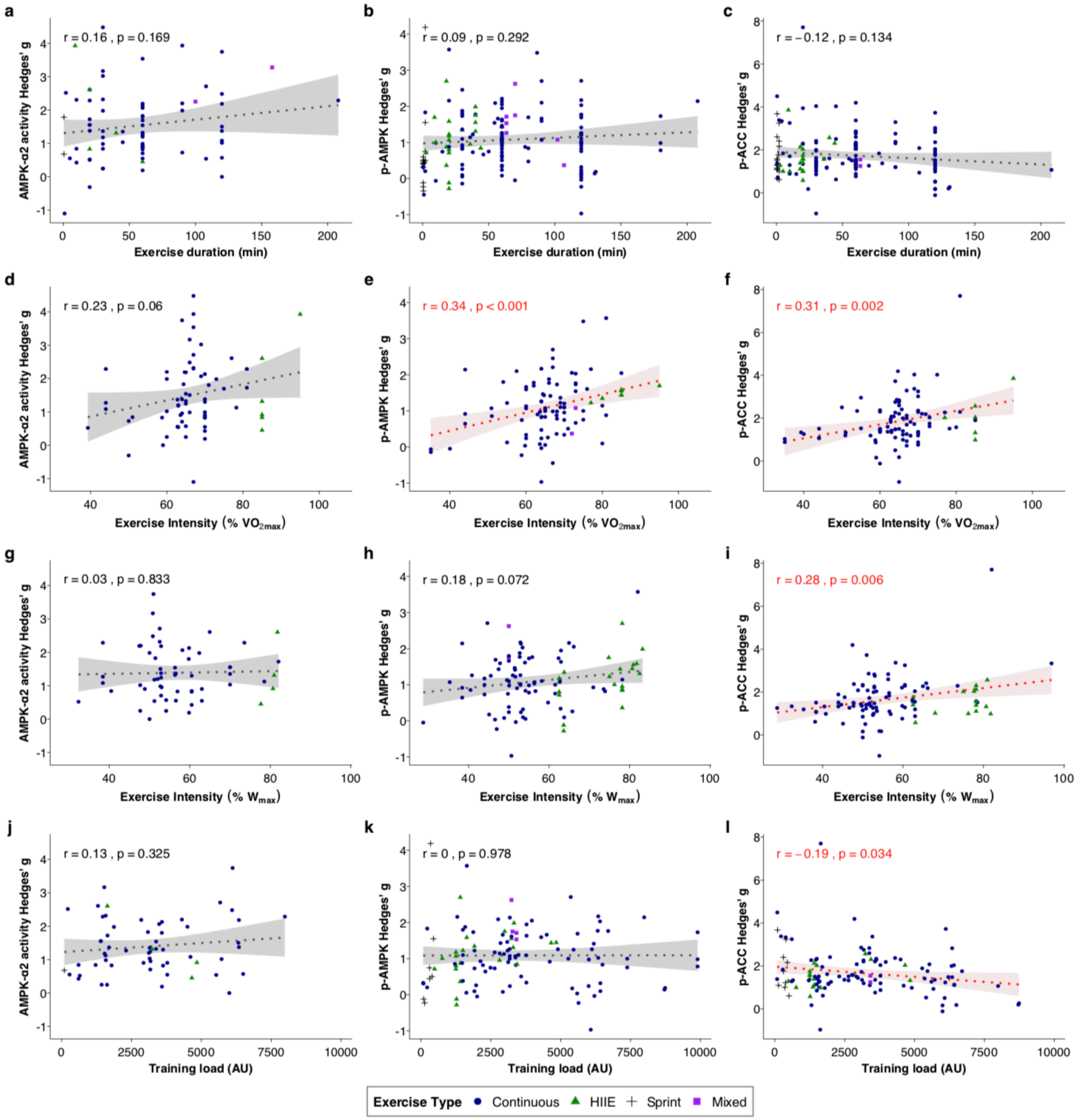

Figure 6.3. Linear correlations between Hedges' g effect size for change in markers of 5' AMP-activated protein kinase (AMPK) activity (pre to post exercise) and exercise intensity, duration, and training load (exercise intensity * duration). Exercise type is depicted separately as continuous exercise (circle), high-intensity interval exercise (HIIE, intermittent bouts > 30 s in duration, triangle), sprint exercise (single or repeated efforts < 30 s, cross), and mixed intensity exercise sessions (square). Shaded areas represent 95% confidence intervals, with significant correlations ($p < 0.05$) highlighted in red.

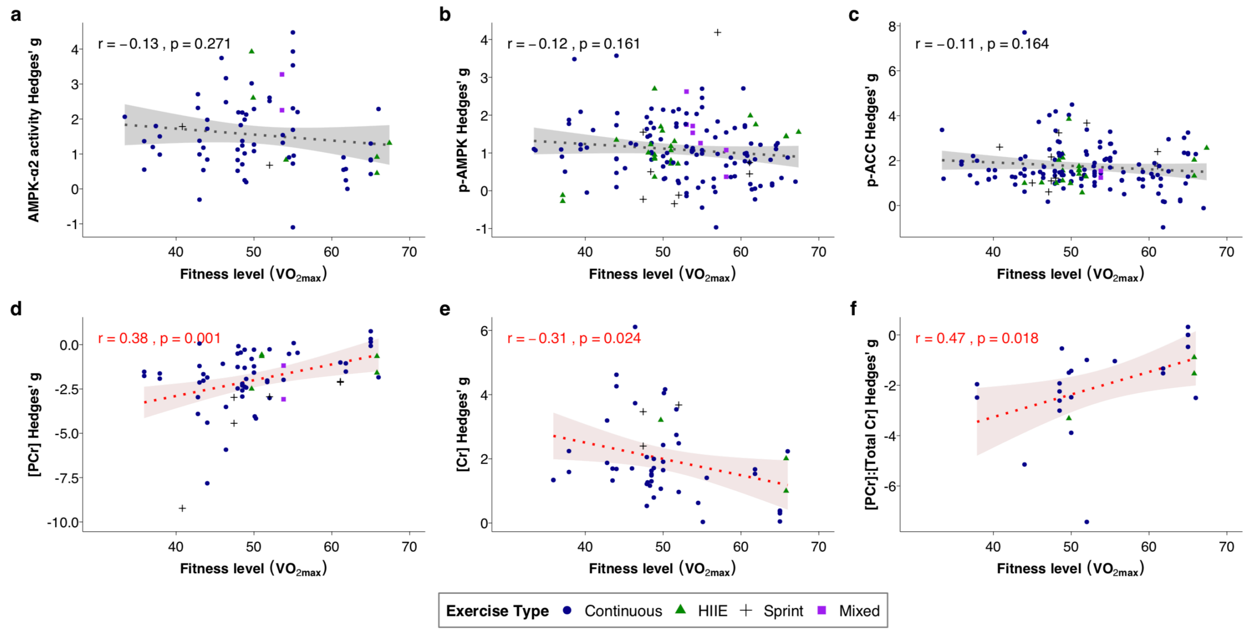

Figure 6.4. Linear correlations between fitness level (as determined by VO2max and expressed as ml kg-1 min-1) and Hedges' g effect size (pre to post exercise) for markers of 5' AMP-activated protein kinase (AMPK) activity (a-c) and effect size (pre to post exercise) for exercise-induced changes in muscle metabolite concentrations (d-f). Exercise type is depicted separately as continuous exercise (circle), high-intensity interval exercise (HIIE, intermittent bouts > 30 s in duration, triangle), sprint exercise (single or repeated efforts < 30 s, cross), and mixed intensity exercise sessions (square). Shaded areas represent 95% confidence intervals, with significant correlations ($p < 0.05$) highlighted in red.

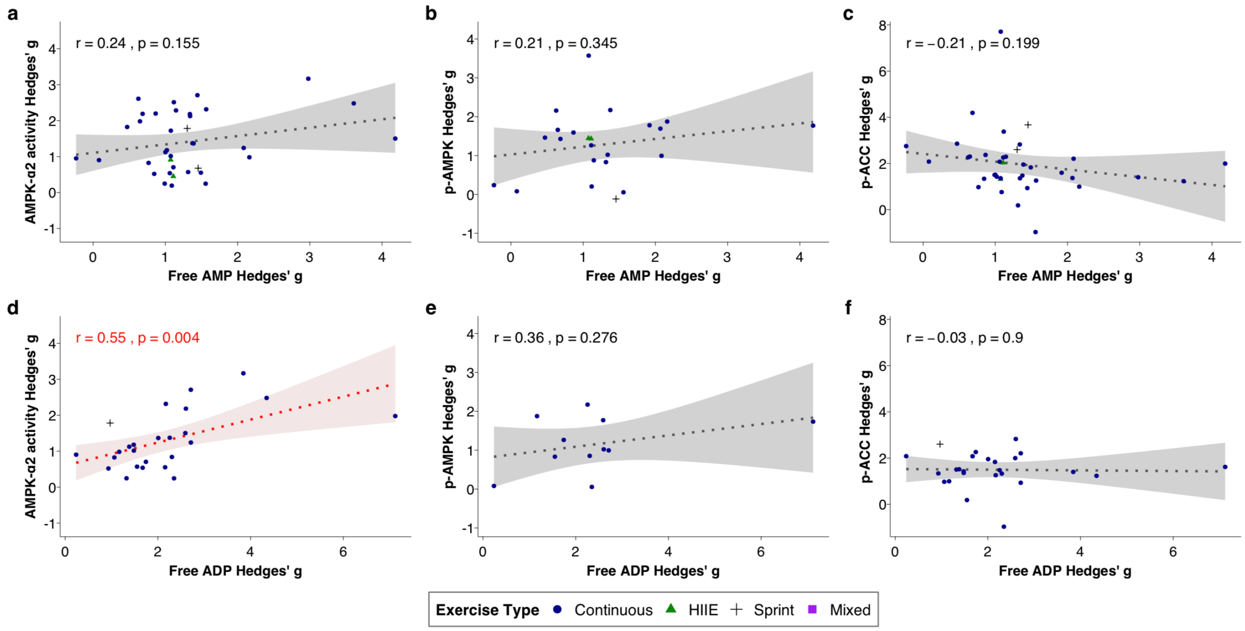

Figure 6.5. Linear correlations between Hedges' g effect size for change in markers of 5' AMP-activated protein kinase (AMPK) activity (pre to post exercise) and allosteric regulators of AMPK. ADP: Adenosine diphosphate, AMP: Adenosine monophosphate. Exercise type is depicted separately as continuous exercise (circle), high-intensity interval exercise (HIIE, intermittent bouts > 30 s in duration, triangle), sprint exercise (single or repeated efforts < 30 s, cross), and mixed intensity exercise sessions (square). Shaded areas represent 95% confidence intervals, with significant correlations ($p < 0.05$) highlighted in red.

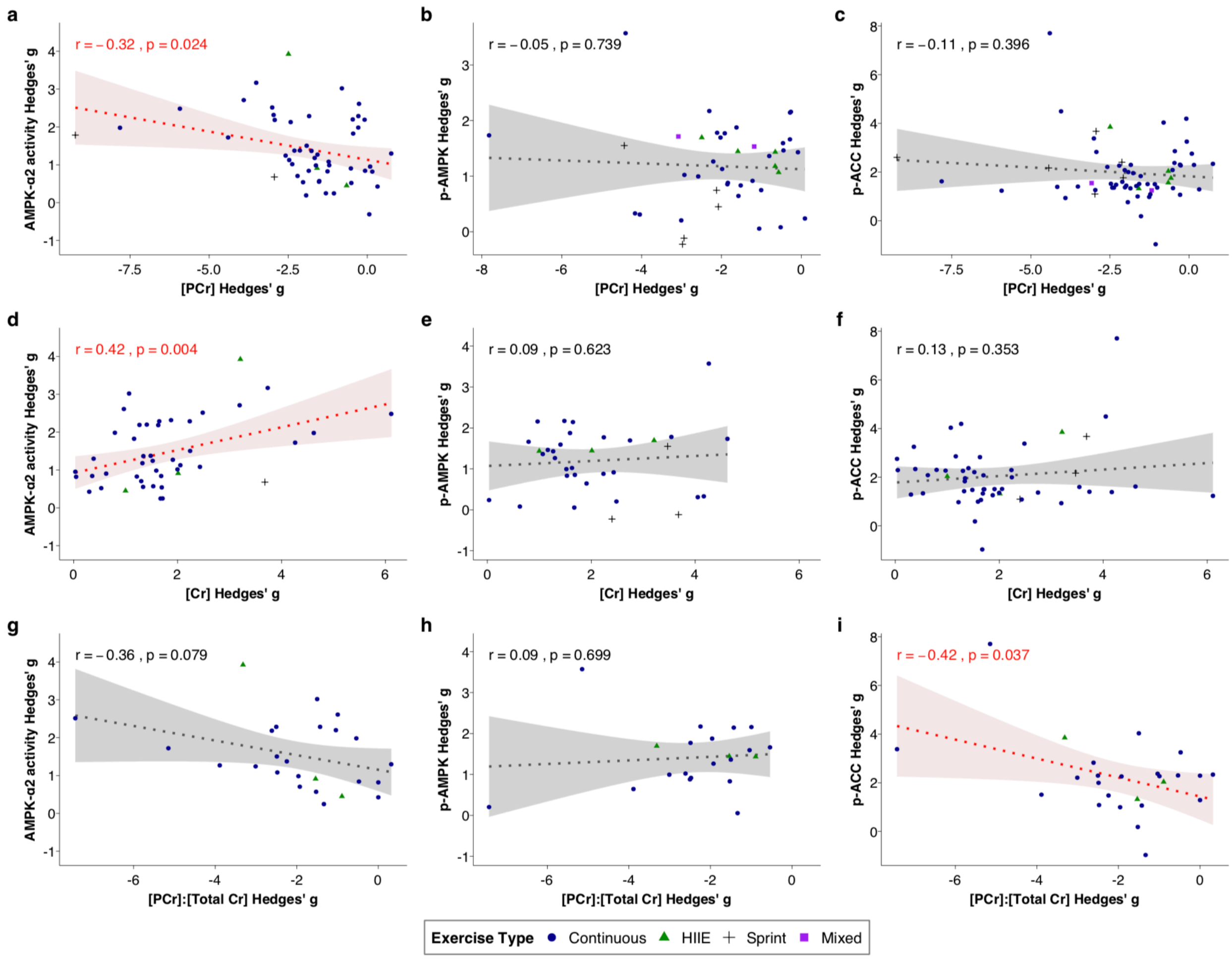

Figure 6.6. Linear correlations between Hedges' g effect size for change in markers of 5' AMP-activated protein kinase (AMPK) activity (pre to post exercise) and intramuscular metabolites. Cr: creatine, PCr: phosphocreatine. Exercise type is depicted separately as continuous exercise (circle), high-intensity interval exercise (HIIE, intermittent bouts > 30 s in duration, triangle), sprint exercise (single or repeated efforts < 30 s, cross), and mixed intensity exercise sessions (square). Shaded areas represent 95% confidence intervals, with significant correlations ($p < 0.05$) highlighted in red.

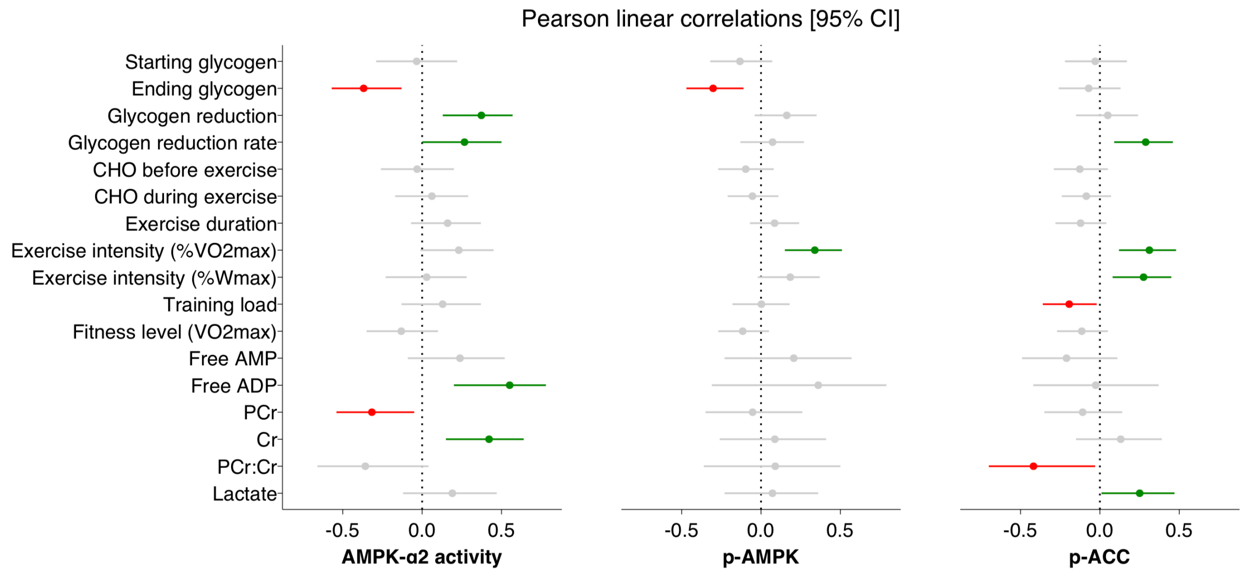

Figure 6.7. Summary of linear correlations between Hedges' g effect size for change in markers of 5' AMP-activated protein kinase (AMPK) activity (pre to post exercise) and factors purported to influence them, shown as Pearson correlation values and 95% confidence intervals. For lactate, phosphocreatine (PCr), creatine (Cr), PCr:Cr ratio, free AMP, and free ADP, calculations were based on the Hedges' g effect size for change from pre to post exercise. Colored lines indicate statistically significant positive (green) and negative (red) correlations.

6.4.2 Markers of AMPK Activation

Significant positive correlations were found between all three markers of AMPK activity, with the strongest (r = .56) being between p-AMPK and AMPK-α2 activity (Fig. 6.8a-c). Because the AMPK-α2/β2/γ3 complex has been identified as the one primarily activated in skeletal muscle during exercise [274], we also examined its relationship to the other markers and found significant correlations with p-ACC and AMPK-α2 activity (Fig. 6.8d-f).

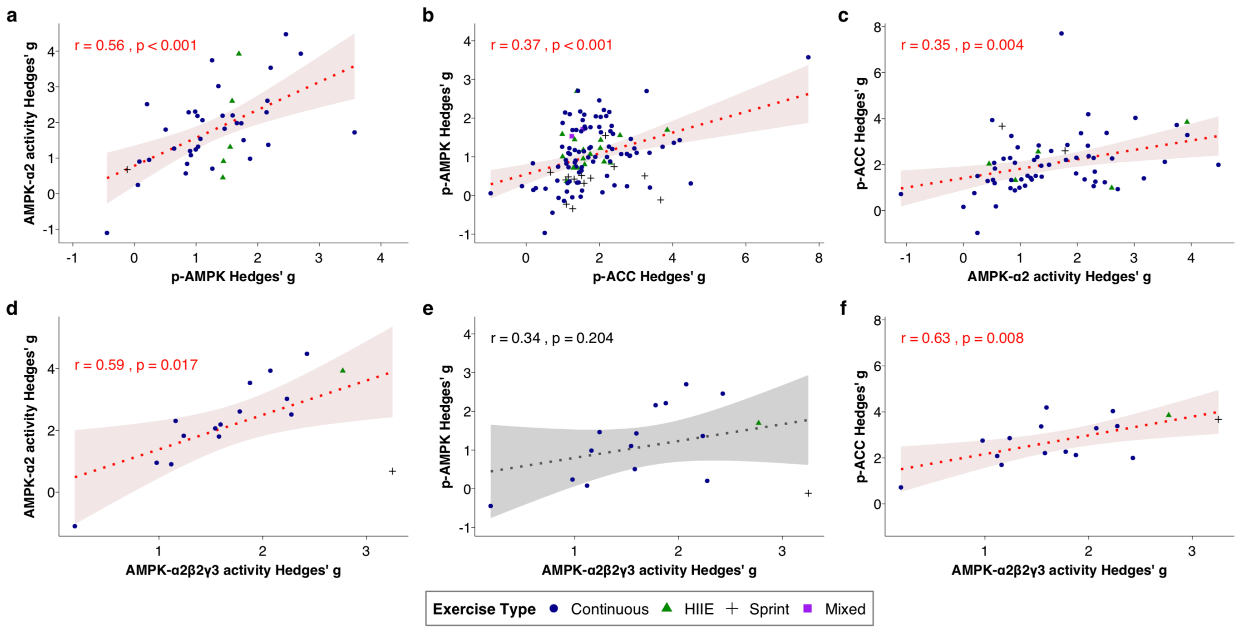

Figure 6.8. Linear correlations between markers of 5' AMP-activated protein kinase (AMPK) activity Hedges' g effect size (pre to post exercise). Panels (a) and (c) are using total AMPK-α2 activity, panels (d-f) are using only the AMPK-α2/β2/γ3 protein complex. Exercise type is depicted separately as continuous exercise (circle), high-intensity interval exercise (HIIE, intermittent bouts > 30 s in duration, triangle), sprint exercise (single or repeated efforts < 30 s, cross), and mixed intensity exercise sessions (square). Shaded areas represent 95% confidence intervals, with significant correlations ($p < 0.05$) highlighted in red.

6.4.3 Multivariable linear mixed models

The best fitting models with and without muscle metabolites are shown in Table 6.1. Standardized coefficients are provided, to allow a better comparison of the relative influence of each factor. The marginal R^2 values for models that include muscle metabolites are considerably higher than the models that do not contain them, indicating a large amount of variance explained by these factors.

Table 6.1. Multivariable linear mixed models that best predict markers of AMPK activity during exercise with (+) and without (-) the inclusion of muscle metabolites

Variable	AMPK-α2 activity (+)	AMPK-α2 activity (-)	p-AMPK (+)	p-AMPK (-)	p-ACC (+)	p-ACC (-) (model 1)	p-ACC (-) (model 2)	p-ACC (-) (model 3)
Starting glycogen[a]				-0.150 (.306)		-0.065 (.499)		
Ending glycogen[a]		-0.384 (< .001)					-0.065 (.522)	
Glycogen reduction[b]								-0.033 (.755)
Fitness level (VO_{2max})				-0.353 (.015)		-0.571 (<.001)	-0.573 (<.001)	-0.605 (<.001)
CHO before (g)				-0.117 (.427)		0.025 (.905)	0.030 (.886)	0.040 (.857)
Exercise intensity (%VO_{2max})		0.332 (.005)	-1.087 (.016)	0.511 (<.001)	-0.234 (.151)	0.187 (.062)	0.173 (.099)	0.194 (.074)
ADP_{free}	-0.443 (.106)		-1.466 (<.001)		-0.309 (.128)			
Cr	0.513 (.027)		1.279 (.008)					
PCr:total Cr	-1.135 (<.001)		-1.131 (<.001)		-1.044 (<.001)			
Lactate (muscle)	-0.652 (.005)							
Marginal R^2	**.81**	**.25**	**.68**	**.30**	**.82**	**.23**	**.23**	**.22**
Conditional R^2	.81	.66		.41		.77	.77	.78
RMSE	.173	.456	.259	.544	.532	.545	.545	.543

Standardized model coefficients, their corresponding p-values (in parentheses), marginal R^2 (variance explained by the fixed factors alone), conditional R^2 (variance explained by both the fixed and random factors), and root mean square error (RMSE) of the best linear mixed models with (+) and without (-) the inclusion of muscle metabolites to explain changes in 5' AMP-activated protein kinase (AMPK) activity during exercise. For p-AMPK (+) and p-ACC (+) it was not possible to estimate conditional R^2 due to the limited number of studies used. For p-ACC (-) three models are included because a single best model could not be determined based on Akaike's information criterion (AIC). CHO: carbohydrate, Cr: creatine, PCr: phosphocreatine. [a] Glycogen in mmol kg^{-1} dry mass [b] Glycogen reduction units are absolute change (mmol kg^{-1} dry mass) from pre- to post-exercise

Figures 6.9–6.11 show the estimated marginal means when exercising across a range of exercise intensities and in relation to all variables included in the models from Table 6.1 without muscle metabolites. The p-ACC values are based on p-ACC (-) model 1 in Table 6.1, with p-ACC (-) models 2 and 3 shown in supplemental Fig. S6.4 and S6.5. For the interested reader, an online app has been created to allow exploration of the data and predict AMPK activation based on the models without muscle metabolites shown in Table 6.1 [354].

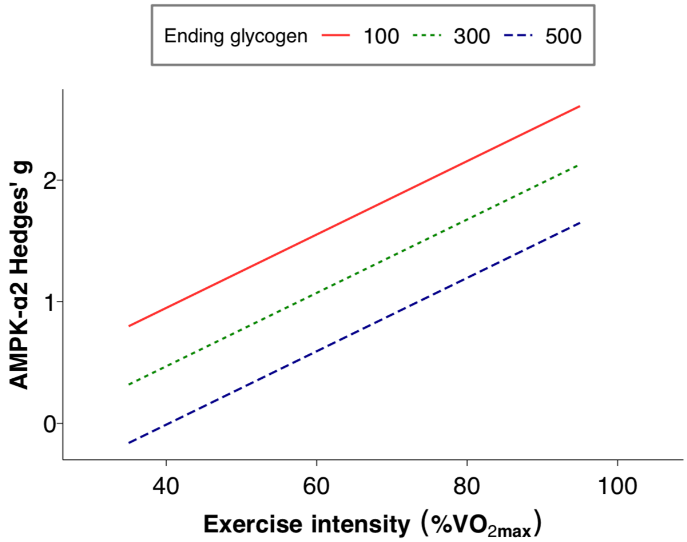

Figure 6.9. Model prediction showing estimated Hedges' g effect sizes for AMPK-α2 activity when exercising at different intensities (as determined by %VO2max) related to three different ending glycogen levels (mmol kg-1 dry mass). Values are based on the regression model in Table 6.1 without muscle metabolites. Using this model, it would be predicted that similar AMPK-α2 activation would occur following exercise at ~50% VO_{2max} that ends with muscle glycogen levels of 100 mmol kg^{-1} dry mass and exercise at ~80% VO_{2max} that ends with muscle glycogen levels of 500 mmol kg^{-1} dry mass.

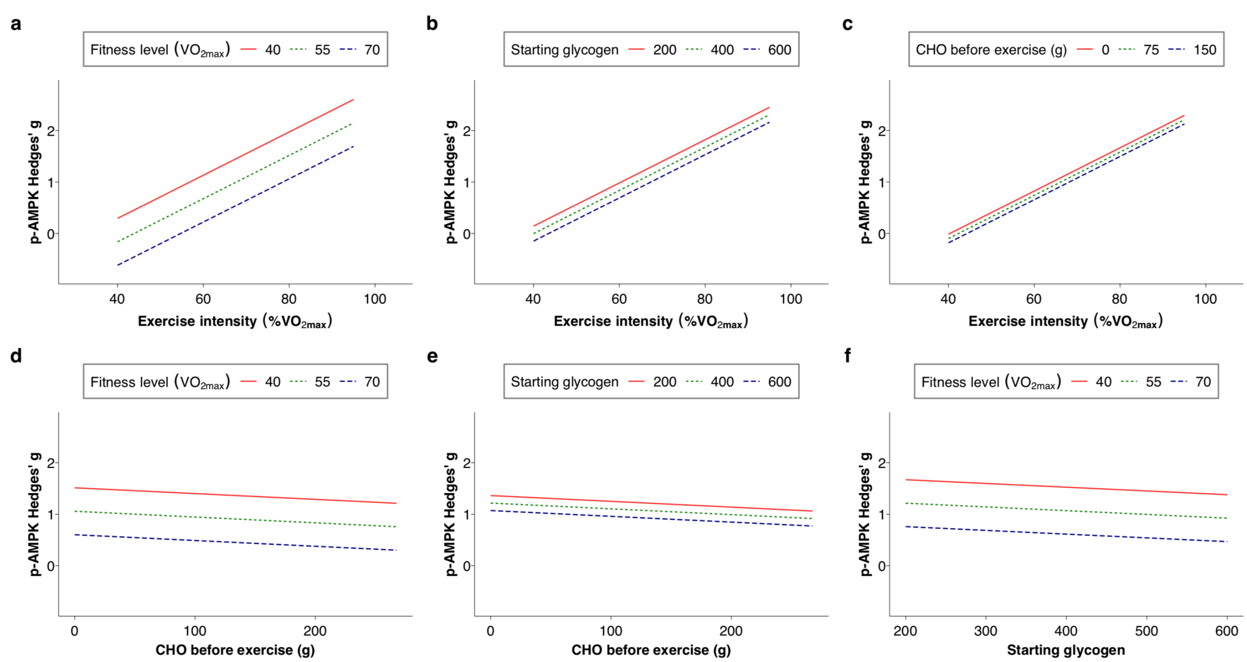

Figure 6.10. Model predictions showing estimated Hedges' g effect sizes for p-AMPK when exercising at different intensities (as determined by %VO2max) related to three different fitness levels (as expressed by VO2max in mL kg-1 min-1, a), starting glycogen levels (mmol kg-1 dry mass, b), and carbohydrate (CHO) intakes before exercise (grams, c), CHO intake before exercise related to fitness level (d) and starting glycogen (e), and starting glycogen related to fitness level (f). Values are based on the regression model in Table 1 without muscle metabolites. Steeper slopes indicate a larger effect of the variable shown on the x-axis, and greater space between lines indicates a larger effect of the variable being plotted. Although linear correlations revealed no relationship between markers of AMPK activation and starting glycogen (Fig. 6.2), fitness level (Fig. 6.4), or carbohydrate ingestion before exercise (supplemental Fig. S6.2), these variables contribute to the best model for p-AMPK (when muscle metabolites are not included). Based on these model predictions, p-AMPK will be lower with higher fitness level (a, d, f), greater CHO intake before exercise (c, d, e), and higher starting levels of muscle glycogen (b, e, f).

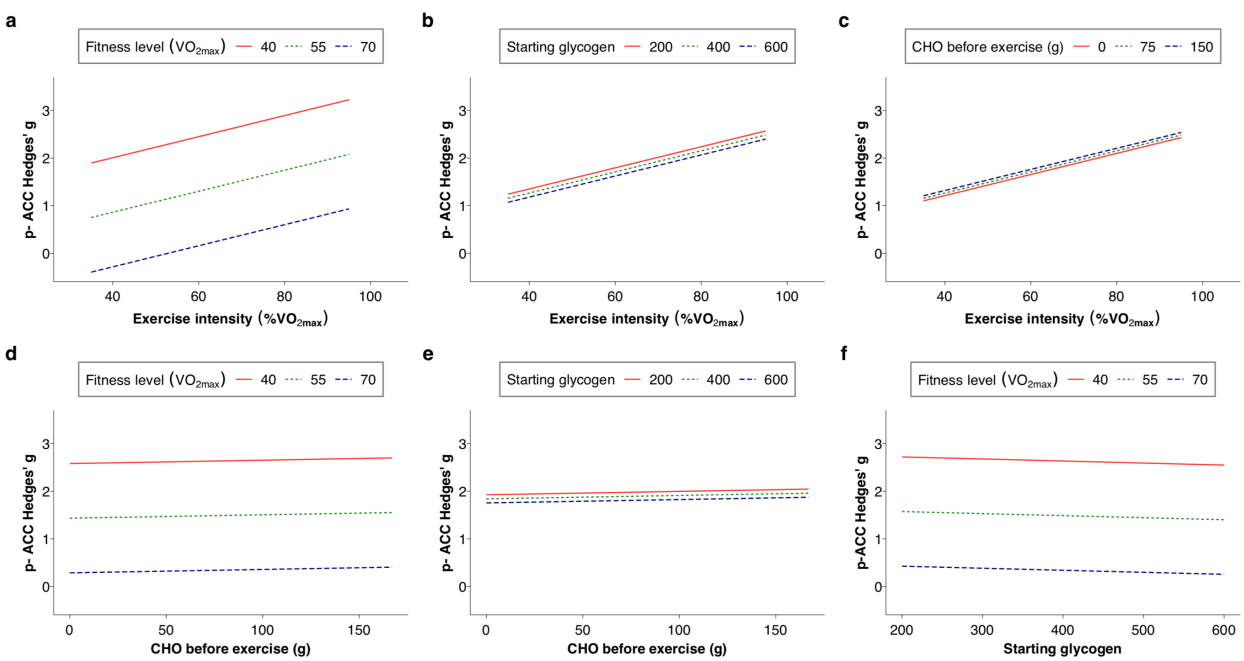

Figure 6.11. Model predictions showing estimated Hedges' g effect sizes for p-ACC when exercising at different intensities (as %VO2max) related to three different fitness levels (as expressed by VO2max in mL kg-1 min-1, a), starting glycogen levels (mmol kg-1 dry mass, b), and carbohydrate (CHO) intakes before exercise (grams, c), CHO intake before exercise related to fitness level (d) and starting glycogen (e), and starting glycogen related to fitness level (f). Values are based on p-ACC (-) model 1 in Table 6.1, with p-ACC (-) models 2 and 3 depicted in supplemental Fig. S6.4 and Fig. S6.5. Steeper slopes indicate a larger effect of the variable shown on the x-axis, and greater space between lines indicates a larger effect of the variable being plotted. These figures demonstrate the far greater influence of exercise intensity and fitness level, compared with muscle glycogen levels, on p-ACC signaling.

6.5 Discussion

The objectives of this analysis were to investigate correlations between factors influencing AMPK activation and commonly reported measures of AMPK activation during exercise, and to formulate linear regression models to determine which factors best explain AMPK activation during exercise. We found statistically significant correlations between AMPK activity and ending glycogen levels, exercise intensity, and post-exercise muscle metabolites (including ADP_{free}, PCr, and Cr) for some, but not all, markers of AMPK activity. No linear correlations were found between starting muscle glycogen level, carbohydrate ingestion before exercise, exercise duration, or fitness level (determined by VO_{2max}) and any marker of AMPK activity; however, these variables did contribute to some of the linear mixed models (Table 1). We also confirmed low to moderate correlations ($r = .35–.56$) between the commonly used markers of AMPK-α2 activity. Finally, the modeling was able to explain between 68–82% of the variance in AMPK activity when including muscle metabolites and exercise intensity, while models excluding the muscle metabolites and focusing on muscle glycogen, carbohydrate ingestion, fitness level, and exercise intensity could only explain 22–33% of the variance in AMPK activity.

6.5.1 Glycogen and Carbohydrate

Muscle glycogen concentrations before, during, and after exercise can influence AMPK signaling via carbohydrate binding modules on the β subunits that alter the conformation of AMPK, and therefore its activity [100, 355]. Although undertaking exercise with reduced muscle glycogen concentrations can increase AMPK activity relative to exercise undertaken with higher muscle glycogen [103, 104], we observed no relationship between starting muscle glycogen concentrations and any markers of AMPK signaling (Fig. 6.2a-c), pointing towards the influence of additional factors. At the same time, significant correlations were found between both ending glycogen and the glycogen reduction during exercise, and markers of AMPK signaling (Fig. 6.2d-l). It is challenging to separate the influence of ending glycogen level, the absolute decrease in glycogen, and the rate of glycogen reduction during exercise. However, very high rates of glycogen breakdown (e.g., > 150 mmol kg^{-1} dry mass min^{-1}) reported during short-duration maximal exercise do not linearly increase AMPK (supplemental Fig. S6.6), suggesting end-exercise glycogen levels and/or the absolute reduction in glycogen during exercise has a greater influence on AMPK activation. Future research is needed to distinguish the relative importance of the absolute glycogen reduction and end-exercise glycogen levels on AMPK activation.

Despite the observed relationships between muscle glycogen and AMPK activity, several lines of evidence support the notion that glycogen concentrations are not the primary factor influencing AMPK signaling. In patients with McArdle's disease (a genetic defect leading to chronically high muscle glycogen stores and an inability to utilize muscle glycogen as a metabolic substrate), AMPK is activated during exercise despite the absence of glycogen breakdown [309]. When exercising at the same absolute intensity in normoxia and hypoxia, there were no significant differences in AMPK signaling despite significantly different levels of net muscle glycogen use [267]. Furthermore, differences in AMPK activation have been observed between trained and untrained subjects despite similar glycogen utilization during exercise [99], and no effects on AMPK activation were seen during exercise undertaken with graded levels of low [105, 106] or high muscle glycogen [266]. Taken together, undertaking exercise with low, compared with high, muscle glycogen can increase AMPK activation during low- to moderate-intensity exercise, and ending glycogen levels appear more influential than starting levels but are not the primary driver of AMPK activity.

Only about one-third of endurance athletes regularly consume carbohydrate before training sessions, and many athletes perform exercise in the overnight-fasted state in an attempt to maximize their training adaptations [21, 22]. Consuming carbohydrate before exercise increases plasma insulin concentrations [59]. Although insulin can reduce AMPK activity [356], our analysis did not find any linear relationship between pre-exercise carbohydrate ingestion and AMPK signaling (supplemental Fig. S6.2). However, the p-AMPK model revealed a negative, albeit non-significant, influence of carbohydrate ingestion on AMPK activation (Fig. 6.10). Studies that have directly compared fed and fasted-state training have largely found no effects on p-AMPK and p-ACC [15, 62, 112, 282, 333], although there have been increases [15] and reductions [112, 282] in AMPK signaling markers in the fed state. Because muscle glycogen breakdown is generally [63, 72-74, 357], although not always [358], similar between fed and fasted-state exercise when starting with similar glycogen levels, similar AMPK activation would be expected. Nonetheless, there are several reasons AMPK activity has the potential to be increased during exercise in the fasted state. Ingestion of large quantities of carbohydrate can increase muscle glycogen within three hours [359], and thus have the potential to alter AMPK activity via increased starting, and therefore ending, muscle glycogen. Additionally, AMPK can be directly activated by long-chain fatty acids [275], which are generally elevated when exercising in the fasted state [225]. However, despite the plausibility of fasted-state training increasing AMPK activity the available evidence in support of this notion is weak.

Likewise, carbohydrate ingestion during exercise does not appear to influence AMPK signaling [42, 62, 86, 333]. It is possible that AMPK activity could be attenuated if muscle glycogen breakdown is reduced. Ingesting carbohydrate during one-legged knee-extensor exercise reduced muscle glycogen breakdown and AMPK-α2 activity [50], but the lack of significant differences in p-AMPK and p-ACC$^{Ser\text{-}221}$, and the low absolute exercise intensity (VO_2 ~12.4 $mL.kg^{-1}.min^{-1}$), minimizes the generalizability of these findings. Although carbohydrate ingestion during exercise can attenuate skeletal muscle AMP_{free} concentrations and the AMP:ATP ratio during exercise, increases in AMP_{free} can still be sufficient to activate AMPK [42]. Furthermore, exercise-induced changes in PCr and ADP are not affected by carbohydrate ingestion during exercise [42, 158]. Therefore, carbohydrate ingestion immediately before and/or during exercise is unlikely to alter skeletal muscle AMPK signaling during exercise.

6.5.2 Exercise Duration and Intensity

Our analysis did not find any relationship between exercise duration and AMPK activation (Fig. 6.3a-c). Some [42, 98, 99, 269, 332], but not all [104, 261, 264, 265, 329], studies taking biopsies at multiple time points have shown increased AMPK activity with exercise duration, during moderate-intensity continuous exercise. It should also be noted, that by focusing our analysis on muscle biopsies performed immediately following exercise some increases in AMPK signaling following low-volume HIIE may have been missed. For example, p-AMPK was not increased immediately following a single 30-s sprint, but elevations have been found 30 min after exercise [280-282]. Additionally, p-ACC$^{Ser\text{-}221 \text{ or } Ser\text{-}79}$ was elevated immediately following a single 30-s sprint, implying some AMPK activation could have been missed if only measuring p-AMPK [280-282, 326].

It has long been established that AMPK activity is increased with exercise intensity [97, 261, 263]. Accordingly, our analysis revealed significant correlations between intensity (as %VO_{2max}) and p-AMPK and p-ACC (Fig. 6.3e-f). A drawback of basing intensity on VO_{2max} is that it excludes many of the interval training studies that prescribed intensity based on W_{max}. Upon converting exercise intensities to %W_{max} the relationships became less clear (Fig. 6.3g-i), possibly because several studies used very short duration HIIE. To this end, we calculated a session training load as the product of intensity (using percent W_{max}) and duration (min). Surprisingly, there still was not a positive relationship between training load and AMPK activity (Fig. 6.3j-l). This could be related to the biopsy sampling time point, discussed above, and/or highlight the importance of other factors beyond training load for AMPK activation. When comparing the influence of absolute vs. relative exercise intensity using hypoxia (111 W, 72% of hypoxia VO_{2max}) and normoxia matched to the same absolute (111 W, 51% of normoxia VO_{2max}) or relative intensity (171 W, 73% of normoxia VO_{2max}), greater AMPK activation, along with a greater muscle energy imbalance, was found during the 73% normoxia trial, demonstrating the importance of the absolute intensity over the relative intensity in the activation of skeletal muscle AMPK during exercise [267]. Overall, exercise intensity, but not duration, is the driving factor for AMPK activation during exercise, which is related to greater disturbances in cellular energy homeostasis.

6.5.3 Fitness Level and Metabolites

Exercise training leads to an improved ability to maintain cellular energy charge during exercise [360]. Trained participants demonstrate smaller exercise-induced increases in lactate and

AMP_{free}, and better preserve the PCr: total Cr ratio [99, 310]. For example, skeletal muscle concentrations of AMP_{free} increased nearly five times more in untrained, compared with trained individuals cycling at 65% VO_{2max}, corresponding with greater AMPK activation [99]. However, differences in AMPK activation with training status may be intensity dependent as similar increases have been reported in untrained and trained subjects following exercise between 75–85% VO_{2max} [310, 313, 327]. This could be because the perturbations in cellular energy charge induced by exercise, rather than total energy flux, are a key factor in AMPK activation [310, 361]. Accordingly, muscle metabolites showed the strongest correlations with AMPK activity and were the best model predictors in our analysis. Furthermore, ADP_{free} remained a significant predictor of AMPK-α2 activity even when removing the influence of exercise intensity and muscle glycogen.

We used VO_{2max} as an indicator of fitness level/training status, but this alone may insufficiently account for short-term training-induced adaptations. Despite negligible changes in VO_{2max}, dramatic improvements in the ability to regulate cellular energy homeostasis and increase muscle glycogen storage capacity can be seen following just 7 to 10 days of training [266, 318, 362]. The reduction in AMPK activation following short-term exercise training coincides with the reduction in exercise-induced alterations in AMP and PCr, but the ratio between AMPK-α2 activity and AMP_{free} during exercise remains [266]. Accordingly, AMPK signaling was attenuated when exercising between ~65–70% VO_{2max} following two [266, 318] and 12 [308] weeks of endurance training, concomitant with improved maintenance of cellular energy charge. However, similar exercise-induced increases in p-AMPK in response to HIIE were found following three weeks of training [304]. Although there were improvements in cellular energy homeostasis following training, the HIIE protocol used (8 x 5 min at 85% VO_{2max}) was sufficient to disturb energy charge and activate AMPK pathways [304]. To summarize, trained athletes have reduced AMPK activation during low- to moderate-intensity exercise, likely due to the improved maintenance of cellular energy homeostasis, but these differences may be minimized with higher-intensity exercise. Additionally, future studies using HIIE should measure changes in AMP_{free}/ADP_{free}, as nearly all studies reporting these measures have used continuous exercise.

6.5.4 Correlations Between Markers of AMPK Activity

Many studies use p-AMPK and/or p-ACC as a marker of AMPK activity, due to the reported correlations between AMPK-α2 activity, p-AMPK, and p-ACC in humans [269] and animals [276]. Although p-ACC is considered a sensitive indicator of skeletal muscle AMPK activity [263, 276], dissociations between markers are commonly observed [103, 112, 264, 282, 310, 311, 324, 326, 330, 334]. The measurement techniques for analyzing AMPK activity indicate the extent of phosphorylation by its kinase and not *in vivo* activity that is subject to additional allosteric activation by AMP [97, 267]. Therefore, it is possible that p-ACC is indeed a more sensitive marker of AMPK activity because it reflects both covalent and allosteric modifications [264]. Elevated p-ACC in the absence of detectable changes in p-AMPK may indicate AMPK activity has been elevated primarily due to allosteric modulation [264], while covalent modifications that induced a measurable change of AMPK-α2 activity or p-AMPK may not be reflected in a further rise in p-ACC [263, 363]. Changes in the PCr:total creatine ratio are likely to influence AMPK activity through its effect on the AMP:ATP ratio via the creatine kinase equilibrium reaction [311], while muscle glycogen can influence AMPK activity through its interaction with the carbohydrate-binding module on the β subunit [100]. Therefore, it is possible that initial increases in AMPK activity during exercise could depend on changes in the AMP:ATP ratio, while subsequent changes are more influenced by the reduction in muscle glycogen content. This time-course dependent activation of AMPK could help to explain some of the contrasting findings with regard to exercise intensity, duration, and differential activation of AMPK signaling makers.

The specific subunit being activated may also be relevant for detecting changes in AMPK activation. In human skeletal muscle only three AMPK heterotrimeric complexes are found α2/β2/γ1 (~65%), α2/β2/γ3 (~20%), and α1/β2/γ1 (~15%) [364]. Our analysis revealed significant correlations between the AMPK-α2/β2/γ3 complex and both AMPK-α2 activity and p-ACC, but not p-AMPK (Fig. 6.8). This finding is in accordance with suggestions that p-AMPK, reflecting both AMPK-α1 and -α2 subunits, would be less sensitive for detecting changes in AMPK activity that are only occurring in the α2/β2/γ3 heterotrimer, while p-ACC may be a good predictor of α2/β2/γ3 activity but may not as closely predict total AMPK activity during exercise [274, 305, 314]. This may be because the AMPK-α2/β2/γ3 complex accounts for only ~20% of AMPK complexes in human skeletal muscle and only ~10% of the total α2 protein pool is phosphorylated during exercise, so changes that occur only in that complex may go undetected [274]. Training-induced reductions in AMPK-α2/β2/γ3 protein content [308, 365] could also help to explain the attenuation of AMPK activity following short and longer-term endurance training [99, 266].

Beyond ACC there are several other known AMPK substrates that are regulated by exercise, which extend AMPK's influence to the regulation of vesicle transport, potassium and chloride transport, calcium homeostasis and excitation contraction coupling, myosin phosphorylation, lipid and glucose transport, and mitochondrial signaling [366]. Our analysis was focused on ACC because it is the most-commonly measured substrate of AMPK and is widely used as a surrogate marker of AMPK. Consideration of other downstream targets of AMPK such as TBC1 domain family members 1 and 4, which regulate cellular transport of GLUT4-containing vesicles, and glycogen synthase, a key regulator of glycogen synthesis [260], would likely give a more complete picture of AMPK activation. Furthermore, several other regulatory phosphorylation sites, in addition to Thr-172, exist on the AMPK complex. These include Ser-485/491 in the α subunit, phosphorylated during insulin stimulation, and Ser-108 and Thr-148 within the glycogen binding domain in the β subunit that regulates binding capacity to glycogen particles as well as kinase activity [260].

6.5.5 Models

To our knowledge, this is the first meta-regression analysis of factors influencing AMPK activity during exercise. We initially aimed to use modifiable factors such as exercise intensity, duration, and muscle glycogen levels. However, without inclusion of the muscle metabolites (e.g., AMP_{free}/ADP_{free}, PCr, Cr) the regression models had considerably less predictive power (marginal R^2 values of 0.23–0.30). This underscores the importance of disturbing cellular energy charge to activate AMPK during exercise and is one of the key findings of this analysis. Our findings are in accordance with studies showing significant relationships between exercise-induced AMPK activity and changes in muscle glycogen, the PCr: total Cr ratio, and Cr [308, 310, 311], and with mathematical modeling suggesting changes in AMPK activity are determined principally by ADP [268]. It has also been suggested that AMP_{free} concentrations, which are not affected by training status, muscle glycogen content, or exercise duration [266], are a key determinant of AMPK activity during exercise [262, 263, 266, 267], although some uncoupling between skeletal muscle AMP_{free} and AMPK activation has been reported [42, 269]. The PCr: total Cr ratio offers another indication of cellular energetic stress [310], but this too can be dissociated from AMPK signaling [266]. Nonetheless, these variables were among the strongest predictors in our modeling. When focusing on the modifiable factors (e.g., exercise intensity/duration and muscle glycogen) and removing the muscle metabolites, the models are weakened considerably highlighting the importance of disturbing cellular energy homeostasis during exercise.

From a practical standpoint, exercising above an individual's critical power would be a way to disturb cellular energy charge and activate AMPK. Critical power demarcates the exercise intensity above which intramuscular metabolic homeostasis cannot be achieved, and occurs between 70–90% VO_{2max}, depending on training status [367]. Critical power is also decreased following extended exercise, irrespective of changes in muscle glycogen [368, 369], which could help to explain why AMPK activation is observed during longer-duration lower-intensity exercise. Model predictions shown in Figs. 6.9–6.11 can be used a guide for maximizing the AMPK response to exercise, and as an estimate of the conditions needed to elicit a minimum amount of AMPK activation.

6.5.6 Limitations

There are several noteworthy limitations to this analysis. Study participants were nearly all male (~95%), with a mean age of 27 years. Although no sex differences have been observed in the AMPK response to high-intensity [255, 281] or submaximal cycling [306, 370, 371], AMPK-α2 activity, p-AMPK, AMP_{free}, and Cr concentrations have increased during submaximal cycling in men but not women [311]. As discussed earlier, limiting our analysis to muscle biopsies taken immediately post-exercise may have obscured some AMPK activation from short-duration sprint interval training, which would be expected to induce large changes in muscle metabolites and can be further elevated 30 min following exercise [280-282]. There is also the possibility of fiber-specific differences in AMPK activation (greater in type-IIx [305, 372]), and indices of whole-muscle AMPK activity may obscure potential fiber-specific changes in AMPK activation [255, 305, 372]. Although this analysis was limited to cycling activity, these findings should be translatable to other forms of aerobic exercise such as running or swimming. This is because the key driver of AMPK activation, disturbing muscular homeostasis, occurs in response to all forms of aerobic exercise performed beyond a certain intensity. However, a similar meta-analysis would be needed to confirm these findings.

Given the importance of proper technique and quality control measures for generating reliable western blot data [373], differences in western blotting protocols between laboratories (often described inadequately) may have impacted the quality of the p-AMPK and p-ACC data used in our analyses. For instance, the strength of the detected signal is dependent on appropriate sample handling/processing (e.g., rapid freezing of tissue and addition of appropriate protease/phosphatase inhibitors during homogenization) and prior determination of an anti-body's linear range of detection (i.e., how well the quantified intensity reflects a change in protein content or post-translational modification). Similarly, different analytical procedures applied to the same western blot data can lead to different statistical outputs [374]. Due to these limitations, it is possible that the magnitude of the reported changes in AMPK or ACC phosphorylation may dissociate from changes in AMPK activation occurring *in vivo*. We limited the inclusion criteria to data obtained from western blots for consistency across data sets. As this method is semi-quantitative, it would be useful for researchers to supplement this data using newer methods such as mass spectrometry-based phosphoproteomic approaches [366]. Other methods such as real-time polymerase chain reaction (PCR) can also be used to measure messenger RNA (mRNA) expression [41].

Despite the inclusion of a large number of studies, our analysis is limited by the number of available data points in several ways. Some correlations may be susceptible to the influence of a small number of data points that sit outside of clustered areas, particularly evident for the muscle metabolites (Fig. 6.5, 6.6). Principle Component Analysis was explored but could not be performed due to a small sample size of the complete data set (i.e., all variables of interest being reported). Similarly, the mixed models only include data points that have all model variables being reported and a limited number of studies have measured the muscle metabolites. Therefore, it is possible with more data the best-fitting models might have included additional variables. Finally, the precise role of AMPK for training-induced mitochondrial and metabolic adaptations is unclear [260], and more research is needed to understand the relationship between acute AMPK activation and longer-term training adaptations.

6.6 Conclusion

Muscle glycogen, fitness level, exercise intensity, and exercise duration each influence AMPK activity during exercise when all other factors are held constant. However, disrupting cellular energy charge (e.g., decreased glycogen, increased free AMP/ADP, and decreased PCr:total Cr ratio) is the most influential factor for AMPK activation during endurance exercise. This could be achieved via short-duration HIIE or lower-intensity continuous training performed to exhaustion. Ending levels of muscle glycogen appear more important than starting levels to increase AMPK activation, while carbohydrate ingestion before or during exercise has minimal influence on AMPK activation. Exercise intensity, but not duration, is correlated with indices of AMPK activity. The commonly used markers of AMPK activation (AMPK-$\alpha 2$ activity, p-AMPK, and p-ACC) are correlated with each other, but p-ACC may be the most sensitive marker for detecting changes in AMPK activity. When possible, it would be advisable for future studies to measure heterotrimer-specific, as well as muscle fiber-specific, changes in AMPK activity. Future research should also strive to include more female participants, and it would be of interest to study AMPK activation in relation to exercise above and below critical power as a demarcation point for the onset of greater changes in muscle metabolites.

7. Factors Influencing Substrate Oxidation During Submaximal Cycling: A Modelling Analysis

The primary reason athletes train in the fasted state relates to the well-established idea that exercise in the fasted state leads to greater fat oxidation than exercise following a carbohydrate-rich breakfast. However, other factors such as habitual diet, exercise duration and intensity, and peri-exercise nutrition intake also influence substrate oxidation, and the relative influence of these factors is unclear. Therefore, this chapter examines factors influencing the respiratory exchange ratio (RER) during continuous exercise and formulate multivariable regression models to determine which factors best explain RER during exercise, as well as their relative influence.

This chapter contains the following publication:

Rothschild, J. A., Kilding, A. E., Stewart, T., & Plews, D. J. (2022). Factors influencing substrate oxidation during submaximal cycling: a modelling analysis. *Sports Medicine*, 52, 2775–2795.

7.1 Abstract

Background Multiple factors influence substrate oxidation during exercise including exercise duration and intensity, sex, and dietary intake before and during exercise. However, the relative influence and interaction between these factors is unclear.

Objectives Our aim was to investigate factors influencing the respiratory exchange ratio (RER) during continuous exercise and formulate multivariable regression models to determine which factors best explain RER during exercise, as well as their relative influence.

Methods Data were extracted from 434 studies reporting RER during continuous cycling exercise. General linear mixed-effect models were used to determine relationships between RER and factors purported to influence RER (e.g., exercise duration and intensity, muscle glycogen, dietary intake, age, and sex), and to examine which factors influenced RER, with standardized coefficients used to assess their relative influence.

Results The RER decreases with exercise duration, dietary fat intake, age, VO_{2max}, and percentage of type I muscle fibers, and increases with dietary carbohydrate intake, exercise intensity, male sex, and carbohydrate intake before and during exercise. The modelling could explain up to 59% of the variation in RER, and a model using exclusively easily modified factors (exercise duration and intensity, and dietary intake before and during exercise) could only explain 36% of the variation in RER. Variables with the largest effect on RER were sex, dietary intake, and exercise duration. Among the diet-related factors, daily fat and carbohydrate intake have a larger influence than carbohydrate ingestion during exercise.

Conclusion Variability in RER during exercise cannot be fully accounted for by models incorporating a range of participant, diet, exercise, and physiological characteristics. To better understand what influences substrate oxidation during exercise further research is required on older subjects and females, and on other factors that could explain additional variability in RER.

7.2. Introduction

Energy production during continuous, submaximal exercise comes primarily from the oxidation of fat and carbohydrate. The respiratory exchange ratio (RER), represents an indirect measure of the skeletal muscle respiratory quotient (RQ) — the quantity of CO_2 produced in relation to O_2 consumed [76]. The RER can be used to estimate the relative contributions of fat and carbohydrate to energy production with higher values equating to increased carbohydrate reliance and lower values representing increased fat reliance [77]. Several factors are known to influence the RER during exercise including exercise duration [80], exercise intensity [78], training status [81], sex [82], dietary intake [83-85], the pre-exercise meal [375, 376], and carbohydrate ingestion during exercise [80, 86]. However, the relative influence and interaction between these factors is unclear. For example, RER decreases with exercise duration (i.e., increased reliance on fat oxidation), but increases with exercise intensity and carbohydrate intake [225], leaving the net effect on RER unclear when multiple factors are being manipulated. Therefore, a better understanding of the factors influencing RER during exercise is needed.

The ability to effectively oxidize fat for fuel, represented by a lower RER, is important for metabolic health [377] and long-duration exercise performance [196, 378], and many athletes attempt to manipulate substrate oxidation during exercise as part of a periodized nutrition and training plan [21, 36]. However, managing substrate oxidation during exercise is challenged by the influence of both modifiable and non-modifiable factors, which may or may not be easily measured (Table 7.1). Previous studies have investigated factors influencing substrate oxidation, but none have considered variables often manipulated by athletes such as the duration or intensity of exercise, the pre-exercise meal, or carbohydrate ingestion during exercise. Goedecke et al. [379] found the most important factors influencing RER during endurance exercise were mitochondrial enzyme activity, muscle glycogen and triglyceride concentrations, dietary fat intake, training volume, and free fatty acid concentrations, which collectively explained 42–56% of the variation in RER during exercise. Distinct from RER, others have studied the determinants of maximal fat oxidation rates and found 34–79% of the variance was related to factors such as maximal oxygen consumption (VO_{2max}), sex, body composition, physical activity level, dietary macronutrient intake, resting fat oxidation, and fasting duration [380-384]. To our knowledge, the relative influence of the modifiable, easily measured factors influencing RER during exercise (e.g., dietary intake before and during exercise, exercise duration, and exercise intensity) has yet to be established. Using multivariable regression models, it would be possible to account for multiple factors influencing RER during exercise and predict the response under various circumstances. Therefore, the purpose of this analysis was to investigate factors influencing the RER during cycling exercise and formulate regression models to determine which factors best explain RER during exercise, their relative influence, and the result of multiple variables being modulated simultaneously. To this end, we performed the largest pooled analyses to date (~3,400 RER observations) of studies examining substrate oxidation during exercise and provide novel insight into the factors influencing fuel selection during endurance exercise.

Table 7.1. Factors influencing RER during exercise and ease of day-to-day modification and measurement

	Easily measured	**Not easily measured**
Easily modified	• Exercise duration [80, 86] • Exercise intensity [81] • Dietary CHO and fat intake [54] • Pre-exercise CHO intake [75] • CHO during exercise [80, 86] • Type of CHO consumed [385] • Energy balance [386] • Pre-exercise meal timing [92] • Cycling cadence [387]	• Muscle glycogen [54, 379] • Muscle triglycerides [379] • Hydration status [388] • Glycemic index [389]
Not easily modified	• Age [390] • Training age [391] • Training volume [379] • Sex [82] • Menstrual phase [392] and status [393] • Fitness level/VO_{2max} [81, 394] • Ventilatory/lactate thresholds [391] • Plasma lactate [379] • Fasting/resting RER [379] • Body composition [395] • Environmental temperature [396] • Altitude [397]	• Type I muscle fiber percentage [379, 387] • Mitochondrial enzymes/proteins [379] • Plasma free fatty acids [379] • Genetic variation [398] • Habitual physical activity levels [382] • Catecholamines [399]

CHO: carbohydrate, RER: respiratory exchange ratio, VO_{2max}: maximal oxygen consumption

7.3. Methods

7.3.1 Eligibility Criteria

Inclusion Criteria

Studies of healthy, adult (> 18 y) humans were included for analysis. Only studies using two-legged cycling exercise were included, due to differences in substrate utilization between cycling and running [284, 400]. Cycling had to be continuous, for at least 5 min in duration and performed at a single exercise intensity. If there were changes in exercise intensity, only the first intensity was included [263, 401-404]. Studies must have been performed in a normoxic, temperate environment (15–25°C), and subjects must not have performed any exercise within 12 h of the trial due to the influence of prior exercise on substrate oxidation [58, 405].

Exclusion Criteria

Participants could have any physical activity level, but people with metabolic disorders were excluded. Children and teenagers were excluded due to differences in substrate utilization compared with adults [406]. Studies using a pre-exercise fasting period > 15 h were excluded to maximize generalizability and practical application. Both fixed-duration and time-to-exhaustion trials were included, but time trials were excluded due to the variability of pacing and intensity.

7.3.2 Search Strategy

A PubMed search was performed on 30 April 2021 and included all publication years up to and including the date the search was conducted, using the following terms: (cycling OR endurance OR exercise OR "prolonged exercise") AND (carbohydrate) AND ("fat oxidation" OR metabolism OR "muscle glycogen" OR "oxygen uptake" OR "substrate oxidation" OR "substrate utilization" OR "carbohydrate oxidation" OR "energy expenditure" OR "skeletal muscle" OR "substrate metabolism” OR “respiratory exchange ratio”) AND (clinical trial [Filter] OR randomized controlled trial [Filter] NOT (diabetes) NOT (running) NOT (treadmill) NOT (resistance). In addition, the reference sections of studies included in this analysis were searched. The titles, abstracts, and full-text articles were independently screened by the lead author (JR). A second author (DJP) was consulted if there was uncertainty about article eligibility. The rationale for excluding articles was documented.

7.3.3 Data Extraction

The following data were extracted from papers meeting the above criteria: RER, duration (min), exercise intensity (%VO_{2max}), sex (% female subjects), daily carbohydrate and fat intake (as percentage of energy intake, and as grams ingested in total and relative to body mass), carbohydrate and fat intake 4-h pre-exercise (g), number of minutes before exercise food was consumed, carbohydrate ingestion during exercise (hourly intake rate as well as the drink composition as percentage glucose, fructose, and other carbohydrate sources), starting and ending muscle glycogen levels (mmol.kg^{-1} dry mass), age (y), training age (y), percentage of type I muscle fibres, BMI, VO_{2max} (ml.kg.min^{-1}), glycemic index of the pre-exercise meal, sample size, and study ID.

The RER had to be reported at multiple time points for exercise lasting longer than 30 min, and/or consist of no more than a 30-min average value. When reported at multiple time points each value was recorded as a separate data point. Substrate oxidation reported in grams per minute was converted to an RER value using equations from Jeukendrup and Wallis [76]. Exercise intensity had to be reported or able to be calculated as a percentage of VO_{2max}. Sex was analyzed as a categorical variable, with the study population considered “male” or “female” if ≥70% of subjects were of one sex, and “mixed” if the split was 30–70%. A categorical variable was created for type of ingestion during exercise and included carbohydrate, fat, protein, water, carbohydrate and protein, and carbohydrate and fat.

Muscle glycogen concentrations before, during, and after exercise were recorded when determined using whole muscle (not fiber-type specific), from muscle biopsies (excluding non-invasive measures such as magnetic resonance spectroscopy). Biopsies must have been performed before and within 30-min post exercise. For studies that took a resting biopsy before providing pre-exercise carbohydrate, starting glycogen concentrations were only recorded for the placebo/control group but ending glycogen levels were recorded for all groups [407, 408]. Studies that depleted glycogen in only one leg prior to an exercise trial were excluded. The conversion factors of Areta and Hopkins [283] were used when glycogen values were reported in units other than $mmol.kg^{-1}$ dry mass.

When studies included multiple groups, data were used if the interventions included factors that were accounted for in the analysis (e.g., differences in carbohydrate ingestion, exercise intensity, or sex) but only the control/placebo groups were used if the intervention arm included a variable not analyzed such as the use of heparin [409, 410], estrogen [411, 412], glucose infusion [413, 414], caffeine [410], alcohol [415], or various dietary supplements [403, 416-427]. Studies that provided protein or fat during exercise were included due to the minimal influence of protein [428-431] or fat [376, 432-437] ingestion on RER, although it is possible that RER values may be less reliable under conditions of increased gluconeogenesis, lipogenesis, or ketogenesis [76, 438]. Interventions that used 5–6 days of a high-fat diet followed by 24-h carbohydrate restoration were excluded due to persisting effects of the high-fat diet on RER [5, 312, 439-442].

7.3.4 Statistical Approach

To find the factors that best explain RER as well as focus on the influence of modifiable and easily measured variables, multiple mixed-effect models were created. All available variables were initially modelled, before focusing specifically on easily measured variables (i.e., excluding glycogen concentration and muscle fiber type but including age, sex, and VO_{2max}), and those that are both easily measured and easily modified, due to the strong emphasis placed by athletes and coaches on the influence of carbohydrate consumption on fat oxidation [21, 22].

Univariable regression analysis was first performed between variables of interest and RER, and the best-fit regression line (linear or polynomial) was established using the likelihood ratio test.

Because data points were collected at multiple time points during exercise in each study, the assumption of independence of residuals is violated. Therefore, we built general linear mixed-effect models to examine how each individual factor was related to RER, specifying study ID as a random intercept using the *lme4* R package [296], and report the marginal R^2, which describes the proportion of variance explained by only the fixed effect.

We then built general linear mixed-effect models to examine which factors were related to RER, with study ID again specified as a random intercept. The following fixed effects were tested: starting muscle glycogen, end-exercise muscle glycogen, exercise duration (min), exercise intensity (%VO_{2max}), daily carbohydrate and fat intake (as percentage of energy intake, total g, and g per kg body mass), carbohydrate and fat intake within 4 h of exercise (g), minutes before exercise carbohydrate was consumed, carbohydrate intake during exercise (g h^{-1}), percentage of glucose and fructose in drinks ingested during exercise, ingestion type during exercise (as a categorical variable), fitness level (VO_{2max}), age, sex, and percentage of type I muscle fibers. Interactions between RER and other fixed effects were explored, and the optimal, best-fitting model was decided based on the likelihood ratio test. The fit of each model was checked by visualizing the Q–Q and other residual plots to ensure approximate residual normality and heteroscedasticity, and outliers were removed based on a composite outlier score using the *performance* R package. Multicollinearity was assessed using the variance inflation factor (VIF), with values > 5 used to indicate excessive collinearity [299]. Model fit is reported as marginal R^2 as well as conditional R^2, which describes the proportion of variance explained by both the fixed and random effects [301], and root mean square error (RMSE). Estimated means were calculated using the *emmeans* package [300]. Descriptive statistics are provided as mean ± SD. All analyses were carried out with R version 4.0.3 (The R foundation for Statistical Computing, Vienna, Austria), with the level of significance set at $p < 0.05$.

7.4. Results

7.4.1 Included Studies

The database search yielded a total of 8052 results. Following the removal of 63 duplicates, 7989 titles and abstracts were screened. A total of 784 full-text articles were screened for eligibility, and data were extracted from 434 studies that met the inclusion criteria. Due to the large influence of daily macronutrient intake on RER, all multivariable models included daily dietary intake as a variable. Consequently, studies not reporting dietary intake during the 24 h prior to exercise were excluded from all multivariable models. Therefore, the univariable analysis includes data from 434 studies (3,498 RER observations) whereas the multivariable models contained data from 106 studies, which included 1,221 participants (21.2% female, mean age 26.5 ± 5.5, range 19–52 years, BMI 23.2 ± 1.3 kg/m^2, VO_{2max} 53.6 ± 9.6 mL kg^{-1} min^{-1}) and 1,104 RER observations, noted fully in the supplemental files.

7.4.2 Correlations

Relationships between RER and the primary factors influencing it are shown in Fig. 7.1. Regression lines and R^2 values are shown, with the best-fit lines for daily carbohydrate intake, starting glycogen, pre-exercise carbohydrate intake, carbohydrate ingestion during exercise, age, percentage of type I muscle fibers, and exercise intensity being curvilinear. For age, the available data shows a negative relationship with RER below age 50 and positive relationship above age 50 (Fig. 7.1g). However, data points above age 52 could not be used in the multivariable modelling due to unreported dietary intake, therefore the models only reflect a negative relationship. A significant positive relationship was also found between RER and the glycemic index of the pre-exercise meal ($R^2 = 0.08$, $p < 0.001$), but the small number of studies reporting this measure (n = 10) precluded its use in the models and thus is not shown. The relationship between exercise duration and RER is shown in Fig. 7.2 stratified by the type of ingestion during exercise. Limited comparisons can be made relating to the effect of each type of nutrient ingestion due to the limited number of data points for some of the variations but are shown to demonstrate the available data and observable trends.

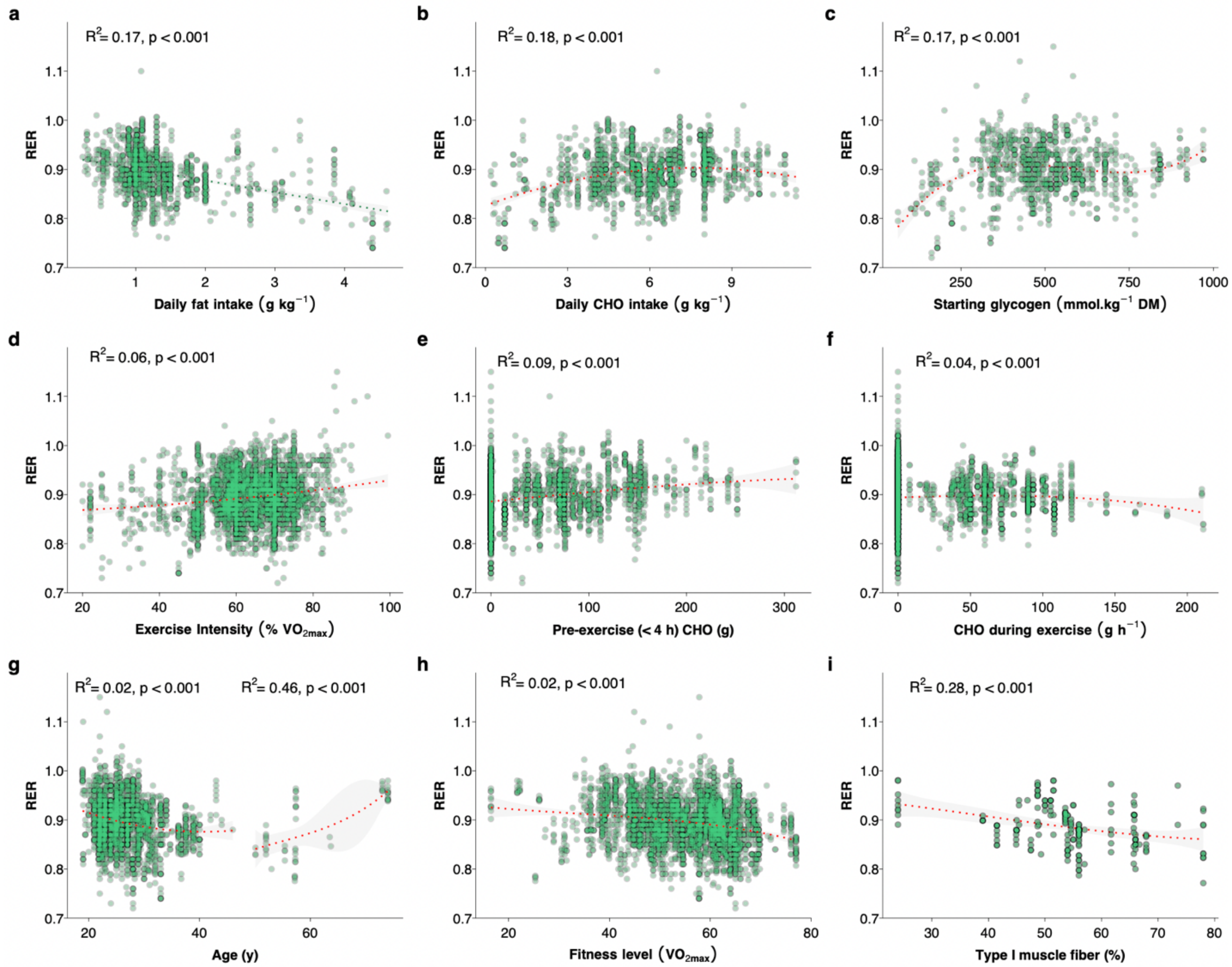

Figure 7.1. Relationships between RER and factors influencing RER. Best-fit regression lines based on univariable mixed effect models are shown, with fit indicated as R2. Best-fit lines using linear regression are shown in green, best-fit lines using polynomial regression and are shown in red. Panel (g) is separated by the natural gap in the data of mean age > or < 50 years. Shaded areas represent 95% confidence intervals. CHO: carbohydrate, DM: dry mass.

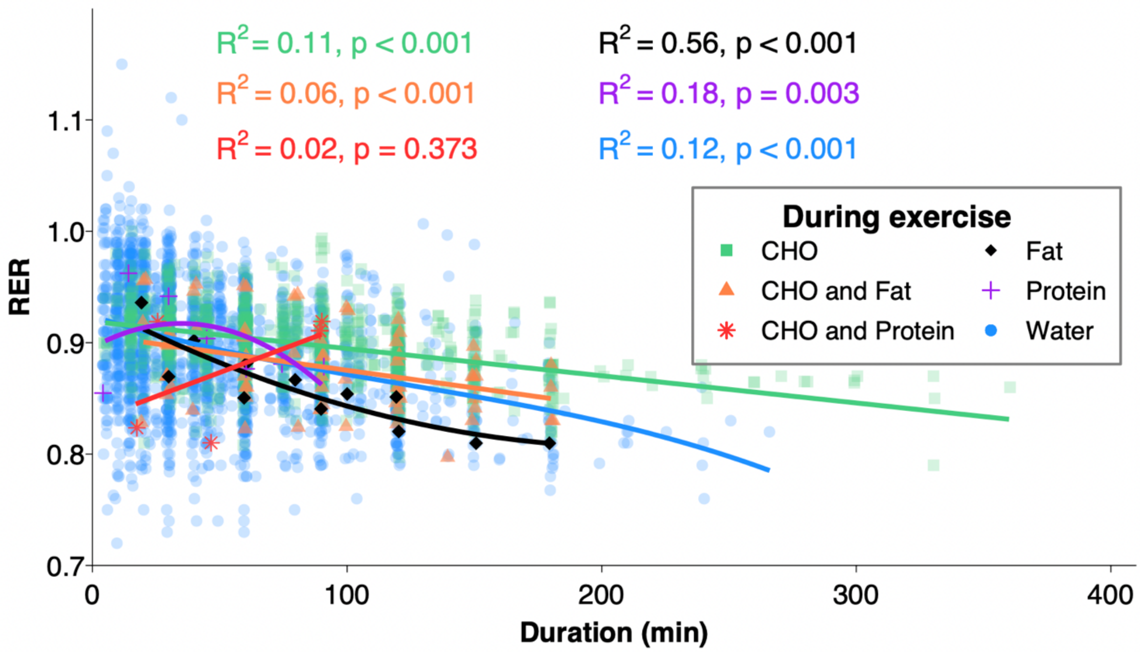

Figure 7.2. Relationship between RER and exercise duration separated by type of ingestion during exercise. Best-fit regression lines based on univariable mixed effects models are shown for each ingestion type during exercise, with fit indicated as R^2 and p-value. CHO: carbohydrate.

7.4.3 Models

The best fitting models are shown in Table 7.2, with the fixed effects explaining up to 59% of the variation in RER. The models show RER decreases with exercise duration, dietary fat intake, age, and VO_{2max}, and increases with dietary carbohydrate intake, exercise intensity, male sex, and carbohydrate intake before and during exercise. Model I, which explains the greatest amount of variance in RER, includes 322 RER observations. Models II (easily measured factors) and III (easily modified factors, for males and females separately) contain more observations but explain a lower proportion of the variation in RER. To visualize the relative influence each variable, standardized model coefficients are shown in Fig. 7.3. Additionally, a model that included the percentage of type I muscle fibers was also made, which explained 56% of the variation in RER, and included daily carbohydrate intake, carbohydrate ingestion during exercise, and exercise intensity (not shown). However, other factors such as sex could not be included in the model with muscle fiber type due to the completeness of available data. Overall, variables with the largest influence on RER are sex and exercise duration, and among the diet-related factors, daily fat and carbohydrate intake has a larger influence than carbohydrate ingestion during exercise.

Table 7.2. Models to explain variation in RER during submaximal cycling.

Variable	Model I	Model II (Easily measured factors only)	Model III (Easily modified factors only - Males)	Model III (Easily modified factors only - Females)
Starting glycogen (mmol kg^{-1} dry mass)	0.0001 (< 0.001)			
Daily CHO intake ($g\ kg^{-1}\ d^{-1}$)		0.0058 (< 0.001)	0.0038 (< 0.001)	0.0105 (< 0.001)
Daily fat intake ($g\ kg^{-1}\ d^{-1}$)	-0.0179 (< 0.001)	-0.0129 (< 0.001)	-0.0172 (< 0.001)	0.0004 (0.962)
Pre-exercise (< 4 h) CHO intake (g)	0.0004 (< 0.001)	0.0002 (< 0.001)	0.0002 (< 0.001)	1.3e-05 (0.852)
CHO during exercise ($g\ h^{-1}$)	0.0003 (< 0.001)	0.0007 (< 0.001)	0.0002 (< 0.001)	-0.0003 (0.043)
Duration (min)	-0.0006 (< 0.001)	-0.0004 (< 0.001)	-0.0004 (< 0.001)	-0.0005 (< 0.001)
Intensity (%VO_{2max})	0.0015 (< 0.001)	0.0008 (< 0.001)	0.001 (< 0.001)	-0.0014 (0.030)
Sex (male)	0.0158 (0.104)	0.0328 (< 0.001)		
Age (y)	-0.0039 (0.037)	-0.0012 (0.030)		
Fitness level (VO_{2max})		-0.0013 (< 0.001)		
Duration * starting glycogen (1) [a]	-0.0003 (0.757)			
Duration * starting glycogen (2) [a]	-0.0021 (< 0.001)			
Duration * CHO during exercise		6.0e-07 (0.191)	4.5e-07 (0.119)	1.4e-05 (< 0.001)
Sex * CHO during exercise		-0.0004 (< 0.001)		
Duration x Sex	0.0003 (0.024)			
Intercept	.859	.912	.843	.924
Marginal R^2	.59	.39	.36	.29
Conditional R^2	.90	.85	.86	.95
RMSE	.018	.018	.018	.013
k	30	99	92	18
Observations	322	1039	903	163

Linear coefficients, their corresponding p-values (in parentheses), marginal R^2 (variance explained by the fixed factors alone), conditional R^2 (variance explained by both the fixed and random effects), root mean square error (RMSE), number of studies (k) and number of observations included in the best-fitting linear mixed models to explain RER during exercise using different factors. [a] (1) and (2) refer to the polynomial terms for starting glycogen

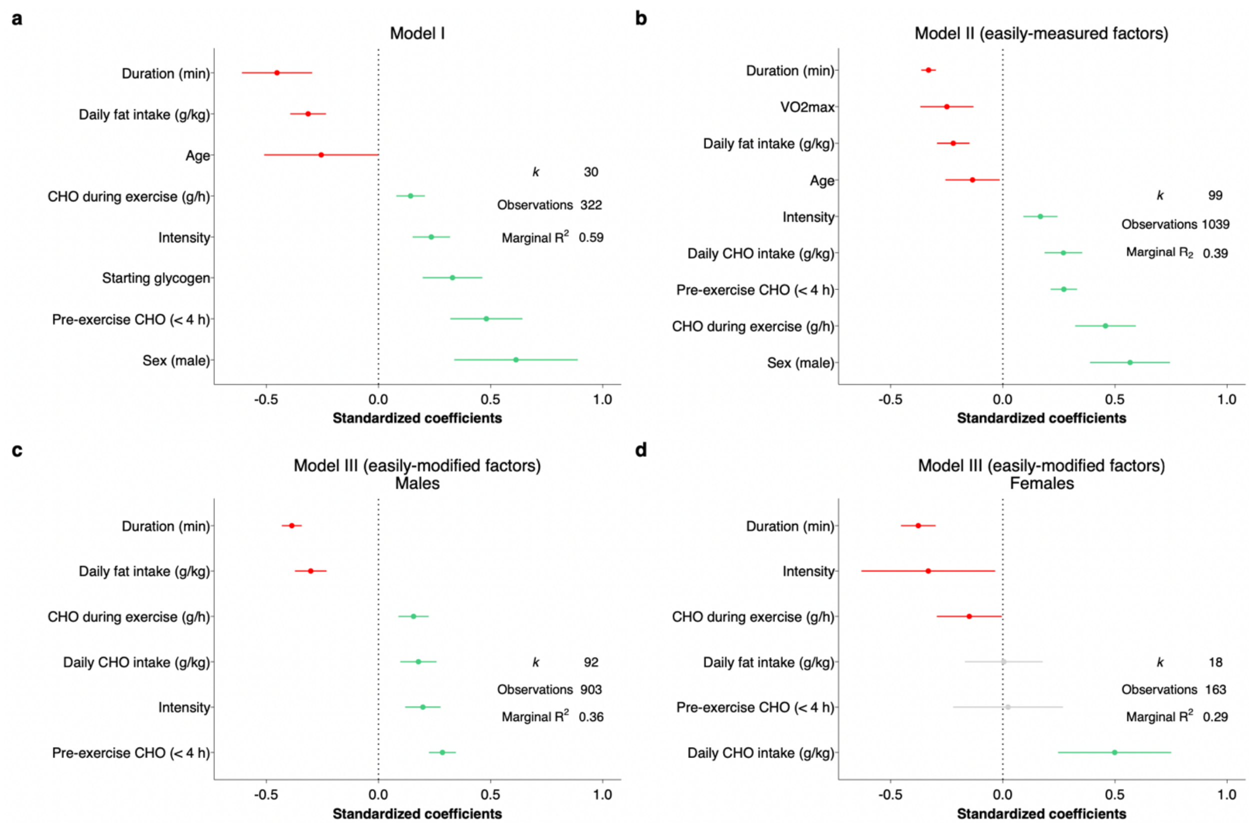

Figure 7.3. Standardized coefficients for model parameters. These figures depict the relative influence each variable has on RER during exercise. For clarity, interaction effects are not shown. Intensity is exercise intensity as $\%VO_{2max}$. Marginal R^2 denotes the variance explained by the fixed factors alone. CHO: Carbohydrate. k: number of studies included. Green bars represent factors that increase RER, red bars indicate factors that decrease RER, lines indicate 95% confidence intervals

7.4.4 Predictions

To visualize the interaction and convergence of multiple factors influencing RER during exercise, estimated marginal means from Model I are shown in Fig. 7.4 in various combinations. The standard error of the estimated means, across the range of values for each fixed effect in Model I, are shown in Fig. 7.5. This represents the confidence in the predicted values shown in Fig. 7.4, and how this confidence changes depending on the value of the fixed effect. When more data points are involved in the calculation of the mean, it tends to lead to smaller standard errors. Therefore, this provides an indication of where the most research has been performed, for each of the variables studied. The value with the lowest standard error is shown in each panel. For the interested reader, an online app has been created to allow exploration of the data and predict RER based on the data used in this analysis [443].

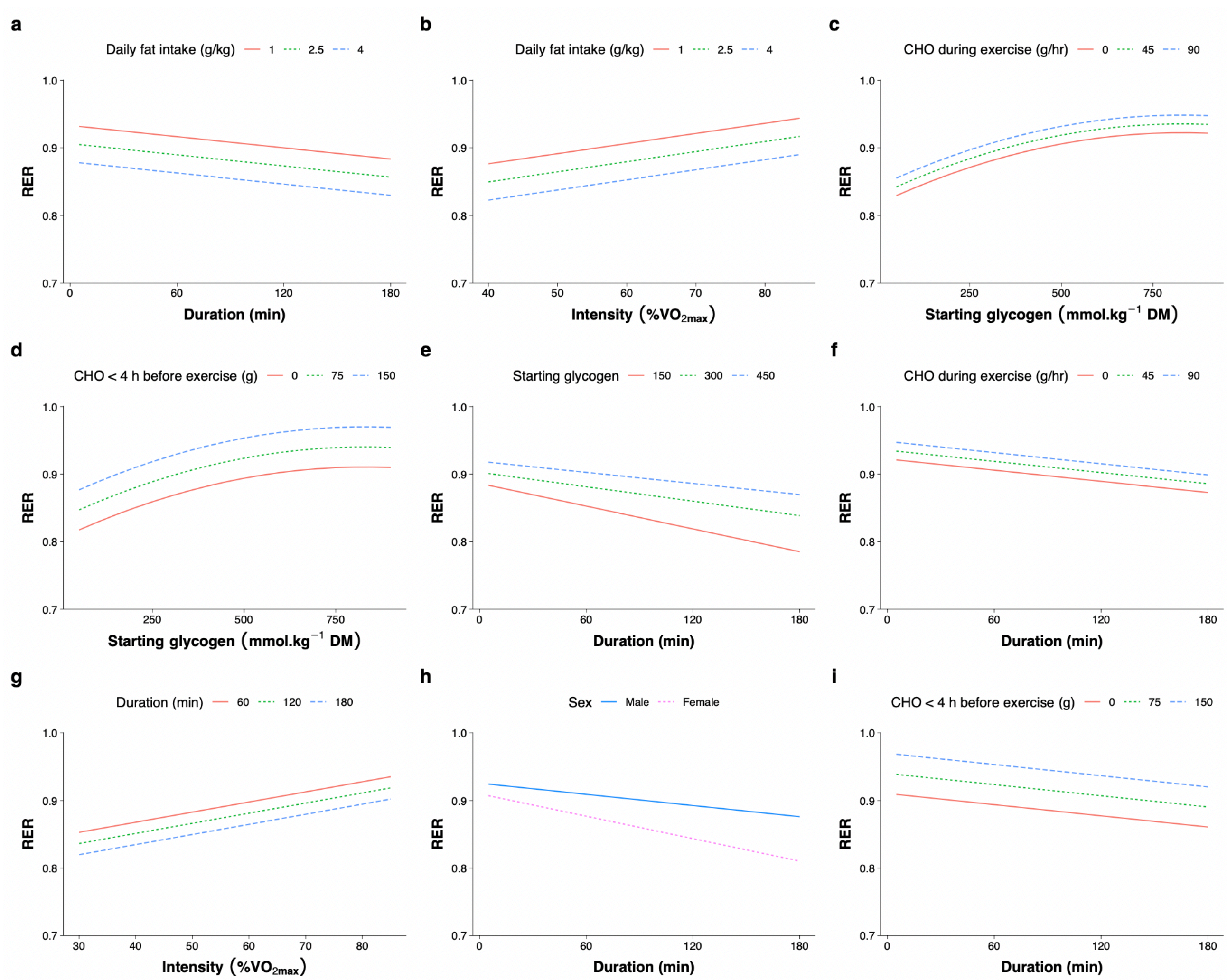

Figure 7.4. Standardized coefficients for model parameters. These figures depict the relative influence each variable has on RER during exercise. For clarity, interaction effects are not shown. Intensity is exercise intensity as $\%VO_{2max}$. Marginal R^2 denotes the variance explained by the fixed factors alone. CHO: Carbohydrate. k: number of studies included. Green bars represent factors that increase RER, red bars indicate factors that decrease RER, lines indicate 95% confidence intervals

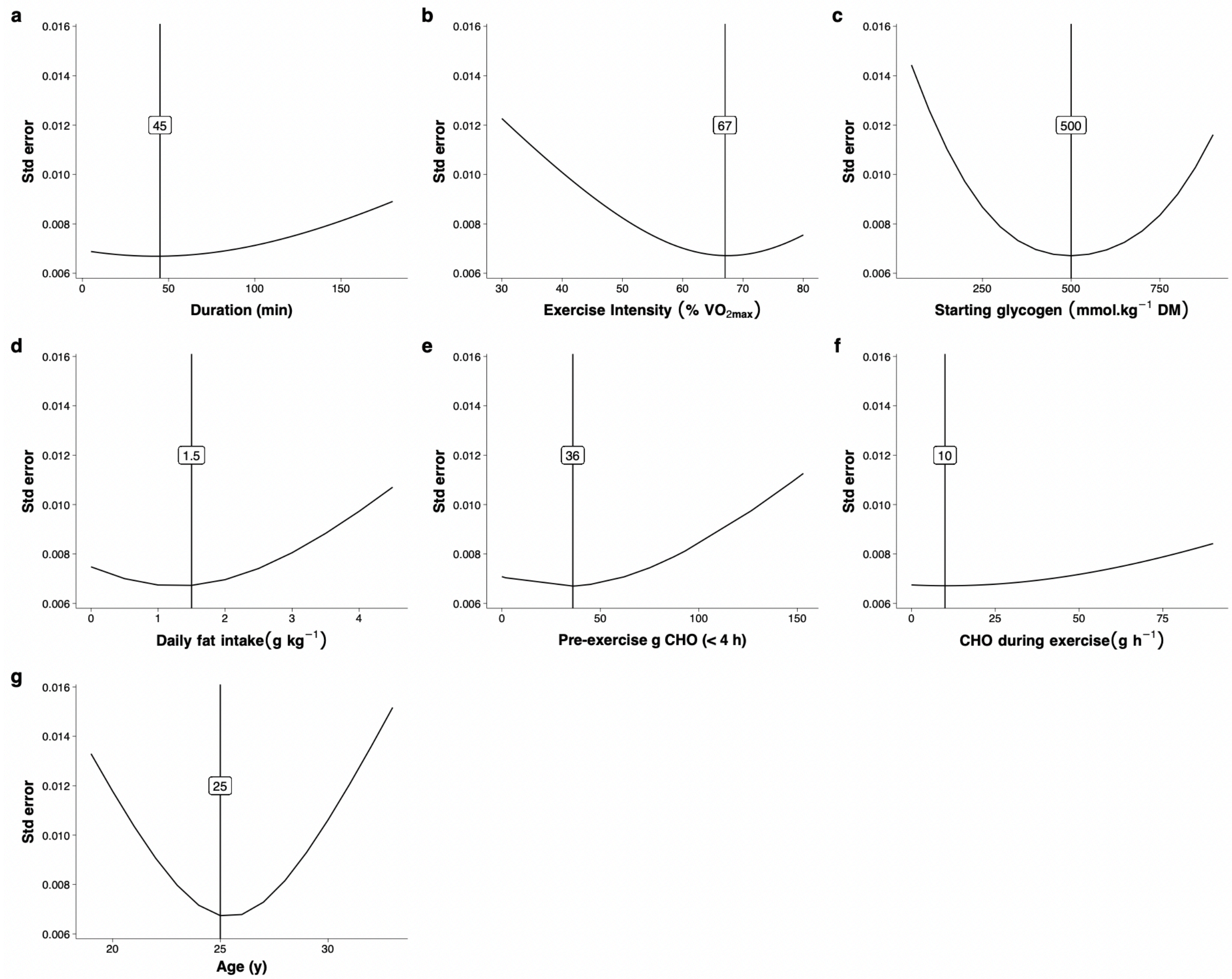

Figure 7.5. Model I Standard Errors. This figure depicts the level of certainty in terms of what we currently know about the effects of these factors on RER (i.e., we can be more confident in the relationship between these factors and RER, when the standard error is lower). The number denoted in each panel indicates the value with the lowest standard error. DM: dry mass

7.5. Discussion

The purpose of this analysis was to investigate factors influencing the RER during cycling exercise, understand their relative influence, and determine how RER is affected when multiple variables are modulated simultaneously. This knowledge is important for athletes and coaches/practitioners who wish to manipulate substrate oxidation during exercise. The key findings are that exercise duration and intensity, age, sex, fitness level, muscle glycogen, and daily dietary intake together explain ~60% of the variation in RER during exercise, indicating a large influence of additional factors, and that daily dietary intake has a larger influence on RER than carbohydrate ingested during exercise. Additionally, the biggest relative determinants of RER during exercise are sex and exercise duration, with pre-exercise carbohydrate intake and daily fat intake also identified as main determinants.

To our knowledge, this is the first large-scale attempt to analyze the determinants of substrate oxidation during exercise using modifiable factors such as exercise intensity, exercise duration, and dietary intake before and during exercise. Goedecke et al. [379] found mitochondrial enzyme activity, muscle glycogen and triglyceride concentrations, dietary fat intake, training volume, and free fatty acid concentrations collectively explained 42–56% of the variation in RER during exercise. Others have studied the determinants of maximal fat oxidation rates and found 34–79% of the variance was related to factors such as VO_{2max}, sex, body composition, physical activity level, 4-d dietary intake, resting fat oxidation, and fasting duration [380-384]. However, these studies did not consider aspects that are routinely modulated by athletes such as the exercise duration or intensity, or pre- and peri-exercise carbohydrate intake.

7.5.1 Modifiable Factors

7.5.1.1 Diet and Starting Glycogen

Our analysis highlights the influence of daily macronutrient intake on RER during exercise, particularly dietary fat intake. It is challenging to distinguish between the influence of dietary carbohydrate and dietary fat intake, as they are often manipulated together. However, there are several reasons why it can be speculated from our findings that daily dietary fat intake may have a greater influence than dietary carbohydrate intake on RER during exercise. The best-fitting model for RER (Model I) included dietary fat intake and was not significantly improved by the inclusion of dietary carbohydrate intake. Furthermore, RER during exercise was decreased more following five days of a high-fat diet, compared with a high-protein diet, when dietary carbohydrate was clamped below 20% of energy intake for both groups [5]. Although a portion of the increased dietary protein intake in that study was likely converted to glucose via gluconeogenesis [444], a short-term increase in dietary fat can decrease the amount of the active form of pyruvate dehydrogenase (PDH) [445], which is the rate-limiting enzyme in carbohydrate metabolism. The rate of glycolysis appears to play a central role in the regulation of fatty acid oxidation [446], and the downregulation of PDH observed following five days of a high-fat diet is not offset by one day of high-carbohydrate intake [442]. Taken together, both dietary fat and dietary carbohydrate influence RER during exercise, but daily dietary fat intake may have a stronger influence.

Bivariate correlations between daily carbohydrate intake and RER suggest a curvilinear relationship (Fig. 1b), which could imply a diminishing influence of dietary carbohydrate on RER past a certain threshold (~4 g kg^{-1}). This is a finding which could be explored in future research to determine if there is a threshold for carbohydrate oxidation to occur based on demand, without being influenced by limitations from an undersupply of carbohydrate, and to investigate the shape of the relationship (i.e., linear or curvilinear) between dietary carbohydrate intake and PDH activity. However, this could also simply reflect fewer data points in the upper ranges of daily carbohydrate intake, particularly because the multivariable regression models were not improved when including the polynomial term (implying other variables could sufficiently explain the differences in RER).

A non-linear relationship was also found between starting muscle glycogen and RER (Fig. 1c), suggesting the influence of glycogen on RER could differ based on concentration, or could again simply reflect fewer data points at the lower and upper ranges. However, unlike daily carbohydrate intake, there was significant model improvement when using the polynomial term and a significant duration*glycogen interaction, which makes sense mechanistically because the rate of glycogen breakdown varies with initial concentration and is reduced with exercise duration [283]. The correlations shown in Fig. 1c and the model predictions shown in Fig. 4c and 4d both suggest a leveling off of the influence of starting muscle glycogen on RER, however the precise breakpoint where this occurs should be investigated in future research.

Dietary carbohydrate intake increases muscle glycogen concentration. Undertaking exercise with higher levels of muscle glycogen can increase RER by increasing muscle glycogenolysis, but does not influence exogenous carbohydrate oxidation rates [54, 447]. Although it could be assumed the influence of the daily carbohydrate intake is solely due to changes in starting muscle glycogen concentrations, other factors are likely involved such as changes in enzyme activity and/or gene expression related to carbohydrate and lipid metabolism seen following short-term high- and low-carbohydrate diets [448-450]. This notion is supported by the persisting effects on RER from 5–6 days of a high-fat diet followed by 24-h carbohydrate restoration, despite similar starting muscle glycogen concentrations [312, 439, 442]. Changes in substrate utilization following two weeks of a high-fat diet have also persisted through three days of a high-carbohydrate diet [451], but one week of a high-carbohydrate diet abolished the increases in fat oxidation observed following a 7-wk high-fat diet [85], indicating the approximate time-decay for changes in enzyme activity.

Studies in this analysis reported dietary intake for an average of 4.5 ± 7.4 (range 1–49) days, but the number of days reported did not influence the models. It seems unlikely that including only longer-term (habitual) dietary intake would have significantly changed the findings, as the decreased RER observed on a low-carbohydrate diet was not different when tested after two days or two weeks [452], or after five days and 15 days [453]. However, diets with extreme short-term variation (e.g., 5–6 days of a high-fat diet followed by 24-h carbohydrate restoration [5, 312, 439-442]) are known to have lingering effects on RER and were therefore excluded from the analysis. Accuracy of dietary reporting is a noteworthy concern when participants are in a free-living situation [454], and likely varies based on the method of dietary control. Some studies simply measured participants' habitual dietary intake [415, 455], whereas others provided short-term [5, 86, 456] or longer-term [85] standardized diets to study participants. Data were extracted from 400+ studies but the majority were not included in the models because they did not report dietary intake, instead reporting that participants were instructed to note and repeat their 24-h dietary intake before each study visit. It would be beneficial for future studies analyzing substrate oxidation during exercise to report daily macronutrient intake.

7.5.1.2 Pre-exercise Meal

Ingesting carbohydrate before exercise increases plasma glucose and insulin levels, reduces hepatic glucose output, and increases skeletal muscle glucose uptake during exercise [59]. This can lower fat oxidation by decreasing plasma free fatty acid availability via insulin-mediated inhibition of lipolysis [60], and also by inhibiting fat oxidation within the muscle due to an increased glycolytic flux [61]. Accordingly, our models highlight the strong influence of pre-exercise carbohydrate on RER during exercise (Fig. 3, 4). In an attempt to increase fat utilization (i.e., decrease RER) during exercise, many endurance athletes train in the overnight-fasted state [21], although evidence on whether the repeated practice of fasted-state training translates to longer-term increases in fat oxidation capacity remains equivocal [225]. More research, using endurance-trained subjects, is needed to determine whether longer-term fasted training increases fat oxidation during continuous exercise, particularly when tested in the carbohydrate-fed state.

There are several aspects related to the pre-exercise meal that may exert influence on RER but were not influential in the final models, likely because of not enough data points. The effect of glycemic index on RER has been equivocal, with lower-index meals resulting in lower [389], higher [457], or similar [135, 458] RER values during exercise, but only three studies meeting the inclusion criteria for our analysis also reported daily dietary intake. The size and timing of the pre-exercise meal may also influence RER, with a higher RER observed following larger meals eaten farther in advance of exercise [92, 225]. Pre-exercise protein ingestion has resulted in similar RER values as fasted-state exercise [16], although this may be influenced by the type of protein and degree of hydrolysation [226], and we found no influence of dietary fat in the pre-exercise meal on RER. Therefore, in our analysis the pre-exercise meal was quantified only by carbohydrate intake, meaning pre-exercise protein and/or fat ingestion, in the absence of carbohydrate, was analyzed in the same way as fasted-state training. However, there is opportunity for future research to further explore pre-exercise protein and its effects on substrate oxidation, including the type of protein, its effects on gluconeogenesis and urea formation [67], and if its influence may be intensity-dependent [375].

7.5.1.3 Peri-exercise Intake

Our analysis revealed a small yet significant influence of carbohydrate intake during exercise on RER. Carbohydrate ingestion during exercise maintains blood glucose levels, carbohydrate oxidation, and RER, and prevents the depletion of liver, but not muscle, glycogen [55, 459-461]. Increasing the rate of carbohydrate ingestion during exercise decreases hepatic glucose output and increases the contribution of exogenous carbohydrate oxidation to total energy contribution in a dose-response manner [462], at least up to the point where gastrointestinal transport of sugars theoretically becomes saturated [463]. However, differences in ingestion rate are not always reflected as differences in RER [86, 462, 464]. The RER is most likely to be influenced after ~1.5–2 h of exercise as endogenous carbohydrate availability declines [80], but differences in RER between carbohydrate and placebo ingestion can be seen earlier in exercise, particularly with very high carbohydrate ingestion [465]. The type of carbohydrate ingested may influence rates of endogenous and exogenous carbohydrate oxidation, but total carbohydrate oxidation (and RER) appears less affected [466-469]. However, future research should examine the differences in carbohydrate type in the context of high (>100 g h^{-1}) ingestion rates, as contrasting findings have been reported [461, 469]. Carbohydrate ingestion may fail to influence RER when exercise intensity is high [470], and/or in untrained participants [471], as RER may already be elevated in these circumstances. However, our models were not significantly improved by interaction effects between carbohydrate ingestion during exercise and either exercise intensity ($p = 0.119$) or VO_{2max} ($p = 0.179$).

The influence of protein and fat ingestion during exercise was explored, but the models were not improved by inclusion of the type of peri-exercise nutrition. Although most studies have reported a minimal influence of protein [428-431] or fat [376, 432-437] ingestion on RER, the RER is typically interpreted based on the assumption of negligible protein oxidation. This assumption could be invalidated in the context of protein ingestion before or during exercise due to increased gluconeogenesis, which could decrease RER irrespective of any change in fat oxidation rate via transfer of the amino group to the urea cycle [76], or by stimulating glucagon secretion which promotes gluconeogenesis and increases fat oxidation [472]. Some evidence suggests high dietary protein intake [444] or protein ingestion during fasted exercise [473] may have a notable effect on gluconeogenesis, and could explain why protein ingestion before or during exercise has been reported to increase fat oxidation in runners [226, 455].

It is also possible that fat intake during exercise, often provided in the form of medium chain triglycerides (MCT), can influence substrate oxidation via an increase in ketogenesis [433, 436]. Unlike long-chain fatty acids, MCTs are rapidly absorbed into the hepatic portal system and transported into the mitochondria independent of transporter proteins [474]. Although the studies included in this analysis have not found an effect of MCT when ingested with carbohydrate, this may be related to the amount provided and the specific exercise context. Reduced carbohydrate oxidation with combined MCT and carbohydrate ingestion compared with carbohydrate ingestion alone during exercise has been observed in one study, but dietary intake was not reported (and thus, not included in the modelling) [475].

A broad look at the influence of various peri-exercise nutrition options can be seen in Fig. 2, but due to the drastically different number of data points for each condition, limited conclusions can be drawn from comparison. Future studies could further explore the influence of these macronutrient combinations on substrate oxidation during exercise, particularly related to protein and/or ketone oxidation.

7.5.1.4 Exercise Duration and Intensity

It is well established that the RER increases with exercise intensity and decreases with exercise duration [78, 81, 476]. A novel finding of this analysis is that the duration of exercise likely exerts a larger influence on RER than the intensity of exercise, shown by the standardized model coefficients (Fig. 3). However, the influence of exercise duration can only be predicted using these models for activities less than ~3 h, as the longest time points reported for the studies included in Model I was 180 min. The longest study that was included in Model III was 360 min [477], but because not all modeled variables were reported it could not be included in Models I–II. Based on Fig. 7.2, which includes all extracted data, it appears RER may level off around 180 min and remain higher when fed carbohydrate compared with water, but further research is needed to confirm this.

Exercise intensity was analyzed as a percentage of VO_{2max} because that is the most widely reported unit in exercise science. This can be problematic because substrate use can vary greatly at a given percentage of VO_{2max} depending on whether someone has a high or low lactate threshold [391, 478]. Therefore, the use of lactate or ventilatory thresholds could be a better reference for exercise intensity [479], and/or could also be included as a model variable in future regression analysis. Although RER should not be used as an index of substrate utilization above 75% VO_{2max} [76], we included all available data points in the models because we modelled RER and not substrate oxidation in absolute values (grams per minute, etc.). This means inferences pertaining to absolute values of substrate oxidation should not be made using this data for exercise intensities > 75% VO_{2max}.

7.5.1.5 Additional Modifiable Factors

Total daily energy intake can also play a role in the RER response during exercise, particularly in the context of low energy availability. Low energy availability describes a mismatch between an athlete's energy intake (diet) and the energy expended in exercise, leaving inadequate energy to support the functions required by the body to maintain optimal health and performance [480]. Endurance athletes with high training volumes are at risk of chronically low energy availability, which can reduce resting metabolic rate and influence the normal metabolic hormonal milieu that may alter the RER response to exercise, as well as influence RER via changes in muscle glycogen concentration [481]. The majority of studies in this analysis standardized dietary intake and had study participants rest before exercise trials, reducing the ability to investigate low energy intake in the models. Future studies investigating variability in RER, as well as for practical application of these findings, should consider the influence of energy availability as a factor that could reduce RER during exercise.

7.5.2 Non-modifiable Factors

7.5.2.1 Sex

Sex is known to influence substrate oxidation and was among the strongest influences on RER in our models. Along with hormonal differences, sex-based differences in lipid storage within the muscle and liver, and in the percentage of type I muscle fibers, can help explain differences in substrate oxidation during exercise [482]. The RER is generally lower for women during submaximal exercise [82], however this is not a universal finding [125, 379, 483-485]. Divergent findings may be related to carbohydrate intake before and during exercise, and/or the duration of exercise. In several studies that did not find sex differences in substrate oxidation the dietary carbohydrate intake was lower in males [379, 483], whereas studies controlling dietary intake have often [486, 487], although not always [484], found a lower RER in females compared with males. Carbohydrate ingestion during exercise can also attenuate sex differences in RER [254, 488].

Our modelling revealed significant interactions between sex and exercise duration (Model I) and between sex and carbohydrate ingestion during exercise (Model II). However, a sex*carbohydrate ingestion during exercise interaction could not be explored in Model I because there were no studies in the analysis that provided exogenous carbohydrate to female subjects while also reporting muscle glycogen concentrations. Although women typically have a greater percentage of type I muscle fibers than men [482], sex could not be included in a model with fiber type percentage due to only one study reporting fiber-type percentage in females [489]. Sex differences in RER would more likely be observed at lower exercise intensities and diminish as the intensity increases and the RER approaches 1.0 [490], but a sex*intensity interaction was left out of the models due to excessive collinearity in the data.

A potential limitation in our analysis is not controlling for menstrual cycle. A lower RER has been reported in the luteal, compared with follicular, phase of the menstrual cycle [491], however, these differences are obscured with carbohydrate ingestion during exercise [492]. Others have found no influence of menstrual phase during 60–75 min of cycling [493-495], or an effect of menstrual phase that only became apparent after 75–90 min of cycling [496]. Increased estrogen concentrations suppress gluconeogenesis [497] and promote increased lipid availability and increased fat oxidation capacity [498], which could decrease RER in the late-follicular phase and luteal phases, although this influence may be antagonized by progesterone making the net effect in the luteal phase dependent on the relative effects of both ovarian hormones [499]. These differences could also be related to muscle glycogen at the start of exercise, which may be lower in the mid-follicular, compared with mid-luteal phase when on a normal/mixed diet (~5 g/kg carbohydrate), but is not different on a high-carbohydrate (8.4 g/kg) diet [493]. However, muscle glycogen sparing during exercise has also been observed in the luteal, compared with follicular phase [500]. It is therefore possible that combining all female cohorts together may be introducing some error in the models, but these effects are likely attenuated in the context of other factors such as daily carbohydrate intake, pre-exercise carbohydrate intake, and carbohydrate ingestion during exercise.

7.5.2.2 Fitness level/VO_{2max}

It is well-established that trained athletes have a lower RER than untrained subjects at a given exercise intensity [81, 394, 501], due to training-induced increases in the ability to oxidize fatty acids, thus sparing muscle glycogen and blood glucose during exercise [168]. A significant negative relationship between VO_{2max} and RER was confirmed in our analysis when considering all 3,498 available data points (Fig. 1h), and the 1039 data points included in Model II ($r = -0.11$, $p = 0.001$), but not among the 322 data points included in Model I ($r = 0.02$, $p = 0.736$). Because the only difference between studies included in a model was whether all factors were reported, and mean VO_{2max} was similar across models, the lack of inclusion into Model I is likely related to the comparatively small number of observations.

As an indicator of fitness level/training status, VO_{2max} has been used to distinguish between trained and untrained participants [341, 484], and is known to increase with endurance training [502]. However, VO_{2max} alone may insufficiently account for short-term training-induced adaptations. Despite negligible changes in VO_{2max}, changes in RER during exercise can be seen following just 7–10 days of training [266, 362]. Furthermore, testing protocols vary and may underestimate someone's true VO_{2max} [292], thus influencing both the relative exercise intensity and our assessment of fitness level. As an alternative measure of fitness status, training age (i.e., number of years performing regular endurance training) could help to explain some of the variability in RER during exercise but was not included in the models due to the limited number of studies reporting this value. An increased training age could be expected to accompany longer-term training adaptations such as an increased lactate threshold and/or higher percentage of type I muscle fibers [391]. Taken together, fitness level, most easily quantified as VO_{2max}, likely has a small yet significant negative influence on RER, but other factors may be more predictive of RER during exercise.

7.5.2.3 Age

Despite the inclusion criteria being open to any studies using adults over the age of 18, the mean age of the study participants included in the models was 26.5 ± 5.5 (range 19–52) years, with the oldest mean participant age in Model I just 33 years and the oldest group of females just 25 years. Older subjects have been studied [503-505] and are included in Fig. 1g, but those studies did not report pre-trial dietary intake and were therefore excluded from the models. Analysis of the relationship between age and RER suggests the potential for a U-shaped curve, with RER decreasing until middle age and increasing thereafter (Fig. 1g). It has been reported that the training-induced increases in maximal fat oxidation rate may be attenuated with aging [506]. This could be related to the decreased mitochondrial oxidative capacity observed in older humans [507-509], and along with an increased glycogen reliance [505] suggest an upward shift in RER during exercise in older individuals that could not be detected in the models. Studies reporting RER during exercise at the same relative intensity between older and younger subjects have been equivocal, and the effects may differ with training status [390, 510, 511]. However, these studies did not control for diet, limiting the conclusions that can be drawn. Adding to the complexity, comparisons between younger and older subjects can be made based on either the same absolute or relative (%VO_{2max}) intensity, yet the ventilatory thresholds occur at a higher percentage of VO_{2max} in trained older cyclists [512]. Although women under 45 years typically have a higher relative rate of maximal fat oxidation compared to men, these differences are not apparent after age 45 [393], possibly related to post-menopausal status characterized by low estrogen concentrations, higher circulating levels of follicle-stimulating hormone, and decreased lean body mass [513]. This suggests the potential for an age * sex interaction that cannot be accounted for in the modelling. Therefore, the findings that RER decreases with age may not translate to older (> 45 y) adults.

7.5.2.4 Fiber Type

We created a model to investigate the effects of muscle fiber type percentage, despite the small number of data points, because of the mechanistic potential to influence RER, and a significant influence of fiber type percentage was found. An increasing percentage of type I muscle fibers would be expected to predispose someone towards a lower RER at rest and during exercise, due to the differences in reliance on oxidative phosphorylation between types I and II muscle fibers [514]. The percentage of type I fibers is known to be higher in trained endurance athletes [515], and is correlated with a higher lactate threshold [516] and negatively correlated with muscle glycogen utilization [517]. However, in untrained subjects no relationship was observed between muscle fiber type composition and RER at rest or during exercise at 55% VO_{2max} [518]. It is possible some degree of endurance training could be needed for type I fiber percentage to help predict RER. Future studies are needed to study the influence of training status and intensity on RER during exercise, in males and especially females as our model could not consider sex as a variable due to the lack of studies in females.

7.5.3 Combined Influence of Factors

From a practical standpoint it is important to understand the net effect of modulating multiple variables at the same time, rather than just in isolation. The relative influence of each variable can be seen in Fig. 7.3 and predicted values when modulating two parameters at once are illustrated in Fig. 7.4. For example, we could expect an RER value during exercise that is ~0.03 units higher when consuming 1 g kg^{-1} per day of dietary fat compared with 2.5 g kg^{-1} per day (Fig. 4a), whereas increasing carbohydrate ingestion during exercise from 45 to 90 g/h would only be expected to increase RER by ~0.01 units (Fig. 7.4f). It can also be expected that someone consuming 1 g kg^{-1} dietary fat per day would have to cycle for 3 h to attain the same RER as someone consuming 2.5 g kg^{-1} per day would attain after just 1 h of cycling (Fig. 7.4a). For the interested reader, we have also created an online dashboard that allows users to simultaneously modulate all parameters to see the influence on predicted RER values [443].

7.5.4 Other Possible Contributing Factors

Body composition has also been thought to influence RER. However, most studies have found no relationship [379, 518-521], although increases [522] and decreases [523] in RER with increasing body fat percentage have been reported. The distribution of body fat (upper vs. lower body) can influence RER during exercise via differing hormonal responses [395], potentially helping to explain some of the divergent findings. Other factors that could influence RER include cycling cadence [387, 524], hydration status [388], short-term exercise training volume [379], genetic variation [398], hyperinsulinemia [525], insulin resistance [526], daily energy and protein intake, protein supplementation during exercise, and pre-exercise glucose levels, but further investigation is needed in these areas.

7.5.5 Technical Factors

Finally, a brief consideration of measurement factors is warranted. The RER represents whole-body substrate utilization, and likely underestimates the RQ at a given work rate, particularly during lower-intensity exercise, due to a dilution effect from other organs that rely more on fat oxidation [527]. At lower intensities the metabolism of non-muscle tissues has a proportionately greater influence on gas exchange and may imply a lower muscle RQ, while the relative proportion of total gas exchange derived from muscle will increase as intensity increases and result in the whole-body RER becoming closer to that of muscle [527]. Although the repeatability of RER measurements during low-intensity exercise has been shown to be very good [528], RER values at exercise intensities >75% VO_{2max} are not reliable due to changes in the size of the bicarbonate pool [76]. Finally, this analysis was performed on group means, rather than individual values. Although not commonly performed in this manner, others have utilized a similar approach [181, 283], which could lead to a higher degree of uncertainty when predicting individual, as opposed to group mean, values. It has also been suggested that modifying factors at the group level may not accurately reflect the modifying effects at the individual level, introducing ecological bias [529]. However, the goal of this analysis was to determine which factors best explain RER during exercise and understand their relative influence, and so the risk of bias can be mitigated by accounting for potentially confounding variables in the analysis [283].

7.5.6 Practical Implications

This modelling can be used by athletes and coaches to gain a better understanding of the convergence of factors influencing substrate oxidation during endurance exercise. Overall, athletes looking to increase fat oxidation during exercise should focus more on daily fat and carbohydrate intake, and to a lesser degree, pre-exercise carbohydrate intake, while being less concerned with carbohydrate ingestion during exercise, particularly as exercise duration extends. Furthermore, the easily measured and easily modifiable factors related to exercise (e.g., exercise duration and intensity, daily macronutrient intake, and pre- and peri-exercise carbohydrate intake) can only explain roughly one-third of the variation in RER during exercise, suggesting most of what dictates RER during exercise cannot be easily controlled by the athlete. However, there are other factors that can be modified such as pre-exercise meal timing and glycemic index, the type of carbohydrate ingested before and during exercise, hydration status, and cycling cadence that are not included in this model due to lack of data. The inclusion of other modifiable factors may indeed strengthen this model, but further research is required. Finally, it is important to remember that substrate oxidation is only one part of the puzzle for athletes, and just because something has little effect on RER does not mean it does not have other implications for performance and adaptations.

7.6. Conclusion

Factors known to influence the RER during exercise, such as exercise duration and intensity, age, sex, fitness level, muscle glycogen, and daily dietary intake, together only explain ~60% of the variation in RER during exercise, and habitual dietary intake has a larger influence on RER than carbohydrate ingested during exercise. More research is needed on older subjects and females, particularly in relation to carbohydrate ingestion during exercise. Future studies should also investigate other potential predictors of RER including the lactate/ventilatory thresholds, training age, genetic markers, and markers of blood glucose and insulin sensitivity, which may help explain part of the remaining ~40% of variance in RER during exercise. Additionally, more research is needed looking at substrate oxidation beyond 4 h of exercise, especially considering the popularity of ultra-endurance events

8. Self-reported dietary intake of endurance athletes during 12 weeks of training

This chapter reports the self-selected dietary intake of endurance athletes across a 12-week period with an emphasis on the relationship between training and carbohydrate intake.

8.1 Abstract

It is recommended that endurance athletes modulate their daily carbohydrate intake according to the demands of training, but there is limited evidence of how this is currently practiced by athletes during real-world, day-to-day training. The purpose of this observational study was to report the dietary intake of endurance athletes across a 12-week period with an emphasis on the relationship between training load and carbohydrate intake. Self-selected training and dietary intake were self-reported using a smartphone app by 55 endurance athletes (62% male) daily for 12 weeks, representing a total of 4,128 days of dietary assessments and 3,535 days of training. Fasted-state training was regularly performed by 63% of athletes and was more common in males ($p = 0.003$). Average daily carbohydrate intake for each athlete ranged from 1.2 to 7.2 g/kg (mean 4.1 ± 1.5). Pearson correlations between training load and daily carbohydrate intake ranged from -0.34 to 0.87. At the group level, a greater amount of variation in daily carbohydrate intake was explained by exercise duration ($R^2 = 0.13$), compared with exercise intensity ($R^2 = 0.03$). Overall, athletes adjust daily carbohydrate intake based on exercise duration, but at the individual level many do not substantially modulate carbohydrate intake based on training load.

8.2 Introduction

Sport nutrition guidelines recommend daily carbohydrate intake be individualized to the athlete, and modulated according to changes in exercise volume [180]. The principle of adjusting daily carbohydrate intake has been referred to as "fueling for the work required" [2], and can be recognized practically by daily fluctuations in carbohydrate intake that consider the demands of the training and competitive schedule. Modulating daily carbohydrate intake relative to an athlete's training has the potential to influence signaling pathways that regulate training-induced skeletal muscle adaptations [2, 181], impact training intensity and exercise capacity [12, 13], manage body composition, and reduce the risk of inadequate energy availability [180]. However, there is limited evidence of how athletes practice it during real-world, day-to-day training. This could be related to several challenges, including athlete adherence to longer-term data collection, difficulty quantifying training loads (particularly when athletes train in multiple exercise modalities), and the lack of any objective method to quantify the relationship between training and dietary carbohydrate intake.

To date, knowledge of athlete nutrition practices in the context of their training has largely been limited to surveys [19-22], case studies [24], or short-duration (1–7 d) observations [25, 26, 530]. Capturing longer-term dietary intake is important to adequately capture day-to-day variability. It has been reported in non-athletes that 31–64 days of dietary tracking are needed to estimate an individual's true average energy and macronutrient intake with 95% confidence [531]. Traditionally, performing long-term data collection has been difficult. However, the wide availability of wearable technologies, smartphone-based diet tracking apps, and web-based fitness platforms has opened up many new possibilities for remote data collection [532]. Indeed, recent sport science studies have been performed entirely [178] or partially [533] in participants' home training environment.

Accordingly, the purpose of this observational study was to report the self-selected dietary intake of endurance athletes across a 12-week period with an emphasis on the relationship between training and carbohydrate intake.

8.3 Methods

8.3.1 Experimental design

This observational study monitored the daily self-reported and self-selected nutrition intake and training of endurance athletes for 12 weeks. Throughout the study, participants could perform any type of exercise and consume any type of diet, provided it was recorded. In addition to diet and exercise, measures of sleep, heart rate variability (HRV), and wellbeing were recorded daily. Data presented herein are from a wider study of endurance training and recovery and are focused on the relationships between dietary intake and training. Results relating to daily recovery have been reported elsewhere (Appendix I). The study was open to male and females aged 18 or older who train at least seven hours per week, use a smartphone to track their dietary intake at least five days per week, capture HRV daily, and track sleep using a wearable device. All study protocols and materials were approved by the Auckland University of Technology Ethics Committee (22/7).

8.3.2 Participants

The study was completed by 55 endurance athletes (62% male, 71.5 ± 10.2 kg, aged 43 ± 9 years), from 10 countries (Supplemental Fig. G1). The primary sports represented were triathlon (67%), running (20%), cycling (11%), and rowing (2%). The self-reported competitive level included professional (4%), elite non-professional (qualify and compete at the international level as an age-group athlete, 35%), high-level amateur (qualify and compete at National Championship-level events as an age-group athlete, 24%), and amateur (enter races but don't expect to win, or train but do not compete, 38%) athletes. Applying the participant classification framework of McKay et al. [534] is currently unclear for age-group competitors, but when considered relative to their respective age-group world ranking/personal bests, the levels correspond with tiers 2/3/4 for amateur, high-level amateur, and elite non-professionals, respectively.

8.3.3 Assessment of self-reported training load

All exercise was recorded in TrainingPeaks software (TrainingPeaks, Louisville, CO, USA). For each session, participants recorded session rating of perceived exertion (sRPE, [535]) using the Borg CR100® scale, which offers additional precision compared with the CR10 scale [536]. Participants were asked to rate the session within 1-h of exercise, but sRPE scores are temporally robust from within minutes to within days following a bout of exercise [535]. For each session, participants also recorded the amount of carbohydrate consumed within the 4-h pre-exercise window.

8.3.4 Assessment of self-reported dietary intake

Participants were instructed to maintain their typical dietary habits and record all calorie-containing food and drink consumed for the duration of the 12-week study. Weighing of food was encouraged, but not mandated, and common issues such as underreporting were discussed before starting the study. Participants were not required to record non-caloric fluid ingestion, micronutrient content, or timing of meals. Dietary intake was self-reported using the MyFitnessPal application (www.myfitnesspal.com). Compliance with dietary tracking was monitored by connecting to participant food logs via MyFitnessPal, and enquiring about any unexpected values (determined both visually and using anomaly detection software [537]. Incomplete days of tracking (1.1 ± 2.5 per participant, range 0 to 12) were removed. To aid compliance, participants were recruited who were already regularly tracking their diet (in several cases daily for 4+ years). Therefore, all participants displayed strong intrinsic motivation for habitual diet tracking.

8.3.5 Data analysis

Training load was calculated for each workout as the product of sRPE and duration of exercise in minutes divided by 10 to account for the 100-point scale, and training monotony was calculated as mean training load divided by standard deviation [538]. Exercise was summed into daily totals for workout duration and training load. Because dietary protein and fat ingestion have minimal effects on substrate oxidation [375], fasted training was defined as consuming < 5 g of carbohydrate in the 4-h pre-exercise window. Athletes performing fasted-state training on at least 15% of training days were considered as regularly performing fasted-state training. For multiple exercise sessions in a single day, a weighted mean based on the duration of each session was used to calculate a single value for pre-exercise carbohydrate ingestion in grams. External load metrics such as heart rate, power, or pace were not collected because many athletes undertake activities that can't be quantified on a common scale such as strength training or swimming, and also because sRPE is a valid and reliable method for calculating training load across modalities [538].

Dietary macronutrient intake was calculated relative to body mass to compare across participants more appropriately. At least 85% of a participant's training and diet must have been logged to be included in the analysis. Four participants were excluded due to poor tracking compliance. Missing values were imputed using multiple linear regression [539]. Participants who did not complete the full 12 weeks due to illness, injury, or drop-out but completed at least 6 weeks of tracking were included in the analysis (n = 12).

8.3.6 Statistical analysis

Descriptive statistics are provided as mean ± SD unless noted. For group-based summary statistics, mean values of each participant were used (e.g., Table 9.1). Univariable regression analysis was performed to examine relationships between carbohydrate intake and training load, exercise duration, and exercise intensity at the group level and for three sub-groups of median daily carbohydrate intake. Low-carbohydrate was considered < 20th percentile (2.6 g/kg, Low-CHO), high-carbohydrate was > 80th percentile (5.2 g/kg, High-CHO), and moderate-carbohydrate was in between (Mod-CHO). The best-fit regression line (linear or polynomial) was established using the likelihood ratio test. Because multiple data points were collected from each participant, the assumption of independence of residuals is violated. Therefore, we built general linear mixed-effect models to examine how factors were related, specifying subject ID as a random intercept using the *lme4* R package, and report the marginal R^2, which describes the proportion of variance explained by only the fixed effect. All models were checked by visualizing the Q–Q and other residual plots to ensure approximate residual normality and heteroscedasticity, using the *performance* R package.

A series of linear models were used to estimate differences in percentage of training days that included fasted-state training, and weekly training volume (hours per week), based on sex (male, female) and competitive level (amateur, high-level amateur, elite non-professional). Contrasts within each sub-group were estimated after adjusting for the other variable, and the Holm adjustment was applied for multiple comparisons. Standardized effect sizes for each contrast were computed based on differences in estimated means using the *emmeans* R package. For sub-group analysis based on competitive level, professional athletes (n = 2) were merged into the elite non-professional group. Pearson's correlation analysis was performed to determine relationships between each participant's average daily carbohydrate intake and the percentage of training sessions performed in the fasted state and weekly training volume. Correlations are interpreted as follows: 0.1–0.3 small, 0.3–0.5 moderate, 0.5–0.7 large, 0.7–0.9 very large, and 0.9–1.0 almost perfect [540].

To determine the number of days of dietary tracking needed to estimate "true" average nutrient intakes for individuals with a given degree of confidence, the calculations of Basiotis et al. [531] were used. Each participant's energy and macronutrient intake over the entire recording period was assumed to reflect their typical intake and day-to-day variability. The number of dietary tracking days needed for their intake to be within 10% of usual intake was estimated as:

$$\text{Number of days} = (Z_a)^2 * (\text{intake SD})^2 / (\text{accuracy level})^2 * (\text{mean intake})^2$$

Z_a represents the Z-value from the Standard Normal Distribution Table at the desired level of statistical significance (0.05), and accuracy level was set at 0.1 (10%). We also calculated the Spearman correlation between number of days needed to estimate carbohydrate intake and the CTI for each athlete. All analyses were carried out with R version 4.0.3 (R foundation for Statistical Computing, Vienna, Austria), with statistical significance at $p < 0.05$.

8.4 Results

8.4.1 Dietary Intake and Training Load

The analysis included 4,128 days of tracking (82 ± 11 days per participant) and 3,535 days of training (69 ± 15 days per participant). Dietary intake and training characteristics are shown in Table 8.1, as well as the number of dietary tracking days needed to estimate true average intake. It was also observed that 63% of athletes regularly trained in the fasted state. Boxplots of daily carbohydrate intake for each participant are shown in Figure 8.1. Daily carbohydrate intake for each participant is shown in Supplemental Figure G2, along with an indication of sessions performed in the fasted state. Boxplots of daily protein, fat, and kcal intake for each participant are shown in Supplemental Figure G3.

Table 8.1. Dietary and training characteristics of participants

Dietary and training characteristics of study participants[1]									
Value	**Kcal (kcal/kg)**	**CHO (g/kg)**	**Protein (g/kg)**	**Fat (g/kg)**	**Weekly training (h)**	**Average daily exercise duration (h)[2]**	**Longest single-day exercise duration (h)**	**Average sRPE (AU)**	**% Training days fasted**
Mean	39.0	4.1	1.9	1.6	11.3	1.9	5.7	35.8	29.0
SD	9.1	1.5	0.4	0.5	4.1	0.5	2.1	10.2	24.4
Low	21.3	1.2	1.1	0.7	4.3	0.9	2.3	15.9	0
High	59.8	7.2	3.2	3.1	23.0	3.3	13.4	72.4	80.6

[1] Values are calculated from mean values for each participant throughout the study period. For example, the 'Low' value of 21.3 kcal/kg refers to the participant with the lowest mean daily kcal intake, not the lowest single-day intake. [2]Values for average daily exercise duration are calculated by excluding days without exercise. [3]Low and high values are considered separately for each of the macronutrients, meaning the lowest/highest values for CHO, protein, and fat. sRPE: session Rating of Perceived Exertion

Table 8.2. Number of dietary tracking days needed to estimate true average intake

Value	Kcal (kcal/kg)	CHO (g/kg)	Protein (g/kg)	Fat (g/kg)
Mean	18	53	22	32
SD	10	63	12	22
Low	1	10	2	9
High	53	413	54	136

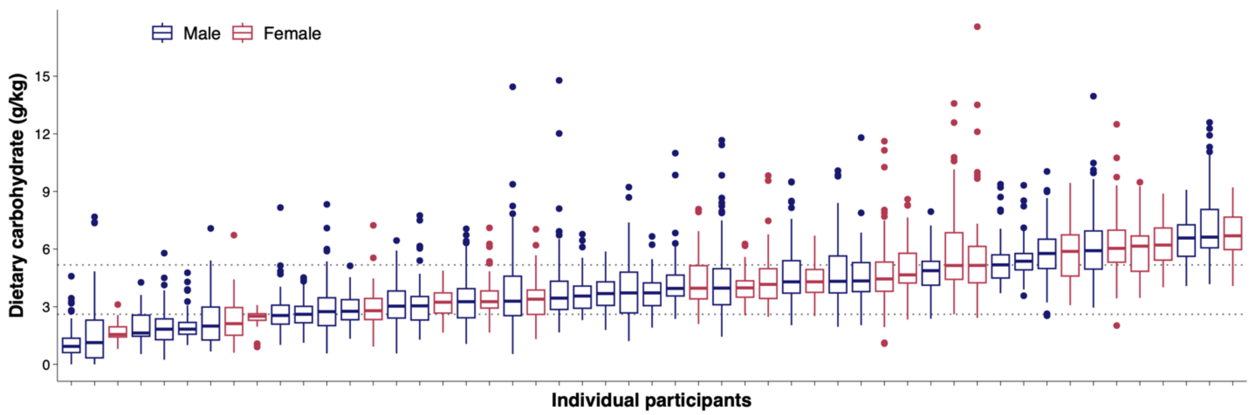

Figure 8.1. Box plot of daily carbohydrate (CHO) intake for each participant, colored by sex. Dotted lines represent median daily CHO intake for the 20th percentile (2.6 g/kg) and 80th percentile (5.2 g/kg).

Relationships between carbohydrate intake (daily and in the 4 h pre-exercise window) and training load, duration, and intensity are shown in Figure 8.2. The coefficient of determination (R^2) was lower for exercise intensity (sRPE) than exercise duration or training load. There was no relationship between daily carbohydrate intake and training load on the day prior, or day following a given training load (R^2 .00–.02, Supplemental Figure G4).

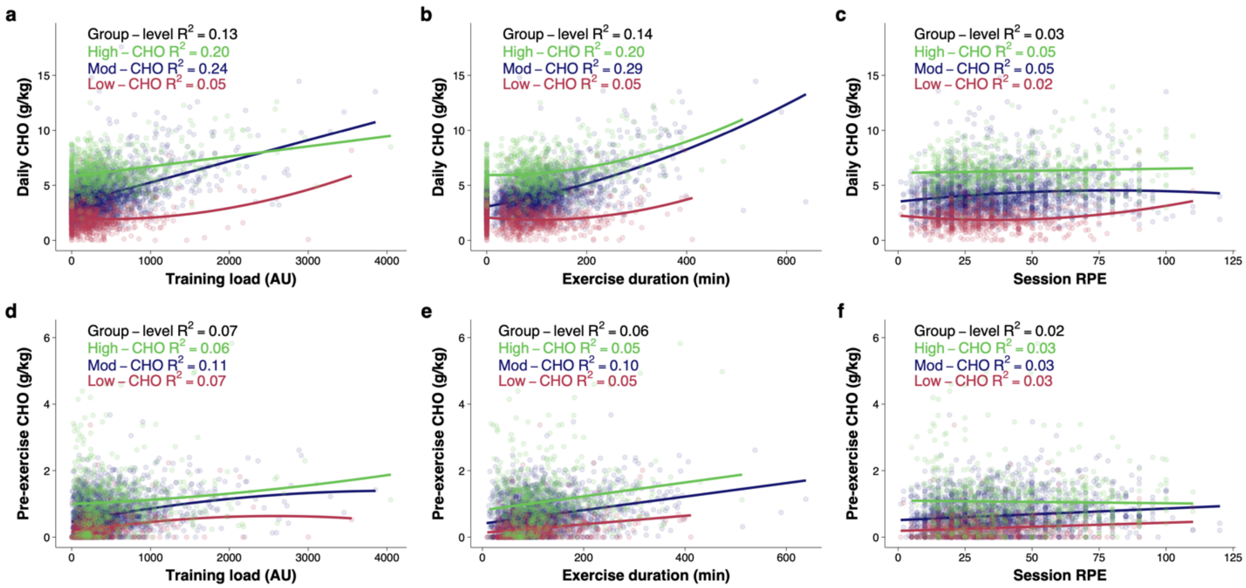

Figure 8.2. Daily carbohydrate (CHO) intake relative to training load (a), exercise duration (b) and exercise intensity as session rating of perceived (RPE, c), and carbohydrate intake within the 4-h pre-exercise window in relation to training load (d), exercise duration (e), and session RPE (f), at the overall group level and separated by daily CHO sub-group. Training load is calculated as the product of session RPE and exercise duration in minutes, divided by 10. Best-fit regression lines based on univariable linear mixed effects models are shown for each diet group, with fit indicated as R^2. To improve visual clarity, a single data point was removed for a day that included 13.4 h of exercise (3 h longer than the next longest exercise duration). AU: arbitrary units

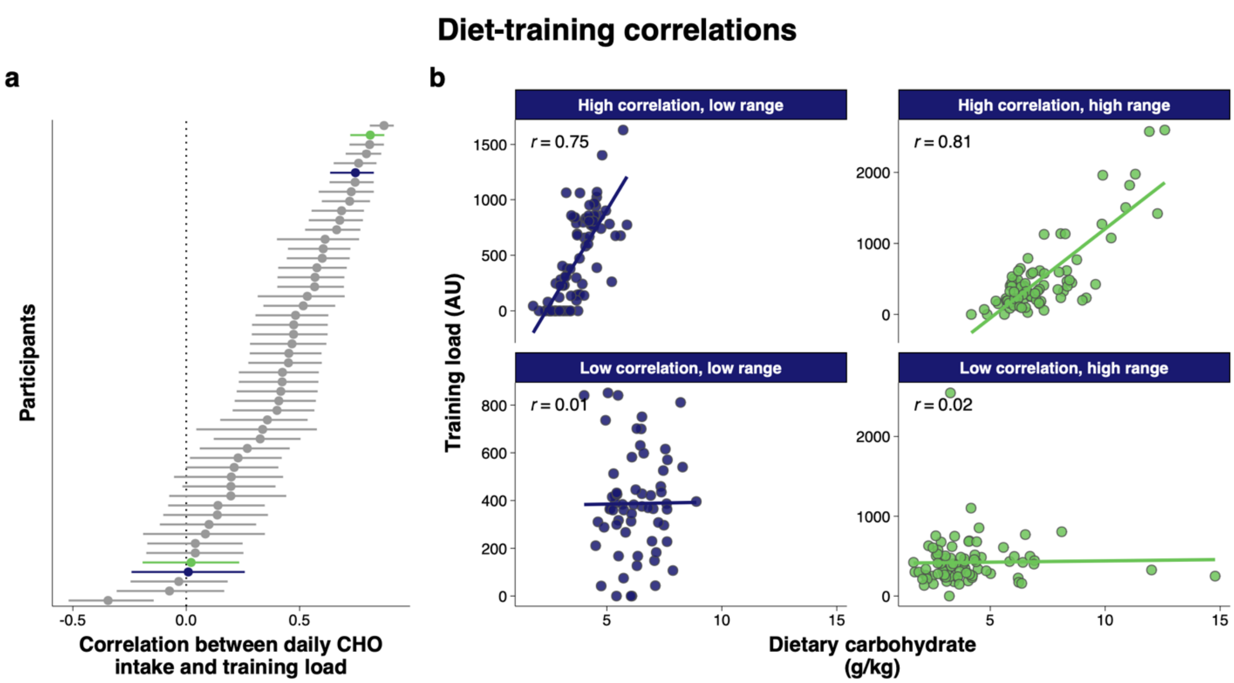

Figure 8.3. Pearson correlation values with 95% confidence intervals are shown between training load and daily carbohydrate (CHO) intake (g/kg) for each participant (a), and example data from participants with combinations of low and high correlations and carbohydrate ranges (b). Colored points in (a) correspond to the data shown in (b). Training load is the product of session rating of perceived exertion and duration in minutes. Range refers to the difference between an athlete's highest and lowest daily carbohydrate intake and can be inferred in (b) as the horizontal distance of the trend line. AU: arbitrary units.

8.4.2 Sub-group analysis

The percentage of training days an athlete performed fasted training was higher for males compared with females, but there were no differences based on competitive level (Figure 8.4). The frequency of fasted training was negatively correlated with average daily carbohydrate intake (Figure 8.5a). Weekly training volume was higher for the highest-level (13.5 ± 3.6 h week) compared with the lowest-level athletes (8.9 ± 3.4 h week) and positively correlated with daily carbohydrate intake (Figure 8.5b), but not different based on sex (Supplemental Figure G7). Training monotony was not different based on sex or competitive level (data not shown).

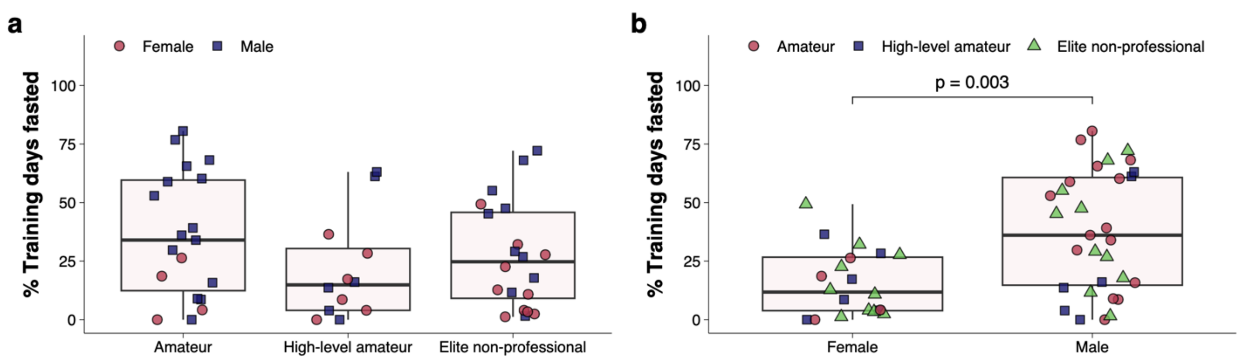

Figure 8.4. Boxplot of percentage of days training in the overnight-fasted state, separated by competitive level (a) and sex (b). Individual participants are shown separated by shape and color based on sex (a) and competitive level (b).

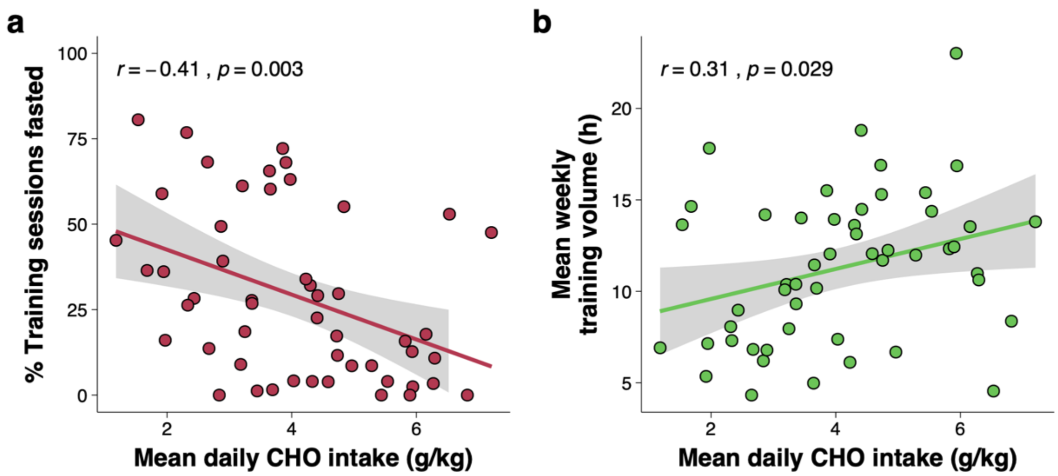

Figure 8.5. Correlations between mean daily carbohydrate (CHO) intake for each participant and percentage of training sessions performed in the fasted state (a) and mean weekly training volume (b).

8.5 Discussion

This study provides novel data on the self-selected dietary intake of endurance athletes across a 12-week period, highlighting the relationships between training and dietary carbohydrate. At the group level we observed athletes adjust daily carbohydrate intake based on exercise duration, and to a lesser degree, intensity, but at the individual level many endurance athletes are not modulating carbohydrate intake based on changes in training, or if they are, the magnitude of adjustment is small.

Prior to this study, knowledge of the day-to-day dietary manipulation of endurance athletes has largely been limited to questionnaires [19, 20, 22], case studies [23, 24], or short-duration (1–7 d) observations [25, 26, 530]. Longer-duration observational studies can help answer questions that would not be feasible to study in a controlled laboratory environment. To this end, we recruited athletes who were habitually tracking their diet and training to obtain longer-term longitudinal data without additional participant burden. Emphasizing the importance of longer-term data collection, our findings suggest ~18–53 days of dietary tracking may be needed for an athlete’s average energy and macronutrient intake to fall within 10% of their 12-wk average intake 95% of the time. Therefore, future studies of dietary intake should strive for this duration or longer.

8.5.1 Dietary Intake

Compared with other studies in triathletes performing similar weekly training volumes [541, 542], daily energy intake was similar (39 vs. 34–37 kcal/kg), whereas carbohydrate intake was lower (4.1 vs. 5.0 g/kg), and protein (1.9 vs. 1.4 g/kg) and fat (1.6 vs. ~1 g/kg) intake were higher. These differences could be related to the increased popularity of lower-carbohydrate diets and/or the wide range of daily carbohydrate intakes observed in our study (Fig. 8.1). It was also found that 62.7% of athletes regularly performed training sessions in the fasted state, a number remarkably similar to the 62.9% of athletes reporting the use of fasted training in a worldwide survey of ~2000 endurance athletes [21].

We observed an overall pattern of carbohydrate intake that increased with training loads, which was more closely related to exercise duration rather than intensity (Fig. 8.2). From this perspective, athletes are generally following the “fuel for the work required” framework [2]. However, at the individual level the relationship between training load and carbohydrate intake varied greatly (Fig. 8.3). Similarly, at the group level athletes trained in the overnight-fasted state on 29% of training days, but at the individual level this ranged from 0–81%, highlighting the need for individualized analysis of diet-training practices.

Our data show athletes are adjusting their daily carbohydrate intake based on exercise duration more than exercise intensity, although it is unclear if this is being done consciously or unconsciously. This finding could be generalizable to the wider population of endurance athletes as study participants were not pre-screened based on any dietary practices nor were they told that the diet-training relationship would be analyzed in this manner. However, by habitually tracking their diet and training it is possible these participants could adjust their diet based on training volume more easily and precisely than someone not tracking their diet. Greater muscle glycogen utilization has been reported during prolonged steady-state running compared with shorter, but higher-intensity, track running sessions in males but not females [543]. Prolonged cycling exercise would also be expected to reduced muscle glycogen to a greater degree than short-duration exercise at higher intensities [283]. However, if the intensity is high enough, similar, or even increased glycogen breakdown can occur during very duration exercise (< 10 min) compared with prolonged low-intensity exercise [283, 544]. Future research is needed to better understand the total carbohydrate requirements of different types of training sessions and training modalities in relation to training load metrics, which could then better inform the practice of matching carbohydrate intake with carbohydrate usage during exercise.

At the group level, the amount of variation in daily carbohydrate intake that could be explained by the sRPE training load measure (13%) was lower than what has been reported in a 7-d study of elite cyclists (36%) using exercise energy expenditure as a training load metric [530]. A study in elite runners and race-walkers compared dietary intake between hard and easy days of training, finding minimal evidence of periodized nutrition practices among females (6.2 vs. 5.8 g/kg carbohydrate on hard vs. easy days, respectively), and no differences among males (7.3 vs. 7.2 g/kg carbohydrate on hard vs. easy days, respectively), despite the majority of athletes reporting an understanding of dietary periodization practices [25]. This supports our findings that exercise intensity may have less influence on dietary carbohydrate intake choices compared with exercise duration. Taken together, it appears many athletes are not following the recommended guidelines of modulating carbohydrate intake based on their training duration and intensity.

Training load can be measured as internal and/or external to the athlete [545]. Internal load reflects the relative physiological stress and disturbance in metabolic homeostasis in response to an external load, which is characterized by objective measures such as distance or power [545]. Internal load has been recommended as the primary measure when monitoring athletes, as it plays a pivotal role in determining training outcomes and can reflect variations in the stress response to a given external load due to other stressors such as extreme temperature, or accumulated training fatigue [545]. The sRPE-based load metric we used provides a measure of internal load, and was chosen as a valid and reliable method for calculating training load across exercise modalities [538], a necessity with multi-sport athletes. We used the CR100® scale, which accounts for the non-linear stimulus-response relationship and overcomes many limitations of other scales [546].

8.5.2 Sub-group analysis

Because many beliefs and practices relating to pre-exercise nutrition vary based on sex, competitive level, and habitual dietary pattern [22], we performed sub-group analysis. The findings echoed survey results reporting a higher prevalence of fasted training among males, and those on low-carbohydrate diets [21], adding further support to the notion that diet-exercise practices vary based on sex, competitive level, and dietary pattern.

8.5.3 Limitations

The most notable limitation to this study relates to self-reported dietary data. Although athletes may underreport dietary intake [547], nearly all previous studies have used short-duration food records rather than smartphone apps. Smartphone-based food diaries lead to better participant compliance compared with paper-based diaries [548], possibly related to features such as the ability to scan barcodes and save commonly-consumed foods. High reliability has been reported with food-tracking apps [549], and in the context of this study it could be argued that reliability and within-participant consistency is more important than between-participant consistency. The MyfitnessPal app has been shown to be accurate for total energy intake, macronutrients, sugar, and fiber [550], but discrepancies may arise from the use of a single, rather than country-specific, food database [551, 552]. It has also been suggested that familiarity with and interest in keeping food records may lead to more reliable estimates of energy intake [553]. All participants were highly motivated and already habitually tracking dietary intake before joining the study. Indeed, mean daily energy intake was similar or higher than other studies in triathletes performing similar weekly training volumes [541, 542], suggesting acceptable reporting accuracy. Moreover, the ecological validity of our study is high, as this is the same type of data that would be received by coaches and nutritionists working with athletes. Despite its limitations, self-report dietary data is considered critical in providing valuable information about dietary patterns and evaluating questions such as whether intakes are consistent with recommendations [554].

8.6 Conclusion

This study utilized a novel approach to monitoring endurance athletes throughout 12 weeks of self-selected training to better understand the current real-world application of dietary periodization. We provide data on day-to-day variability in carbohydrate intake and show that many endurance athletes are not following current sport nutrition guidelines to periodize carbohydrate intake based on training load, or if they are, the magnitude of adjustment is small. Our findings also suggest that future observational studies of dietary intake should strive for ~18–53 days of dietary tracking for an athlete's average energy and macronutrient intake to fall within 10% of their true average intake.

9. The Influence of Dietary Carbohydrate on Perceived Recovery Status Differs at the Group and Individual Level – Evidence of Nonergodicity Among Endurance Athletes

This chapter examines the relationship between daily carbohydrate intake and perceived recovery status, and if group-level statistics can be generalized to individual athletes.

This chapter contains the following publication:

Rothschild J, Stewart T, Kilding A, Plews D. The influence of dietary carbohydrate on perceived recovery status differs at the group and individual level – evidence of nonergodicity among endurance athletes (under review).

9.1 Abstract

Purpose: Research findings are typically reported at the group level but applied to individuals. However, an emerging issue in sports science concerns nonergodicity — whereby group-level data cannot be generalized to individuals. The purpose of this study was to determine if the relationship between daily carbohydrate intake and perceived recovery status displays nonergodicity.

Methods: Fifty-five endurance athletes recorded daily measures of self-selected dietary intake, training, sleep, and subjective wellbeing for 12 weeks. We constructed linear models to measure the influence of daily carbohydrate intake on perceived recovery status while accounting for training load, sleep duration, sleep quality, and muscle soreness. Using linear model coefficients for carbohydrate intake we tested whether the distributions (mean and SD) differed at the group and individual levels (indicating nonergodicity). Additionally, a decision tree was created to explore factors that could provide an indication of an individual athlete's relationship between carbohydrate intake and perceived recovery status.

Results: Mean values were not different between group- and individual-level analyses, but SDs at the individual level were ~2.4 times larger than at the group level, indicating nonergodicity. Model coefficients for carbohydrate intake were negative for three participants, positive for four participants, and non-significant for 37 participants. The Kappa value measuring accuracy of the decision tree was 0.52, indicating moderate prediction accuracy.

Conclusion: For most individuals, carbohydrate intake did not influence recovery status. However, the influence of dietary carbohydrate intake on daily recovery differs at the group and individual level. Therefore, practical recommendations should be based on individual-level analysis.

9.2. Introduction

Studies in sports science are typically conducted and reported at the group level yet applied at the individual level. However, it has been increasingly questioned whether group-level results can generalize to individuals [555-558], as group-level findings could conceal relevant inter-individual variability in response to a training stimulus or intervention [559]. This could lead to sub-optimal training or nutrition prescriptions for an individual athlete. When the group-level variability of data does not resemble the individual-level variability, or when individual-level variability exhibits changing variance over time, the data is non-ergodic [560, 561]. Nonergodicity could lead studies to overestimate the accuracy of aggregated statistical estimates and in turn, the generalizability of conclusions between the group and individual. In light of this, nonergodicity has been suggested as a threat to human subjects research [557].

Sports nutrition guidelines recommend carbohydrate intake be modulated according to changes in exercise volume [180], with the intention of optimizing training adaptation while ensuring adequate recovery. Under-fueling can cause low energy availability and impaired recovery [562], while over-fueling can cause weight gain and potentially attenuate desired training adaptations [1]. It is commonly reported that perceived ratings of wellness and recovery are sensitive to fluctuations in training load [563, 564], and sleep duration [565]. However, the influence of dietary intake on daily recovery during endurance training is less understood. During short-term periods of intensified endurance training, increasing energy and carbohydrate intake may attenuate symptoms of overreaching [566-569], although it is unclear if this relationship between carbohydrate intake and daily recovery extends over longer time periods and/or across a range of training volumes in a practical setting.

To determine ergodicity of a given data set, a Cattell data box can be used as previously described by Molenaar and Campbell [560] and Neumann et al. [555]. This can be visualized as a 3-dimensional box with time, measured variables, and individual subjects as the dimensions. For group-level analysis of a variable of interest, a single time point is pooled across all subjects (e.g., all subjects on day 1), repeated for each additional time point, and summarized (e.g., mean, standard deviation (SD), confidence intervals (CI), etc.). For individual-level analysis the variable is analyzed across all time points separately for each subject and then summarized. If the structure of the group- and individual-level data differ (e.g., statistics of central tendencies, variations, and/or correlations of time series data), the process is considered non-ergodic and results obtained from standard analysis at the group level cannot be applied to the individual [560].

Nonergodicity is relevant in the context of nutrition and training, as evidence-based practitioners and athletes often apply group-level research findings to the individual [180, 570]. Therefore, the purpose of this study was to examine the relationship between daily carbohydrate intake and perceived recovery status and determine if group-level statistics can generalize to individual athletes. To do so, 55 endurance athletes recorded daily measures of self-selected nutrition intake, exercise training, sleep habits, and subjective wellbeing for 12 weeks. We constructed linear models to measure the influence of daily carbohydrate intake on perceived recovery status the following morning while accounting for other factors such as training load, sleep, and muscle soreness. Using the model coefficient for carbohydrate intake we tested whether the distributions (mean and SD) differed at the group and individual levels. As an exploratory analysis, we also created a decision tree model to understand general traits of athletes that would predict a positive, negative, or non-significant model coefficient for carbohydrate intake. This could serve as the next step in understanding individual level-differences and provide a direction for coaches and practitioners to make better decisions to support the individual athlete's needs.

9.3. Methods

9.3.1 Study design

Self-selected nutrition intake, exercise training, sleep habits, and subjective wellbeing of endurance athletes were monitored daily over a 12-week period. Throughout the study period, participants were free to perform any type of exercise and consume any type of diet. Results presented herein are from a wider study of endurance training and recovery. Data related to carbohydrate periodization [571] and machine learning predictions [572] have been reported elsewhere. The study was open to male and females aged 18 or older who train at least seven hours per week, were using a smartphone app to track their dietary intake at least five days per week, captured HRV daily, and tracked sleep using a wearable device. All study protocols and materials were approved by the Auckland University of Technology Ethics Committee (22/7), and all participants provided informed consent prior to starting the study.

9.3.2 Participants

Fifty-five endurance athletes (61.8% male, aged 42.6 ± 9.1 years, training 11.6 ± 3.9 hours per week) took part in the study. The primary sports represented were triathlon (67.3%), running (20.0%), cycling (10.9%), and rowing (1.8%). The self-reported competitive level included professional (2.6%), elite non-professional (qualify and compete at the international level as an age-group athlete, 34.6%), high-level amateur (qualify and compete at National Championship-level events as an age-group athlete, 25.6%), and amateur (enter races but don't expect to win, or train but do not compete, 37.2%) athletes.

9.3.3 Assessment of self-reported exercise

All exercise was recorded in Training Peaks software (TrainingPeaks, Louisville, CO, USA). Each session was noted for modality (e.g., bike, run, swim), duration, and session rating of perceived exertion (sRPE [535]) using the Borg CR100® scale, which offers additional precision compared with the CR10 scale [573]. Participants were instructed to rate their perceived effort for the whole training session within 1-h of exercise, although sRPE scores are temporally robust from minutes to days following a bout of exercise [535].

9.3.4 Assessment of self-reported dietary intake

Participants were instructed to maintain their typical dietary habits and record all calorie-containing food and drink consumed for the duration of the 12-week study, using the MyFitnessPal application (www.myfitnesspal.com) [550]. Compliance with dietary tracking was monitored by connecting to participant food logs via MyFitnessPal, and enquiring about any unexpected values (determined both visually and using anomaly detection software [537]). Incomplete days of tracking (2.2 ± 4.6% of days per participant) were removed from the data. To aid compliance, participants were recruited who were already regularly tracking their diet (in several cases daily for 4+ years), and so all participants displayed strong intrinsic motivation for habitual diet tracking.

9.3.5 Assessment of sleep and subjective wellbeing

Nightly sleep duration was recorded using wearable devices, which included Oura ring, Whoop strap, Applewatch, Fitbit, and Garmin models as previously described [572]. These consumer-grade devices offer adequate accuracy in detecting sleep-wake times, but not sleep staging [574-578]. Each morning participants answered four questions related to subjective wellbeing based on the recommendations of Hooper and Mackinnon [579]. The perceived recovery status (PRS) scale [580] was used to measure overall recovery with athletes manually typing a number into Training Peaks software. The 100-point version of the scale was used, which has been shown to provide more accurate measures of recovery than the 10-point scale [573]. In addition, ratings of life stress (1–7), sleep quality (1–7), and muscle soreness (1–10) were also recorded into the software each morning. Participants were familiarized with all scales prior to starting the study.

9.3.6 Data preparation

Training load was calculated for each workout as the product of sRPE and duration of exercise in minutes [538], divided by 10 to account for the 100-point scale, and summed into daily totals. External load metrics such as heart rate, power, or pace were not collected because many athletes undertake activities that can't be quantified on a common scale such as strength training, yoga, or swimming without a HR monitor, and also because the sRPE is considered to be a valid and reliable method for calculating training load across modalities [538]. Seven-day rolling measures for training monotony (a measure of day-to-day variability in the weekly training load, calculated as average daily load divided by the standard deviation) and training strain (product of total weekly training load and training monotony) were calculated [538]. A sleep index score was calculated as the product of sleep duration and sleep quality [581]. Dietary macronutrient intake was converted to a relative intake (g per kg body mass) to allow appropriate comparison between athletes.

Participants were excluded from the analysis if they were training on average less than 6 h per week (n = 8) or did not log at least 85% of the required data points (n = 3). Participants who did not complete the full 12 weeks due to illness, injury, or drop-out but completed at least 6 weeks of tracking were included in the analysis (n = 11). Among participants included in the analysis (n = 44), 2.4 ± 1.7 % of data points were missing. Missing values were imputed at the individual level using multiple linear regression and nearest neighbor algorithms for diet and training measures and using median values for other variables [539].

9.3.7 Analysis

Following the recommendations of previous studies [555, 557], we extracted a subset of data that was symmetrical (i.e., an equal number of participants and observations per participant) to equalize statistical power for analysis at the group and individual levels. Because we had 44 participants in the final analysis, 44 consecutive days were chosen beginning with day 8 to allow for an accurate calculation of training strain (which reflects the previous 7 days of training). Repeated measures correlation [582] was used at the group level to examine the bivariate relationship between the morning (AM) PRS score and prior day carbohydrate intake. Pearson or Spearman correlations, depending on normality of the data as determined by the Shapiro-Wilk test, were used to examine the bivariate relationship between the AM PRS score and prior day carbohydrate intake for each individual.

Previous studies of ergodicity have focused on comparisons of univariate distributions and bivariate correlations [555, 557]. However, the relationship between diet and recovery is likely also dependent on other factors relating to training and sleep. To account for this, linear regression models were constructed with AM PRS score specified as the dependent variable, and prior day carbohydrate intake (g/kg), prior day training load, training strain (encompassing the previous 7 days), muscle soreness, and sleep index specified as independent variables. These variables were chosen because they had the highest importance scores in our predictive modeling study [572]. The model coefficient for carbohydrate intake was the primary variable of interest. For group level analysis, models were made for all 44 athletes together on day 1 and repeated for each of the 44 days with the results summarized across days (as mean, SD, and 95% CIs). For individual-level analysis, a separate model was created for each athlete, and the results were then summarized. However, data at the individual level are a time series, which refers to a sequence of data points at equally spaced points in time and ordered chronologically [583]. Time series data cannot be analyzed with common techniques such as linear modeling if the day-to-day observations are correlated with observations at previous time points (i.e., auto-correlated) and are not independent of each other, as key assumptions of linear regression are violated [584]. Autoregressive Integrated Moving Average (ARIMA) models are commonly used in time series analysis to account for these issues [583]. Therefore, for individual-level analyses we obtained the model coefficient for carbohydrate intake by constructing ARIMA models using the Hyndman-Khandakar algorithm for automatic ARIMA modelling [585]. Ergodicity can be confirmed if the mean and SD at the group and individual levels were not significantly different [560]. R-squared (R^2) was also calculated as an overall measure of model fit.

To explore characteristics which might inform the individual responses to carbohydrate intake, a decision tree model was created to predict the classification of statistical significance for the model coefficient of prior day carbohydrate intake from the individual ARIMA models (non-significant, significantly positive, or significantly negative). To determine statistical significance, 95% CIs for the unstandardized regression coefficients were calculated, and values were considered significant if the CIs did not cross zero. The coefficients were organized into these three categories with practical application in mind. That is, coaches or nutritionists might benefit more from knowing if/how an individual responds to carbohydrate in this context, rather than getting a predicted model coefficient for the individual athlete.

Variables used in the decision tree model were age, training age, competitive level, primary sport, sex, BMI, percentage of training days performing fasted-state training, and average values of daily kcal intake (kcal/kg), daily carbohydrate, fat, and protein intake (g/kg), carbohydrate monotony (mean daily intake/SD), weekly training volume (h), training monotony, and training strain. All available data points were used for the decision tree models (n = 3,588, 81.5 ± 10.4 days per participant), rather than the 44-d subset used to compare group vs. individual responses to obtain the most accurate picture of each individual's characteristics. Modeling was performed in R using the Tidymodels ecosystem [586]. Hyperparameters were tuned using 100 bootstrap resamples and model accuracy was established using 500 bootstrap resamples. Class imbalances were handled by up-sampling prior to tuning. Cohen's Kappa was used as the primary accuracy measure due to the imbalanced, multi-class nature of the outcome variable. Kappa accounts for the accuracy that would be generated simply by chance, producing values between −1 and 1. We interpret these values using the guidelines of Landis and Koch [587], with values of 0–0.20 considered slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial, and 0.81–1 as almost perfect. In addition, we report positive predictive value and negative predictive value [588]. All analyses were carried out with R version 4.0.3 (The R foundation for Statistical Computing, Vienna, Austria). Descriptive statistics are provided as mean ± SD.

9.4 Results

During the 44-d period selected for the primary analysis, average participant training volume was 11.9 ± 3.4 h per week. Mean daily dietary intake was 39.4 ± 9.0 kcal/kg, 4.0 ± 1.6 g/kg carbohydrate, 1.9 ± 0.4 g/kg protein, and 1.7 ± 0.6 g/kg fat. Average sleep duration was 7.5 ± 0.7 hours per night. Bivariate repeated-measures correlation at the group level revealed a significant negative relationship between AM PRS and carbohydrate ingestion the prior day (r = -0.09, 95% CI -0.14 to -0.05, $p < 0.001$), but this relationship varied considerably among individuals (Fig. 9.1).

After accounting for prior day training load, 7-day training strain, muscle soreness, and sleep index via linear modeling, model coefficients for carbohydrate intake were negative for three participants (7%), positive for four participants (9%), and non-significant for 37 participants (87%,

Fig. 9.2). Mean values for model coefficients were similar between the group and individual (evidenced by overlapping CIs), whereas SDs were different, (i.e., non-overlapping CIs) indicating nonergodicity (Fig. 9.3). Non-ergodicity was also observed in the overall model accuracy. Mean R-squared values were 0.32 (95% CI 0.29 to 0.35), and 0.40 (95% CI 0.35 to 0.45), for the group and individual models, respectively, and SD values were 0.11 (95% CI 0.09 to 0.13) and 0.18 (95% CI 0.14 to 0.22) for the group and individual models, respectively.

A decision tree was created to explore potential factors that could provide coaches or practitioners with an indication of an athlete's relationship between carbohydrate intake and perceived recovery status (Fig. 9.4). The Kappa value was 0.52, indicating a moderate level of agreement. Positive predictive value was 0.44, and negative predictive value was 0.87. A confusion matrix of actual and predicted classes is shown in Figure 9.5.

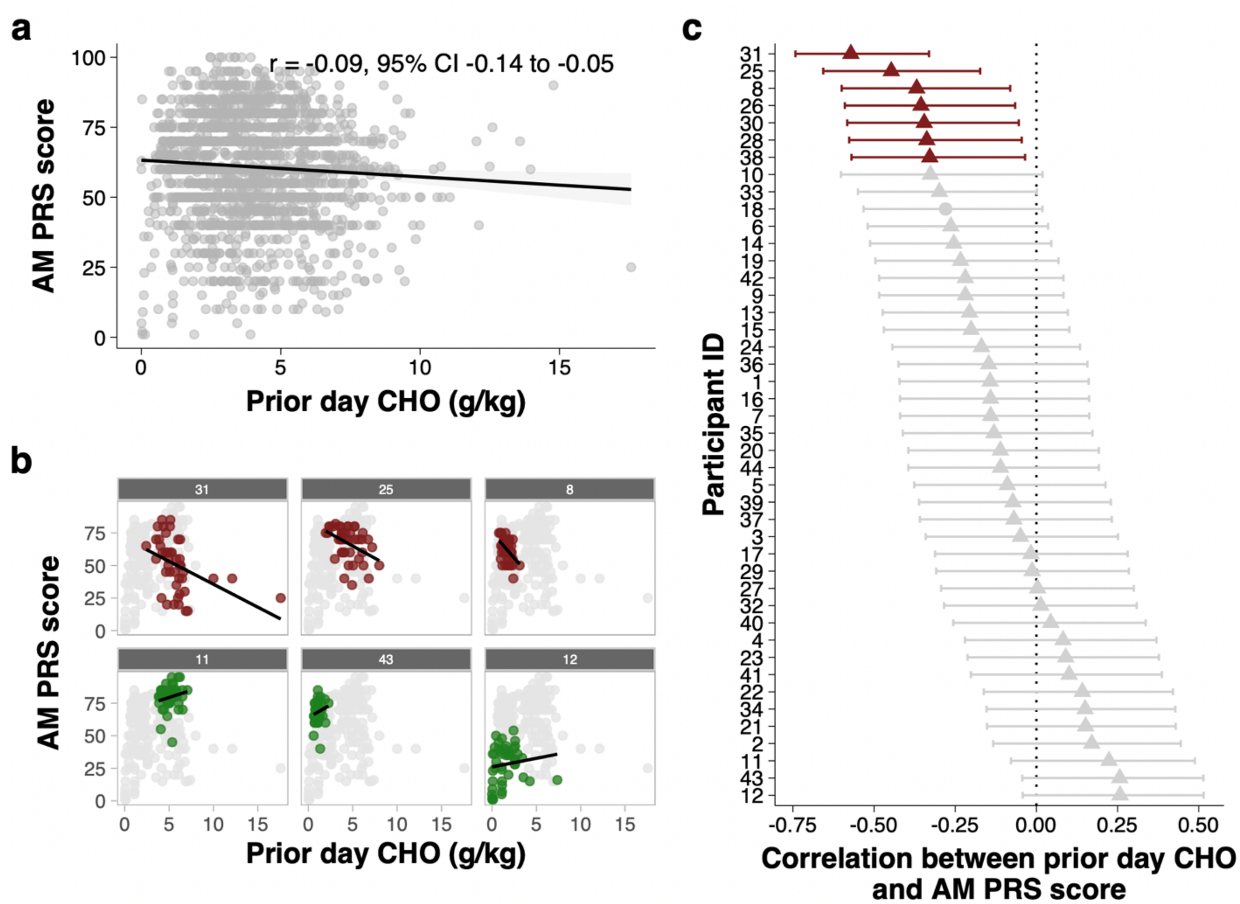

Figure 9.1. Bivariate correlations between AM perceived recovery status (PRS) and carbohydrate ingestion the prior day (g/kg). Values given in (a) used repeated-measures correlation analysis. Panel (b) shows Pearson (circle) or Spearman (triangle) correlation values and 95% confidence intervals for each participant, colored based on statistical significance ($p < 0.05$). Panel (c) shows example scatterplots for the participants with the three highest and three lowest correlation values. Numbers at the top of each panel in (c) relate to the participant ID shown in (b). The light grey points in (c) depict all points for the 6 participants shown in (c), with each individual's points shown in color (red for negative correlation values and green for positive)

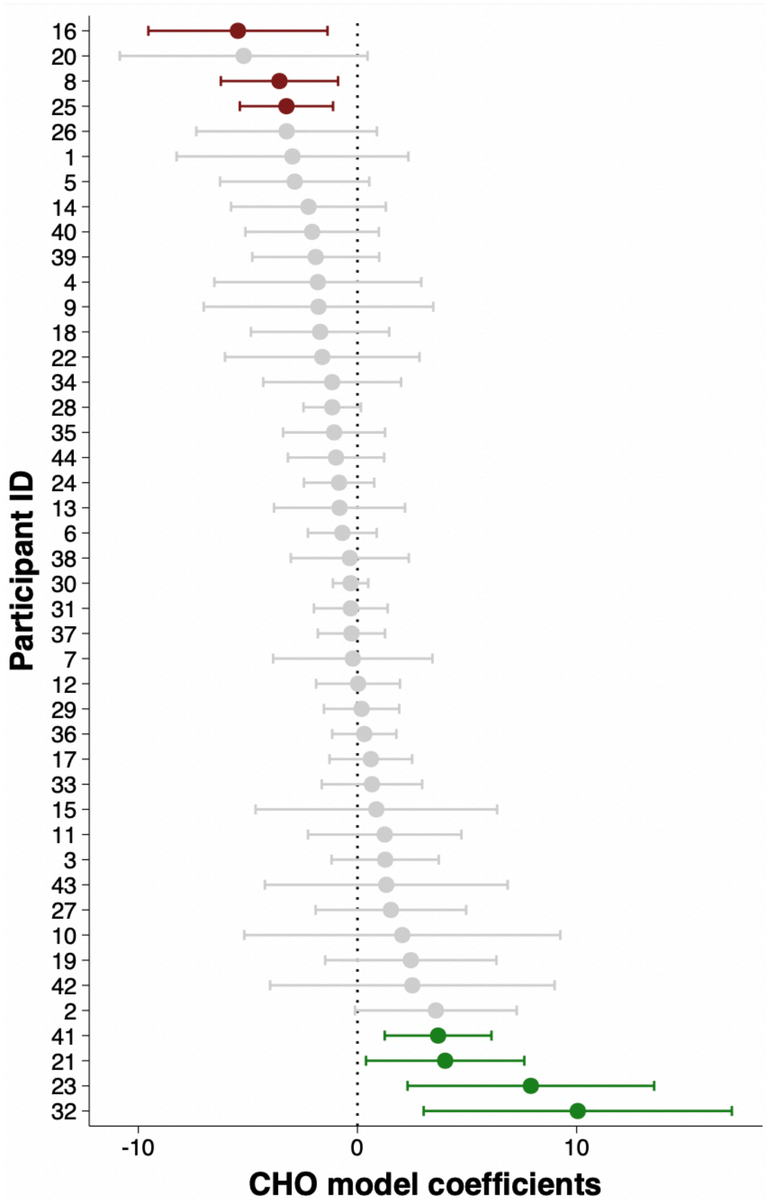

Figure 9.2. Individual participant model coefficients with 95% Confidence Intervals for the effect of prior day carbohydrate (CHO) intake (g/kg) on AM Perceived Recovery Status (PRS) score after accounting for prior day training load, 7-d training strain, muscle soreness, sleep index (product of sleep duration and sleep quality), using Autoregressive Integrated Moving Average (ARIMA) modeling. This can be interpreted as a change of AM PRS in the amount shown on the x-axis for every 1 g/kg increase in daily CHO intake, after holding everything else constant. Green indicates statistically significant positive values, red indicates statistically significant negative values, and grey indicates non-significant values ($p > 0.05$)

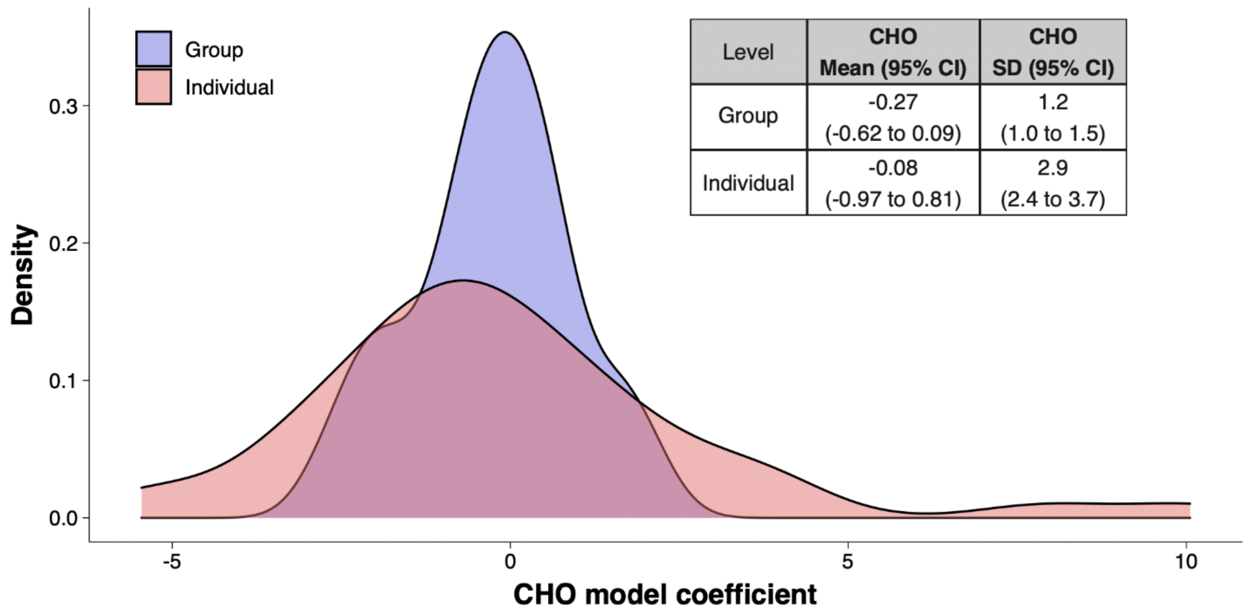

Figure 9.3. Density plots of the model coefficients for the effect of prior day carbohydrate (CHO) intake (g/kg) on AM Perceived Recovery Status (PRS) score after accounting for prior day training load, 7-d training strain, muscle soreness, and sleep index (product of sleep duration and sleep quality). Inset table shows mean, SD, and 95% confidence intervals for model coefficients for carbohydrate intake from group and individual level modeling

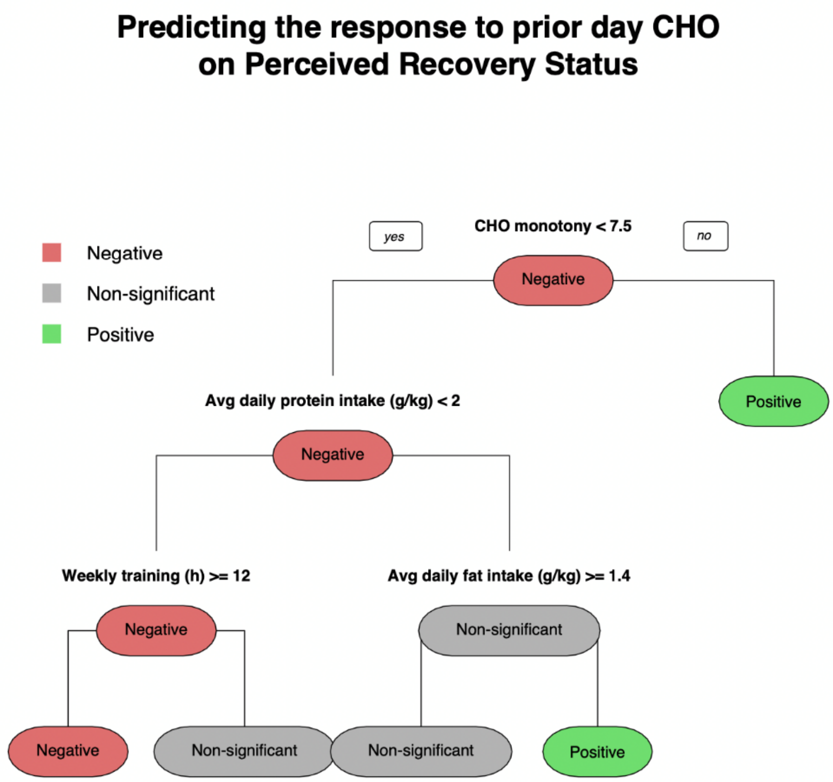

Figure 9.4. Decision tree predicting the response to prior day carbohydrate (CHO) intake on AM Perceived Recovery Status. Each node indicates the predicted class (negative, non-significant, or positive model coefficients). At each level, following the node to the left corresponds to yes, and following the node to the right corresponds to no

Figure 9.5. Confusion matrix of actual and predicted classes for the decision tree model predicting the response to prior day carbohydrate ingestion on AM Perceived Recovery Status. Values shown in the dark green boxes indicate the number of correct predictions for each class

9.5. Discussion

The aim of this study was to examine the relationship between daily carbohydrate intake and perceived recovery status and determine if group-level statistics can be generalized to individual athletes. The main outcomes are 1) the data are non-ergodic, meaning group-level findings cannot be generalized to the individual, 2) daily carbohydrate intake does not influence perceived recovery status the following morning for most athletes, after accounting for other influential variables such as training load, muscle soreness, and sleep, 3) for those that are affected the influence can be positive or negative, and 4) we build upon previous work using bivariate correlations to include linear model coefficients and offer a method for understanding the individual responses through a decision tree algorithm.

We observed a large discrepancy between inter- and intra-individual variation (i.e., nonergodicity), as SDs at the individual level were ~2.4 times larger than at the group level. This means there would be a difference when computing statistics by first averaging the data before the calculations versus first calculating the statistics for each individual before averaging these results [558]. Furthermore, mean values may be misleading when determining the influence of carbohydrate intake on AM PRS. At the group level, a traditional interpretation would suggest carbohydrate has minimal influence on AM PRS after accounting for the other variables. Although this would be true for most athletes (~87% of our participants), model coefficients were positive for 9% and negative for 7% of our participants (Fig. 9.2). This means the individual, rather than the group, should be placed at the level of analysis to avoid wrong conclusions [558].

Previous studies have used bivariate correlations to explore ergodicity [555, 557]. In this context, bivariate correlations could be misleading because athletes often increase carbohydrate intake on days with higher training loads [571]. Because of the multifactorial nature of day-to-day recovery, we created linear models to account for these additional factors while focusing the analysis on daily carbohydrate intake. Subjective muscle soreness and sleep index were included because they are two of the most important factors predicting AM PRS scores, as reported by us [572] and others [563, 564, 581]. Training strain was included in the model to account for potential residual fatigue from the previous seven days of training. Training strain (the product of training load and training monotony) is high when high training loads are combined with low variability of load, and low when athletes complete either low training loads or have regular variation in training [538]. Together, these variables account for a substantial amount of the variance in PRS scores and allow a more focused look at the influence of carbohydrate intake.

The beliefs and practices surrounding nutrition and training vary widely among athletes [21, 22]. Although it could be tempting to try and find unifying answers to some of the contrasting beliefs held by athletes (e.g., the positive or negative influence of fasted-state training or increasing carbohydrate intake), the current study underscores the idea that what’s best for one athlete may not be best for another. It is also noteworthy that athletes in this study were undertaking self-selected training programs, and results cannot be generalized to short-term periods of intensified training, where increasing energy and/or carbohydrate intake has been shown to attenuate symptoms of overreaching [566-569].

As a way of translating the interindividual variability from a statistical concept to practical application, a decision tree model was created. Variables such as age, sex, BMI, competitive level, training volume, and habitual dietary patterns were included to better understand what traits or qualities might be related to a certain response to carbohydrate intake. Although interpretation of the decision tree is challenged by the small number of athletes presenting significant model coefficients for carbohydrate intake and the inability of the model to accurately predict positive coefficients (Fig. 9.5), it can serve as a starting point for understanding how an athlete might be expected to respond to carbohydrate intake. The most important variables were carbohydrate monotony, followed by average daily protein intake. Among athletes with low carbohydrate monotony scores (i.e., larger daily variations in carbohydrate intake), those with a lower average daily protein intake were likely to have a negative response to carbohydrate intake whereas those with a higher daily protein intake were more likely to have a non-significant effect of carbohydrate intake on AM PRS score (Fig. 9.4). Athletes with a higher daily fat intake and those training less than 12 h per week were also less likely to be influenced by changes in daily carbohydrate intake (Fig. 9.4). The model displayed moderate accuracy (Kappa value of 0.52), although the ability of the model to learn from the data was challenged by the small and imbalanced data set. As shown in Figure 9.5, negative and non-significant outcomes were able to be predicted very well, but the model did not accurately predict any positive responders. Nevertheless, we feel this approach can be adopted by others who wish to better understand individual responses to a given intervention or stimulus.

There are several limitations to this study, primarily related to the use of self-report measures. Data integrity was checked based on the number of missing values, and by looking for unexpected values. However, it is possible that participants did not always enter data as accurately as possible. There is also the risk of bias in reporting if an athlete is aware that a coach or a researcher will be seeing their data, answering based on what they think is desirable. In addition, the limited number of data points, particularly with unbalanced classes, made training and interpreting the decision tree model challenging.

9.6 Conclusion

Our findings suggest the influence of dietary carbohydrate intake on daily recovery differs at the group and individual level. Therefore, inferences may not be generalized from the group to the individual, and practical recommendations should be based on individual analysis. Furthermore, at the group level, the previous day's carbohydrate intake did not influence the perceived recovery status of athlete training ~12 h per week. This research also adds to the literature around ergodicity in sports science, an emerging concept that should be routinely considered as part of the statistical analysis process. Future research in athletes should focus on individual responses to better understand the relationship between nutrition, training, and recovery for each athlete.

10. Discussion and Conclusion

This chapter provides a summary of the key findings of the thesis discussed in the context of the aims outlined in Chapter 1, and as an addition to the pre-existing literature, separated by the three main themes (stated beliefs and practices of athletes, scientific validity of common beliefs reported by athletes, and actual pre-exercise nutrition practices of athletes, Fig. 1.2). This is followed by practical applications, limitations, recommendations for future research, and an overall conclusion.

10.1 Discussion

This thesis provides a unique contribution to the field by filling several gaps in the understanding of the diet-training relationship in endurance athletes. By conducting a survey of a large sample of athletes along with a combination of observational, acute crossover, and modeling studies, this thesis offers a more comprehensive understanding of the beliefs and practices of athletes and the impact of their pre-exercise nutrition choices on performance, fat oxidation, hunger, and molecular signaling. It also offers novel insights into the relationship between dietary carbohydrate intake and training loads and provides evidence to support or refute commonly held beliefs about the role of pre-exercise nutrition in endurance training and performance.

Key findings from this thesis include:

1. The prevalence of fasted-state training in a large sample of non-professional endurance athletes (63%), which was previously unknown (Chapter 3)
2. The pre-exercise meal has minimal influence on work capacity, perceived exertion, oxidative stress, hunger, or AMPK activation during a 1-h mixed-intensity exercise session (Chapters 5–6)
3. Most of what dictates substrate oxidation during exercise cannot be easily controlled by athletes on a daily basis (Chapter 7)
4. Daily carbohydrate intake has minimal influence on perceived recovery status in the context of a self-selected diet and training program (Chapter 9)

The overarching aim of this thesis was to better understand the pre-exercise nutrition beliefs and practices of endurance athletes by investigating: (i) stated beliefs and practices of athletes, (ii) scientific validity of common beliefs reported by athletes, and (iii) actual pre-exercise nutrition practices of athletes. The following sections will reflect on each of these aspects, putting the thesis in context with the literature in the field.

10.1.1 Stated Beliefs and Practices of Athletes

The first section of the thesis contributes to the fields by examining the stated beliefs and practices of athletes relating to pre-exercise nutrition. Prior to this work, limited data were available describing what athletes eat before training or how they view the role of nutrition, most of which was in the context of small cohorts of elite athletes [20, 24, 27]. Others have reported the dietary intake and practices of endurance athletes but have focused primarily on macronutrient intake and meal frequency [183-186], leaving a gap in the understanding of dietary intake specifically in relation to daily exercise sessions.

To determine the self-reported beliefs and practices relating to pre-exercise nutrition intake among endurance athletes of varying ages and competitive levels, an anonymous online survey was developed and circulated internationally via social media and email (Appendix A). To optimize the number and diversity of respondents, the survey was designed to be succinct (~10 min to complete) and open to a wide range of athletes (anyone ≥18 years old, who had been training at least 1 year, regularly completing at least four exercise sessions per week, and had participated in at least one organized endurance event of any distance at any time). This resulted in 1,950 athletes from 57 countries completing the survey. There was a balance of male (49%) and female (51%) athletes, but an uneven distribution of competitive levels that was skewed towards recreational athletes who either train but don't race or enter age-group races but don't expect to win (61.3% of respondents). The remaining athletes qualify and compete at the national level (27.3%) or international level (9.6%) as age-group athletes or were professional athletes (1.7%). This work was conducted prior to the participant classification framework proposed by McKay et al. [534]. Classification of age-group competitors within that framework remains challenging, but when considered relative to their respective age-group world ranking/personal bests the levels can be estimated as tiers 2/3/4 for amateur, high-level amateur, and elite non-professionals, respectively [534]. Although data were stratified by sex and competitive level, results from this survey are likely to be most applicable to non-professional athletes.

In the survey, 63% of endurance athletes reported the use of fasted-state training. This finding is important because it was previously unknown how common fasted-state training was among non-elite endurance athletes. The top reasons for training in the fasted state were related to increasing fat utilization, gut comfort, and convenience, whereas the top reasons for avoiding fasted training were not feeling like it helps their training, feeling bad and not performing well, and getting too hungry during exercise.

A striking observation was the discordant beliefs surrounding the use of fasted training, based on a series of questions using a Likert scale. For example, 43% agreed and 23% disagreed with the statement “Skipping breakfast will allow me to burn more fat during my workout”, something that is well established to be true [11]. In addition, 26% agreed and 51% disagreed with the statement “The quality of my workout is the same whether I eat or do not eat beforehand”. In hindsight, providing context on the type or duration of workout would have improved the question, as exercise performance is generally improved by the pre-exercise feeding for longer (> 60 min) but not shorter-duration exercise [10], a finding echoed in Chapter 5 of this thesis.

Beyond fasted training, the survey also revealed less than half of athletes vary their pre-exercise nutrition choices based on workout duration, workout intensity, or mode of workout. This is relevant because it suggests many athletes may not be following best-practice guidelines of providing high carbohydrate availability when it is important to exercise with high quality and/or at high intensity [180]. In addition, very few athletes reported ever consuming a low-carbohydrate meal prior to training, despite research suggesting potential utility for athletes who don’t want to train in a fasted state but still want to retain some of the physiological qualities of fasted-state training [16, 17]. It was also found that nearly all factors measured relating to pre-exercise nutrition intake vary by sex, competitive, level, and/or habitual dietary pattern.

Taken together, the survey provides important information on the beliefs and practices of a large group of endurance athletes, while also highlighting several areas of conflicting beliefs. This emphasized the need for research examining the effects of pre-exercise nutrition choices on workout quality, fat oxidation, hunger, and molecular signaling, which are explored in the second section of the thesis.

10.1.2 Scientific Validity of Common Beliefs Reported by Athletes

To further interrogate the reported beliefs of athletes, the thesis includes an acute crossover study and two modeling analyses. In the crossover study (Chapter 5), trained cyclists completed a mixed-intensity training session following each of three pre-exercise nutrition options: a carbohydrate-rich breakfast, a protein-rich breakfast, and fasting. This study tested several assertions commonly made by athletes, including that the workout will feel harder, they would feel hungrier, and they wouldn't perform as well in the fasted state, as well as exploring the influence of a pre-exercise protein-rich (low-carbohydrate) meal on these factors. The two modeling studies (Chapters 6 and 7) use existing literature to explore and make inferences about the role of pre-exercise nutrition on AMP-activate protein kinase (AMPK) signaling and substrate oxidation during exercise.

10.1.2.1 Rating of Perceived Exertion

To examine how three different pre-exercise nutrition choices impacted the perception of exercise, rating of perceived exertion (RPE) was collected at multiple time points during and after an acute exercise bout (Chapter 5). Participants performed 20 minutes of submaximal cycling followed by 6 × 3 min high-intensity intervals. No differences in RPE were observed at any time point during exercise. Overall session RPE (sRPE) was not different between trials ($p = 0.076$), but there was a trend for carbohydrate (7.9 [95%CI 7.5, 8.2]) to be lower than fasted (8.3 [95% CI 7.9, 8.5], $p = 0.101$), suggesting the potential for reduced sRPE that may become apparent with greater sample sizes or a different exercise structure. Others have also reported no difference in RPE during short-duration (~25 min) [143], or longer-duration (90 min) [140, 589] interval training in the fasted or fed state. In contrast, a lower RPE was reported during a 90-min intermittent running protocol following ingestion of a low glycemic index meal compared to placebo, although there were no differences between low- and high-glycemic meals [140]. A lower RPE has also been reported during ~45 min of variable intensity exercise following a high-carbohydrate meal compared to both fasted and low-carbohydrate meals [145], although muscle glycogen levels were likely higher in the high-carbohydrate trial. In sum, RPE may be modestly affected by pre-exercise feeding status in some contexts, but future research and/or meta-regression is needed for a better understanding of who might be affected and in what context.

10.1.2.2 Performance and Exercise Capacity

As shown in Chapter 3, many athletes believe that fasting will decrease the quality of their training session, although this is most likely to occur only as the exercise duration extends beyond ~ 1 h [10]. It is possible that the belief of diminished work capacity during fasted-state training comes from observations of decreased power during interval training performed with reduced muscle glycogen concentrations [163, 219]. However, exercise performed in the overnight-fasted state is undertaken with reduced hepatic but not muscle glycogen [8], and so performance differences between fed- and fasted-state exercise would not be expected to appear until liver glycogen is depleted. Accordingly, work capacity during interval training sessions lasting less than 1 h has been similar in the fed or fasted state in trained (Chapter 5) and untrained [143] participants, as well as in the context of repeated 6–15 s sprint cycling intervals [141, 590]. In contrast, others have shown benefit of pre-exercise carbohydrate ingestion on exercise capacity tests lasting ~5–10 min, with results dependent on the size and timing of the meal. For example, improved performance was observed when consuming 32 g of carbohydrate 30-min, but not 120-min, before exercise [144], and when consuming 250 g, but not 42 g of carbohydrate, 3.5 h before exercise [145]. However, in the latter study there were likely differences in starting muscle glycogen as it has been previously shown that consuming 175 g of carbohydrate increased muscle glycogen by 33 mmol kg dry weight in three hours [359]. Taken together, performance impairments would not be expected based on feeding status during exercise lasting less than ~ 1 h, with the possible exception of short-duration, non-intermittent high-intensity exercise and/or when muscle glycogen concentrations are not equivalent.

10.1.2.3 Substrate Oxidation

It is well established that exercising in the fasted state allows higher levels of fat oxidation than exercise performed in the carbohydrate-fed state during low-to-moderate intensity exercise [11]. This was confirmed in Chapters 5 and 7, and it was also shown in Chapter 5 that pre-exercise protein ingestion resulted in fat oxidation similar to what was observed in the fasted state. Pre-exercise protein ingestion has been studied considerably less than pre-exercise carbohydrate, but others have also found a metabolic response from protein ingestion that more closely resembles the overnight-fasted state than the carbohydrate-fed state [16, 17, 136, 237]. This is relevant for the many athletes who cite increased fat oxidation as a primary reason for performing fasted-state training, who now have an alternative to fasted training that can also help reduce the risk of low energy availability [481]. However, this approach is currently utilized by only a small number of athletes. As reported in Chapter 4, only 27% of athletes reported ever consume low-carbohydrate food/drink before exercise.

Many factors beyond the pre-exercise meal can influence fuel selection and substrate oxidation during exercise, including the exercise duration and intensity, muscle glycogen concentrations, carbohydrate ingestion during exercise, daily macronutrient intake, and others (Table 7.1). When all else is held constant, the influence of each of these factors is not controversial (e.g., the respiratory exchange ratio (RER) goes up with carbohydrate ingestion and exercise intensity, and down with exercise duration). However, all else is rarely held constant with athletes living and training in a dynamic environment that bears little resemblance to a controlled laboratory setting. In attempt to untangle the web of factors influencing substrate oxidation during exercise, a modeling analysis was performed on data extracted from 400+ studies (Chapter 7). The key findings were that exercise duration and intensity, age, sex, fitness level, muscle glycogen, and daily dietary intake together explained only ~60% of the variation in RER during exercise, indicating a large influence of additional factors, and that daily dietary intake has a larger influence on RER than carbohydrate ingested during exercise. It was also found that the factors which can easily be modified on a daily basis by athletes (e.g., dietary intake, exercise intensity/duration) could only explain 36% of the variation in RER. This suggests many athletes trying to micro-manage their fuel selection during training sessions might be doing so without the desired effect. To better translate these findings, an online dashboard was created that allows users to simultaneously modulate all parameters to see the influence on predicted RER values (https://rothschild.shinyapps.io/RER_dashboard/).

The strong influence of daily dietary fat intake on substrate oxidation could be related to the ability of high-fat diets to decrease the amount of the active form of pyruvate dehydrogenase (PDH) [445], which is the rate-limiting enzyme in carbohydrate metabolism, and/or increase gene expression and the abundance of proteins involved in fatty-acid transport such as cluster of differentiation (CD) 36 [448] and fatty acid transport proteins 1 and 4 [591]. Recent research from our lab [592] has revealed these and other proteins involved in fatty-acid transport can explain a substantial degree of the variation (~61–87%) in fat oxidation rates during exercise.

10.1.2.4 Skeletal Muscle Signaling

Some athletes avoid carbohydrate ingestion before or during exercise out of fear of attenuating the training response. This belief is likely related to the increasing awareness around the use of carbohydrate periodization as a potential method for augmenting the signaling response to exercise [2]. However, strategies undertaken to reduce muscle vs. liver glycogen content may often be conflated. To investigate the influence of pre-exercise carbohydrate intake on muscle signaling, consideration of the multiple pathways influencing skeletal muscle adaptations to endurance training is needed. Among the key signals are changes in cellular energy status (i.e., AMP:ATP ratio), contraction-induced changes in mechanical strain, increased calcium flux, increased reactive oxygen species, and the availability of endogenous carbohydrate and free fatty acids (FFA) [39, 40, 94].

Reactive oxygen species play a direct role in regulating the response to acute exercise and are critical for longer-term exercise training adaptations [128, 129, 215]. It is conceivable that the oxidative stress response to exercise may be influenced by food consumed prior to the exercise session, but there is a paucity of data. With this in mind, F_2-Isoprostanes were measured in the acute study (Chapter 5), which are one of the preferred markers for the detection of organism-wide oxidative stress [250]. However, no effect of nutrition or exercise on levels of F_2-Isoprostanes was found. It was unexpected to not find an increase in F_2-Isoprostanes following exercise as most, but not all, studies have reported exercise-induced increases [250]. This result could be due to the well-trained status of the participants [252], the short-duration exercise protocol (~ 60 min), or the high interindividual variability in the response [251]. Future studies could investigate other measures of exercise-induced oxidative stress which might be sensitive to pre-exercise nutrition intake.

In addition to oxidative stress signaling, the calcium-dependent and contraction-induced signaling pathways also appear unlikely to be significantly influenced by pre-exercise nutrition intake or muscle glycogen concentrations [68, 105, 112, 113, 375]. Therefore, the energy-sensing and substrate-sensing pathways are the most likely ways in which pre-exercise nutrition could influence the training response.

Changes in the AMP:ATP ratio activate the energy-sensing AMPK, with repeated AMPK activation leading to a range of beneficial adaptations associated with endurance training including increases in glucose uptake, glycolytic flux, fat oxidation, and mitochondrial biogenesis [96]. Like substrate oxidation, many factors influence AMPK activation during exercise including exercise intensity, fitness level, muscle glycogen content, and pre-exercise carbohydrate intake. The modeling analysis in Chapter 6 suggests carbohydrate intake in the 4 h prior to exercise is unlikely to negatively influence AMPK activation after accounting for other important variables such as intensity, duration, and muscle glycogen content, among others.

The FFA signaling pathways regulate transcription of genes encoding proteins involved in muscle lipid metabolism [118], and are among the most likely pathways to be affected by pre-exercise nutrition choices. It is thought that this could occur via direct or indirect targeting of peroxisome proliferator-activated receptors by fatty acid ligands, by fatty acid-induced NAD^+-stimulated activation of sirtuin 1 and/or fatty acid-mediated activation of AMPK [118]. Studies showing benefits from fasted-state training compared with training in the carbohydrate-fed state, particularly relating to fat oxidation capacity, often provided a very large amount of carbohydrate before (e.g., 120–160 g) and during (1 g/kg) training sessions [43, 45, 157], which would be expected to drastically attenuate FFA levels during exercise [65]. Further support for the importance of FFA signaling can be found in studies of twice-daily training. Although differences in muscle glycogen levels are often the primary aspect of differentiation between groups training once vs. twice daily (i.e., the second session being performed with reduced glycogen levels), several studies reporting improvements in fat oxidation and mitochondrial markers from twice-daily training limited participants to only water ingestion between the sessions [9, 12, 163]. This is relevant because FFA are increased during exercise and increased even further if no food is ingested in the hours following exercise [118]. Therefore, the second session would be commenced not only with reduced muscle glycogen but also with elevated FFA. Moreover, a study comparing once vs. twice daily training with both groups having low glycogen for the second session found an increase in genes involved in fat oxidation to be increased only in the twice-daily training group [172], highlighting the potential importance of training in close proximity (1–2 h apart) with elevated FFA. Future research should attempt to separate the influence of elevated FFA and reduced muscle glycogen in the context of twice-daily training.

10.1.2.5 Hunger and Gut Comfort

Many athletes perform fasted-state training to improve gut comfort during exercise, yet one of the most common reasons for avoiding training in the fasted state is related to hunger (Chapter 3). To further investigate this area, subjective ratings of hunger and gut comfort were obtained using a visual analog scale before and after an acute bout of exercise following a carbohydrate-rich meal, a protein-rich meal, and exercise in the fasted state (Chapter 5). In all conditions, hunger decreased following exercise. The reduction in hunger is likely related to the high blood lactate accumulation achieved during the interval exercise, which is thought to play a role in exercise-induced appetite suppression [244]. In line with this, other research has found sprint interval exercise reduced appetite during exercise more than continuous endurance exercise [245]. However, in the context of moderate-intensity exercise, a carbohydrate-rich pre-exercise meal reduced hunger compared with fasted-state exercise [246]. Therefore, the belief held by some athletes that they may get too hungry during fasted-state training is more likely applicable to lower-intensity steady-state training, rather than high-intensity interval training, at least in the context of exercise lasting ≤ 1 h.

Protein has been shown to increase the risk of gut discomfort during exercise in some [247, 248], but not all [16], studies. In Chapter 5, gut discomfort was low before exercise (~12 out of 100) and increased after exercise only following ingestion of the protein-rich breakfast. However, the increase in gut discomfort was modest (mean post-exercise values ~21 out of 100), and there was a large inter-individual variation. This suggests pre-exercise protein ingestion can be an effective strategy for those who would prefer to eat before exercise while maintaining higher levels of fat oxidation, and those with high training volumes who struggle to achieve adequate energy availability, but tolerance should be considered on an individual basis. Future studies should measure indices of gut integrity and damage (e.g., intestinal fatty acid-binding protein, lipopolysaccharide-binding protein, and soluble CD 14), in addition to relying on self-reported of gut comfort and hunger.

10.1.3 Actual Pre-exercise Nutrition Practices of Athletes

This thesis provides novel insight into the day-to-day nutrition practices of endurance athletes in real-world training environments, particularly in relation to their daily training load. Prior to this thesis, knowledge of athlete nutrition practices in the context of the diet-training relationship has largely been limited to surveys [19, 20], case studies [24], or short-duration (7 d) observations of elite athletes [25, 27]. Knowledge of diet-training practices of non-elite athletes as well as in sports other than running has been limited, and there has been a lack of studies describing longer time periods during real-world, day-to-day training. This is likely related to several challenges including athlete adherence to longer-term data collection, difficulty quantifying training loads (particularly when athletes train in multiple exercise modalities), and the lack of any objective method to quantify the relationship between training and dietary carbohydrate intake.

The observation that many athletes and coaches regularly track dietary intake, training metrics, heart rate variability (HRV), sleep, and subjective wellbeing measures gave rise to the idea of utilizing this type of data in a field-based observational research project. Recruiting participants who were already tracking this data on their own volition could make it feasible to collect data across longer time periods by overcoming many of the limitations related to participant burden that occur when a large amount of self-report data collection is required. To this end, self-selected nutrition intake, exercise training, sleep habits, HRV, and subjective wellbeing was recorded by 55 endurance athletes daily for 12 weeks (Chapters 8–9). Analysis of this data led to the creation of a novel method to quantify the relationship between training load and dietary carbohydrate intake (Appendix J), a description of athlete nutrition practices with an emphasis on how carbohydrate intake is adjusted in relation to training (Chapter 8), and investigation of the influence of carbohydrate intake on perceived recovery status with an emphasis on nonergodicity (Chapter 9), a phenomenon in which group group-level findings cannot be applied at the individual level [555].

10.1.3.1 Daily Nutrition Intake of Endurance Athletes

Chapter 8 details the self-reported daily dietary intake of a large group of endurance athletes across a 12-week timespan. There was a large variation in dietary intake among participants, with average daily carbohydrate intake ranging from 1.2–7.2 g/kg, average daily protein intake ranging from 1.1–3.2 g/kg, and average daily fat intake ranging from 0.7–3.1 g/kg. These values are generally in line with other studies reporting 2–7 days of dietary intake in triathletes performing a similar volume of weekly training (Table 10.1).

Table 10.1. A comparison of reported dietary intakes of triathletes training 8–13 h per week. Values shown are mean ± SD. CHO: carbohydrate

Study	Subjects	Days tracking	Method	Training h week	Kcal per kg	CHO g/kg	Protein g/kg	Fat g/kg
Nogueira and Da Costa 2004 [213]	9 females	2	24-h recall	8.0 ± 4.2	42.4 ± 11.1	5.9 ± 2.6	1.6 ± 0.5	1.3 ± 0.4
Nogueira and Da Costa 2004 [213]	29 males	2	24-h recall	10.0 ± 4.7	51.7 ± 15.1	7.3 ± 2.9	2.0 ± 0.6	1.6 ± 0.7
Worme et al., 1990 [541]	50 males	3	Food diary	10.9	37.3 ± 1.6	5.1 ± 0.2	1.4 ± 0.2	1.2 ± 0.3
Worme et al., 1990 [541]	21 females	3	Food diary	12.4	36.5 ± 2.2	4.9 ± 0.3	1.4 ± 0.3	1.2 ± 0.2
Frentsos and Baer 1997 [542]	4 males 2 females	7	Food diary	11	33.6	5.0 ± 2.3	1.3 ± 0.4	0.8 ± 0.1
Rothschild thesis (Chapter 8)	34 males 21 females	82.3 ± 10.5	Smartphone food diary	11.3 ± 4.1	39.0 ± 9.1	4.1 ± 1.5	1.9 ± 0.4	1.6 ± 0.5

Perhaps most remarkably, 62.7% of athletes performed at least 15% of training sessions in the fasted state, a number essentially identical to the 62.9% of athletes reporting the use of fasted training in the survey (Chapter 3). A negative relationship was also observed between average daily carbohydrate intake and the frequency of training sessions performed in the fasted state, a finding that also echoes the survey results (Chapter 3). It is regretted that the online survey from Chapters 3–4 was not also given to the participants in the remote tracking study. This would have allowed a comparison between the stated beliefs and actual practices within the same group of endurance athletes.

Despite its limitations (discussed in Chapters 8–9, and section 10.3), self-report dietary data can provide valuable information about dietary patterns [554]. The decision to rely entirely on athlete self-report data was made to allow longer collection periods to be logistically feasible while also maximizing ecological validity, as this is the type of data collected by coaches and nutritionists. Nearly all previous studies reporting athlete intake during training have used time periods of 1–9 days. This could be problematic based on the findings in Chapter 8 that ~18–53 days of dietary tracking may be needed for an athlete's average energy and macronutrient intake to fall within 10% of their 12-wk average intake 95% of the time. This value was established using the approach of Basiotis et al. [531], who reported similar values of 27–71 days of tracking needed to accurately reflect the true average intake for an individual [531]. Therefore, it is possible that despite a greater degree of supervision in the dietary tracking process, prior studies might have been too short to adequately capture and describe athlete dietary practices, particularly in relation to their training volume.

10.1.3.2 Fueling for the Work Required

Sport nutrition guidelines recommend carbohydrate intake be modulated according to changes in exercise duration and intensity [180]. This has been referred to as "fueling for the work required" [2]. Put most simply, an athlete should eat more carbohydrate on harder/longer training days and less carbohydrate on easier/shorter training days. However, additional nuanced and strategic approaches to carbohydrate manipulation can also be used, such as twice-daily training without carbohydrate restoration between sessions [12], and an overnight sleep-low approach [165].

In the survey (Chapters 3–4), 10% of athletes identified as following a periodized carbohydrate dietary pattern, which was defined as habitually lower carbohydrate and then increase before key training or habitually higher carbohydrate and then restrict before key training sessions. A survey of elite runners and race walkers by Heikura et al. (2018) found 22% of athletes reported following a periodized-carbohydrate diet [20], a number consistent with the 26.5% of professional athletes who reported following that dietary approach in the survey from Chapters 3–4.

The observational study (Chapter 8) offered a unique opportunity to observe how athletes are adjusting both their pre-exercise and total daily carbohydrate intake based on their training duration and intensity. At the group level, there were small to moderate positive relationships between daily carbohydrate intake and training load (calculated as sRPE * duration, $r = 0.35$), exercise duration (r = 0.36), and exercise intensity (r = 0.17), and between pre-exercise carbohydrate intake and training load (r = 0.22), exercise duration (r = 0.22), and exercise intensity (r = 0.14). This suggests that overall, athletes are making small to moderate adjustments to their intake based more upon the exercise duration rather than intensity. In contrast, there was no relationship between carbohydrate intake the day before (r = 0.01) or after (r = -0.02) a given training load, suggesting athletes are not planning ahead with, or catching up on, their carbohydrate intake the day before or after a given training load. Furthermore, although there was a positive relationship at the group level, examination at the participant level revealed a very wide range of correlations between carbohydrate intake and training load (-0.34 to 0.87). Few other studies have reported correlations between self-selected carbohydrate intake and training load, but a correlation of 0.60 was reported between daily carbohydrate intake and exercise energy expenditure in a 7-d study of elite cyclists [530].

Data from Chapter 8 also showed that at the individual level, 39% of athletes displayed significantly higher pre-exercise carbohydrate intake before long vs. short sessions (defined as longer or shorter than 90 min), and 37% of athletes had significantly higher pre-exercise carbohydrate intake before hard vs. easy sessions (defined as an sRPE above or below the midway point of each individual's highest and lowest sRPE recorded during the study period). These findings are fairly consistent with survey responses in Chapter 4, where fewer than half of respondents reported eating differently prior to a training depending on the duration (48%) or intensity (39%) of the upcoming session, and in surveys of elite runners and race walkers where 51–53% of athletes reported eating more carbohydrate-rich foods before key training sessions [19, 20]. Considered together, this thesis provides additional data building upon recently published literature [19, 20, 25] that suggests many athletes are not following dietary strategies that modulate carbohydrate intake based on the volume or intensity of their training.

Reasons why more athletes are not following contemporary periodization strategies could include lack of knowledge, or a lack of understanding by athletes of how to implement such practices. Indeed, it has been reported that despite awareness of dietary periodization strategies by elite athletes, only modest evidence was found of its practice [25]. There also has not been an objective method of quantifying the dietary periodization, which means athletes and practitioners have no way of knowing "how much" of it they are or should be doing compared with others.

10.1.3.3 Carbohydrate Intake and Daily Recovery

During short-term periods of intensified endurance training, increasing energy and carbohydrate intake may attenuate symptoms of overreaching [566-569]. However, less is known about the relationship between carbohydrate intake and daily recovery over longer time periods and across a range of training volumes. Chapter 9 used a novel approach to investigate the relationship between daily carbohydrate intake and perceived recovery status the following day. Rather than simply looking at correlations between carbohydrate intake and recovery scores, which would not account for important factors such as training load, multivariable regression models were used to account for several of the key moderating factors. To my knowledge, this is the first time such an approach has been used in this context, building upon analytical methods used in psychology and adjacent areas of sports science [555, 557, 558].

Multivariable linear models were constructed with AM perceived recovery status (PRS) score specified as the dependent variable, and prior day carbohydrate intake (g/kg), prior day training load, training strain (encompassing the previous 7 days), muscle soreness, and sleep index (subjective sleep quality * duration) specified as independent variables. These variables were chosen because they had the highest importance scores in the predictive modeling study using the same data (Appendix I). The model coefficient for carbohydrate intake was the primary variable of interest. Prior day energy intake was considered for inclusion in the modeling but was deemed unnecessary because of the strong correlation between daily energy and carbohydrate intake ($r = 0.76$), which increases the subsequent risks of multicollinearity. After accounting for the other factors, there was no significant effect of prior-day carbohydrate on AM PRS at the group level. However, investigation at the individual level revealed a mix of positive, negative, and non-significant model coefficients for carbohydrate intake. This suggested the potential for nonergodicity, a phenomenon in which group-level findings cannot be applied to the individual level [555].

It is commonly assumed that the average response of representative samples allow predictions about individual members of those samples [561]. However, non-ergodic data can overestimate the accuracy of aggregated statistical estimates and have major implications for how we understand the consistency between group and individual correlations [557]. Because of the potential for misleading results, researchers should check for nonergodicity when working with intensive repeated-measures data (also known as intensive longitudinal data), which refers to data that have been collected on many occasions across many individuals [557].

In the context of intensive repeated-measures data, a Cattell data box can be used to determine ergodicity [555, 593], as described in Chapter 9. This can be visualized as a 3-dimensional box with time, measured variables, and individual subjects as the dimensions (Figure 10.1). Group- and individual-level analyses are performed by “slicing” the data either horizontally or vertically. If the structure of the group- and individual-level data differ (e.g., statistics of central tendencies, variations, and/or correlations of time series data), the process is considered non-ergodic and results obtained from standard analysis at the group level cannot be applied to the individual [560]. An important note when applying this method is that the number of observations must equal the number of subjects to allow consistent statistical power at the group and individual levels. This approach is reminiscent, yet distinct from statistical approaches aimed at determining “responders” and “non-responders” to a given intervention [594].

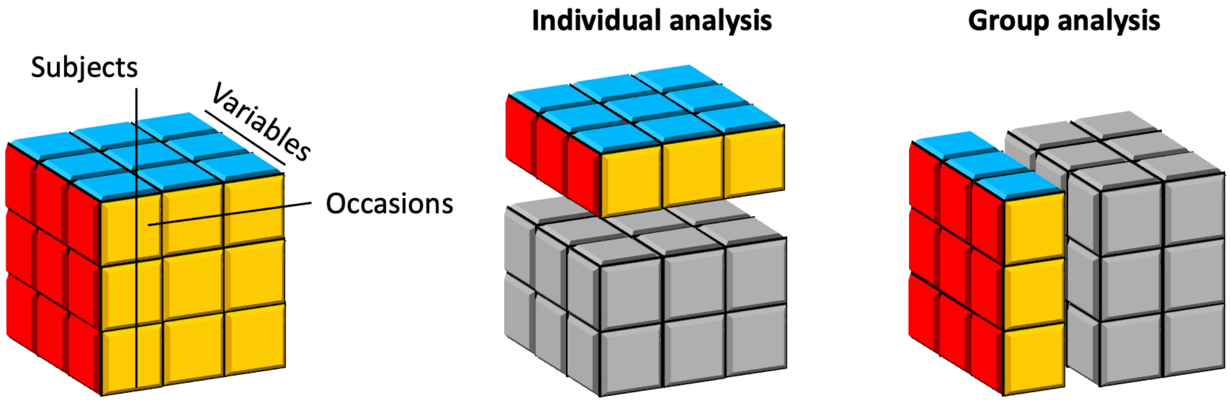

Figure 10.1. The Cattell data box, adapted from [555]. For individual-level analysis the variable of interest is analyzed across all time points separately for each subject (e.g., subject 1 on all days, subject 2 on all days, etc.) and then summarized. For group-level analysis, a single time point is pooled across all subjects and repeated for each additional time point (e.g., all subjects on day 1, all subjects on day 2, etc.), and summarized (e.g., mean, standard deviation, confidence intervals, etc.).

Mean values at the group and individual levels were both centered around zero, indicating no effect of prior-day carbohydrate on AM PRS scores. However, the standard deviations were significantly different at the group and individual levels, indicating a different distribution and data that are nonergodic and group results cannot be generalized to individuals. At the individual level, the model coefficients for ~20% of athletes indicated either a positive or negative relationship between carbohydrate intake and AM PRS score (Fig. 9.2). Only one other sport science study has thus far applied this method, also finding nonergodicity in the relationship between sRPE-training load and perceived recovery in team sport athletes [555].

From an applied perspective, a practitioner wants to know how their athlete responds rather than simply knowing there is a wide range of variation. To investigate if athletes with a given response (e.g., positive, negative, or non-significant) could be categorized or grouped in any way, a machine learning decision tree model was created (Fig. 9.4). Variables used to train the decision tree were age, training age, competitive level, primary sport, sex, BMI, percentage of training days performing fasted-state training, and average values of daily kcal intake (kcal/kg), daily carbohydrate, fat, and protein intake (g/kg), carbohydrate monotony (mean daily intake/SD), weekly training volume (h), training monotony, and training strain. The decision tree displayed moderate accuracy when tested using bootstrap resampling [595], and indicated the most important variable was carbohydrate monotony. For those that displayed higher monotony scores (i.e., had less variation in their daily carbohydrate intake), carbohydrate could be expected to have a positive influence on AM PRS score the following day. For those with lower monotony values, daily protein and fat intake were indicative of how someone would respond to carbohydrate (e.g., those with higher daily protein and fat intakes would not be influenced by carbohydrate, whereas those with higher protein and lower fat intakes would have a positive response to carbohydrate). Most importantly, perhaps, this approach offers a method for coaches and practitioners to dive deeper into their observed data and create actionable insights.

10.2 Practical Application

Each study in this thesis was designed with a focus on practical applications for endurance athletes, nutritionists, coaches, and researchers. Athletes striving to increase fat oxidation during exercise should focus on increasing exercise duration and/or daily fat intake, while reducing pre-exercise carbohydrate intake (Chapter 7). This is particularly relevant for long- and ultra-distance endurance athletes. Although carbohydrate intake during exercise is often minimized by athletes aiming to increase fat oxidation, this has a smaller influence than many other factors and is also unlikely to influence AMPK signaling occurring in response to exercise (Chapter 6). Therefore, carbohydrate intake during exercise could be a useful strategy to allow greater exercise durations to be performed without compromising some of the key molecular signals, particularly when exercise is undertaken with reduced muscle glycogen stores.

Activation of AMPK during exercise is most influenced by disrupting cellular energy homeostasis. This means that any type of high-intensity interval training (i.e., exercise performed above critical power/ maximal metabolic steady state) would be expected to activate AMPK irrespective of nutrition-related factors. Although it is often suggested that high-quality training should be performed in the fed state, exercise sessions lasting ~1 h can be performed in the overnight-fasted state without an expected decline in exercise capacity (Chapter 5). This could be particularly relevant for non-professional athletes who might schedule early-AM training sessions around work and family commitments. In contrast, for athletes performing longer-duration interval training sessions and/or very high training loads, the potential downsides of training in the overnight-fasted state may outweigh any potential benefit. Taken together, the duration and intensity of the exercise session should be considered when considering the best pre-exercise nutrition choices, along with the personal preferences of each individual athlete, as described in Figure 10.2.

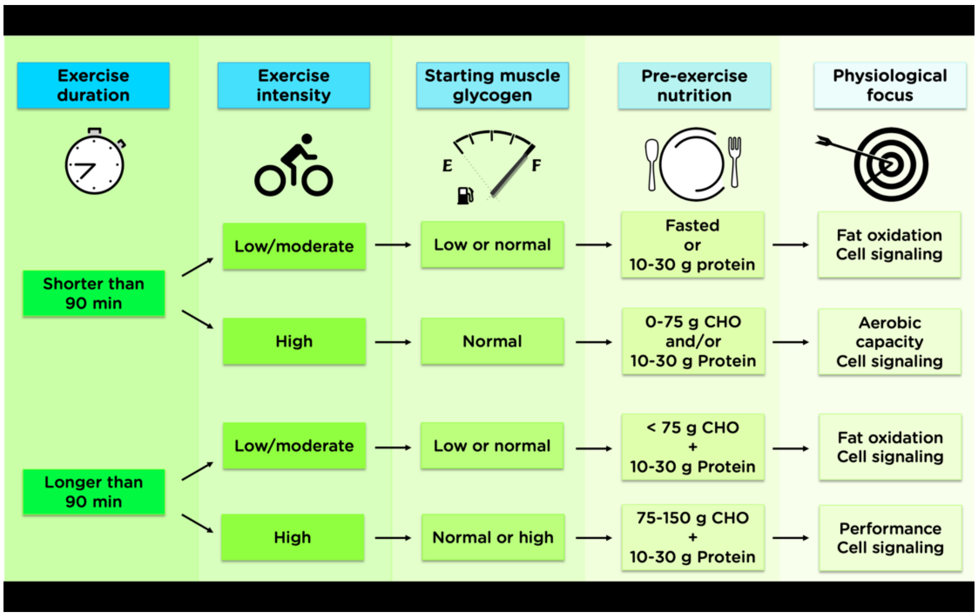

Figure 10.2. Practical application of pre-exercise nutrition to optimize training adaptations. The duration and intensity of the exercise session should be considered when considering the best pre-exercise nutrition choices. Before shorter duration exercise sessions that focus on lower intensity steady-state training, it may be beneficial to withhold carbohydrate (CHO), while there is little evidence supporting CHO restriction before high-intensity exercise. When consuming less than ~75 g CHO, food choices before HIIT can be left to personal preference. For longer duration exercise (>90 min), there is little evidence to suggest fasted-state training offers any additional benefit, although this is still practiced by approximately one-third of endurance athletes (Chapter 3). Ingesting less than ~75 g CHO is unlikely to impair mitochondrial signaling adaptations from longer-duration, low-intensity exercise, while consuming 75–150 g CHO prior to extended high-intensity exercise is suggested to increase endogenous fuel storage.

For nutritionists and coaches, it is important to acknowledge that pre-exercise nutrition beliefs and practices vary by sex, habitual dietary pattern, and competitive level, and recommendations should be considered accordingly (Chapters 3–4). When practitioners are working with athletes seeking to increase fat oxidation during exercise, Chapters 5 and 7 provide actionable information including highlighting the underutilized potential of pre-exercise protein ingestion, and the findings that carbohydrate intake during exercise might not be as detrimental to fat oxidation or molecular signaling as some might believe. It is also important, particularly for athletes competing in shorter-duration (< 3 h) endurance events, to consider strategies which prioritize carbohydrate oxidation over fat oxidation [596].

Chapter 5 also highlighted that short-duration (~ 1 h) interval training sessions commenced with normal muscle glycogen levels can be effectively performed in the overnight-fasted state without a decline in performance or increase in hunger. The descriptive approaches used in Chapter 8 can also be used by practitioners to visualize and analyze dietary intake from an individual athlete in relation to their training load to better understand the correlation between load and intake, how much the intake varies between days of high and low carbohydrate intake, and the day-to-day variability in the athlete's intake. For practitioners working in a team setting, the methods used in Chapter 9 can be used to distinguish individual vs. group responses to a given intervention or research question.

From a research perspective, this thesis adds to the emerging literature examining pre-exercise protein ingestion as a viable option for athletes, which should be followed-up with longer-term studies looking at the adaptive response compared with fasted-state training. This thesis also utilized a novel approach in creating a scalable method of collecting longer-term longitudinal data from athletes by combining smartphone apps and online training software (Chapters 8–9). This method could be used and/or built upon in future studies. This is particularly relevant based on the findings in Chapter 9 suggesting 18–53 days of dietary tracking may be needed for an athlete's average energy and macronutrient intake to fall within 10% of their 12-wk average intake 95% of the time. Additionally, the use of multivariable linear models in the investigation of nonergodicity in the data (Chapter 9) can easily be adapted for future studies capturing intensive longitudinal data.

10.3 Limitations

This section will focus on the overarching limitations of the thesis, as limitations of each study have been discussed in each chapter.

10.3.1 Performance Measures

The absence of data surrounding performance or training adaptations precludes the reader from knowing which pre-exercise nutrition strategy might be “best” for a given context. A 3-week training study was designed for this purpose and commenced but was paused due to Covid-19 restrictions before any conclusions could be drawn. Preliminary findings are reported in Appendix H.

10.3.2 Survey Limitations

The survey (Chapters 3–4) provided valuable insight into many of the practices and beliefs relating to the dietary intake of endurance athletes. However, it should be considered if the questions were biased such that fasted training was positioned as the desirable strategy or status quo. There were several questions about why the athlete might undertake fasted training, but the options around avoiding it focused on the absence of benefits, rather than allowing the athlete to state that they deliberately consumed carbohydrate for specific benefits (e.g., better fueling/performance, better energy availability, practice of intended competition strategies). It would have been more desirable for the questions around carbohydrate intake prior to training sessions to have provided opportunities for more in-depth interrogation.

10.3.3 Self-report Data

The biggest limitation for the remote monitoring study relates to the use of self-report measures. Training metrics were typically recorded via GPS-based cycling/running computers, but there was a large reliance on athletes to self-report subjective measures of wellbeing as well as objective measures like dietary intake. The accuracy of self-reported dietary intake can be impacted by several factors including the observation effect (people decrease food intake when asked to report their diet [597]), missing foods (hidden ingredients such as sauces, or simply forgetting to record something), poor estimation of portion sizes, inability to weigh/measure food when dining out, and/or not capturing enough days of dietary tracking to estimate true intake.

Participants were recruited who were already tracking the desired metrics routinely, to avoid any major additional burden and increase both compliance and accuracy of the dietary tracking. Although athletes have been shown to underreport dietary intake [547], nearly all previous studies have used short-duration food records rather than smartphone apps. Smartphone-based food diaries lead to better participant compliance compared with paper-based diaries [548], possibly related to features such as the ability to scan barcodes and save commonly-consumed foods. To my knowledge, the accuracy and reliability of dietary tracking in athletes habitually tracking their intake has yet to be studied, and in hindsight an evaluation of this would have been beneficial prior to the observational study. The remote food photography (snap-n-send) method could have been used alongside the app-based tracking, but this method does not appear to offer additional precision [598]. Importantly, any error measurement attained from days when subjects were sending pictures of their food is not likely to be applicable to other days when they were not sending pictures.

10.3.4 Training Load Measures and Carbohydrate Utilization

A large focus of Chapter 8 is on the relationship between training load and dietary carbohydrate intake. To accommodate athletes training across a variety of endurance and strength training modalities the sRPE-training load (the product of sRPE and exercise duration) measure was used. This provides a measure of internal training load that is considered valid and reliable for calculating training load across exercise modalities [538, 545]. However, it has yet to be established if the relationship between carbohydrate utilization and sRPE-training load (or between carbohydrate utilization and external load measures such as total work done) is consistent across exercise modalities and intensities. Limiting the study to cyclists training with a power meter would have allowed for calculation of external load measures as well an examination of dietary carbohydrate intake in relation to both internal and external load. In addition, a study examining the relationship between carbohydrate utilization and both internal and external load measures would have been valuable to perform prior to the remote monitoring study.

10.3.5 Actual vs. Reported Practices

Inclusion of the survey from Chapters 3–4 into each of the other studies (Chapters 5 and 8) would have allowed for better comparison of reported beliefs vs. observed practices.

10.4 Future research

This thesis has opened a number of additional questions relating to training adaptations, dietary tracking, and athlete monitoring. Each of these will be discussed in this section, with a summary provided in Table 10.2.

10.4.1 Training Adaptations

Findings from the survey (Chapters 3–4) highlighted the need to further investigate the influence of fasted training on training adaptations in a real-world training context. It was shown in Chapter 5 that pre-exercise protein ingestion allows similar levels of fat oxidation compared with fasted-state training, without influencing performance or RPE, but its influence on longer-term training adaptations, compared with carbohydrate ingestion and fasted training, are not well established. To mimic real-world application, future studies should use a mix of fasted and fed-state training, ideally isolating the intervention during only high-intensity training sessions (with intake before low-intensity sessions standardized), or the inverse. This is the approach taken in Appendix H, where the intervention is used only before low-intensity training with a standardized snack provided before high-intensity sessions. Although there are not enough participants to draw conclusions, the possibility of impaired adaptations in the carbohydrate group appears plausible (Figures H2, H3). The potential for sex differences in response to fasted training should also be investigated, particularly as fasted-state training is currently utilized more by males than females. Potential mechanisms of action, particularly relating to the role of FFA signaling, should also be investigated if beneficial training adaptations are observed from fasted-state or protein-fed training.

10.4.2 Dietary Tracking

As used in Chapters 8–9 of this thesis, modern technology has opened new opportunities for collecting data across longer time horizons outside of the controlled laboratory environment. To be valuable in research settings the data must be valid and reliable. It can be hypothesized that quality of dietary tracking using a smartphone app would be higher in people who track their intake routinely, but this has yet to be tested. Several aspects should be considered, including compliance (i.e., percentage of eating occasions recorded), accuracy (compared against known quantities of pre-measured meals, against freely chosen foods that can be weighed independently, and against doubly labeled water for daily energy intake), and reliability (how consistent are measurements relative to other measurements by the same person).

10.4.3 Training Monitoring

The relationship between training load and carbohydrate utilization across different exercise intensities, durations, and modalities has not been well characterized. It would be of interest to compare several measures of training load (e.g., sRPE-training load, total work done during cycling, etc.) during both steady-state and interval training sessions to determine how well these measures relate to carbohydrate use during exercise. Based on the findings from Chapter 7, it is likely that other information such as VO_{2max}, sex, exercise duration, mean power output, and power output and/or percentage of VO_{2max} at the second ventilatory threshold would dramatically increase the prediction accuracy of a regression equation predicting total carbohydrate use during exercise.

10.4.4 Acute Intake

Extending the observations from Chapter 5 of differences in gut comfort following different pre-training meals, further investigation of dietary intake on markers of gut function [599] is warranted. This could include a potential protective role of carbohydrate and investigation into the role of protein.

Table 10.2 Summary of future research directions.

Training Adaptations	Training Monitoring

• How does pre-training protein ingestion compare with carbohydrate and fasted state training influence performance improvements?
  o Training should include a mix of fasted and fed-state training, ideally isolating the intervention during only high-intensity training sessions (with intake before low-intensity sessions standardized), or the inverse.
• Sex differences in the response to fasted training
• How is the role of FFA signaling in endurance adaptations?
• Does fasted-state training increase fat oxidation when tested in a fed state?
• Sleep-low variations of AM training
  o Pre-training protein
  o During-training carbohydrate
  o Pre-training carbohydrate
	• The relationship between training load and carbohydrate utilization across different exercise intensities, durations, and modalities
	  o Compare several measures of training load (e.g., sRPE-training load, total work done during cycling, etc.) during both steady-state and interval training sessions to determine how well these measures relate to carbohydrate use during exercise.

Acute Intake	**Dietary Tracking**
• Does pre-training dietary intake influence markers of gut function (e.g., I-FABP, LBP, sCD14, gram-negative bacterial endotoxin)?	• Do habitual trackers display increased tracking accuracy, reliability, and/or adherence?

FFA: free fatty acid, I-FABP: intestinal fatty acid-binding protein, LBP: lipopolysaccharide-binding protein, sCD14: soluble cluster of differentiation, sRPE: session rating of perceived exertion

10.5 Conclusions

The majority (~63%) of endurance athletes perform exercise in the overnight fasted state, but beliefs and practices relating to pre-exercise nutrition differ based on sex, competitive level, and habitual dietary pattern. Generally, the pre-exercise meal has little effect on work capacity, perceived effort, oxidative stress, or hunger during exercise lasting ~1 h, but its importance increases as exercise duration extends. Exercise in the fasted state can increase fat oxidation and influence FFA-related signaling pathways, though it has little influence on AMPK activation. Finally, for most individuals, daily carbohydrate intake does not influence recovery status the following morning when accounting for training load, sleep, and muscle soreness. These findings contribute to the sport nutrition literature and can be used by nutritionists, coaches, and athletes to make better-informed pre-exercise fueling choices.

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Appendices

Appendix A: Survey used for Chapters 3–4

Q1 What is your age?

Q2 What is your sex?

- Male
- Female
- Prefer not to say

Q5 What is your country of current residence?

Q6 What are the primary sports you participate in? (select all that apply)

- Triathlon
- Running
- Cycling
- Swimming
- Rowing
- Cross-country skiing
- Other

Q7 What other sports?

Q8 What is the most typical duration of the competitive events you train for?

- < 10 min
- 10-30 min
- 30–60 min
- 1-3 hrs
- 3-12 hrs
- 12-24 hrs
- 24 hrs

Q63 How many races per year do you compete in?

- None
- 1-2
- 3-5
- 5-10
- >10

Q10 Which of the following best describes your competitive level?

- Professional
- Elite non-professional (qualify and compete at the International level)
- High-level amateur (qualify and compete at National Championship-level events)

- Amateur (enter races but don't expect to win
- Recreational (train but do not participate in competitions)

Q11 Number of years training? *[This refers to training at a level where weekly training volume has been at least 5 hours/week, including weight training and recovery treatments]*

- < 3 years
- 3-6 years
- 6-10 years
- >10 years

Q12 Do you consistently follow a specific dietary plan? (select all that apply)

- Vegan (avoid all animal products)
- Vegetarian (include some dairy or eggs)
- Pescatarian (vegetarian that also consume fish)
- Paleo (avoid grains, legumes, and dairy products)
- Paleo for athletes (Paleo but with more carbs around training sessions)
- High carbohydrate (>50% dietary intake)
- High-protein, low-carb (>2.2 g protein per kg body weight, less than 130 g carbohydrate)
- Low-carb, high-fat (LCHF; less than 130 g carbohydrate)
- Periodised Carb (habitually lower carb and then increase carbs before key training, OR habitually high carb and then restrict carbs before key training)
- Gluten-free
- Low-FODMAP
- Other:
- No, I do not follow any special dietary plan

Q71 Please enter your best performance times from any of the following competitive triathlon distances that you regularly train for and compete in (personal-best time over the past three years - please enter as hh:min)

- Olympic distance
- 70.3/ Half-Ironman
- 140.6/ Full-Ironman

Q72 Please enter your best performance times from any of the following competitive running distances that you regularly train for and compete in (personal-best time over the past three years)

- 1500 m (min : sec)
- 5 km (min : sec)
- 10 km (min : sec)

- Half-marathon (21.1 km/ 13.1 mi) (hh : min)
- Marathon (42.2 km/ 26.2 mi) (hh : min)
- Ultra-marathon (50 km) (hh : min)
- Ultra-marathon (50 mi) (hh : min)
- Ultra-marathon (100 km) (hh : min)
- Ultra-marathon (100 mi) (hh : min)

Q77 If you train using wattage, what is your functional threshold power (FTP; which can be calculated as 95% of your highest 20-min power) [if no, skip to next question]

- Watts
- Watts per kg

Q73 Please enter your best performance times from any of the following competitive swim distances that you regularly train for and compete in (personal-best time over the past three years)

- 400 m Freestyle (min : sec)
- 800 m Freestyle (min : sec)
- 1500 m Freestyle (min : sec)
- 5 km (Open water) (hh : min)
- 10 km (Open water) (hh : min)
- 25 km (Open water) (hh : min)

Typical Weekly Training - Please answer using your most typical training schedule, thinking of the past 12 months

Q14 How many hours per week do you currently train?

- Less than 7
- 7-9
- 10-15
- 16-20
- 21-30
- >30

Q15 How many exercise sessions per week do you typically perform?

- 3-4
- 5-8
- 9-12
- >12

Q16 How many exercise sessions per week do you start before 9 am?

- 0
- 1-2
- 3-4

- 5-7

Q18 How many exercise sessions per week do you start between 9 am and 3 pm?
- 0
- 1-2
- 3-4
- 5-7
- 8-12
- >12

Q17 How many exercise sessions per week do you start between 3 pm and 7 pm?
- 0
- 1-2
- 3-4
- 5-7
- >7

Q20 How many exercise sessions per week do you start after 7 pm?
- 0
- 1-2
- 3-4
- 5-7

Q21 Thinking of your training over the past 12 months, do you ever intentionally train in the fasted state (e.g. train first thing in the morning without having eaten any food – if you drink coffee-only, consider that as fasted-state training)?
- No, I do not train in the fasted state
- Yes, I complete some/all of my training sessions in the fasted state

Q68 For a morning workout, what dictates if you train in the fasted or fed state? (check all that apply)
- The length/duration of the workout
- The intensity of the workout
- How early the workout is
- How hungry I am
- Whether or not I have food available to eat
- Other:

Q31 Why do you avoid fasted training? (Check all that apply)
- I feel terrible during the training session and perform badly so it's not worth it
- I find I get sick or injured more easily
- I get too hungry during workouts

- I do not feel it helps my training overall
- Someone told me not to do it
- I'd never even heard of doing that
- I don't know
- Other

Q23 Before fasted training sessions, do you consume any type of caffeinated beverage?

- No, I drink water only
- Yes, I always/almost always have coffee (or other caffeinated beverage) prior to exercise (>75% of workouts)
- Yes, but not all the time

Q25 How often do you train in the AM-fasted state? (e.g. train first thing in the morning without having eaten any food – if you drink coffee-only, consider that as fasted-state training)

- Once in a while, but not every week
- 1-2x per week
- 3-4x per week
- 5-7x per week

Q26 Why do you train in the AM-fasted state? (Check all that apply)

- I find it helps my overall training
- It helps my ability to utilise fat as a fuel source
- I can't actually tell, but I believe it's supposed to help my overall training
- I like to train on an empty stomach because of gut comfort
- It means I can get up later and then go straight to training without wasting time on eating
- Weight loss
  Someone told me to do so
- Other:

Q28 If you have been told that training in the fasted state is good for you, where did you hear it from? (Check all that apply)

- Another athlete
- Coach
- Nutritionist Physiologist
- Social media/online
- Other:
- I haven't been told that

Q30 How long have you trained in a fasted state?

- Less than 1 year

- 1-3 years
- More than 3 years

Q60 If you do eat breakfast before a morning workout, does it vary based on the **duration** of the workout?

- Yes, I'll eat more before longer workouts and less before shorter workouts
- No, I eat pretty much the same thing before any workout
- I never eat before workouts so it doesn't matter to me

Q62 If you do eat breakfast before a morning workout, does it vary based on the **intensity** of the workout?

- Yes, I'll eat more before hard workouts and less before easy workouts
- Yes, I'll eat less before hard workouts and more before easy workouts
- No, I eat pretty much the same thing before any workout
- I never eat before workouts so it doesn't matter to me

Q61 If you do eat breakfast before a morning workout, does it vary based on the **type** of exercise you're doing? (e.g. swimming vs. running vs. cycling, etc.)

- Yes, I will eat more or less depending on which sport I'm doing
- No, I eat pretty much the same thing before any workout
- I never eat before workouts so it doesn't matter to me

Q31 How often do you consume carbohydrate-containing food or drinks (bread, bar, fruit, juice, etc.) prior to a morning exercise session?

- Never or almost never
- Some days but not always
- Before almost every workout

Q32 How often do you consume a low-carbohydrate breakfast prior to a morning exercise session? (e.g. eggs and avocado, or protein powder, *without* carbohydrates such as bread, cereal, or juice)

- Never or almost never
- Some days but not always
- Before almost every workout

Q33 How often do you consume caffeine (including coffee or other sources) prior to a morning exercise session, regardless of whether you are fasting or have eaten something?

- Never or almost never
- Some days but not always
- Before almost every workout

Please answer the following questions only in relation to workouts that are **shorter** than 90 minutes in duration

Q35 What is the minimum amount of time you would wake up before a morning workout that was less than 90 min in duration?

- Less than 15 min before
- 15-30 min before
- 30-60 min before\
- > 60 min before

Q36 Before a morning workout less than 90-min in duration, would you usually eat anything?

- Yes
- Maybe
- No

Q37 What would you eat?

Q58 Before a workout less than 90-min in duration, how far in advance would you eat?

- Less than 15 min before
- 15-30 min before
- 30-60 mins before
- 60 mins before

Q40 What would you eat if you do eat?

Please answer the following questions only in relation to workouts that are **longer** than 90 minutes in duration

Q46 What is the minimum amount of time you would wake up before a morning workout that was longer than 90 min in duration?

- Less than 15 min before
- 15-30 min before
- 30-60 min before
- 60 min before

Q47 Before a morning workout longer than 90-min in duration, would you usually eat anything?

- Yes
- Maybe
- No

Q48 What would you eat?

Q59 Before a workout longer than 90-min in duration, how far in advance would you eat?

- Less than 15 min before
- 15-30 min before
- 30-60 min before
- 60 mins before

Q51 What would you eat if you do eat?

Q50 There are no right or wrong answers in this section, we want to learn how people think about their nutrition as it relates to exercise training

Q51 If I have an early morning workout, I would rather not have a full stomach so I don't eat anything

- Strongly agree
- Somewhat agree
- Neither agree nor disagree
- Somewhat disagree
- Strongly disagree

Q52 If I have an early morning workout, I always eat something small beforehand like a banana, piece of toast, or a bar

- Strongly agree
- Somewhat agree
- Neither agree nor disagree
- Somewhat disagree
- Strongly disagree

Q53 If I have an early morning workout, I will always wake up early so that I have enough time to eat a full breakfast beforehand

- Strongly agree
- Somewhat agree
- Neither agree nor disagree
- Somewhat disagree
- Strongly disagree

Q54 The quality of my workout is the same whether I eat or do not eat beforehand

- Strongly agree
- Somewhat agree
- Neither agree nor disagree
- Somewhat disagree
- Strongly disagree

Q55 Skipping breakfast will allow me to get a better workout

- Strongly agree
- Somewhat agree
- Neither agree nor disagree
- Somewhat disagree
- Strongly disagree

Q56 Skipping breakfast will allow me to burn more fat during my workout

- Strongly agree
- Somewhat agree
- Neither agree nor disagree
- Somewhat disagree
- Strongly disagree

Q57 Please select any of the following dietary supplements that you take prior to training sessions? (within 1 hr before starting exercise)

- Coffee or tea
- Caffeine-containing pre-workout drinks, or caffeine pills/powder/gum
- Multi-vitamin
- Vitamin B12
- Vitamin C
- Vitamin E
- Fish oil/omega-3
- Creatine
- Sodium bicarbonate (baking soda)
- Beta-alanine
- Dietary nitrate (beetroot juice)
- Protein powder

Q78 Are there any other supplements that you take prior to training?

Appendix B: Supplemental Figures from Chapter 4

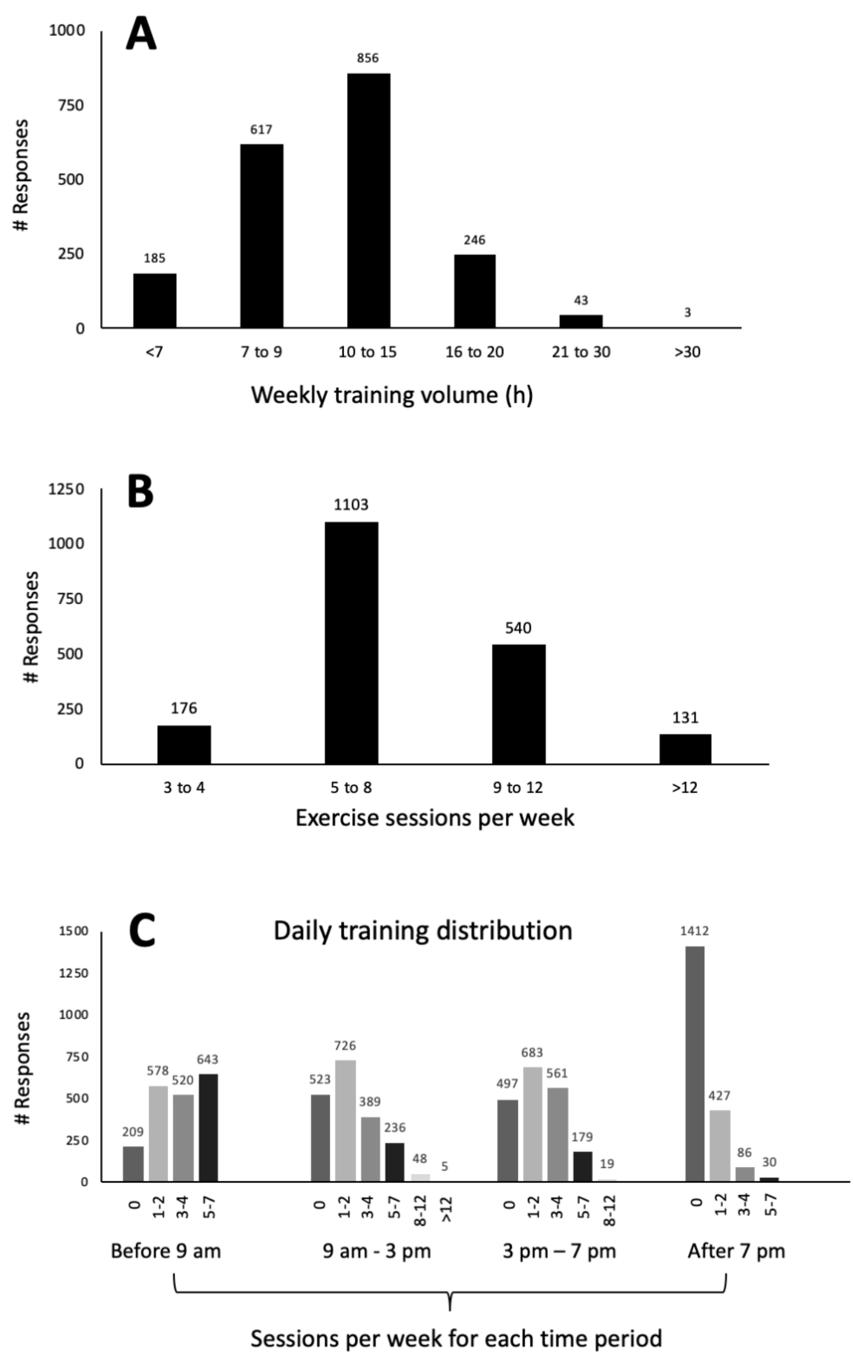


Figure S4.1. Weekly training hours per week (A), number of exercise sessions per week (B), and daily distribution of training sessions (C). n = 1,950.

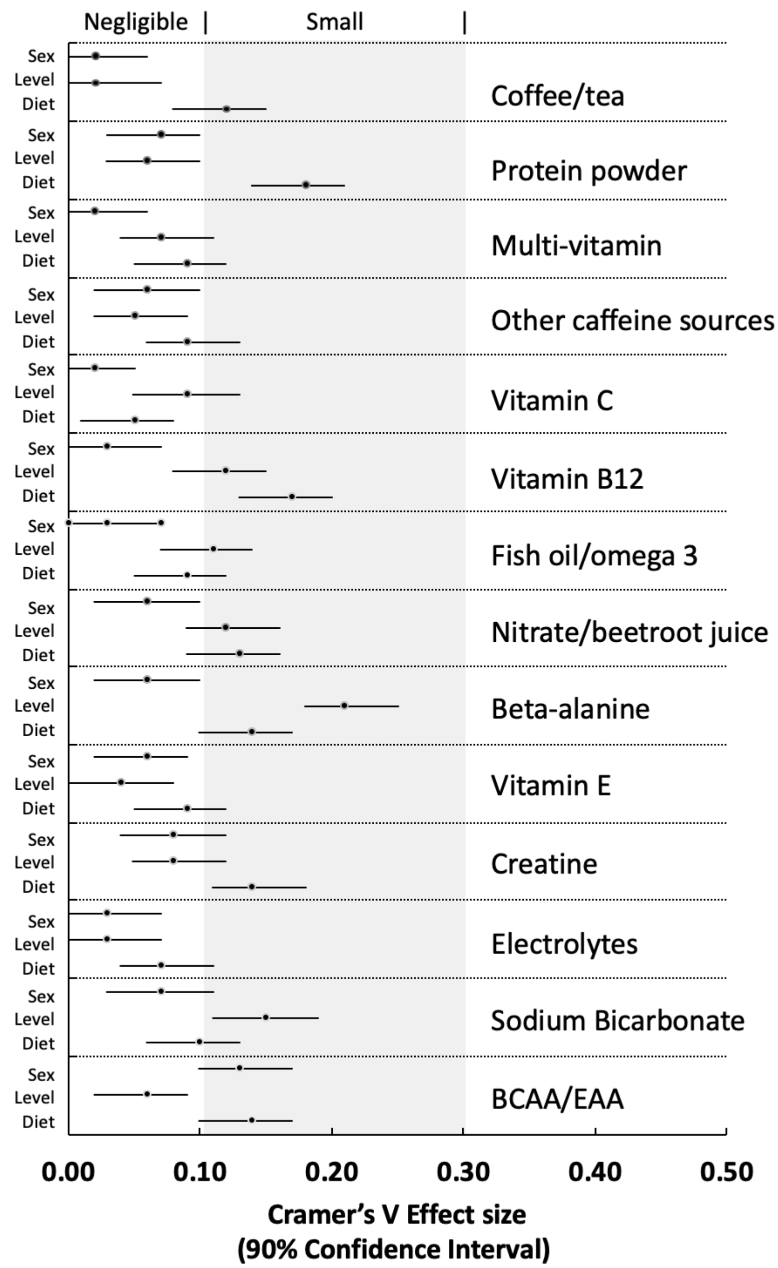


Figure S4.2. Sub-group interactions (as Cramer's V effect size with 90% Confidence Interval) for use of dietary supplements within 1 h before exercise. Shaded area represents a "small" effect, to the left of each shaded area represents a "negligible" effect.

Appendix C: Supplemental Tables from Chapter 4

Table S4.1. Eating patterns before workouts shorter and longer than 90 min by sex, competitive level, and habitual dietary pattern

	Before a morning workout less than 90-min in duration, would you usually eat anything?			**Before a morning workout longer than 90-min in duration, would you usually eat anything?**		
	Yes	**No**	**Maybe**	**Yes**	**No**	**Maybe**
Total	**n = 662 (33.9%)**	**n = 779 (39.9%)**	**n = 509 (26.1%)**	**n = 1297 (66.5%)**	**n = 253 (13.0%)**	**n = 400 (20.5%)**
Sex						
Male	n = 268 (28.2%)	n = 433 (45.5%)	n = 251 (26.4%)	n = 546 (57.4%)	n = 171 (18.0%)	n = 235 (24.7%)
Female	n = 393 (39.5%)	n = 346 (34.8%)	n = 256 (25.7%)	n = 749 (75.3%)	n = 82 (8.2%)	n = 164 (16.5%)
	(X^2 (2, n = 1,947) = 32.5, p < 0.001, φc = 0.13 [90% CI 0.10, 0.17])			**(X^2 (2, n = 1,947) = 74.9, p < 0.001, φc = 0.20 [90% CI 0.16, 0.23])**		
Competitive level						
Amateur	n = 384 (32.1%)	n = 502 (42.0%)	n = 309 (25.9%)	n = 799 (66.9%)	n = 157 (13.1%)	n = 239 (20.0%)
High-level amateur	n = 191 (35.8%)	n = 199 (37.3%)	n = 143 (26.8%)	n = 356 (66.8%)	n = 68 (12.8%)	n = 109 (20.5%)
Elite non-professional	n = 69 (36.7%)	n = 73 (38.8%)	n = 46 (24.5%)	n = 116 (61.7%)	n = 28 (14.9%)	n = 44 (23.4%)
Professional	n = 18 (52.9%)	n = 5 (14.7%)	n = 11 (32.4%)	n = 26 (76.5%)	n = 0 (0.0%)	n = 8 (23.5%)
	(X^2 (6, n = 1,950) = 14.2, p = 0.027, φc = 0.06 [90% CI 0.04, 0.09]) **Amateur different from professional**			(X^2 (6, n = 1,950) = 7.3, p = 0.184, φc = 0.04 [90% CI 0.01, 0.08])		
Habitual dietary pattern						
No dietary plan	n = 371 (38.8%)	n = 342 (35.7%)	n = 244 (25.5%)	n = 697 (72.8%)	n = 92 (9.6%)	n = 168 (17.6%)
LCHF	n = 12 (6.3%)	n = 147 (77.0%)	n = 32 (16.8%)	n = 42 (22.0%)	n = 80 (41.9%)	n = 71 (37.2%)
Periodised carb	n = 39 (20.4%)	n = 76 (39.8%)	n = 76 (39.8%)	n = 118 (61.8%)	n = 23 (12.0%)	n = 50 (26.2%)
Vegetarian	n = 51 (37.2%)	n = 44 (32.1%)	n = 42 (30.7%)	n = 90 (65.7%)	n = 13 (9.5%)	n = 34 (24.8%)
High carbohydrate	n = 62 (45.3%)	n = 37 (27.0%)	n = 38 (27.7%)	n = 105 (76.6%)	n = 9 (6.6%)	n = 23 (16.8%)
Pescatarian	n = 32 (26.2%)	n = 54 (44.3%)	n = 36 (29.5%)	n = 77 (63.1%)	n = 18 (14.8%)	n = 27 (22.1%)
Gluten-free	n = 37 (31.6%)	n = 42 (35.9%)	n = 38 (32.5%)	n = 89 (76.1%)	n = 13 (11.1%)	n = 15 (12.8%)
High-protein, low-carb	n = 29 (26.1%)	n = 61 (55.0%)	n = 21 (18.9%)	n = 59 (53.2%)	n = 26 (23.4%)	n = 26 (23.4%)
Paleo	n = 27 (32.5%)	n = 35 (42.2%)	n = 21 (25.3%)	n = 54 (65.1%)	n = 16 (19.3%)	n = 13 (15.7%)
Vegan	n = 28 (34.1%)	n = 30 (36.6%)	n = 24 (29.3%)	n = 54 (65.9%)	n = 8 (9.8%)	n = 20 (24.4%)
	(X^2 (18, n = 2,128) = 179.9, p < 0.001, φc = 0.21 [90% CI 0.18, 0.23]) **NP different from PC** **LCHF different from all others** **PC different from HC, HP** **HC different from HP**			**(X^2 (18, n = 2,128) = 251.0, p < 0.001, φc = 0.24 [90% CI 0.22, 0.27])** **NP different from HP** **LCHF different from all others** **HC different from HP**		

Interactions shown as X^2 goodness of fit, p-value, Cramer's V. Significant interactions ($p < 0.05$) in bold. Dietary pattern abbreviations - LCHF: low-carb, high-fat; HC: high-carbohydrate; HP: high-protein, low-carb; NP: no dietary plan; PC: periodised carbohydrate. Cramer's V effect sizes interpreted for sex as 0-0.1 (negligible) and 0.1-0.3 (small), and for competitive level and diet as 0-0.07 (negligible), 0.07-0.21 (small), and 0.21-0.35 (medium).

Table S4.2. Wake-up timing before workouts shorter and longer than 90 min by sex, competitive level, and habitual dietary pattern

	What is the minimum amount of time you would wake up before a morning workout that was less than 90 min in duration?				**What is the minimum amount of time you would wake up before a morning workout that was longer than 90 min in duration?**				**If I have an early morning workout, I will always wake up early so that I have enough time to eat a full breakfast beforehand**		
	< 15 min	**15-30 min**	**30-60 min**	**> 60 min**	**< 15 min**	**15-30 min**	**30-60 min**	**> 60 min**	**Agree**	**Neutral**	**Disagree**
Total	**n = 221 (11.3%)**	**n = 709 (36.4%)**	**n = 694 (35.6%)**	**n = 326 (16.7%)**	**n = 74 (3.8%)**	**n = 427 (21.9%)**	**n = 812 (41.6%)**	**n = 637 (32.7%)**	**n = 581 (29.8%)**	**n = 225 (11.5%)**	**n = 1144 (58.7%)**
Sex											
Male	n = 123 (12.9%)	n = 354 (37.2%)	n = 316 (33.2%)	n = 159 (16.7%)	n = 45 (4.7%)	n = 241 (25.3%)	n = 385 (40.4%)	n = 281 (29.5%)	n = 265 (27.8%)	n = 117 (12.3%)	n = 570 (59.9%)
Female	n = 97 (9.7%)	n = 354 (35.6%)	n = 377 (37.9%)	n = 167 (16.8%)	n = 28 (2.8%)	n = 186 (18.7%)	n = 425 (42.7%)	n = 356 (35.8%)	n = 314 (31.6%)	n = 107 (10.8%)	n = 574 (57.7%)
	(X^2 (3, n = 1,947) = 7.69, p = 0.053, φc = 0.06 [90% CI 0.03, 0.10])				**(X^2 (3, n = 1,947) = 20.9, p < 0.001, φc = 0.10 [90% CI 0.07, 0.14])**				(X^2 (2, n = 1,947) = 3.7, p = 0.160, φc = 0.04 [90% CI 0.01, 0.08])		
Competitive level											
Amateur	n = 126 (10.5%)	n = 426 (35.6%)	n = 446 (37.3%)	n = 197 (16.5%)	n = 41 (3.4%)	n = 249 (20.8%)	n = 513 (42.9%)	n = 392 (32.8%)	n = 343 (28.7%)	n = 139 (11.6%)	n = 713 (59.7%)
High-level amateur	n = 63 (11.8%)	n = 205 (38.5%)	n = 182 (34.1%)	n = 83 (15.6%)	n = 18 (3.4%)	n = 125 (23.5%)	n = 228 (42.8%)	n = 162 (30.4%)	n = 165 (31.0%)	n = 54 (10.1%)	n = 314 (58.9%)
Elite non-professional	n = 28 (14.9%)	n = 71 (37.8%)	n = 55 (29.3%)	n = 34 (18.1%)	n = 14 (7.4%)	n = 49 (26.1%)	n = 61 (32.4%)	n = 64 (34.0%)	n = 58 (30.9%)	n = 25 (13.3%)	n = 105 (55.9%)
Professional	n = 4 (11.8%)	n = 7 (20.6%)	n = 11 (32.4%)	n = 12 (35.3%)	n = 1 (2.9%)	n = 4 (11.8%)	n = 10 (29.4%)	n = 19 (55.9%)	n = 15 (44.1%)	n = 7 (20.6%)	n = 12 (35.3%)
	(X^2 (9, n = 1,950) = 17.3, p = 0.044, φc = 0.05 [90% CI 0.02, 0.09]) Post-hoc NS				**(X^2 (9, n = 1,950) = 23.9, p = 0.005, φc = 0.06 [90% CI 0.03, 0.10]) Amateur different from Elite**				(X^2 (6, n = 1,950) = 10.6, p = 0.102, φc = 0.05 [90% CI 0.02, 0.09])		
Habitual dietary pattern											
No dietary plan	n = 128 (13.4%)	n = 366 (38.2%)	n = 322 (33.6%)	n = 141 (14.7%)	n = 41 (4.3%)	n = 215 (22.5%)	n = 404 (42.2%)	n = 297 (31.0%)	n = 310 (32.4%)	n = 124 (13.0%)	n = 523 (54.6%)
LCHF	n = 21 (11.0%)	n = 77 (40.3%)	n = 68 (35.6%)	n = 25 (13.1%)	n = 10 (5.2%)	n = 51 (26.7%)	n = 91 (47.6%)	n = 39 (20.4%)	n = 17 (8.9%)	n = 12 (6.3%)	n = 162 (84.8%)
Periodised carb	n = 14 (7.3%)	n = 72 (37.7%)	n = 75 (39.3%)	n = 30 (15.7%)	n = 2 (1.0%)	n = 43 (22.5%)	n = 83 (43.5%)	n = 63 (33.0%)	n = 51 (26.7%)	n = 22 (11.5%)	n = 118 (61.8%)
Vegetarian	n = 16 (11.7%)	n = 46 (33.6%)	n = 60 (43.8%)	n = 15 (10.9%)	n = 4 (2.9%)	n = 33 (24.1%)	n = 59 (43.1%)	n = 41 (29.9%)	n = 40 (29.2%)	n = 16 (11.7%)	n = 81 (59.1%)
High carbohydrate	n = 14 (10.2%)	n = 44 (32.1%)	n = 51 (37.2%)	n = 28 (20.4%)	n = 5 (3.6%)	n = 26 (19.0%)	n = 52 (38.0%)	n = 54 (39.4%)	n = 59 (43.1%)	n = 16 (11.7%)	n = 62 (45.3%)

Pescatarian	n = 7 (5.7%)	n = 45 (36.9%)	n = 48 (39.3%)	n = 22 (18.0%)		n = 3 (2.5%)	n = 30 (24.6%)	n = 44 (36.1%)	n = 45 (36.9%)		n = 32 (26.2%)	n = 14 (11.5%)	n = 76 (62.3%)
Gluten-free	n = 8 (6.8%)	n = 34 (29.1%)	n = 51 (43.6%)	n = 24 (20.5%)		n = 3 (2.6%)	n = 18 (15.4%)	n = 49 (41.9%)	n = 47 (40.2%)		n = 43 (36.8%)	n = 12 (10.3%)	n = 62 (53.0%)
High-protein, low-carb	n = 14 (12.6%)	n = 46 (41.4%)	n = 34 (30.6%)	n = 17 (15.3%)		n = 6 (5.4%)	n = 24 (21.6%)	n = 53 (47.7%)	n = 28 (25.2%)		n = 23 (20.7%)	n = 7 (6.3%)	n = 81 (73.0%)
Paleo	n = 5 (6.0%)	n = 27 (32.5%)	n = 33 (39.8%)	n = 18 (21.7%)		n = 2 (2.4%)	n = 8 (9.6%)	n = 37 (44.6%)	n = 36 (43.4%)		n = 20 (24.1%)	n = 6 (7.2%)	n = 57 (68.7%)
Vegan	n = 10 (12.2%)	n = 25 (30.5%)	n = 31 (37.8%)	n = 16 (19.5%)		n = 2 (2.4%)	n = 21 (25.6%)	n = 30 (36.6%)	n = 29 (35.4%)		n = 22 (26.8%)	n = 14 (17.1%)	n = 46 (56.1%)
	(X^2 (27, n = 2,128) = 38.5, p = 0.070, φc = 0.08 [90% CI 0.04, 0.11])					**(X^2 (27, n = 2,128) = 44.5, p = 0.009, φc = 0.08 [90% CI 0.05, 0.12]) LCHF different from Paleo**					**(X^2 (18, n = 1,950) = 93.8, p <0.001, φc = 0.15 [90% CI 0.11, 0.18]) No dietary plan vs. High-protein LCHF vs. all except high-protein and Paleo High-carb vs. High-protein**		

Interactions shown as X^2 goodness of fit, p-value, Cramer's V. Significant interactions ($p < 0.05$) in bold. LCHF: low-carb, high-fat; NS: not statistically significant. Cramer's V effect sizes interpreted for sex as 0-0.1 (negligible) and 0.1-0.3 (small); for competitive level and diet as 0-0.07 (negligible), 0.06-0.17 (small), and 0.17-0.29 (medium).

Table S4.3. Food timing before workouts shorter and longer than 90 min by sex, competitive level, and habitual dietary pattern

	Before a workout less than 90-min in duration, how far in advance would you eat?					**Before a workout longer than 90-min in duration, how far in advance would you eat?**			
	< 15 min	**15-30 min**	**30-60 min**	**> 60 min**		**< 15 min**	**15-30 min**	**30-60 min**	**> 60 min**
Total	**n = 136 (11.6%)**	**n = 419 (35.9%)**	**n = 425 (36.4%)**	**n = 188 (16.1%)**		**n = 86 (5.1%)**	**n = 413 (24.4%)**	**n = 721 (42.7%)**	**n = 470 (27.8%)**
Sex									
Male	n = 64 (12.3%)	n = 169 (32.6%)	n = 190 (36.6%)	n = 96 (18.5%)		n = 52 (6.7%)	n = 204 (26.2%)	n = 311 (39.9%)	n = 212 (27.2%)
Female	n = 71 (11.0%)	n = 249 (38.5%)	n = 235 (36.3%)	n = 92 (14.2%)		n = 34 (3.7%)	n = 209 (23.0%)	n = 408 (44.9%)	n = 258 (28.4%)
	(X^2 (3, n = 1,166) = 6.55, p = 0.088, φc = 0.07 [90%CI 0.03, 0.12])					**(X^2 (3, n = 1,688) = 11.5, p = 0.009, φc = 0.08 [90% CI 0.04, 0.12])**			
Competitive level									
Amateur	n = 84 (12.2%)	n = 242 (35.1%)	n = 261 (37.8%)	n = 103 (14.9%)		n = 48 (4.6%)	n = 254 (24.6%)	n = 454 (43.9%)	n = 278 (26.9%)
High-level amateur	n = 38 (11.4%)	n = 128 (38.3%)	n = 117 (35.0%)	n = 51 (15.3%)		n = 26 (5.6%)	n = 118 (25.5%)	n = 196 (42.4%)	n = 122 (26.4%)
Elite non-professional	n = 13 (11.3%)	n = 43 (37.4%)	n = 36 (31.3%)	n = 23 (20.0%)		n = 10 (6.3%)	n = 38 (23.8%)	n = 59 (36.9%)	n = 53 (33.1%)
Professional	n = 1 (3.4%)	n = 6 (20.7%)	n = 11 (37.9%)	n = 11 (37.9%)		n = 2 (5.9%)	n = 3 (8.8%)	n = 12 (35.3%)	n = 17 (50.0%)
	(X^2 (9, n = 1,168) = 16.3, p = 0.061, φc = 0.07 [90% CI 0.02, 0.12])					(X^2 (9, n = 1,690) = 15.2, p = 0.087, φc = 0.05 [90% CI 0.01, 0.09])			
Habitual dietary pattern									
No dietary plan	n = 78 (12.7%)	n = 227 (37.0%)	n = 215 (35.1%)	n = 93 (15.2%)		n = 46 (5.3%)	n = 216 (25.1%)	n = 389 (45.2%)	n = 210 (24.4%)
LCHF	n = 6 (13.6%)	n = 17 (38.6%)	n = 15 (34.1%)	n = 6 (13.6%)		n = 3 (2.7%)	n = 34 (30.6%)	n = 50 (45.0%)	n = 24 (21.6%)
Periodised carb	n = 9 (7.8%)	n = 46 (40.0%)	n = 42 (36.5%)	n = 18 (15.7%)		n = 10 (6.0%)	n = 51 (30.4%)	n = 55 (32.7%)	n = 52 (31.0%)
Vegetarian	n = 6 (6.5%)	n = 37 (39.8%)	n = 36 (38.7%)	n = 14 (15.1%)		n = 4 (3.2%)	n = 32 (25.8%)	n = 56 (45.2%)	n = 32 (25.8%)
High carbohydrate	n = 9 (9.0%)	n = 31 (31.0%)	n = 35 (35.0%)	n = 25 (25.0%)		n = 6 (4.7%)	n = 22 (17.2%)	n = 50 (39.1%)	n = 50 (39.1%)
Pescatarian	n = 8 (11.8%)	n = 23 (33.8%)	n = 27 (39.7%)	n = 10 (14.7%)		n = 7 (6.7%)	n = 22 (21.2%)	n = 45 (43.3%)	n = 30 (28.8%)
Gluten-free	n = 2 (2.7%)	n = 36 (48.0%)	n = 27 (36.0%)	n = 10 (13.3%)		n = 4 (3.8%)	n = 26 (25.0%)	n = 45 (43.3%)	n = 29 (27.9%)
High-protein, low-carb	n = 6 (12.0%)	n = 19 (38.0%)	n = 19 (38.0%)	n = 6 (12.0%)		n = 6 (7.1%)	n = 24 (28.2%)	n = 34 (40.0%)	n = 21 (24.7%)
Paleo	n = 6 (12.5%)	n = 12 (25.0%)	n = 19 (39.6%)	n = 11 (22.9%)		n = 0 (0.0%)	n = 10 (15.2%)	n = 32 (48.5%)	n = 24 (36.4%)
Vegan	n = 10 (19.2%)	n = 11 (21.2%)	n = 22 (42.3%)	n = 9 (17.3%)		n = 6 (8.2%)	n = 18 (24.7%)	n = 22 (30.1%)	n = 27 (37.0%)
	(X^2 (27, n = 1,258) = 31.9, p = 0.234, φc = 0.09)					**(X^2 (27, n = 1,824) = 44.3, p = 0.019, φc = 0.09 [90% CI 0.05, 0.13]) LCHF different from all others except high-protein High-carb different from high-protein**			

Interactions shown as X^2 goodness of fit, p-value, Cramer's V. Significant interactions ($p < 0.05$) in bold. Cramer's V effect sizes interpreted for sex as 0-0.1 (negligible) and 0.1-0.3 (small); for competitive level and diet as 0-0.07 (negligible), 0.06-0.17 (small), and 0.17-0.29 (medium).

Table S4.4. Differences in pre-exercise supplementation practices of endurance athletes based on sex, competitive level, and habitual dietary pattern

Which supplements do you take *before* training, at least some of the time		**Coffee/tea**	**Protein powder**	**Multi-vitamin**	**Other caffeine sources**	**Vitamin C**	**Vitamin B12**	**Fish oil/omega 3**	**Nitrate/beetroot juice**	**Beta-alanine**	**Vitamin E**	**Creatine**	**Electrolytes**	**Sodium Bicarbonate**	**BCAA/EAA**
	Total	**n = 1488 (76.3%)**	**n = 576 (29.5%)**	**n = 474 (24.3%)**	**n = 396 (20.3%)**	**n = 365 (18.7%)**	**n = 345 (17.7%)**	**n = 336 (17.2%)**	**n = 277 (14.2%)**	**n = 166 (8.5%)**	**n = 148 (7.6%)**	**n = 141 (7.2%)**	**n = 123 (6.3%)**	**n = 87 (4.5%)**	**n = 85 (4.4%)**
Sex	Male	n = 734 (77.1%)	n = 252 (26.5%)	n = 241 (25.3%)	n = 216 (22.7%)	n = 184 (19.3%)	n = 157 (16.5%)	n = 176 (18.5%)	n = 155 (16.3%)	n = 98 (10.3%)	n = 87 (9.1%)	n = 89 (9.3%)	n = 68 (7.1%)	n = 57 (6.0%)	n = 15 (1.6%)
	Female	n = 751 (75.5%)	n = 324 (32.6%)	n = 232 (23.3%)	n = 179 (18.0%)	n = 180 (18.1%)	n = 187 (18.8%)	n = 160 (16.1%)	n = 121 (12.2%)	n = 68 (6.8%)	n = 61 (6.1%)	n = 52 (5.2%)	n = 55 (5.5%)	n = 30 (3.0%)	n = 70 (7.0%)
	p-value Cramer's V [90% CI]	p = 0.400 φc = 0.02 [0.0, 0.06]	**p = 0.003 φc = 0.07 [0.03, 0.10]**	p = 0.304 φc = 0.02 [0.0, 0.06]	**p = 0.010 φc = 0.06 0.02, 0.10]**	p = 0.484 φc = 0.02 [0.0, 0.05]	p = 0.183 φc = 0.03 [0.0, 0.07]	p = 0.160 φc = 0.03 [0.0, 0.07]	**p = 0.009 φc = 0.06 [0.02, 0.10]**	**p = 0.006 φc = 0.06 [0.02, 0.10]**	**p = 0.012 φc = 0.06 [0.02, 0.09]**	**p < 0.001 φc = 0.08 [0.04, 0.12]**	p = 0.143 φc = 0.03 [0.0, 0.07]	**p = 0.002 φc = 0.07 [0.03, 0.11]**	**p < 0.001 φc = 0.13 [0.10, 0.17]**
Competitive level	Amateur	n = 909 (76.1%)	n = 353 (29.5%)	n = 277 (23.2%)	n = 223 (18.7%)	n = 203 (17.0%)	n = 178 (14.9%)	n = 194 (16.2%)	n = 129 (10.8%)	n = 57 (4.8%)	n = 82 (6.9%)	n = 77 (6.4%)	n = 78 (6.5%)	n = 32 (2.7%)	n = 46 (3.8%)
	High-level amateur	n = 407 (76.4%)	n = 148 (27.8%)	n = 132 (24.8%)	n = 122 (22.9%)	n = 109 (20.5%)	n = 110 (20.6%)	n = 85 (15.9%)	n = 105 (19.7%)	n = 60 (11.3%)	n = 45 (8.4%)	n = 35 (6.6%)	n = 28 (5.3%)	n = 27 (5.1%)	n = 24 (4.5%)
	Elite non-professional	n = 144 (76.6%)	n = 58 (30.9%)	n = 49 (26.1%)	n = 45 (23.9%)	n = 39 (20.7%)	n = 43 (22.9%)	n = 42 (22.3%)	n = 36 (19.1%)	n = 37 (19.7%)	n = 17 (9.0%)	n = 23 (12.2%)	n = 15 (8.0%)	n = 23 (12.2%)	n = 11 (5.9%)
	Professional	n = 28 (82.4%)	n = 17 (50.0%)	n = 16 (47.1%)	n = 6 (17.6%)	n = 14 (41.2%)	n = 14 (41.2%)	n = 15 (44.1%)	n = 7 (20.6%)	n = 12 (35.3%)	n = 4 (11.8%)	n = 6 (17.6%)	n = 2 (5.9%)	n = 5 (14.7%)	n = 4 (11.8%)
	p-value Cramer's V [90% CI]	p = 0.865 φc = 0.02 [0.0, 0.07]	**p = 0.050 φc = 0.06 [0.03, 0.10]**	**p = 0.013 φc = 0.07 [0.04, 0.11]**	p = 0.118 φc = 0.05 [0.02, 0.09]	**p = 0.002 φc = 0.09 [0.05, 0.13]**	**p < 0.001 φc = 0.12 [0.08, 0.15]**	**p < 0.001 φc = 0.11 [0.07, 0.14]**	**p < 0.001 φc = 0.12 [0.09, 0.16]**	**p < 0.001 φc = 0.21 [0.18, 0.25]**	p = 0.332 φc = 0.04 [0.0, 0.08]	**p = 0.005 φc = 0.08 [0.05, 0.12]**	p = 0.547 φc = 0.03 [0.0, 0.07]	**p < 0.001 φc = 0.15 [0.11, 0.19]**	p = 0.097 φc = 0.06 [0.02, 0.09]
	Post hoc testing		post hoc NS	Amateur and HLA < Pro		Amateur < Pro	Amateur < all others	Amateur and HLA < Pro	Amateur < HLA and Elite	Amateur < all others; HLA < Elite and Pro		post hoc NS		Amateur < Elite and Pro; HLA < Elite	
Habitual dietary	No dietary plan	n = 696 (72.7%)	n = 233 (24.3%)	n = 214 (22.4%)	n = 185 (19.3%)	n = 173 (18.1%)	n = 124 (13.0%)	n = 151 (15.8%)	n = 103 (10.8%)	n = 52 (5.4%)	n = 61 (6.4%)	n = 51 (5.3%)	n = 46 (4.8%)	n = 29 (3.0%)	n = 32 (3.3%)
	LCHF	n = 166 (86.9%)	n = 55 (28.8%)	n = 37 (19.4%)	n = 36 (18.8%)	n = 32 (16.8%)	n = 30 (15.7%)	n = 38 (19.9%)	n = 31 (16.2%)	n = 23 (12.0%)	n = 13 (6.8%)	n = 18 (9.4%)	n = 13 (6.8%)	n = 17 (8.9%)	n = 11 (5.8%)
	Periodised carb	n = 162 (84.8%)	n = 92 (48.2%)	n = 53 (27.7%)	n = 58 (30.4%)	n = 43 (22.5%)	n = 45 (23.6%)	n = 45 (23.6%)	n = 40 (20.9%)	n = 29 (15.2%)	n = 20 (10.5%)	n = 26 (13.6%)	n = 14 (7.3%)	n = 12 (6.3%)	n = 14 (7.3%)

	Vegetarian	n = 106 (77.4%)	n = 44 (32.1%)	n = 38 (27.7%)	n = 29 (21.2%)	n = 29 (21.2%)	n = 39 (28.5%)	n = 23 (16.8%)	n = 22 (16.1%)	n = 11 (8.0%)	n = 13 (9.5%)	n = 9 (6.6%)	n = 9 (6.6%)	n = 5 (3.6%)	n = 6 (4.4%)
	High carbohydrate	n = 102 (74.5%)	n = 37 (27.0%)	n = 36 (26.3%)	n = 36 (26.3%)	n = 25 (18.2%)	n = 29 (21.2%)	n = 23 (16.8%)	n = 33 (24.3%)	n = 22 (16.1%)	n = 5 (3.6%)	n = 8 (5.8%)	n = 8 (5.8%)	n = 9 (6.6%)	n = 2 (1.5%)
	Pescatarian	n = 97 (79.5%)	n = 43 (35.2%)	n = 36 (29.5%)	n = 18 (14.8%)	n = 21 (17.2%)	n = 25 (20.5%)	n = 19 (15.6%)	n = 24 (19.7%)	n = 12 (9.8%)	n = 13 (10.7%)	n = 10 (8.2%)	n = 7 (5.7%)	n = 6 (4.9%)	n = 9 (7.4%)
	Gluten-free	n = 88 (75.2%)	n = 47 (40.2%)	n = 32 (27.4%)	n = 22 (18.8%)	n = 23 (19.7%)	n = 36 (30.8%)	n = 27 (23.1%)	n = 21 (17.9%)	n = 15 (12.8%)	n = 13 (11.1%)	n = 10 (8.5%)	n = 9 (7.7%)	n = 4 (3.4%)	n = 17 (14.5%)
	High-protein, low-carb	n = 88 (79.3%)	n = 49 (44.1%)	n = 35 (31.5%)	n = 24 (21.6%)	n = 21 (18.9%)	n = 24 (21.6%)	n = 15 (13.5%)	n = 17 (15.3%)	n = 11 (9.9%)	n = 12 (10.8%)	n = 18 (16.2%)	n = 13 (11.7%)	n = 7 (6.3%)	n = 4 (3.6%)
	Paleo	n = 67 (80.7%)	n = 32 (38.6%)	n = 20 (24.1%)	n = 19 (22.9%)	n = 19 (22.9%)	n = 18 (21.7%)	n = 20 (24.1%)	n = 13 (15.7%)	n = 12 (14.5%)	n = 10 (12.0%)	n = 16 (19.3%)	n = 8 (9.6%)	n = 5 (6.0%)	n = 8 (9.6%)
	Vegan	n = 59 (72.0%)	n = 22 (26.8%)	n = 12 (14.6%)	n = 17 (20.7%)	n = 13 (15.9%)	n = 28 (34.1%)	n = 10 (12.2%)	n = 18 (22.0%)	n = 7 (8.5%)	n = 4 (4.9%)	n = 5 (6.1%)	n = 6 (7.3%)	n = 1 (1.2%)	n = 4 (4.9%)
	p-value Cramer's V [90% CI]	**p <0.001 φc = 0.12 [0.08, 0.15]**	**p <0.001 φc = 0.18 [0.14, 0.21]**	p = 0.055 φc = 0.09 [0.05, 0.12]	**p = 0.035 φc = 0.09 [0.06, 0.13]**	p = 0.856 φc = 0.05 [0.01, 0.08]	**p <0.001 φc = 0.17 [0.13, 0.20]**	p = 0.07 φc = 0.09 [0.05, 0.12]	**p <0.001 φc = 0.13 [0.09, 0.16]**	**p <0.001 φc = 0.14 [0.10, 0.17]**	**p = 0.044 φc = 0.09 [0.05, 0.12]**	**p <0.001 φc = 0.14 [0.11, 0.18]**	p = 0.222 φc = 0.07 [0.04, 0.11]	**p = 0.019 φc = 0.10 [0.06, 0.13]**	**p = <0.001 φc = 0.14 [0.10, 0.17]**
	Post hoc testing	NP < LCHF, PC	NP < GF, HP PC > NP, LCHF, HC		NP < PC		NP < PC, GF, VGN; LCHF < VGN		NP < PC, HC	NP < HC	Post hoc NS	NP < PC		NP < LCHF	NP < GF

Significant interactions (p < 0.05) in bold. BCAA/EAA: Branched chain amino acids/ essential amino acids; HLA: high-level amateur; Pro: professional. Dietary pattern abbreviations - LCHF: low-carb, high-fat; GF: gluten-free; HC: high-carbohydrate; HP: high-protein, low-carb; NP: no dietary plan; PAL: paleo; PC: periodised carbohydrate; PESC: pescatarian; VEG: vegetarian; VGN: vegan. NS: not statistically significant. Cramer's V effect sizes interpreted as 0-0.1 (negligible), 0.1-0.3 (small).

Appendix D: Supplemental Tables from Chapter 5

Table S5.1. Estimated means and 95% confidence intervals (CI) for heart rate during submaximal exercise, which included 4 x 5-min stages at a power equivalent to 60%, 80%, and 100% of the first ventilatory threshold (VT) (VT60, VT80, VT100, respectively), and 20% of the difference between VT and Wmax (VTΔ20).

Treatment	Intensity	Estimated mean	Standard Error	Degrees of freedom	Lower 95% CI	Upper 95% CI
Carbohydrate	VT60	58.58	1.37	10.26	55.54	61.62
Fasted	VT60	56.76	1.24	10.54	54.02	59.50
Protein	VT60	58.90	1.41	10.41	55.77	62.03
Carbohydrate	VT80	66.23	1.37	10.26	63.19	69.27
Fasted	VT80	64.40	1.24	10.54	61.67	67.14
Protein	VT80	66.54	1.41	10.41	63.41	69.68
Carbohydrate	VT100	73.64	1.37	10.26	70.60	76.68
Fasted	VT100	71.82	1.24	10.54	69.08	74.56
Protein	VT100	73.96	1.41	10.41	70.83	77.09
Carbohydrate	VTΔ20	80.98	1.37	10.26	77.94	84.02
Fasted	VTΔ20	79.16	1.24	10.54	76.42	81.90
Protein	VTΔ20	81.30	1.41	10.41	78.17	84.43

Table S5.2. Estimated means and 95% confidence intervals (CI) for gross efficiency during submaximal exercise, which included 4 x 5-min stages at a power equivalent to 60%, 80%, and 100% of the first ventilatory threshold (VT) (VT60, VT80, VT100, respectively), and 20% of the difference between VT and Wmax (VTΔ20).

Treatment	Intensity	Estimated mean	Standard Error	Degrees of freedom	Lower 95% CI	Upper 95% CI
Carbohydrate	VT60	18.97	0.31	11.99	18.29	19.66
Fasted	VT60	19.16	0.31	11.87	18.48	19.85
Protein	VT60	18.83	0.31	11.87	18.15	19.52
Carbohydrate	VT80	20.34	0.31	11.99	19.65	21.02
Fasted	VT80	20.53	0.31	11.87	19.84	21.21
Protein	VT80	20.20	0.31	11.87	19.51	20.88
Carbohydrate	VT100	21.14	0.31	11.99	20.46	21.82
Fasted	VT100	21.33	0.31	11.87	20.65	22.01
Protein	VT100	21.00	0.31	11.87	20.32	21.68
Carbohydrate	VTΔ20	21.62	0.31	11.99	20.94	22.31
Fasted	VTΔ20	21.81	0.31	11.87	21.13	22.50
Protein	VTΔ20	21.48	0.31	11.87	20.80	22.17

Table S5.3. Estimated means and 95% confidence intervals (CI) for rating of perceived exertion during submaximal exercise, which included 4 x 5-min stages at a power equivalent to 60%, 80%, and 100% of the first ventilatory threshold (VT) (VT60, VT80, VT100, respectively), and 20% of the difference between VT and Wmax (VTΔ20).

Treatment	Intensity	Estimated mean	Standard Error	Degrees of freedom	Lower 95% CI	Upper 95% CI
Carbohydrate	VT60	8.55	0.23	28.35	8.07	9.03
Fasted	VT60	8.46	0.23	27.23	7.99	8.93
Protein	VT60	8.39	0.23	27.23	7.91	8.86
Carbohydrate	VT80	10.30	0.23	28.35	9.83	10.78
Fasted	VT80	10.22	0.23	27.23	9.74	10.69
Protein	VT80	10.14	0.23	27.23	9.67	10.62
Carbohydrate	VT100	12.09	0.23	28.35	11.61	12.57
Fasted	VT100	12.00	0.23	27.23	11.53	12.48
Protein	VT100	11.93	0.23	27.23	11.46	12.40
Carbohydrate	VTΔ20	13.91	0.23	28.35	13.43	14.39
Fasted	VTΔ20	13.82	0.23	27.23	13.35	14.30
Protein	VTΔ20	13.75	0.23	27.23	13.27	14.22

Table S5.4. Estimated means and 95% confidence intervals (CI) for respiratory exchange ratio during submaximal exercise, which included 4 x 5-min stages at a power equivalent to 60%, 80%, and 100% of the first ventilatory threshold (VT) (VT60, VT80, VT100, respectively), and 20% of the difference between VT and Wmax (VTΔ20).

Treatment	Intensity	Estimated mean	Standard Error	Degrees of freedom	Lower 95% CI	Upper 95% CI
Carbohydrate	VT60	0.88	0.01	13.39	0.86	0.90
Fasted	VT60	0.84	0.01	13.50	0.82	0.86
Protein	VT60	0.86	0.01	14.88	0.84	0.87
Carbohydrate	VT80	0.91	0.01	13.39	0.89	0.93
Fasted	VT80	0.88	0.01	13.50	0.86	0.90
Protein	VT80	0.90	0.01	14.88	0.88	0.91
Carbohydrate	VT100	0.92	0.01	13.39	0.91	0.94
Fasted	VT100	0.90	0.01	13.50	0.88	0.92
Protein	VT100	0.92	0.01	14.88	0.90	0.93
Carbohydrate	VTΔ20	0.95	0.01	13.39	0.93	0.97
Fasted	VTΔ20	0.94	0.01	13.50	0.92	0.95
Protein	VTΔ20	0.94	0.01	14.88	0.93	0.96

Table S5.5. Estimated means and 95% confidence intervals (CI) for fat oxidation (g/min) during submaximal exercise, which included 4 x 5-min stages at a power equivalent to 60%, 80%, and 100% of the first ventilatory threshold (VT) (VT60, VT80, VT100, respectively), and 20% of the difference between VT and Wmax (VTΔ20).

Treatment	Intensity	Estimated mean	Standard Error	Degrees of freedom	Lower 95% CI	Upper 95% CI
Carbohydrate	VT60	0.38	0.04	15.11	0.30	0.45
Fasted	VT60	0.51	0.05	14.12	0.41	0.61
Protein	VT60	0.46	0.03	15.75	0.39	0.53
Carbohydrate	VT80	0.34	0.04	15.11	0.27	0.42
Fasted	VT80	0.47	0.05	14.12	0.37	0.57
Protein	VT80	0.41	0.03	15.75	0.34	0.49
Carbohydrate	VT100	0.35	0.04	15.11	0.27	0.42
Fasted	VT100	0.46	0.05	14.12	0.36	0.56
Protein	VT100	0.39	0.03	15.75	0.31	0.46
Carbohydrate	VTΔ20	0.28	0.04	15.83	0.21	0.36
Fasted	VTΔ20	0.36	0.05	14.49	0.26	0.47
Protein	VTΔ20	0.30	0.03	15.75	0.23	0.37

Table S5.6. Estimated means and 95% confidence intervals (CI) for carbohydrate oxidation (g/min) during submaximal exercise, which included 4 x 5-min stages at a power equivalent to 60%, 80%, and 100% of the first ventilatory threshold (VT) (VT60, VT80, VT100, respectively), and 20% of the difference between VT and Wmax (VTΔ20).

Treatment	Intensity	Estimated mean	Standard Error	Degrees of freedom	Lower 95% CI	Upper 95% CI
Carbohydrate	VT60	1.41	0.13	17.05	1.13	1.69
Fasted	VT60	1.08	0.12	18.49	0.84	1.32
Protein	VT60	1.23	0.12	18.28	0.99	1.48
Carbohydrate	VT80	2.05	0.13	17.05	1.77	2.33
Fasted	VT80	1.73	0.12	18.49	1.49	1.97
Protein	VT80	1.91	0.12	18.28	1.66	2.15
Carbohydrate	VT100	2.62	0.13	17.05	2.34	2.90
Fasted	VT100	2.31	0.12	18.49	2.07	2.55
Protein	VT100	2.56	0.12	18.28	2.31	2.80
Carbohydrate	VTΔ20	3.43	0.13	17.80	3.15	3.71
Fasted	VTΔ20	3.18	0.12	19.42	2.93	3.42
Protein	VTΔ20	3.39	0.12	18.28	3.15	3.64

Table S5.7. Holm-adjusted p-values for all contrasts during submaximal exercise, which included 4 x 5-min stages at a power equivalent to 60%, 80%, and 100% of the first ventilatory threshold (VT) (VT60, VT80, VT100, respectively), and 20% of the difference between VT and Wmax (VTΔ20). Significant ($p < .05$) p-values are noted in bold.

Treatment	Intensity	Heart rate	Gross efficiency	Rating of perceived exertion	Respiratory exchange ratio	Fat oxidation (g/min)	Carbohydrate oxidation (g/min)
Carbohydrate	VT60	0.037	0.125	1.000	0.000	0.014	0.017
Fasted	VT60	0.682	0.171	0.992	0.004	0.035	0.197
Protein	VT60	0.000	0.003	1.000	0.061	0.197	0.197
Carbohydrate	VT80	0.037	0.125	1.000	0.004	0.018	0.021
Fasted	VT80	0.682	0.171	0.992	0.088	0.069	0.243
Protein	VT80	0.000	0.003	1.000	0.088	0.157	0.243
Carbohydrate	VT100	0.037	0.125	1.000	0.039	0.034	0.025
Fasted	VT100	0.682	0.171	0.992	0.442	0.236	0.559
Protein	VT100	0.000	0.003	1.000	0.085	0.101	0.054
Carbohydrate	VTΔ20	0.037	0.125	1.000	0.333	0.227	0.116
Fasted	VTΔ20	0.682	0.171	0.992	0.551	0.627	0.759
Protein	VTΔ20	0.000	0.003	1.000	0.483	0.227	0.118

Appendix E: Supplemental Figures from Chapter 6

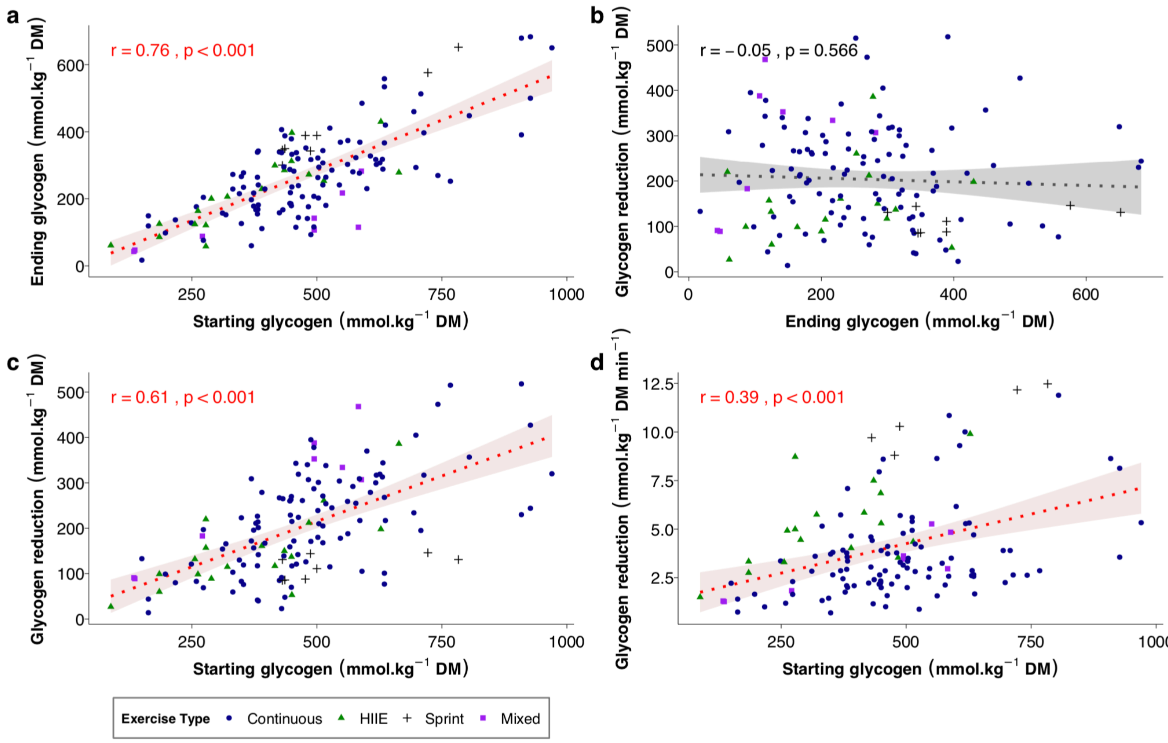


Figure S6.1. Linear correlations between Hedges' g effect size for change in markers of 5' AMP-activated protein kinase (AMPK) activity (pre to post exercise) and starting, ending, and changes in muscle glycogen concentration. Panel (c) refers to absolute glycogen reduction from pre- to post-exercise (mmol kg^{-1} DM), panel (d) refers to the rate of glycogen reduction (mmol kg^{-1} DM per min). DM: dry mass. Exercise type is depicted separately as continuous exercise, high-intensity interval exercise (HIIE, intermittent bouts > 30 s in duration), sprint exercise (single or repeated efforts < 30 s), and mixed intensity exercise sessions. Shaded areas represent 95% confidence intervals, with significant correlations ($p < .05$) highlighted in red.

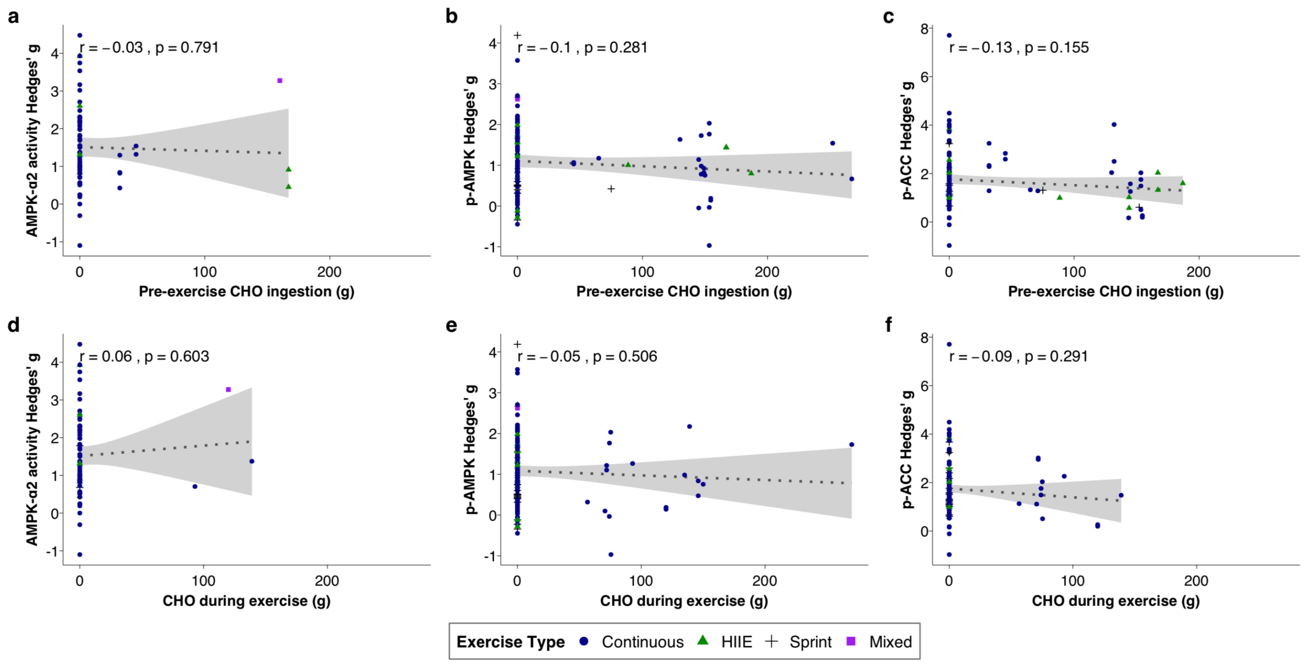


Figure S6.2. Linear correlations between Hedges' g effect size for change in markers of 5' AMP-activated protein kinase (AMPK) activity (pre to post exercise) and amount of carbohydrate intake within 4 h of beginning exercise (a–c) and carbohydrate during exercise (d–f). CHO: carbohydrate, DM: dry mass. Exercise type is depicted separately as continuous exercise, high-intensity interval exercise (HIIE, intermittent bouts > 30 s in duration), sprint exercise (single or repeated efforts < 30 s), and mixed intensity exercise sessions. Shaded areas represent 95% confidence intervals, with significant correlations (p < .05) highlighted in red.

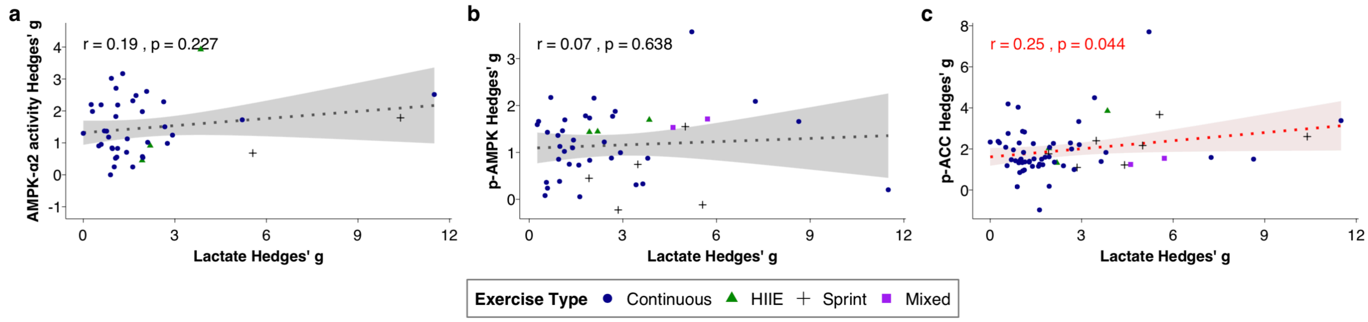


Figure S6.3. Linear correlations between Hedges' g effect size for change in markers of 5' AMP-activated protein kinase (AMPK) activity (pre to post exercise) and intramuscular lactate. Exercise type is depicted separately as continuous exercise, high-intensity interval exercise (HIIE, intermittent bouts > 30 s in duration), sprint exercise (single or repeated efforts < 30 s), and mixed intensity exercise sessions. Shaded areas represent 95% confidence intervals.

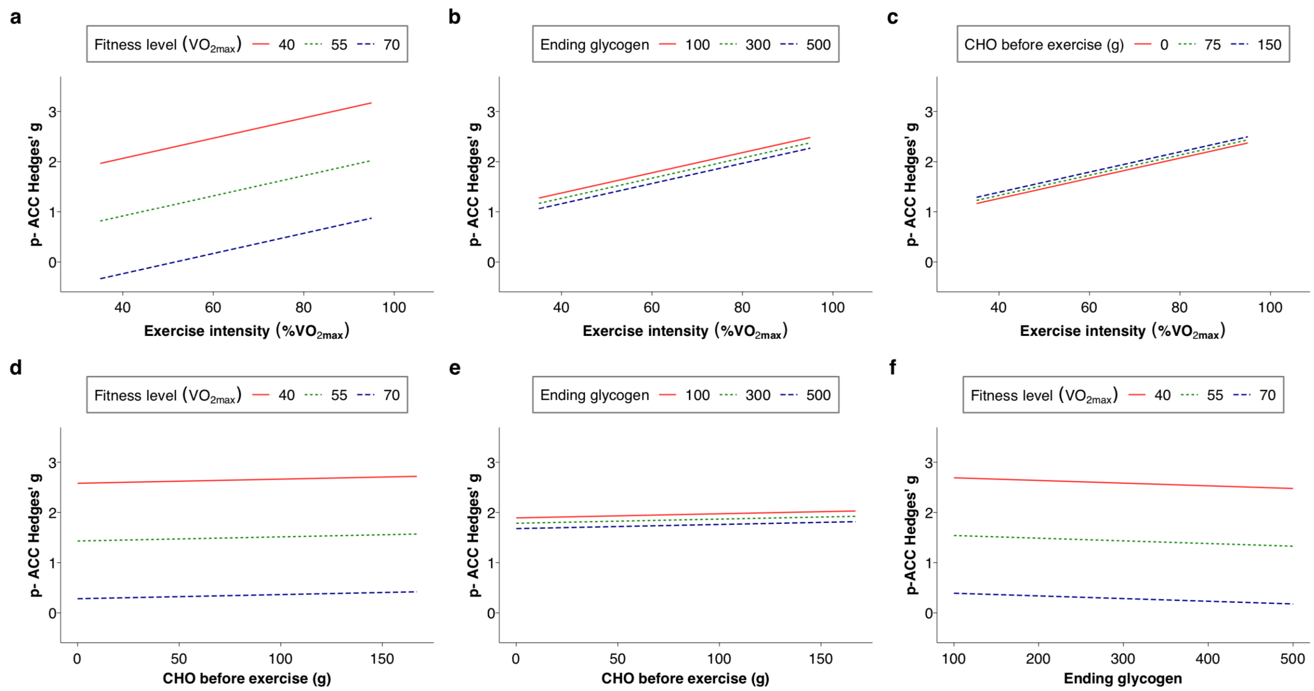


Figure S6.4. Model predictions showing estimated Hedges' g effect sizes for p-ACC when exercising at different intensities (as $\%VO_{2max}$) related to fitness level (as VO_{2max} in mL kg^{-1} min^{-1}, a), ending glycogen level (mmol kg^{-1} dry mass, b), and carbohydrate (CHO) intake before exercise (grams, c), CHO intake before exercise related to fitness level (d) and ending glycogen (e), and ending glycogen related to fitness level (f). Values are based on p-ACC (-) model 2 in Table 1. Steeper slopes indicate a larger effect of the variable shown on the x-axis, and greater space between lines indicates a larger effect of the variable being plotted. These figures demonstrate the far greater influence of exercise intensity and fitness level, compared with muscle glycogen levels, on p-ACC signaling.

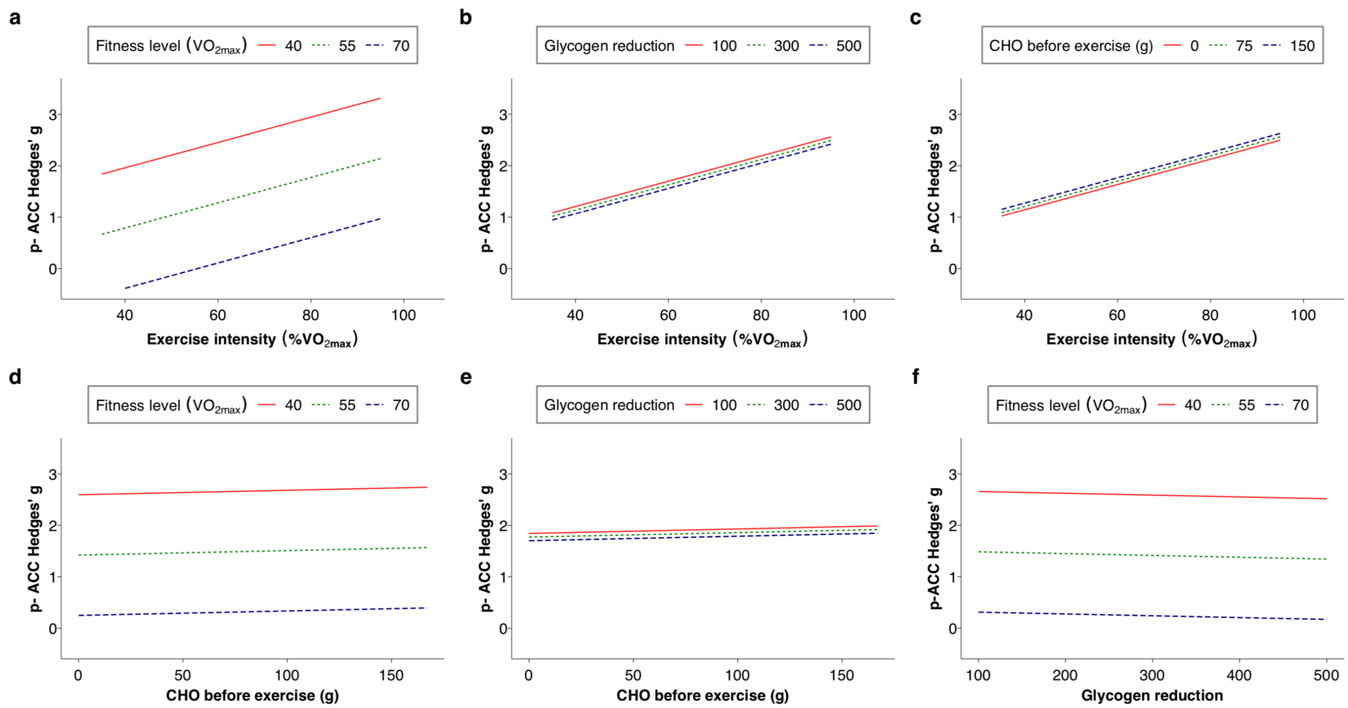


Figure S6.5. Model predictions showing estimated Hedges' g effect sizes for p-ACC when exercising at different intensities (as %VO_{2max}) related to fitness level (as VO_{2max} in mL kg^{-1} min^{-1}, a), glycogen reduction (mmol kg^{-1} dry mass, b), and carbohydrate (CHO) intake before exercise (grams, c), CHO intake before exercise related to fitness level (d) and glycogen reduction (e), and glycogen reduction related to fitness level (f). Values are based on p-ACC (-) model 3 in Table 1. Steeper slopes indicate a larger effect of the variable shown on the x-axis, and greater space between lines indicates a larger effect of the variable being plotted. These figures demonstrate the far greater influence of exercise intensity and fitness level, compared with muscle glycogen levels, on p-ACC signaling.

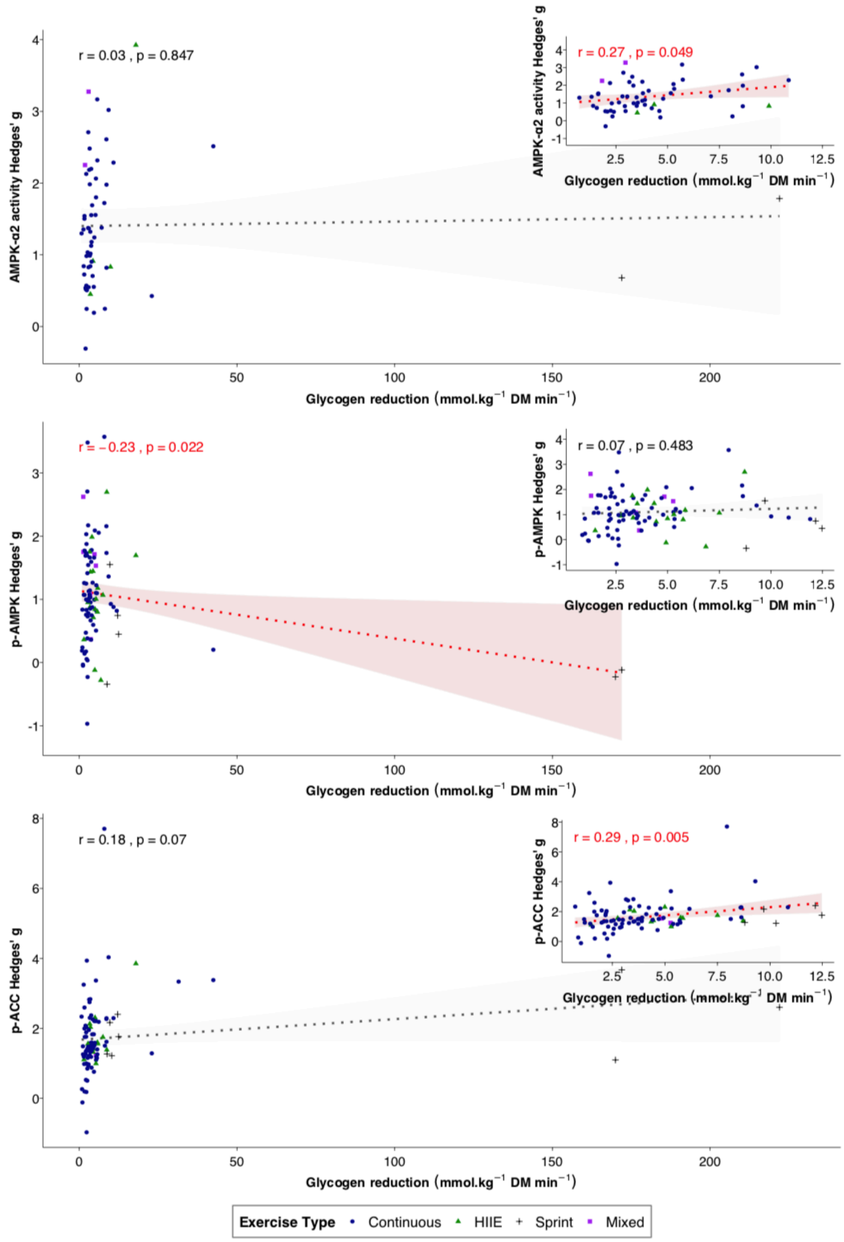


Figure S6.6. Linear correlations between Hedges' g effect size for changes from pre to post exercise for 5' AMP-activated protein kinase (AMPK) α-2 activity (top panel), p-AMPK (middle panel), and p-ACC (bottom panel), and rate of muscle glycogen breakdown (mmol kg^{-1} dry mass (DM) min^{-1}). The large panels show all data points, insets remove outliers (> 17 mmol kg^{-1} DM min^{-1}), typically observed during sprint exercise or short-duration maximal efforts. Exercise type is depicted separately as continuous exercise, high-intensity interval exercise (HIIE, intermittent bouts > 30 s in duration), sprint exercise (single or repeated efforts < 30 s), and mixed intensity exercise sessions. Shaded areas represent 95% confidence intervals, with significant correlations ($p < 0.05$) highlighted in red.

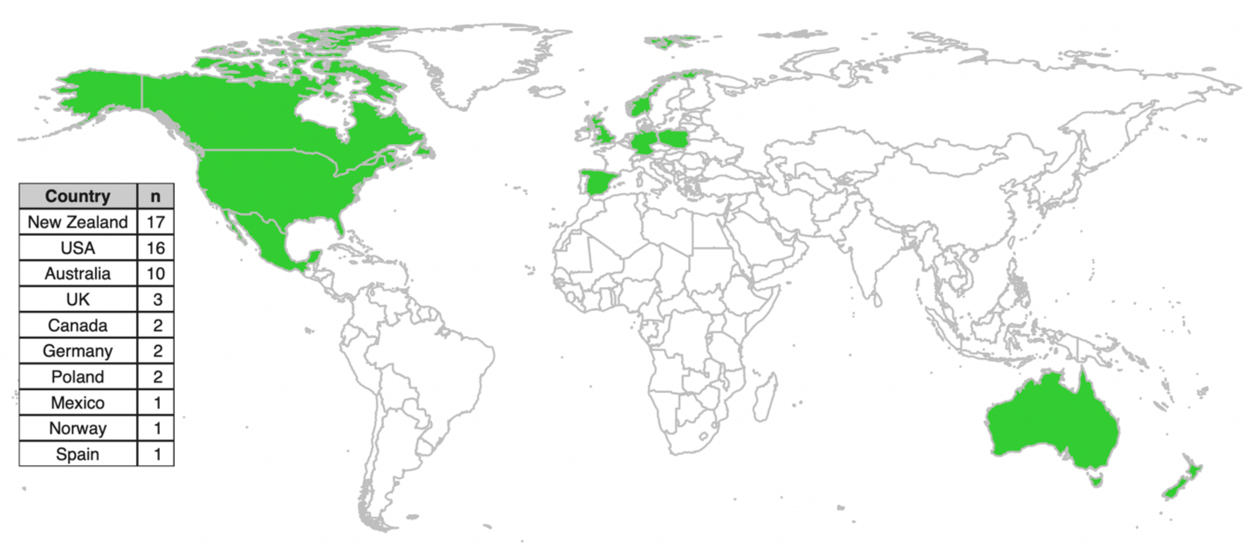
Appendix F: Supplemental Table from Chapter 6

Table S6.1. Zero-order and partial Pearson's correlations between markers of AMPK activity and ADP and muscle glycogen

	AMPK-α2	p-AMPK	p-ACC
ADP_{free} (zero-order correlations)	r = .55 P = .004	r = .36 p = .276	r = -.03 p = .900
ADP_{free} (partial correlations removing glycogen reduction)	r = .49 p = .017	r = .14 p = .724	r = -.04 p = .846
ADP_{free} (partial correlations removing exercise intensity)	r = .52 p = .011	r = .26 p = .465	r = .02 p = .927
Glycogen reduction[a] (zero-order correlations)	r = .37 p = .003	r = .18 p = .087	r = .05 p = .646
Glycogen reduction[a] (partial correlations removing ADP_{free})	r = .23 p = .287	r = .25 p = .525	r = .20 p = .372
Glycogen reduction[a] (partial correlations removing exercise intensity)	r = .29 p = .045	r = -.09 p = .524	r = .04 p = .762
Ending glycogen (zero-order correlations)	r = -.37 p = .004	r = -.32 p = .002	r = -.08 p = .455
Ending glycogen (partial correlations removing ADP_{free})	r = -.44 p = .033	r = -.52 p = .150	r = -.06 p = .784
Ending glycogen (partial correlations removing exercise intensity)	r = -.33 p = .021	r = -.16 p = .236	r = -.06 p = .637

Zero-order and partial Pearson's correlations between Hedges' g effect size for change in markers of 5' AMP-activated protein kinase (AMPK) activity (pre to post exercise) and free ADP (ADP_{free}) with and without the influence of changes in muscle glycogen and exercise intensity, and absolute glycogen reduction during exercise and ending glycogen with and without the influence of ADP_{free} and exercise intensity. Significant correlations ($p < .05$) in bold. [a] Expressed as mmol per kg dry mass.

Appendix G: Supplemental Figures from Chapter 9



Country	n
New Zealand	17
USA	16
Australia	10
UK	3
Canada	2
Germany	2
Poland	2
Mexico	1
Norway	1
Spain	1

Figure G1 Location of study participants.

Figure G2. Daily carbohydrate intake (g/kg) for each day of the study, separated by participant and color-coded according to if training was performed in the overnight-fasted (light green circles) or fed (dark blue circles) state.

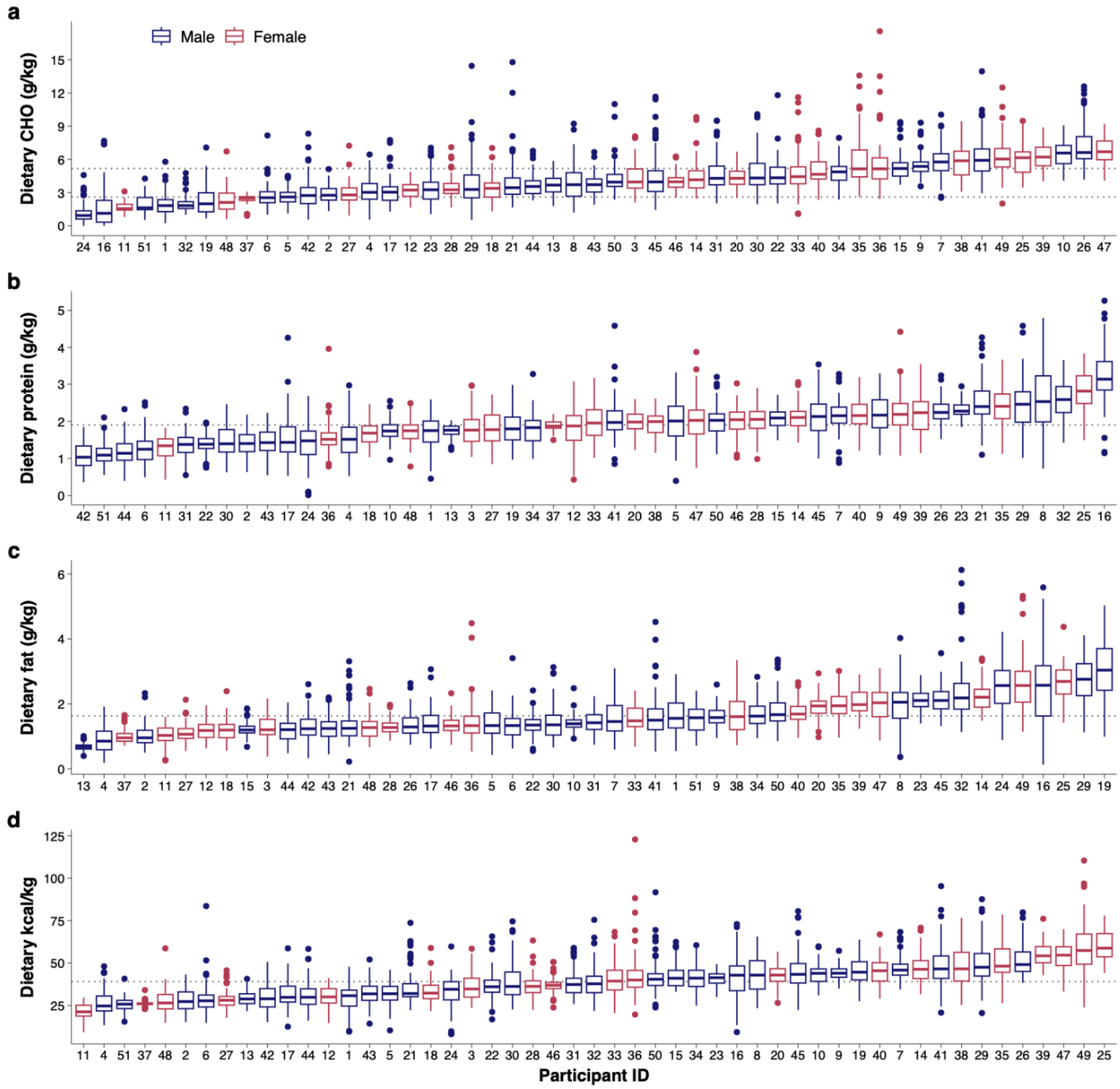


Figure G3. Box plot of daily macronutrient and energy intake for each participant, colored by sex. Dotted lines in (a) represent a median daily CHO intake for the 20th percentile (2.6 g/kg) and 80th percentile (5.2 g/kg), and in (b–d) represent the mean intake.

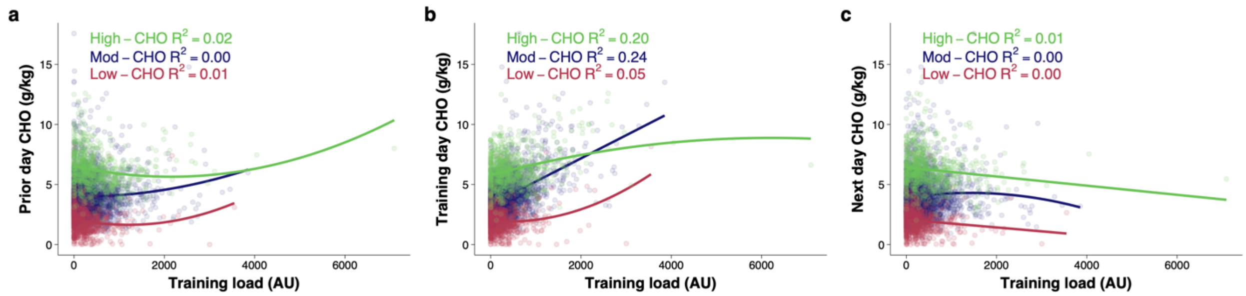


Figure G4. Daily carbohydrate (CHO) intake relative to training load for the day before (a), day of (b) and day following (c) a given training load (calculated as the product of session rating of perceived exertion (sRPE) and exercise duration in minutes) divided by 10, separated by habitual diet. Best-fit regression lines based on univariable linear mixed effects models are shown for each diet group, with fit indicated as marginal R^2. AU: arbitrary units.

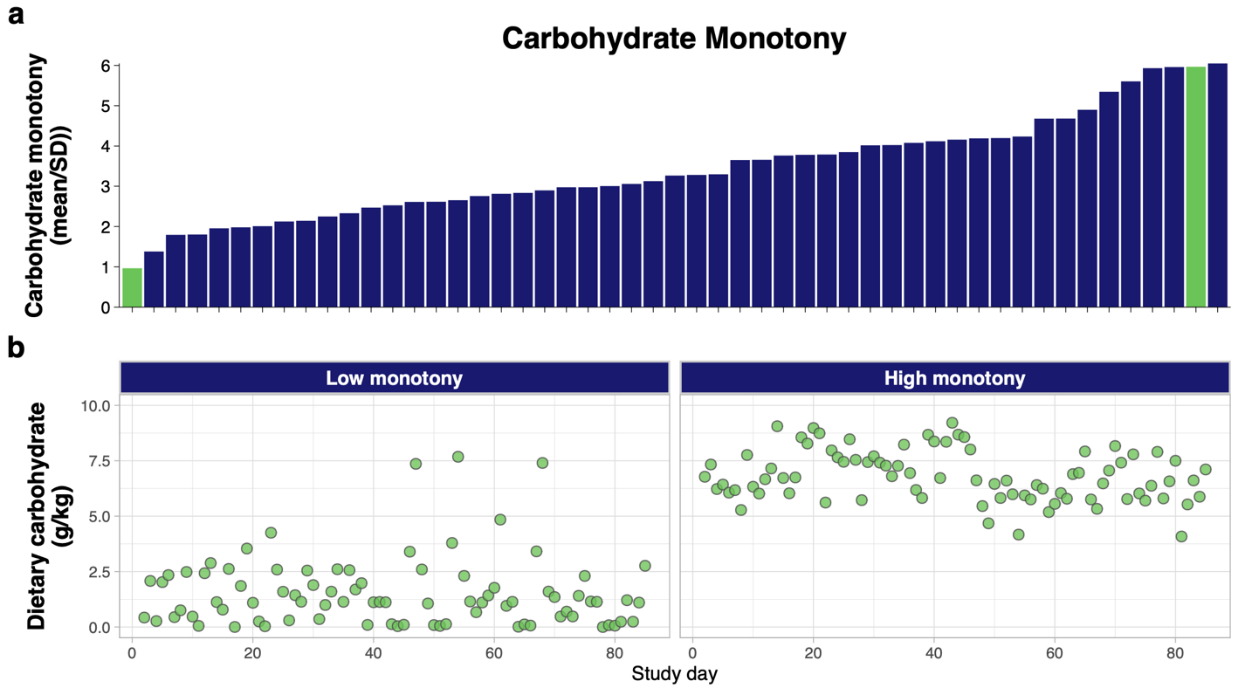


Figure G5. Carbohydrate monotony scores for each participant (a), and example data from participants with low and high monotony scores (b). Grey bars in (a) correspond to the data shown in (b). For (b), the x-axis represents the sequential days of the study tracking period

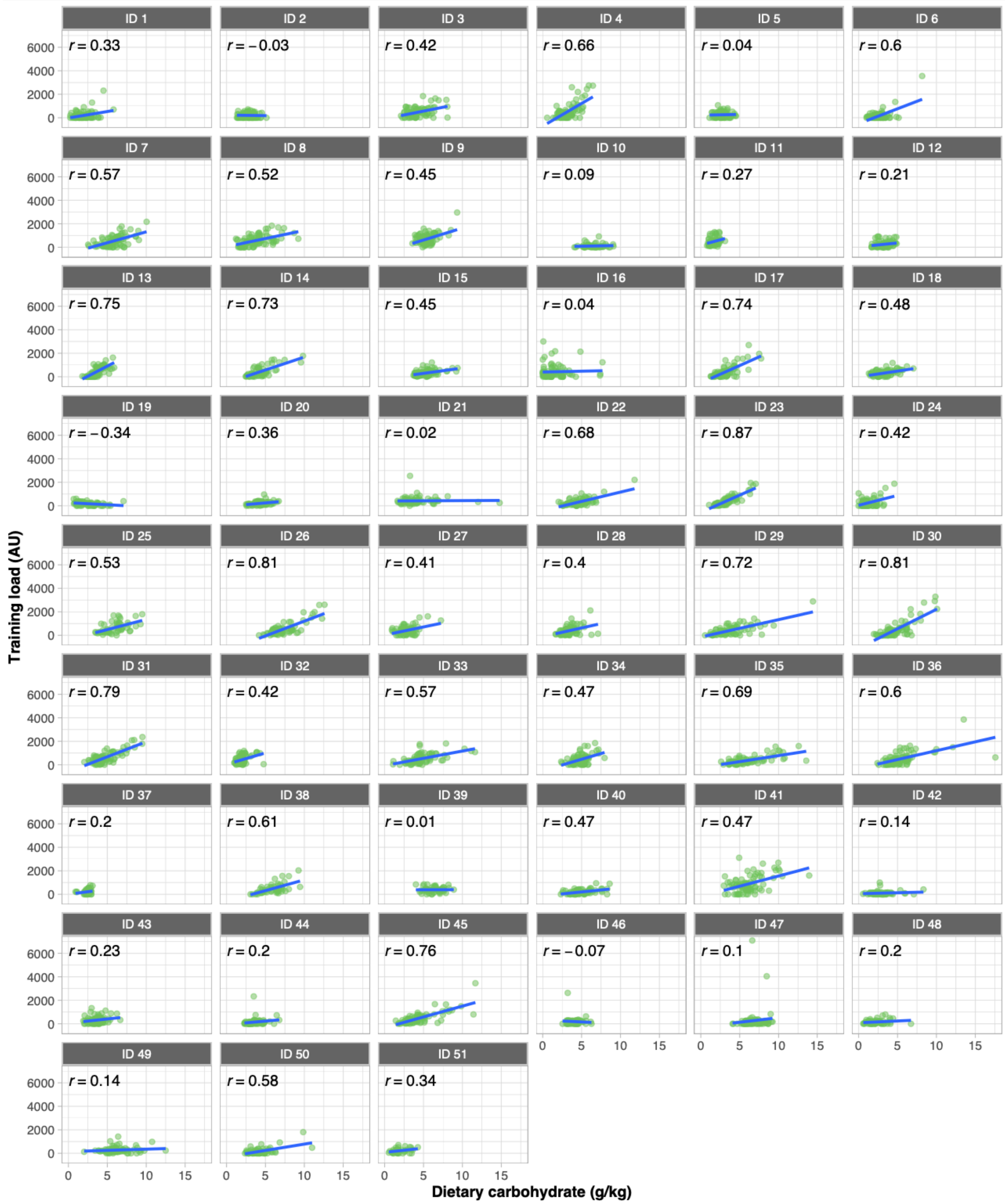


Figure G6. Relationships between daily carbohydrate intake (g/kg) and training load (product of session RPE and duration), for each participant. Pearson correlations are shown for each participant.

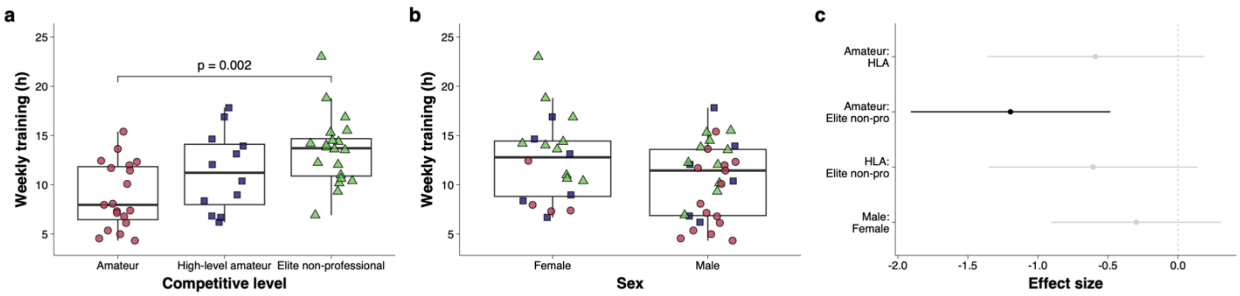


Figure G7. Boxplot of training volume, separated by competitive level (a) and sex (b). Individual participants are shown separated by shape and color. AU: arbitrary units. Effect sizes (c) shown in black correspond to pairwise comparisons with significant p-values after adjusting for multiple comparisons.

Appendix H: The influence of pre-exercise carbohydrate or protein ingestion on the metabolic and performance adaptations to steady-state endurance training in males

This 3-week training study was planned as a major part of the thesis. The abrupt COVID shutdown in August 2021 halted progress at a point when 4 participants had completed the study and 4 others were in progress. The 4 that were in progress finished the study remotely, using their home cycle trainer setups to perform the post-training time trial but missing the final biopsy and in-lab testing. Six additional participants were scheduled to begin the study in August/September, which also had to be cancelled. By December, we were still not allowed into the lab, and it was unclear what the lab access would be over the subsequent months. With the information available at the time, the choice was made to pause data collection and shift focus to another study in 2022 which could be performed remotely (Chapters 8–10). Therefore, this section consists of the methods and preliminary results from the 8 participants.

H.1 Introduction

A powerful interaction exists between dietary intake and exercise training. Accumulating research has shown that strategies which alter nutrient availability before and during endurance exercise can also impact training adaptations by enhancing or blunting the cellular responses to the exercise-induced perturbations [188]. Specific strategies to alter nutrient availability can include exercising in the overnight-fasted state or following a low- or high-carbohydrate (CHO) breakfast. An obvious, but important distinction, is that fasted training reduces overall energy and nutrient availability while low- and high-CHO meals provide energy intake before exercise. This can trigger different cell signaling responses that are responsive to the availability of CHO [3], fatty acids [5], and/or amino acids [6].

We recently reported nearly two-thirds of endurance athletes (63%) perform at least some training in the overnight-fasted state [21]. Despite the large number of athletes who report performing at least some training sessions in an overnight-fasted state, limited data are available on training adaptations as a result of exercise performed in the fasted state, particularly in trained athletes. Terada et al. [156] conducted the only study using trained cyclists, who performed 4 weeks of sprint interval training (SIT) either in the overnight-fasted state or while ingesting CHO before and during exercise, and found that the fasted group had a greater improvement in high-intensity aerobic endurance capacity, though neither group had improvements in VO_{2max}. Other studies using untrained participants have used 4 to 6-wk moderate-intensity continuous endurance training interventions performed either in the fasted state or with CHO ingestion before and during exercise with variable results [225]. Similar improvements in VO_{2max} and time-trial performance have been reported with fasted or fed training, while maximal rates of fat oxidation have been increased more with fasted training [157]. Interpreting the available evidence is challenging due to the use of untrained subjects in all but one of the training studies. Untrained participants can more easily show improvements following training, and may have overshadowed some of the subtle, yet significant, effects of fasted vs. fed training. For example, 2 to 6 weeks of HIIT in untrained and recreationally-active people has increased VO_{2max} by 7-9% [600, 601], and led to increased capillarization [602], improved skeletal muscle mitochondrial function [600, 603], and faster VO_2 kinetics [604]. However, in trained subjects, similar effects on VO_{2max} [605, 606], VO_2 kinetics [607], and mitochondrial function [606] have not been observed. Taken together, many athletes are performing exercise in the overnight-fasted state, but research in trained subjects is needed before evidence-based nutrition recommendations can be made.

An alternative to training in the overnight-fasted state is to consume a breakfast that includes protein but not CHO. This can reduce feelings of hunger [608], while potentially reducing muscle protein breakdown and still allow high levels of fat oxidation during exercise [16, 18, 375]. Aird et al. [171] reported similar training-induced increases in mitochondrial enzymatic activity and exercise performance in untrained participants following a 3-wk SIT program performed in the fasted state or following the ingestion of pre-exercise hydrolyzed or concentrated whey protein. Because overnight fasting reduces liver glycogen [8], it is also possible that ingesting amino acids (via protein consumption) prior to exercise may provide additional gluconeogenic precursors and aid in the maintenance of blood glucose during exercise, particularly compared with exercise in the fasted state. This may become more relevant as the duration of exercise extends, as performance differences between fed and fasted exercise become apparent beyond ~60 min of exercise [10]. Additionally, providing energy intake in the form of protein before exercise may be important for athletes doing a high volume of training, as exercising in the overnight-fasted state could more likely lead to a negative energy balance, which can be associated with hormonal and immune dysfunction [14], and pre-exercise protein intake can provide a more favorable amino acid profile during exercise compared with exercising in the fasted state [609]. In contrast to the large number of studies comparing CHO to a placebo prior to endurance exercise, there are no studies to date that have compared steady-state training performed in the fasted state with both CHO-rich and protein-rich pre-exercise meals.

Therefore, the aim of this study was to determine the effects of pre-exercise CHO or protein ingestion and fasted-state exercise on training adaptations (physiological, metabolic, and performance) following 3 weeks of steady-state endurance training. Study outcomes were intended to be both performance-based (e.g. time-trial performance) and mechanistic (e.g. changes in the activity of muscle oxidative enzymes), which have both shown to be improved following one [610], two [600, 604, 611], and three [612, 613] weeks of training. We hypothesized all three groups having similar improvements in VO_{2max}, ventilatory threshold, and time-trial performance, and fat oxidation will be increased most in the fasted group and least in the CHO group.

H.2. Methods

To determine the impact of pre-exercise nutrition strategies on the adaptations to endurance training, a 3-wk training protocol was utilized with four low-intensity training sessions per week performed following one of three different pre-exercise nutrition options (carbohydrate-rich meal, protein-rich meal, or fasted training), and two high-intensity interval training sessions per week performed following a standardized mixed-macronutrient breakfast.

Participants reported to the laboratory on 13 occasions over six weeks as follows: three pre-testing days separated by 48-72 h, one resting muscle biopsy prior to training, six in-lab training sessions (two times per week for three weeks), followed by two post-testing days separated by 48-72 h and one muscle biopsy after completing the training sessions. The study overview is shown in Table H1.

Visit 1. After obtaining written informed consent and completing a health screening, a graded exercise test was performed to determine maximal oxygen consumption (VO_{2max}). Participants cycled on an electronically-braked cycle ergometer (Excalibur Sport, Lode BV, Groningen, The Netherlands) at 60 W for three minutes followed by a 30 W per minute increase until volitional fatigue. Expired gas was collected and analyzed continuously using a computerized metabolic system with mixing chamber (TrueOne2400, ParvoMedics, Sandy, UT, USA), with the VO_{2max} recorded as the highest 15-s average. Peak power (W_{max}) was determined by the workload in the last completed stage plus the workload relative to the time spent in the last incomplete stage [power of completed stage + (30*(seconds at uncompleted stage/60)]. The first ventilatory threshold (VT_1) was identified as the work rate where the ventilatory equivalent for oxygen ($\dot{V}E.\dot{V}O_2^{-1}$) began to increase in the absence of changes in the ventilatory equivalent for carbon dioxide ($\dot{V}E.\dot{V}CO_2^{-1}$), and the second ventilatory threshold (VT_2) was identified as the first work rate at which $\dot{V}E.\dot{V}O_2^{-1}$ and $\dot{V}E.\dot{V}CO_2^{-1}$ increased alongside a reduction in $PetCO_2$ [614], with 15 W deducted to account for the lag in VO_2 during the incremental test [229].

Following a 10-min rest period, participants performed a familiarization 30-min time trial, where they were asked to produce as many watts as possible. This was performed using the Lode ergometer set to linear mode, with resistance set so that each participant's preferred cycling cadence generated a power equivalent to 95% of VT_2 as determined during the graded exercise test. Participants were instructed to increase/decrease their cadence to increase/decrease power. During all testing sessions participants will be blinded to their heart rate (HR) and power but were allowed to see the elapsed time during the time-trial.

Visit 2. Upon arrival to the lab in the overnight-fasted state, participants were given a standardized pre-testing meal providing 0.75 g/kg CHO, 0.13 g/kg protein, and 0.13 g/kg fat (peanut butter and jam sandwich). Thirty minutes after ingestion of the meal, participants began the sub-maximal cycling portion of the testing which included 3 x 5-min stages at a power equivalent to 60%, 80%, and 100% of VT_1 (VT 60, VT80, VT100, respectively), to measure substrate oxidation, energy expenditure, heart rate (HR), and perceived exertion (RPE). Expired gas was continuously measured, with average values during the final two minutes of each stage analyzed. Intensity was normalized based around the VT to reduce inter-subject variability in the physiological and perceived responses to exercise compared with using a percentage of VO_{2max} [232]. Relative exercise intensity for the three stages was 42.5 ± 2.8, 51.5 ± 4.3, and 61.0 ± 5.8 $\%VO_{2max}$ for VT 60, VT80, and VT100, respectively. Following a 5 min rest, they were asked to perform a 30-min time-trial. Prior to this session, participants were given a pre-measured standardized CHO-based food (e.g. rice or pasta) to consume with dinner containing 1.5 g per kg CHO. Participants were asked to refrain from exercise, caffeine, and alcohol 24 h before each visit and kept a 24 h food log to replicate dietary intake prior to each testing day. Instructions on keeping a food log were provided.

Visit 3. Participants reported to the AUT Millennium Sports Medicine clinic to receive a muscle biopsy obtained from the vastus lateralis using the microbiopsy technique [615].

Visit 4. To estimate technical and biological day-to-day variability, the same protocol for visit 2 was repeated and the average of the two pre-testing values was used as the baseline value [616].

Following baseline testing, participants were randomized into one of three pre-exercise nutrition groups:

- CARB – 1 g/kg CHO (e.g., 20 g jellybeans + 40 g white bread + ~500 mL CHO-drink)
- PROTEIN - 0.45 g/kg protein + 0.24 g/kg fat (e.g., ~25 g whey protein + ~ 35 g peanuts)
- FASTED – 500 mL water

*Training intervention***.** During the 3-week training intervention, participants performed four steady-state training sessions per week according to their treatment group (CARB, PROTEIN, or FASTED), and two high-intensity interval sessions per week following a standardized breakfast (Table H1).

Participants were provided all food in advance of each session, to be consumed 20-30 minutes prior to starting exercise. All continuous sessions were prescribed based on a HR of 95-100% VT1, with the four "intervention" sessions performed for 90 minutes on Monday, Wednesday, and Friday, and 120 minutes on a weekend day. During the 120-min sessions all participants consumed a mixed macronutrient snack 1 h into the exercise session (Frooze balls, 27 g CHO, 8 g protein, 19 g fat, Revive foods, New Zealand). All workouts were noted for duration, HR, and Watts from the participant's cycling computer, as well as session RPE (sRPE) using a 10-point scale [234].

Interval training sessions were performed twice weekly in the lab, with participants consuming the same mixed macronutrient snack ~30 mins before training (Frooze balls, 27 g CHO, 8 g protein, 19 g fat, Revive foods, New Zealand). After a 15-min self-selected warm up, the main workout was performed with the instruction to complete as much work during each interval as possible.

A protein bar (Horley's Protein 33 energy bar, 20 g protein, 20 g CHO, 4 g fat, Horley's, New Zealand) was provided and consumed following each of the four steady-state training sessions. This was done to ensure each group received at least a minimal amount of post-exercise CHO and protein ingestion, which has been shown to stimulate muscle protein synthesis rates during recovery from endurance exercise [617, 618], and either augment [619, 620] or have no impact on [621] endurance training adaptations. Participants were allowed to perform up to four hours per week of additional training (cycle, run, or swim) at a HR below VT_1, which was noted for time, HR, and sRPE.

Post testing. Post testing was performed in the same manner as pre-testing but without the additional familiarization trial, between three and seven days following the final training session and with 48 h between tests.

Table H1. Schedule for the 6-wk study.

Week	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
1		GXT + TT Familiarization			45 min VT1	30-min TT	
2				Biopsy	45 min VT1	30-min TT	Rest
3	90 min VT1	HIIT: 6x3 min	90 min VT1	SIT: 4x30 s	90 min VT1	120 min VT1	Rest
4	90 min VT1	HIIT: 7x3 min	90 min VT1	HIIT: 10x1 min	90 min VT1	120 min VT1	Rest
5	90 min VT1	HIIT: 8x3 min	90 min VT1	SIT: 4x30 s	90 min VT1	120 min VT1	Rest
6	Rest	GXT	90 min VT1	Biopsy	45 min VT1	30-min TT	

Weeks 1 and 2 are pre-testing weeks, weeks 3–5 are the main training block, and week 6 is post-testing. GXT: Graded exercise test, HIIT: High-intensity interval training, SIT: Sprint interval training, TT: Time trial. Sessions performed in the lab are highlighted in green.

Statistics

Due to the limited sample size only descriptive statistics are provided, shown as mean ± SD unless noted otherwise.

H.3. Results

Participants were classified as Tier 2 according to the framework established by McKay et al. [534]. Baseline characteristics are shown in Tables H2, H3, and H4, and dietary intake for two days during the training block is shown in Table H5. Figure H1 shows mean sRPEfor each participant, and Figures H2 and H3 show changes in performance-related metrics from pre- to post-intervention.

Table H2. Baseline characteristics of all study participants (n = 8)

	Age	VO_{2max} (mL/kg/min)	VO_{2max} (L/min)	Peak power (W)	Peak power (W/kg)	Weekly Training (h)
Mean	38.4	54.9	4.07	376	5.1	8.8
SD	11.2	5.4	0.35	42	0.7	1.7
Range	21–54	48.2–62.0	3.5–4.6	330–450	4.3–6.0	7–11

Table H3. Baseline characteristics of study participants separated by group.

	Age	VO_{2max} (mL/kg/min)	Peak power (W)	Peak power (W/kg)	Time-trial (W)	Time-trial (W/kg)	Peak fat ox (g/min)	Avg weekly training (h)	N
Carbohydrate	34.5 ± 2.1	55.7 ± 8.9	399 ± 72	5.2 ± 1.2	289 ± 62	3.7 ± 1.0	0.5 ± 0.0	9.5 ± 2.1	2
Fasted	39 ± 17	54.5 ± 4.6	361 ± 51	5.1 ± 0.6	248 ± 30	3.5 ± 0.4	0.3 ± 0.0	8.7 ± 2.1	3
Protein	40.3 ± 12	54.9 ± 6.3	376 ± 15	5 ± 0.6	265 ± 12	3.5 ± 0.5	0.5 ± 0.1	8.3 ± 1.5	3

Table H4. Baseline dietary intake of study participants separated by group.

	Kcal/d	Carb (g/kg)	Carb (%)	Protein (g/kg)	Protein (%)	Fat (g/kg)	Fat (%)	N
Carbohydrate	2950 ± 23	5.1 ± 0.0	51.5 ± 0.7	1.6 ± 0.2	17.0 ± 2.8	1.2 ± 0.4	31.5 ± 2.1	2
Fasted	2363 ± 469	4.2 ± 0.5	51.7 ± 4.7	1.7 ± 0.4	20.3 ± 2.3	1.0 ± 0.4	28 ± 4.6	3
Protein	3062 ± 1283	4.4 ± 2.2	43.7 ± 7.1	2 ± 0.3	22.3 ± 9.3	1.5 ± 0.8	32.7 ± 2.1	3

Table H5. Dietary intake of study participants midway through training, separated by group.

	Kcal/d	Carb (g/kg)	Carb (%)	Protein (g/kg)	Protein (%)	Fat (g/kg)	Fat (%)	N
Carbohydrate	2928 ± 221	4.4 ± 1.0	42.3 ± 10.0	1.6 ± 0.1	17.3 ± 2.4	1.5 ± 0.2	35.5 ± 2.1	2
Fasted	2432 ± 190	3.5 ± 0.6	40 ± 1.4	2.2 ± 0.4	26 ± 8.5	1.4 ± 0.4	34 ± 7.1	2
Protein	3027 ± 743	4.2 ± 2.5	40.5 ± 16	2 ± 0.3	20.8 ± 3.6	1.6 ± 0.2	39.7 ± 15	3

Values reflect the average of one "endurance" training day and one "HIIT" training day

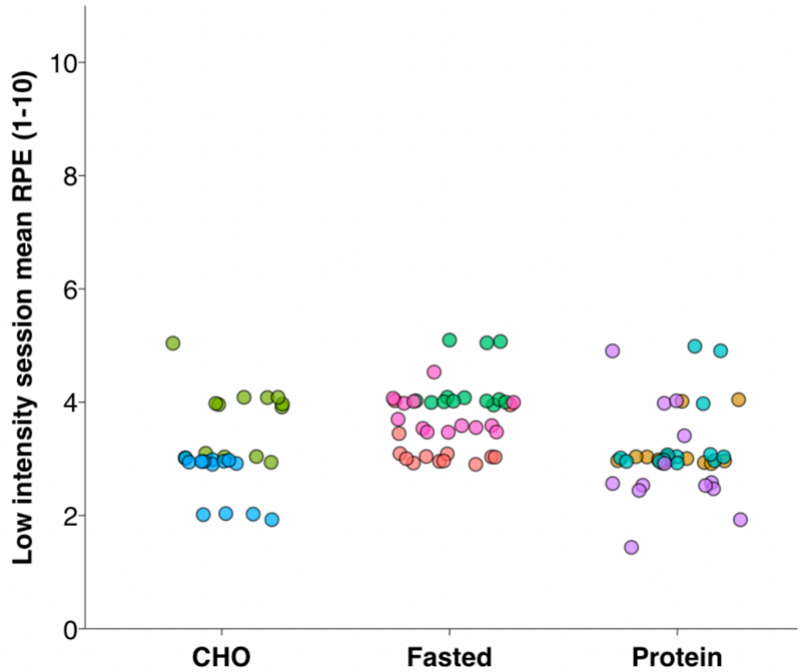


Figure H1. Individual data points for session rating of perceived exertion (RPE) during low-intensity training sessions, separated by group and colored by participant.

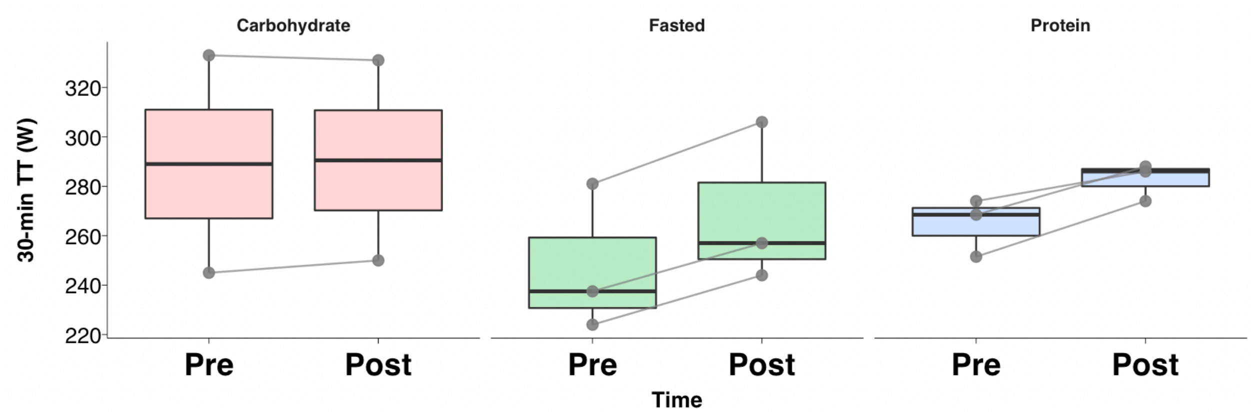


Figure H2. Box plot with individual data points of 30-minute time-trial (TT) performance before (Pre) and after (Post) 3 weeks of training, separated by group.

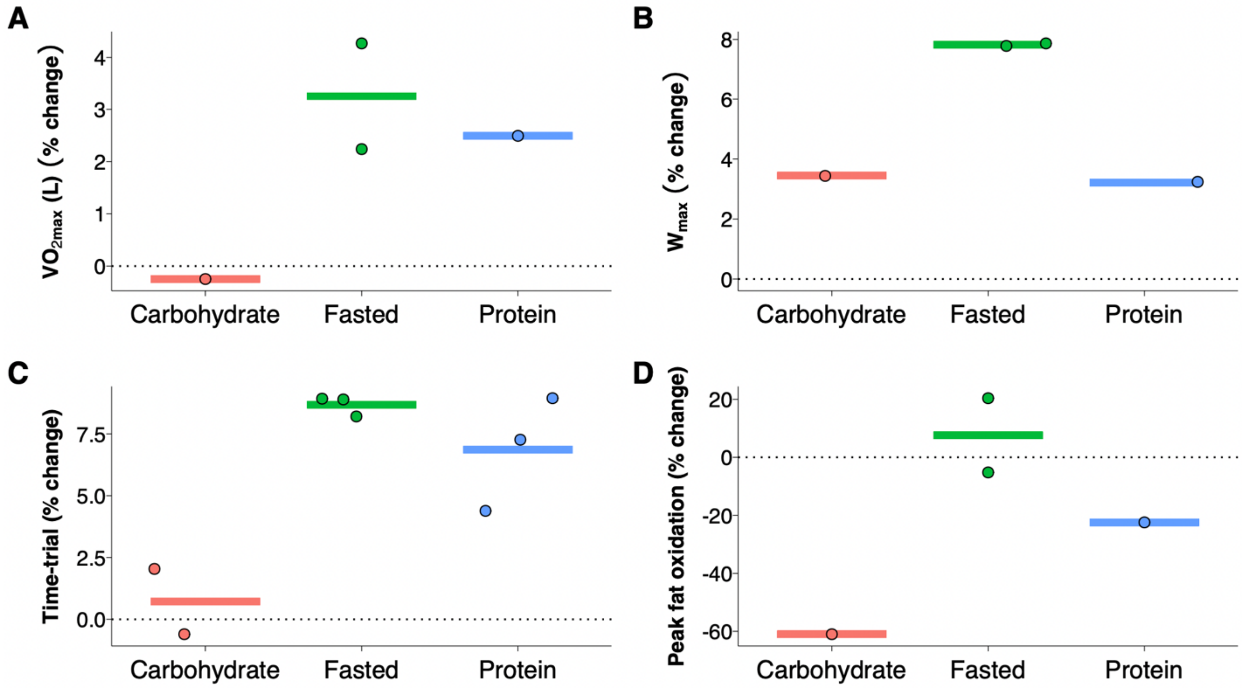


Figure H3. Mean and individual data points for percentage change pre- to post-training in VO_{2max} (A), peak power (W_{max}, B), time-trial performance (C), and peak fat oxidation during the graded exercise test (D), separated by group. Dashed lines represent no change from pre to post.

Appendix I. Predicting Daily Recovery During Long-term Endurance Training Using Machine Learning Analysis

This section includes an additional study from the data collected for Chapters 9–10, where the goal was to predict measures of daily recovery status using machine learning techniques.

This chapter contains the following publication:

Rothschild J, Stewart T, Kilding A, Plews D. A Predicting daily recovery during long-term endurance training using machine learning analysis (under review).

I.1 Abstract

Purpose: The aim of this study was to determine if machine learning models could predict the perceived morning recovery status (AM PRS), training feeling during exercise (exercise TF), and daily change in heart rate variability (HRV change) of endurance athletes based on training, dietary intake, sleep, HRV, and subjective wellbeing measures.

Methods: Self-selected nutrition intake, exercise training, sleep habits, HRV, and subjective wellbeing of 40 endurance athletes was monitored daily for 12 weeks (3,325 days of tracking). Global and individualized models were constructed using nine machine learning techniques and combined into an ensemble model at the group level, and with a single best algorithm chosen for individualized models. Model performance was compared with a baseline intercept-only model.

Results: Prediction error (root mean square error [RMSE]) was lower than baseline for the group models (12.1 vs. 17.5, 13.1 vs. 14.7, and 0.25 vs. 0.30 for AM PRS, exercise TF, and HRV change, respectively). At the individual level prediction accuracy outperformed the baseline model but varied greatly across participants (RMSE range 5.5 to 23.6, 5.7 to 18.2, and 0.05 to 0.52 for AM PRS, exercise TF, and HRV change, respectively).

Conclusion: Daily recovery measures can be predicted based on commonly measured variables, with a small subset of variables providing most of the predictive power. However, at the individual level the key variables may vary, and additional data may be needed to improve prediction accuracy.

I.2. Introduction

Coaches and athletes routinely monitor a range of metrics with the hope of gaining insight into how an athlete is responding to their training. These can include measures of training load (duration and intensity), heart rate variability (HRV), sleep, diet, and daily measures of subjective wellbeing, among others [622]. Despite careful planning, there can still be large discrepancies between the training stimulus prescribed by coaches and experienced by athletes [623]. Improved understanding of an athlete's training response could allow a training plan to be better tailored to an individual's needs, and help minimize the risks of non-functional overreaching, illness, and/or injury [624].

Training load refers to the combination of training volume and intensity, and can be measured and classified as either external or internal [545]. External training loads are characterized by measures such as distance, power, or speed, whereas internal loads reflect the relative physiological strain represented by heart rate (HR), blood lactate, and session rating of perceived exertion (sRPE) [624]. Internal load has been recommended as the primary measure when monitoring athletes, as it plays a pivotal role in determining training outcomes and can reflect variations in the stress response to a given external load due to other stressors such as extreme temperature, or accumulated training fatigue [545]. Coaches often use subjective wellness ratings by athletes for monitoring purposes, which are sensitive to fluctuations in training load [625]. However, much of the research on the relationship between training load, sRPE, and wellness has been in team sports and not endurance sports and has not accounted for potential interactions between training load, sleep, and dietary intake.

From a nutrition perspective, athletes and sports nutritionists are continually challenged to balance the nutritional demands of training while also optimizing body composition and promoting skeletal muscle adaptation. Increasing energy and carbohydrate intake during periods of intensified endurance training can attenuate symptoms of overreaching [568], yet many athletes routinely train in an overnight-fasted state and/or restrict carbohydrate intake before exercise [21]. The interaction between dietary intake and training quality in the context of longer-term, self-selected training and nutrition intake has not been well characterized. Although logistically challenging, investigating longer-term dietary intake during endurance training would help elucidate the role of self-selected nutrition intake on daily recovery during endurance training. The increased availability of valid and user-friendly mobile food-tracking apps can help facilitate data collection while minimizing disruption to an athlete's training and lifestyle.

The relationship between training, diet, sleep, and other lifestyle factors is complex, as many factors converge which may have non-linear and/or temporal relationships, with one often influencing the other. This underscores the need for more advanced tools for understanding athlete readiness and wellbeing. Machine learning techniques have been increasingly used in sports science, particularly in the context of multi-factorial data such as predicting injuries [626], training feeling scores [627], and subjective wellbeing [628], as well as in nutrition research to model complex nutrient interactions and address confounding variables [629]. However, to our knowledge machine learning has yet to be used to predict an endurance athlete's perceived recovery or HRV based on a combination of factors routinely monitored by athletes and coaches. Therefore, the goal of this study was to predict perceived AM recovery status, wellbeing during exercise, and daily change in HRV based on training metrics, dietary intake, sleep, HRV, and subjective wellbeing. Secondary aims were to highlight the most important variables for accurate prediction, and to examine the influence of factors that can tangibly be manipulated by coaches and athletes. It is hoped that such information can allow coaches to focus on a subset of variables with the strongest predictive power.

I.3. Methods

I.3.1 Study design

This observational study monitored the daily self-selected nutrition intake, exercise training, sleep habits, HRV, and subjective wellbeing of endurance athletes for 12 weeks. Throughout the study period, participants were free to perform any type of exercise and consume any type of diet. Measures of diet, training, sleep, HRV, and subjective wellbeing were recorded daily. Models were created for three primary outcome variables — two subjective measures (AM Perceived

Recovery Status (PRS) score, and Training Feeling (TF) during exercise score), and an objective measure of change in resting HRV from the previous day (HRV change). The study was open to male and females aged 18 or older who train at least seven hours per week, were using a smartphone app to track their dietary intake at least five days per week, capture HRV daily, and track sleep duration using a wearable device. All study protocols and materials were approved by the Auckland University of Technology Ethics Committee (22/7), and all participants provided informed consent prior to starting the study. Data collected from the same athletes related to training load and carbohydrate periodization have been reported elsewhere [571].

I.3.2 Participants

Fifty-five endurance athletes (61.8% male, aged 42.6 ± 9.1 years, training 11.6 ± 3.9 hours per week) took part in the study. The primary sports represented were triathlon (67.3%), running (20.0%), cycling (10.9%), and rowing (1.8%). The self-reported competitive level included professional (2.6%), elite non-professional (qualify and compete at the international level as an age-group athlete, 34.6%), high-level amateur (qualify and compete at National Championship-level events as an age-group athlete, 25.6%), and amateur (enter races but don't expect to win, or train but do not compete, 37.2%) athletes.

I.3.3 Assessment of self-reported exercise

All exercise was recorded in Training Peaks software (TrainingPeaks, Louisville, CO, USA). Each session was noted for modality (e.g., bike, run, swim), total time, and session rating of perceived exertion (sRPE, [535]) using the Borg CR100® scale, which offers additional precision compared with the CR10 scale [573]. Participants were instructed to rate their perceived effort for the whole training session within 1-h of exercise, although sRPE scores are temporally robust from minutes to days following a bout of exercise [535]. As an indicator of the type of feedback that occurs between athletes and coaches on a daily basis, participants also rated a subjective (TF) score from 0–100 using a customized scale based on the Perceived Recovery Status (PRS) scale [580]. Athletes were instructed to consider how they felt during the training session, which was distinct from the sRPE. For example, someone could feel very good during a hard workout and very poor during an easy workout, or vice-versa. Additionally, participants noted the amount of carbohydrate (in grams) consumed within the 4-h pre-exercise window.

I.3.4 Assessment of self-reported dietary intake

Details of dietary assessment have been described elsewhere [571]. Briefly, participants were instructed to maintain their typical dietary habits and record all calorie-containing food and drink consumed for the duration of the 12-week study, using the MyFitnessPal application (www.myfitnesspal.com). Due to previous habitual use, three participants used the Cronometer application (www.cronometer.com) and one participant used the Carbon application (www.joincarbon.com). Incomplete days of tracking (2.2 ± 4.6% of days per participant) were removed from the data, and analysis of the calorie intake trend over time was performed for each participant as an additional check of compliance as previously described [571]. Four participants were excluded from the analysis due to the detection of a downward trend in daily calorie intake that could not be explained by changes in training load or body weight.

I.3.5 Assessment of resting HRV and sleep

Resting HRV was recorded daily, and analyzed using the natural logarithm of the square root of the mean sum of the squared differences (Ln rMSSD) between R–R intervals [630]. For participants using Oura ring (Oura Health, Oulu, Finland) or Whoop straps (Whoop, Inc., Boston, USA) nocturnal HRV was used, whereas measurements were taken upon waking for those using the HRV4Training (www.hrv4training.com), Elite HRV (Elite HRV, Inc., Asheville, USA), or ithlete (HRV Fit Ltd. Southampton, UK) smartphone apps. High correlations have been reported between nocturnal and morning HRV measurements [631]. Nightly sleep duration was recorded using wearable devices, which included Oura ring, Whoop strap, Applewatch, Fitbit, and Garmin models. These consumer-grade devices offer adequate accuracy in detecting sleep-wake times, but not sleep staging [574, 575, 577, 578].

I.3.6 Assessment of subjective wellbeing

Each morning participants answered four questions related to subjective wellbeing, which have been shown to respond consistently to training-induced stress [632]. The PRS scale [580] was used to measure overall recovery with athletes manually typing a number into Training Peaks software. The 100-point version of the scale was used, which has been shown discriminate between answers better than the 10-point scale [573]. In addition, ratings of life stress (1–7), sleep quality (1–7), and muscle soreness (1–10) were also recorded into the software each morning. Participants were familiarized with all scales prior to starting the study. In addition, participants were asked to record their body mass at least one time per week.

I.3.7 Data preparation

Training load was calculated for each workout as the product of sRPE and duration of exercise in minutes [538], divided by 10 to account for the 100-point scale. Exercise was summed into daily totals for workout duration and training load, along with coded variables for modality of workout (e.g., swim, bike, run, strength, other) and if any training was performed in the fasted state. Because dietary protein and fat ingestion have minimal effects on substrate oxidation [633], fasted training was defined as consuming < 5 g of carbohydrate in the 4-h pre-exercise window. For multiple exercise sessions in a single day, a weighted mean based on the duration of each session was used to calculate a single daily value for pre-exercise carbohydrate ingestion and TF score. External load metrics such as HR, power, or pace were not collected because many athletes undertake activities that can't be quantified on a common scale such as strength training, yoga, or swimming without a HR monitor. This was deemed acceptable because sRPE is considered to be a valid and reliable method for calculating training load across modalities [538]. Seven-day rolling measures for training monotony (a measure of day-to-day variability in the weekly training load, calculated as average daily load divided by the standard deviation) and training strain (product of total weekly training load and training monotony) were calculated [538]. Exponentially weighted 7-d moving averages of training load, HRV, and resting HR were calculated to account for residual effects of recent training [634]. A sleep index value was calculated as the product of sleep duration and subjective sleep quality [581].

Participants were excluded from the analysis if they trained an average of less than 6 h per week (n = 8) or did not log at least 85% of the required data points (n = 3). Participants who did not complete the full 12 weeks due to illness, injury, or drop-out but completed at least 6 weeks of tracking were included in the analysis (n = 11). Among participants included in the analysis (n = 40), 2.5 ± 1.7 % of data points were missing. Missing values were imputed at the individual level using multiple linear regression and nearest neighbor algorithms for diet and training measures, and using median values for other variables [539].

To increase the available options for modeling and interpretation, the data were transformed from a time series into independent observations. A time series is a sequence of data points at equally spaced points in time and ordered chronologically. Time series data cannot be analyzed with common techniques such as linear modeling if the day-to-day observations are correlated with observations at previous time points (i.e., auto-correlated) and are not independent of each other, as key assumptions of linear regression are violated [584]. To account for this, a process of Markov unfolding [635] was used. This is based on the Markov assumption, whereby the values in any state are influenced only by the values of the immediately preceding or a small number of immediately preceding states [636]. Data were analyzed for autocorrelation, and it was determined that a maximum of seven previous days could have a relevant influence on a given day's data. This makes logical sense, as many behavioral and training schedules follow a weekly cycle. The process of Markov unfolding entails copying the columns of the original dataset, shifting them down by one row, and stacking them as new columns on the right of the dataset (labeled as lag 1). This is repeated with shifts of 2–n, where n is the number of previous days to be included. The first n rows from the beginning of the dataset are discarded, as there are missing values for some of the lags. This results in a dataset that is a few rows shorter, but n + 1 times wider than the original dataset and the observations can be treated as totally independent, allowing the use of any modeling approach that assumes independent data. This approach to making the dataset ~7x wider can result in the curse of dimensionality, whereby the test error tends to increase as the dimensionality of the problem (i.e. the number of predictors) increases

[584], but this may be mitigated by the use of algorithms which use regularization to conduct feature selection [539]. It should be noted that the variables created as 7-d rolling averages would allow the previous 14 days of information to be provided to the model (i.e., a 7-d average from 7 days ago). All analyses were carried out with R version 4.0.3 (The R foundation for Statistical Computing, Vienna, Austria). Descriptive statistics are provided as mean ± SD.

I.3.8 Models

A series of models were built for the three outcomes of interest — AM PRS score, exercise TF score, and daily change in HRV, at both the group level (full dataset) and for each individual participant. To reduce multicollinearity, highly correlated predictors (Pearson correlation > 0.85) were removed from the dataset prior to training each model by removing the one with the largest mean absolute correlation with the rest of the data [539]. For each outcome, models were made using the primary subset of variables (MAIN), and a subset of variables that can tangibly be manipulated by athletes/coaches (ACTIONABLE). Included variables are shown in Table I1. At the group level, ensemble models (described below) for each outcome were made using the two variable subsets (MAIN and ACTIONABLE). For comparison with the ensemble models, three linear regression models were created — a least absolute shrinkage and selection operator (LASSO) regression model using the MAIN set of variables, a linear mixed model using the 5 variables with the highest importance scores from the MAIN ensemble as fixed effects and participant ID specified as a random effect (Top 5 from group MAIN), and an intercept-only model as a baseline comparison that was simply predicting the mean value. The LASSO was chosen as a linear model that can be used with a large number of variables due to its built-in feature selection process, the mixed model was chosen to determine how a very limited subset of variables would perform, and the intercept-only baseline was used to establish a realistic upper bound for the root mean squared error (RMSE), as useful prediction models should have lower RMSE values. At the individual level, in addition to the MAIN and ACTIONABLE models, linear models were made consisting of the 5 variables with the highest importance from the MAIN group model (Top 5 from group MAIN), the 5 variables with the highest importance from their own respective MAIN model (Top 5 from individual MAIN), and an intercept-only model as a baseline comparison.

For group models, data were split into a training set (75%) and a testing set (25%). To avoid data leakage [637], all observations from a given participant were assigned to either the training or testing set, and preprocessing steps such as standardization and removal of highly correlated variables were performed only using the training set. Nine different learning algorithms, including parametric and non-parametric methods, were trained for each model using the *Tidymodels* ecosystem in R. These included three linear regression models with regularization (Ridge, LASSO, and LASSO with interaction terms), three non-linear regression models (Multivariate Adaptive Regression Spline (MARS), Support Vector Machine (SVM), and K-Nearest Neighbors (KNN)), two ensembles of decision trees (XGBoost and Light GBM), and a single layer neural network (NNET). Ten-fold cross-validation was repeated five times for tuning parameter optimization, and the tuned models were combined into a stacked ensemble using the *Stacks* R package. Stacking is a method that takes the outputs of many models and combines them to generate a new ensemble model [638]. Predictions from each candidate in the ensemble are weighted based on a stacking coefficient, generated by the betas of a LASSO regression model fitting the true outcome with the predictions given in the data stack. Model performance (RMSE and R-squared) was calculated using the hold-out (testing) dataset. Variable importance (a measure of the strength of the relationship between observed values of the variable and the observed response) for the group ensemble models was determined using a permutation-based approach, which measures a feature's importance by calculating the increase of the model's prediction error after permuting the feature [639]. In addition to variable importance, partial dependence profiles were created to aid model interpretation, which show how the expected value of a model prediction changes after accounting for the average effect of all other variables [639].

The individual models used the same algorithms mentioned above, except for the NNET. Ten-fold cross-validation was repeated ten times, with the best algorithm and parameter set chosen based on the lowest RMSE. Accuracy metrics were calculated using 500 bootstrap resamples. Variable importance was calculated using a model-based approach [640], and scaled so the total importance summed to 1. Because individual models could use different algorithms for each participant, scaling the importance allowed a summarization of importance across different model types by taking the mean values. To compare performance among the five types of individual models (best model MAIN, best model ACTIONABLE, Top 5 from group MAIN, Top 5 from individual MAIN, and baseline intercept-only model), a linear mixed model was used, with RMSE from each model used as the dependent variable, model type specified as a fixed effect, and participant ID specified as a random effect. Estimated means were calculated using the *Emmeans* R package, and comparisons made using the Tukey test.

Table I1. Overview of variables included in the modeling

Category	Variables
Training	Exercise duration (min), modality, fasted training (yes/no), number of workouts per day, number of consecutive training days, session rating of perceived exertion (sRPE, highest for a single session each day and a duration-based weighted average for the day), training load (TL; min x sRPE), 7-d exponentially weighted and non-weighted moving average of TL, 7-d highest single-day TL, training monotony (weekly mean TL/weekly SD), training strain (weekly load x monotony), training feeling (TF), day of the week
Dietary	Total kcal, carbohydrate (CHO, g/kg), fat (g/kg), protein (g/kg), pre-exercise CHO (g), 3-d and 7-d moving averages of CHO, fat, protein, and kcal intake, 7-d moving average standard deviation of daily CHO intake and CHO monotony (weekly mean intake/ weekly SD)
Sleep	Sleep duration (hours), *sleep index (sleep duration x quality),* 7-d moving average sleep duration and *sleep index*
Subjective measures	*Perceived Recovery Status (PRS)*, soreness, life stress, *sleep quality*
Non-exercise	*Resting HRV* and *resting HR* (daily, change from previous day, and 7-d moving averages of each)
Planned interactions	**AM PRS:** 7-d average TL * 3-d average CHO intake 7-d training monotony * 3-d average CHO intake **Exercise TF**: Pre-exercise CHO intake * TL Prior day CHO intake * prior day TL 7-d average CHO intake * 7-d average TL **HRV:** Prior day TL * sleep duration Prior day TL * prior day AM PRS score
Subject characteristics	Participant ID, age, HRV app, sleep app, percentage of missing data, competitive level, primary sport, training age, body weight
Top 5 Variables from group MAIN	**AM PRS:** AM PRS 1 and 2 days ago, soreness, life stress, sleep quality **Exercise TF**: AM PRS, AM PRS 2 days ago, pre-exercise CHO, training strain 7 days ago, exercise duration (min)

	HRV: 7-d avg HRV change 1 day ago, HRV change 1, 2, and 7 days ago, HRV 1 day ago

Italics indicate variables that were removed from the ACTIONABLE models.

I.4. Results

A total of 3,325 days of tracking were included in the analysis (83.1 ± 9.6 per participant). Average participant training volume was 11.9 ± 3.5 h per week. Mean daily dietary intake was 38.9 ± 8.6 kcal/kg, 4.0 ± 1.5 g/kg carbohydrate, 1.9 ± 0.4 g/kg protein, and 1.7 ± 0.5 g/kg fat. Average sleep duration was 7.5 ± 0.7 hours per night. Values for the three main outcomes were 61.7 ± 18.5, 62.2 ± 15.7, and 0.0 ± 0.3 for AM PRS, exercise TF, and HRV change, respectively. MAIN group models demonstrated improved accuracy compared with the baseline model (Table I2). Accuracy of the individual models was improved compared with the baseline models (Table I3) but varied more than 5-fold across participants (Figure I1). Figures I2–4 show the ten variables with the highest importance from the group modeling for AM PRS (Figure I2), Exercise TF (Figure I3), and HRV change (Figure I4), as well as a scatterplot comparing predicted vs. actual values (inset into each figure), and partial dependence plots showing how the expected value of a model prediction changes based on these variables. Figure I5 shows the ten variables with the highest mean importance scores across all participants for the individual MAIN models.

Table I2. Accuracy of Group Models

Outcome	Model	Variables	RMSE [95% CI]	R^2
AM PRS	Ensemble	MAIN	12.1 [11.5, 12.7]	0.52
AM PRS	LASSO	MAIN	12.9 [12.3, 13.6]	0.45
AM PRS	LMM	Top 5 from MAIN Ensemble	13.6 [13.0, 14.3]	0.41
AM PRS	Ensemble	ACTIONABLE	16.4 [15.6, 17.3]	0.16
AM PRS	Baseline	Intercept only	17.5 [16.7, 18.4]	NA
Exercise TF	Ensemble	MAIN	13.1 [12.4, 13.8]	0.23
Exercise TF	LMM	Top 5 from MAIN Ensemble	13.1 [12.4, 13.8]	0.22
Exercise TF	LASSO	MAIN	13.2 [12.5, 13.9]	0.20
Exercise TF	Baseline	Intercept only	14.7 [14.0, 15.5]	NA
Exercise TF	Ensemble	ACTIONABLE	14.9 [14.1, 15.7]	0.02
HRV change	Ensemble	MAIN	0.25 [0.24, 0.26]	0.40
HRV change	LASSO	MAIN	0.25 [0.24, 0.26]	0.41
HRV change	LMM	Top 5 from MAIN Ensemble	0.26 [0.25, 0.27]	0.33

HRV change	Baseline	Intercept only	0.30 [0.29, 0.32]	NA
HRV change	Ensemble	ACTIONABLE	0.32 [0.31, 0.34]	0

AM PRS: AM Perceived Recovery Status, Exercise TF: Exercise Training Feeling score, LASSO: linear regression model with regularization, LMM: Linear Mixed Model with participant ID specified as a random effect, RMSE: Root Mean Squared Error, in units of the original measurement (0–100 for AM PRS and Exercise TF, and Ln rMSSD for HRV change).

Table I3. Accuracy of Individual Models

Outcome	Model	Variables	RMSE [a]	R^2
AM PRS	Linear	Top 5 from individual MAIN	12.1 ± 4.3[a]	0.31 ± 0.17
AM PRS	Linear	Top 5 from group MAIN Ensemble	12.4 ± 4.1[a]	0.28 ± 0.16
AM PRS	*	MAIN	12.8 ± 4.2[ab]	0.23 ± 0.13
AM PRS	*	ACTIONABLE	13.3 ± 4.4[b]	0.19 ± 0.11
AM PRS	Baseline	Intercept only	14.2 ± 4.3[c]	NA
Exercise TF	Linear	Top 5 from individual MAIN	11.9 ± 3.6[a]	0.23 ± 0.10
Exercise TF	*	ACTIONABLE	12.6 ± 3.3[b]	0.11 ± 0.07
Exercise TF	Baseline	Intercept only	12.7 ± 3.3[b]	NA
Exercise TF	*	MAIN	12.8 ± 3.8[b]	0.12 ± 0.07
Exercise TF	Linear	Top 5 from group MAIN Ensemble	12.8 ± 3.6[b]	0.14 ± 0.11
HRV change	Linear	Top 5 from individual MAIN	0.20 ± 0.09[a]	0.59 ± 0.15
HRV change	*	MAIN	0.23 ± 0.11[b]	0.41 ± 0.19
HRV change	Linear	Top 5 from group MAIN Ensemble	0.24 ± 0.09[b]	0.37 ± 0.09
HRV change	Baseline	Intercept only	0.30 ± 0.12[c]	NA
HRV change	*	ACTIONABLE	0.31 ± 0.12[c]	0.08 ± 0.05

AM PRS: AM Perceived Recovery Status, Exercise TF: Exercise Training Feeling score, RMSE: Root Mean Squared Error, in units of the original measurement (0–100 for AM PRS and Exercise TF, and Ln rMSSD for HRV change). *For MAIN and ACTIONABLE models, values are from the single best-performing algorithm (LASSO: 30%, SVM: 30%, XGBoost: 23%, Light GBM: 12%, KNN: 4%, Ridge: 1%, and MARS: 0.4% of models). All metrics were established using 500 Bootstrap resamples. [a] Within each outcome, models not sharing any letter are significantly different by the Tukey test at the 5% level of significance.

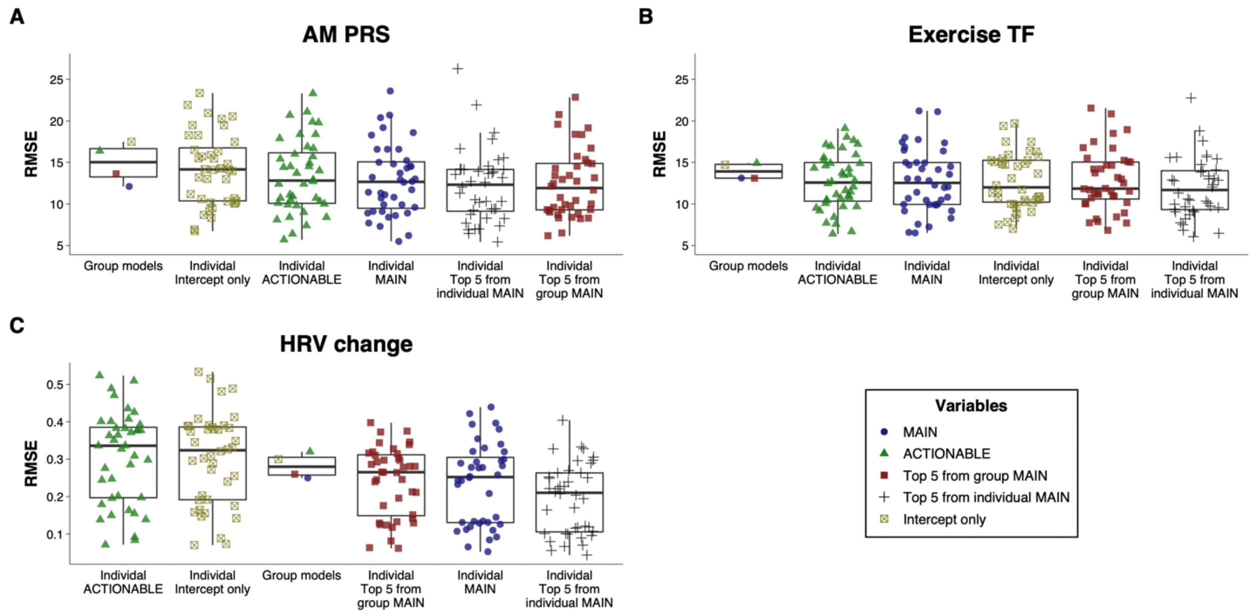


Figure I1. Root mean squared error (RMSE) of the group and individual models, separated by the variable set included in the model and ordered by mean RMSE values. For group models, MAIN and ACTIONABLE models represent the ensemble model, and "Top 5 from MAIN" represents a linear mixed model with the top 5 features from the MAIN group model based on variable importance scores. For individual models, "Top 5 from group MAIN" represents a linear model with the same top 5 features from the MAIN group model, and "Top 5 from individual MAIN" represents a linear model with the top 5 features from each participant's MAIN model. RMSE values were determined using out-of-sample data for group models and using 500 Bootstrap resamples for individual models.

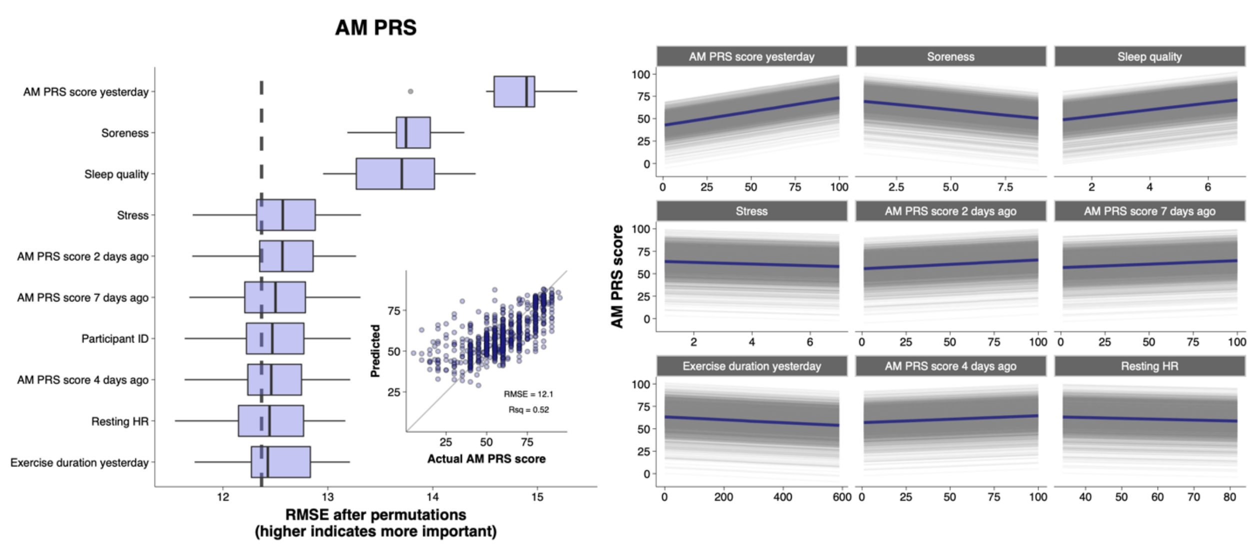


Figure I2. AM PRS group model results from the best performing model (ensemble using MAIN variables). Top 10 most important variables based on permutation-based feature importance are shown in a boxplot, along with a scatterplot of actual vs. predicted values on an out-of-sample dataset (inset), and partial dependence plots for the top 9 continuous variables (right), where colored lines represent the average of all observations shown individually as the grey lines. The vertical dashed line in the boxplot represents the full model RMSE from the training dataset.

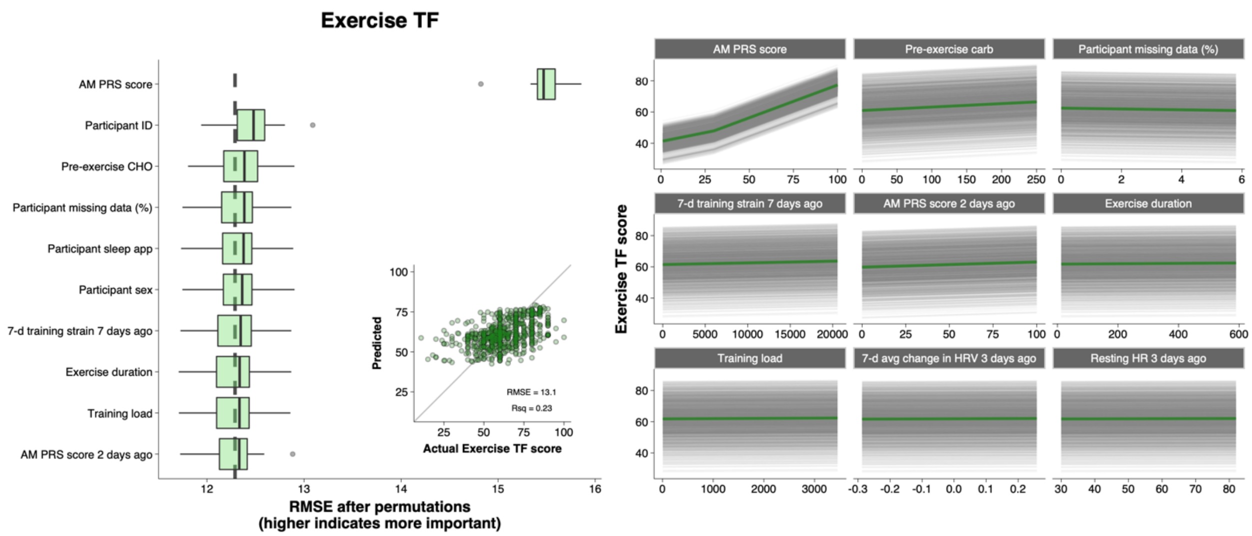


Figure I3. Exercise TF group model results from the best performing model (ensemble using MAIN variables). Top 10 most important variables based on permutation-based feature importance are shown in a boxplot, along with a scatterplot of actual vs. predicted values on an out-of-sample dataset (inset), and partial dependence plots for the top 9 continuous variables (right), where colored lines represent the average of all observations shown individually as the grey lines. The vertical dashed line in the boxplot represents the full model RMSE from the training dataset.

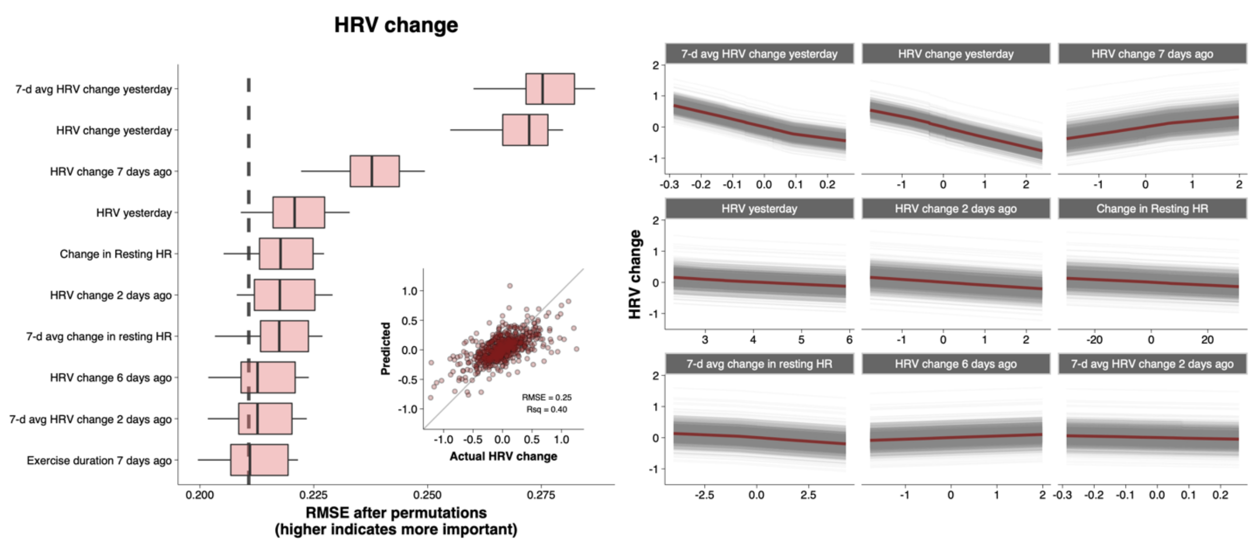


Figure I4. HRV change group model results from the best performing model (ensemble using MAIN variables). Top 10 most important variables based on permutation-based feature importance are shown in a boxplot, along with a scatterplot of actual vs. predicted values on an out-of-sample dataset (inset), and partial dependence plots for the top 9 continuous variables (right), where colored lines represent the average of all observations shown individually as the grey lines. The vertical dashed line in the boxplot represents the full model RMSE from the training dataset.

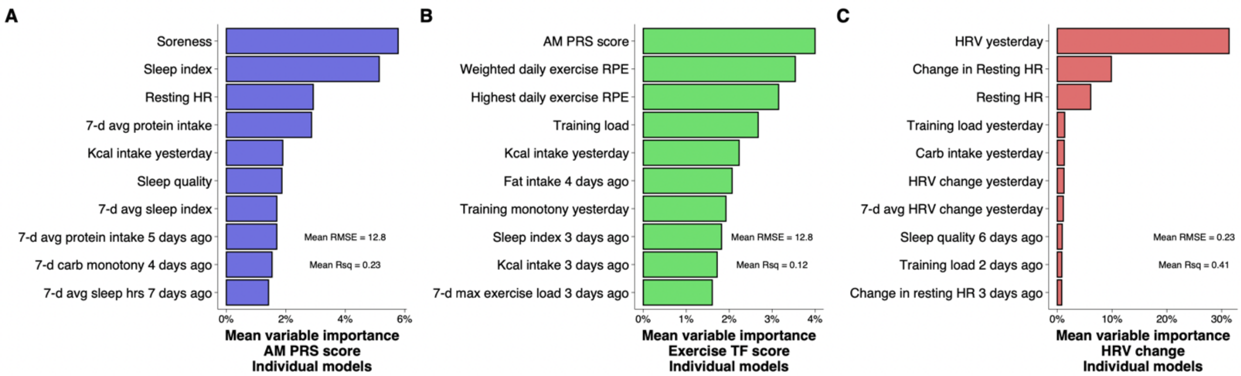


Figure I5. Variables with the highest mean variable importance scores from the individual MAIN models for AM PRS (A), Exercise TF (B), and HRV change (C) models. Root mean squared error (RMSE) and R-squared (Rsq) values shown are the average of the individual MAIN models.

I.5. Discussion

Athlete monitoring can help coaches better understand how an athlete is adapting to a training program, and minimize the risk of developing non-functional overreaching, illness, and/or injury [624]. This study utilized a novel approach to monitoring endurance athletes throughout 12 weeks of self-selected training to better understand the factors that can predict an athlete's day-to-day recovery and wellbeing. Key findings from this study are 1) day-to-day recovery measures can be predicted based on commonly measured variables, 2) a small subset of variables offers similar predictive capability as the full dataset, 3) predictive accuracy varies greatly at the individual level, and 4) remote monitoring of multiple training, diet, sleep, and recovery measures can be performed throughout longer-term training in real-world environments.

I.5.1 Model Performance

All models constructed using the MAIN variables outperformed the baseline model, demonstrating utility of the tracked variables. Unexpectedly, performance at the group level of the ACTIONABLE models was poor, indicating they alone do not offer any added value for predicting the recovery markers used in our study. As shown in the scatterplots in Figures 2–4, prediction accuracy was generally worse for scores on the upper and lower ends, likely due to the small number of extremely low or high values with which to train the models. At the individual level, the most striking finding was the large degree of variation in model performance (Figure I1). This suggests key variables may be missing from the models that could disproportionately affect some athletes more than others. For example, alcohol intake, acute illness, and the menstrual cycle are known to influence HRV [641]. Moreover, participants spent only ~10% of their waking hours engaged in exercise, indicating the potential for many non-exercise factors to influence recovery and wellness such as walking, job-related physical activity/stress, massage, sauna, and/or ice baths. Future studies could expand on the current work by accounting for some or all of these additional factors.

At the group level, the ensemble models displayed the best overall performance, but these algorithms can be computationally expensive and slow to run. Because of this, two linear regression models were constructed as practical alternatives. The LASSO regression model is well suited to handle a large number of predictors because it uses regularization to reduce estimated coefficients towards zero [584], essentially removing non-needed variables from the model. We also used a linear mixed model of the top 5 variables based on variable importance scores, with participant ID included as a random effect. Although the ensemble outperformed the other models for AM PRS score, the LASSO and linear mixed model performed well on out-of-sample data and the three models had roughly the same accuracy for exercise TF and HRV change (Table I2).

When constructing individual-level MAIN and ACTIONABLE models, the single best model from the suite of machine learning algorithms was chosen as the accepted model. Two linear regression models were then made, using the top 5 variables from the group and individual MAIN models. The best performance was achieved from the linear models with the top 5 individual variables (Table I.3), highlighting the importance of a very small subset of variables that coaches and practitioners could pay closer attention to. Importantly, the difference in performance between linear models of the top 5 from individual and top 5 from group models highlights the fact that the most important variables for each athlete will be different (Table I.3). From a practical perspective, a prudent approach might be to start by monitoring a wide array of variables, and reduce the number based on feedback from initial models.

I.5.2 Variable Importance

The variable importance calculations of the group-level models revealed a small number of variables having a disproportionately large influence on prediction accuracy (Figures I2–4). This finding is corroborated by the generally good performance of the linear mixed models, which included only the top 5 variables, and implies the ability for coaches and practitioners to focus on just a few of the many variables that are routinely monitored. These include muscle soreness, life stress, and sleep quality for AM PRS scores, pre-exercise CHO, training strain, exercise duration, and AM PRS for exercise TF scores, and the changes in HRV over recent days for predicting the current day's change in HRV. However, when trying to predict at the individual level, the chosen variables should be specific to the individual. The aggregated importance scores from the individual models shown in Figure I.5 are far more diverse than at the group level, supporting the notion that the most important variables vary among different athletes. For example, among two participants with the lowest RMSE values for the AM PRS models, the most important variables were muscle soreness, prior-day PRS scores, and prior-day protein intake for one athlete, while the top variables for another athlete were all related to sleep (prior night, previous nights, and 7-d rolling averages). The importance of the individual differences is further evidenced by the improved performance (2–17% improvement in RMSE) of the individual linear models that used the individual's top 5 variables compared with the top 5 group variables (Table 3).

I.5.3 Explain vs. Predict

The priority of a statistical model can be to explain (i.e., test causal explanations), predict (new or future observations), or describe the data structure in a compact manner [642]. The focus of this analysis was on predictive power, for several reasons. The observational nature of our data from free-living environments is better suited to predictive modeling, whereas laboratory-controlled experimental data are better for explanatory modeling [642]. In the context of a large dataset with complex relationships, predictive modeling can help uncover potential new causal mechanisms and lead to the generation of new hypotheses [642]. This is reflected in the variable importance scores, particularly for HRV change, where few of the top predictors could be thought of as having any causal role. However, new hypotheses could be generated relating to a potential reversion to the mean effect for HRV, for example, based on the negative relationship between the top predictors and the daily change in HRV (Figure I.4, partial dependence plot). From a practical perspective, use of these models should be limited to communicating the expected values for an athlete on a given day, rather than suggesting ways to modulate the variables of interest.

I.5.4 Athlete monitoring

Direct monitoring of training and fatigue responses is common in high-performance sport environments [622]. Better understanding of an athlete's response to training and recovery could help coaches improve the effectiveness of a training program. However, it is challenging to control for, or even account for, the large number of variables potentially influencing an athlete's response to training, particularly over longer time frames. Observational studies can help to answer questions that would not be feasible to study in a controlled laboratory environment. A strength of this study design is the length of monitoring period, which allowed athletes to capture a range of daily and weekly training volumes. Advances in technology have also opened far more opportunities to gather valid and reliable data from athletes in their home training environments [643, 644]. Although dietary intake can often be underreported, nearly all previous studies have used short-duration food records rather than smartphone apps. It has been suggested that familiarity with and interest in keeping food records may lead to more reliable estimates of energy intake [553], and in our study all participants were already habitually recording dietary intake using a smartphone app. Although this approach to gathering data would not suit all athletes, many are accustomed to daily tracking of a wide range of data, and it is likely that a model-based analytical approach could offer valuable insight.

I.5.5 Machine Learning

Machine learning has been increasingly used in sports science, often for predicting injuries [626], but also for predicting training feeling scores [627], and subjective wellbeing [628]. Machine learning algorithms can be criticized for their lack of transparency, particularly when combined in an ensemble as we did in this study. This approach was chosen to optimize prediction accuracy, with linear models constructed as a transparent alternative. Indeed, the ensemble models achieved the best performance, but the linear models also performed nearly as well (Tables I2 and I3). This finding is echoed by a systematic review showing no performance benefit of machine learning over logistic regression for clinical prediction models [645]. However, in our study the complex models played a critical role in being used to identify the top variables for the linear models.

I.5.6 Limitations

Limitations of this study relate to the observational and uncontrolled nature of the data collection, the large number of variables collected, and the potential for important factors to have not been collected. Participants were required to record their training, diet, sleep, HRV, and subjective wellbeing daily for 12 weeks. We specifically recruited people who were already doing this routinely, as this approach would not be practical for all athletes. Data integrity was checked based on the number of missing values, and by looking for trends in dietary reporting that could not be explained by changes in training load or body weight. Nonetheless, it is possible that participants did not always enter data as accurately as possible. There is also the risk of bias in reporting if an athlete is aware that their coach or a researcher will be seeing their data, answering based on what they think is desirable. Despite capturing a wide range of variables, we only had a single measure of internal training load and no measure of external load. This was done to accommodate athletes training across a variety of endurance and strength training modalities. Future research in single-sport athletes (e.g., cyclists or runners) would allow additional load metrics like HR, total work, or distance to be more easily factored into the modeling. In addition, energy availability, alcohol intake, and menstrual cycle tracking would be desirable metrics to include. Future work could also benefit from using continuous sliding scales for subjective wellbeing measures that would allow decimal places to be recorded, rather than the 7- or 10-point integer scales built-in to the training monitoring software. This was the reason we used the 100-point, rather than 10-point PRS scale [573]. Finally, no performance measures were captured, leaving the ultimate utility of this approach unclear.

I.6. Perspective

To our knowledge, this is the first study of its kind to track this diverse range of self-selected and self-reported training of endurance athletes. Findings from this study, and the approach used, can enable coaches and athletes to better understand and focus on the few key measures which can offer an outsized amount of predictive capability. Although the prediction accuracy could likely be improved by capturing additional variables of interest, the current predictions offer information that is practically relevant. For example, an RMSE value of 12 from our model using the 100-point scale would translate to an average error of 1.2 when using a 10-point wellbeing scale, providing a coach with a useful gauge of an athlete's readiness. These data also reveal the importance of looking into factors affecting each athlete, rather than applying group-level findings to the individual. Importantly, use of these models should be limited to communicating the expected values for an athlete on a given day, rather than suggesting ways to modulate the variables of interest. This approach can also be combined with domain knowledge to individualize key metrics for athlete monitoring and evaluation.

Appendix J. A Carbohydrate Training Index to quantify fueling for the work required

This chapter is a commentary highlighting the need for a method of quantifying how an athlete adjusts their daily carbohydrate intake in relation to their training load and presenting a novel Carbohydrate Training Index (CTI) — a single metric to capture how tightly an athlete's carbohydrate intake is adjusted based on their training load, the magnitude of adjustment, and how frequently these adjustments occur.

This chapter contains the following publication:

Rothschild J, Morton J, Stewart T, Kilding A, Plews D. A Carbohydrate Training Index to quantify fueling for the work required (under review)

J.1 Abstract

Contemporary sports nutrition guidelines recognize that endurance athletes should modulate their daily carbohydrate (CHO) intake according to the demands of their training and competitive schedule. This concept, referred to as "fueling for the work required", has gained traction among athletes and researchers over recent years. Although the concept is easy for an athlete to understand, the degree to which the diet and training are modulated is likely to vary greatly among athletes. However, objective assessments of the dietary variation occurring with respect to an athlete's training load are not readily available. We believe establishing a method to quantify this relationship would allow researchers and practitioners to better understand how an athlete is varying their CHO intake based on training, perform measurable dietary interventions, and improve accuracy when characterizing athlete practices. In this article we introduce a novel Carbohydrate Training Index (CTI) — a single metric to capture how tightly an athlete's CHO intake is adjusted based on training load, the magnitude of adjustment, and how frequently these adjustments occur. The CTI is calculated as r * range / monotony, where r represents the correlation between daily training load and CHO intake, range is the difference between the highest and lowest single-day CHO intake (g/kg), and monotony is average daily CHO intake divided by the standard deviation. We also provide examples of the CTI in practice, using data obtained from professional cyclists during a Grand tour. The CTI represents a promising tool that that can be used by researchers, coaches, and athletes to quantify diet-training practices and make comparisons within and between individuals.

J.2. Introduction

Contemporary sports nutrition guidelines recommend carbohydrate intake be individualized to the athlete and their event, and modulated according to changes in exercise volume [180]. This is commonly known as "fueling for the work required" [2]. At its simplest level, fueling for the work required can be recognized practically by daily fluctuations in total carbohydrate intake that consider the physical demands of the training and competitive schedule. These purposeful alterations to daily carbohydrate intake have the potential to modulate cell signaling pathways that regulate training-induced skeletal muscle adaptations [2, 181], influence training intensity and exercise capacity [12, 13, 163], manage body composition, and reduce the risk of inadequate energy availability [480]. Despite the strong theoretical rationale for modulating dietary carbohydrate intake, there is limited evidence of how such practices are currently practiced by athletes during real-world, day-to-day training, likely related in part to the lack of any objective way of quantifying it. To date, knowledge of athlete practices has largely been limited to surveys [19-22], case studies [23, 24], or short-duration (3–7 d) observations [25, 26].

Although the concept of fueling for the work required is easy for an athlete to understand, the degree to which the diet and training are modulated is likely to vary greatly among athletes. It is also possible that many athletes who are aware of this approach may be unclear if, or how, they are implementing it. For example, it has been reported that despite an awareness of dietary periodization strategies they were not widely implemented by elite athletes during a period of intensified training [25]. This suggests there may be a gap between what athletes are doing and the current best-practice recommendations. Combined with the lack of precision in questionnaires (e.g., simply asking someone if they adjust carbohydrate intake based on their planned training sessions), there is a need for an objective method of quantifying the degree of dietary variation occurring with respect to an athlete's training load.

Accordingly, the aim of this article is to highlight key aspects of "fueling for the work required" from a theoretical and applied perspective. We first present rationale for why a method to quantify an athlete's practice of fueling for the work required is needed. We then outline the nutrition- and training-based factors that can be quantified, leading to the introduction of a novel Carbohydrate Training Index (CTI) — a single metric to capture how tightly an athlete's carbohydrate intake is adjusted based on their training load, the magnitude of adjustment, and how frequently these adjustments occur. We also provide examples of the CTI in practice, using data obtained from professional cyclists during a Grand tour.

J.3. Why should we measure the diet-training relationship?

An ability to quantify how an athlete is fueling for their required training load would be of benefit to practitioners, researchers, and athletes. Practitioners working with athletes currently have no way to objectively look at an athlete's diet and quantify how they are adjusting their intake based on training. Consequently, the ability to prescribe or assess any intervention that aims to match an athlete's diet more closely to their training volume becomes challenging. For researchers, a quantifiable approach would allow the dietary practices of athletes to be characterized more accurately. While questionnaires can provide valuable information across groups of athletes [21], there are limitations related to the qualitative and binary nature of the questions (e.g., do you adjust carbohydrate intake based on planned training sessions, do you eat more on hard training days or less on easy training days). For example, athletes with more extreme changes in dietary intake would be classified in the same way as athletes who might make subtle day-to-day variations [530], and some athletes may think they adjust their intake based on training, but objective measurement could suggest otherwise [25]. Measuring the diet-training relationship would also allow studies to be designed to investigate the influence of fueling for the work required on training and recovery outcomes and allow for a common metric of comparison across studies investigating variations in carbohydrate intake with training. Finally, the individual athlete may wish to compare their own diet-training practices to those of other athletes. Taken together, quantifying how an athlete modulates their dietary carbohydrate intake based on their training would allow practitioners, researchers, and athletes to assess if/how the fuel for the work required framework is being followed, from the individual to the group level.

J.4. What should we measure?

To begin quantifying an athlete's fueling in relation to their training load, several factors can be considered relating to how tight the relationship is, how extreme the highs and lows are, and how often it gets modulated.

J.4.1 Measures of Training Load

Any measure of fueling for the work required needs, by definition, a measure of work performed. Training load can be measured and classified as either internal and/or external, based on the measurable aspects occurring internally or externally to the athlete [545]. Internal load reflects the relative physiological strain and disturbance in homeostasis of the metabolic processes in response to an external load, which is characterized by objective measures such as distance, power, or speed [624, 646]. Due to the wide availability of cycling power meters, total energy expenditure during exercise (measured as kJ or kcal) is a sensible option when measuring training load for cyclists. However, very large correlations have been reported across multiple measures of training load in cyclists during racing and training, suggesting other metrics such as session rating of perceived exertion (sRPE), Lucia training impulse, and training stress score (TSS) could also be used [647]. Because the relevance of training load is primarily related to the relationship between training load and dietary intake (described in section 8.4.2), the use of other load measures such as distance for runners or swimmers, or GPS-based metrics in team sports could also theoretically be used. Measures of internal load could also be explored in this context. Internal load plays a pivotal role in determining training outcomes and can reflect variations in the stress response to a given external load due to other stressors such as extreme temperature, or accumulated training fatigue [545]. Most notably, the sRPE provides a valid and reliable method for calculating training load across exercise modalities [538], a necessity for multi-sport athletes.

J.4.2 Correlation Between Intake and Training Load

The correlation between carbohydrate intake and training load can offer insight into how tightly an athlete matches their intake with their training. Higher values indicate a tighter relationship between increasing training load and increasing dietary carbohydrate intake. Correlations are typically measured using the Pearson product–moment correlation coefficient, in the case of normally distributed data, or the Spearman rank correlation coefficient, for data that are non-normally distributed and can potentially include influential outliers [648]. As shown by the simulated data in Figure J.1, correlation values can be calculated based on the relationship between daily carbohydrate intake and training load. The correlation provides a useful measure of how tightly an athlete links their intake with their training but does not offer any insight into how wide the adjustments are between the high and low volume days. Figure J.1 depicts two athletes with similar training loads and similar correlation values, but a clear difference in how they manage their fueling. This discrepancy can be addressed by also considering the range of daily carbohydrate intake by an athlete.

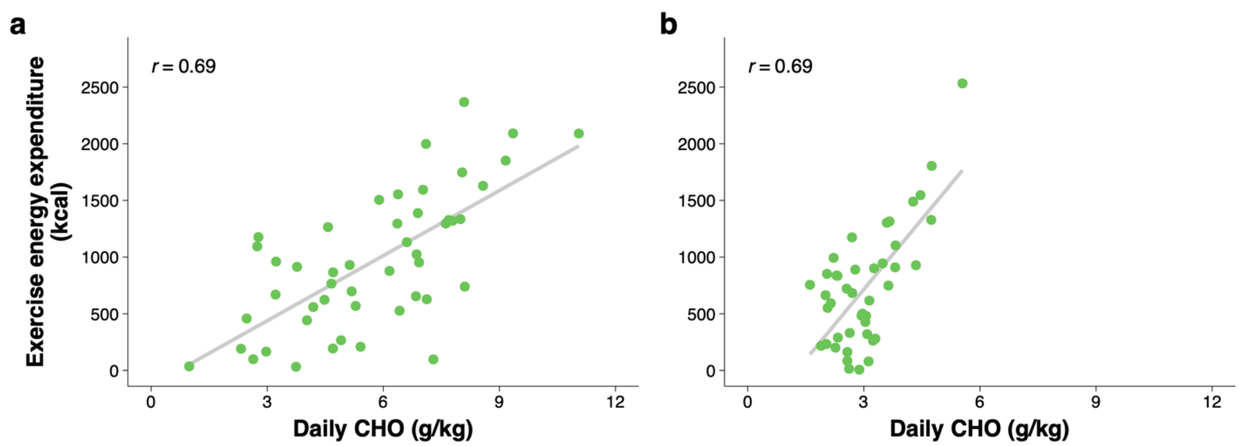


Figure J.1. Example correlations between daily dietary carbohydrate (CHO) intake and exercise energy expenditure. Despite similar training volumes and correlation values (shown as r values on each panel), hypothetical athlete (a) varies their intake to a much greater degree than athlete (b)

J.4.3 Range of Daily Carbohydrate Intake

For some athletes, increasing carbohydrate intake on a high-volume training day might simply mean adding a banana or potato to their meals, while for others it might lead to a 2–3x increase in daily carbohydrate intake. If they are consistent over time, their correlation values would be similar despite a large practical difference (Fig. J.1). Therefore, quantifying the range of daily carbohydrate intake can provide a measure of how extreme the changes are between high- and low-volume training days. When calculated relative to body weight, this range value can be compared with any other athlete.

Together, the correlation and range values can provide a great deal of useful information about the coordination between an athlete's diet and training load. However, it is also of interest to know how frequently these changes in diet and training occur. For example, someone could very closely match their intake and training, yet perform very similar types of training that would not warrant large changes in carbohydrate intake. This discrepancy is highlighted with simulated data shown in Figure J.2.

J.4.4 Carbohydrate Monotony

In the context of measuring training load, training monotony is calculated as the daily mean training load divided by the standard deviation (SD) [649]. This concept can be applied to quantifying carbohydrate intake, with carbohydrate monotony calculated as average daily carbohydrate intake (g/kg) divided by the SD. This monotony measure decreases as the daily variation in carbohydrate intake increases. Example athletes with high and low monotony scores are shown in Figure J.2. Although carbohydrate monotony scores will be dependent on the training plan, they still provide a useful measure for monitoring the variability in an athlete's daily carbohydrate intake.

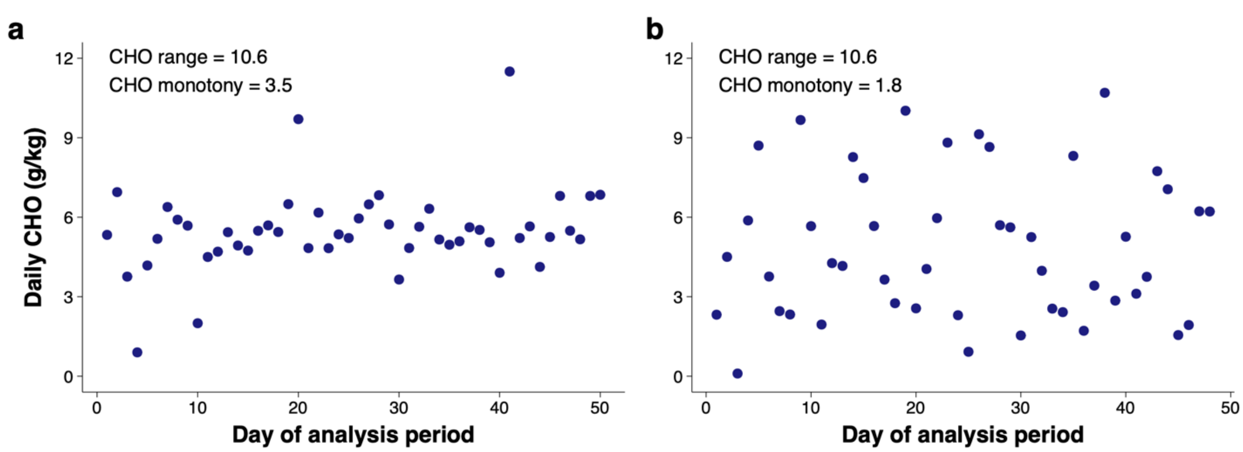


Figure J.2. Example carbohydrate (CHO) monotony and range scores of two hypothetical athletes. Despite a similar range of CHO intake (highest minus lowest daily intake values), athlete (a) displays greater monotony than athlete (b)

J.5. Carbohydrate Training Index

In light of the above rationale, we propose a novel Carbohydrate Training Index (CTI) as a single value that could capture the combination of how closely an athlete modulates their carbohydrate intake based on training load, the degree of day-to-day variation in carbohydrate intake, and how frequently these modulations occur, as:

CTI = *r* * range / monotony

Where *r* represents the Pearson correlation value between daily training load and daily carbohydrate intake (g/kg), range is calculated as the difference between the highest and lowest single-day CHO intake (g/kg), and monotony is calculated as average daily carbohydrate intake (g/kg) divided by the standard deviation. Simulated CTI values are shown in Figure 8.3, across a range of inputs. Based on published and unpublished data from elite and non-elite endurance athletes, we interpret CTI scores using the following guidelines:

< 1.0: no evidence of dietary carbohydrate periodization

– 2.0: evidence of moderate dietary carbohydrate periodization

2.0 – 3.5: evidence of a high degree of dietary carbohydrate periodization

> 3.5: evidence of extreme dietary carbohydrate periodization

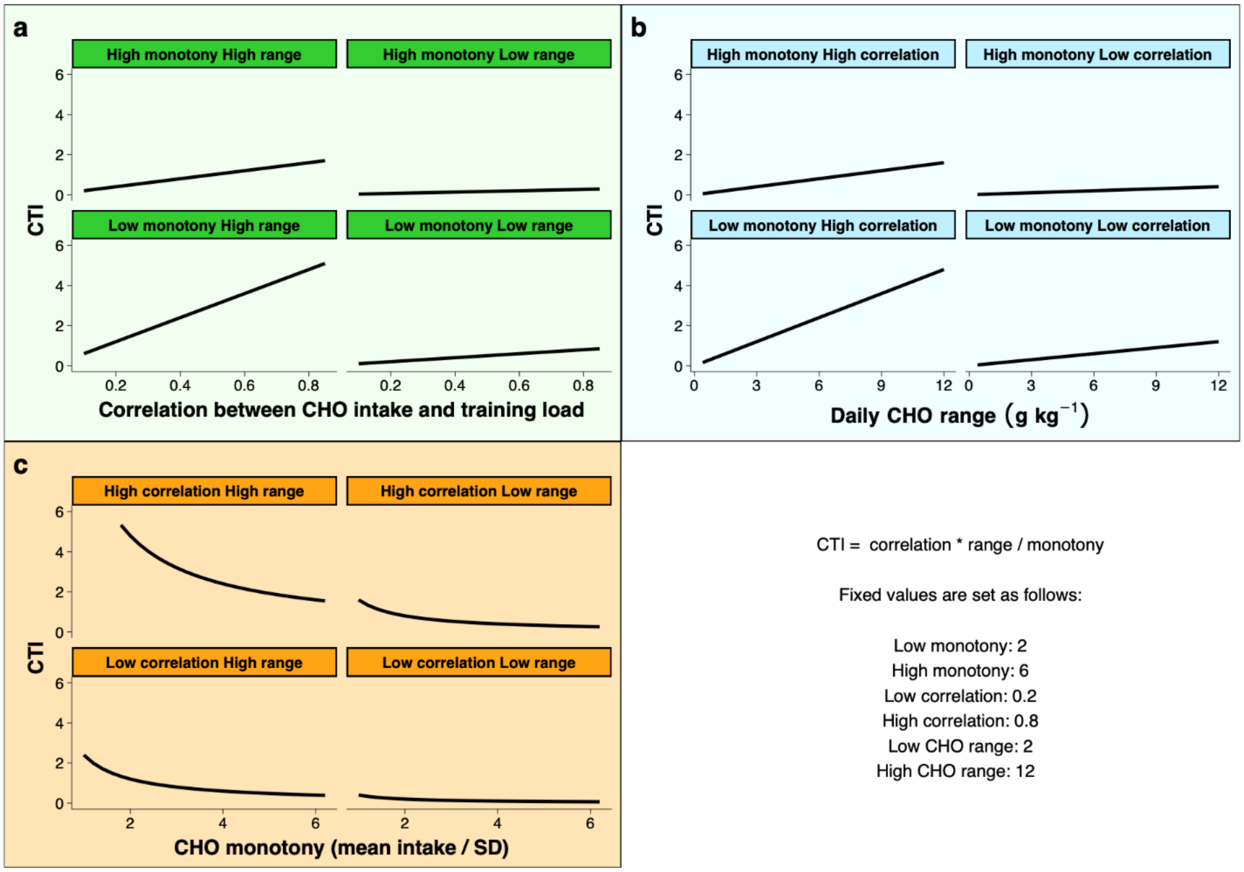


Figure J.3. Simulated Carbohydrate Training Index (CTI) values across a range of correlations (a), carbohydrate (CHO) intake ranges (b), and CHO monotony values (c)

Being a composite of three variables, one could achieve the same CTI score in a variety of different ways (Fig. J.3). Therefore, at the individual level interpretation should be informed by the context of the athlete's training load (e.g., large training volumes would be needed to achieve a high range of carbohydrate intake), habitual dietary pattern (e.g., lower-carbohydrate athletes may struggle to achieve a high range), and personal preferences. The length of observation period could also influence the achievable CTI values. A very high CTI (> 3.5) may more likely be attained during a short period of time such as a cycling Grand Tour, where the energy expenditure can vary greatly across stages and rest days. This allows for a large range of carbohydrate intake within a short time, lowering the monotony score. Additionally, few recreational athletes could achieve the workload of professional cyclists, particularly during a Grand Tour, thereby placing a *de facto* limit on their available carbohydrate intake range. To ease calculations and provide additional examples, a free online app has been created where the user can calculate the CTI by entering daily training load and dietary carbohydrate intake values [650].

J.6. CTI in Practice

Figure J.4 demonstrates the CTI using previously reported data from professional cyclists during a Grand tour [23, 651]. Both cyclists were working with sports nutritionists intentionally following the fuel for the work required principles. Expectedly, both athletes display high CTI values reflecting the combination of high correlations between intake and energy expenditure, and large range in daily intakes.

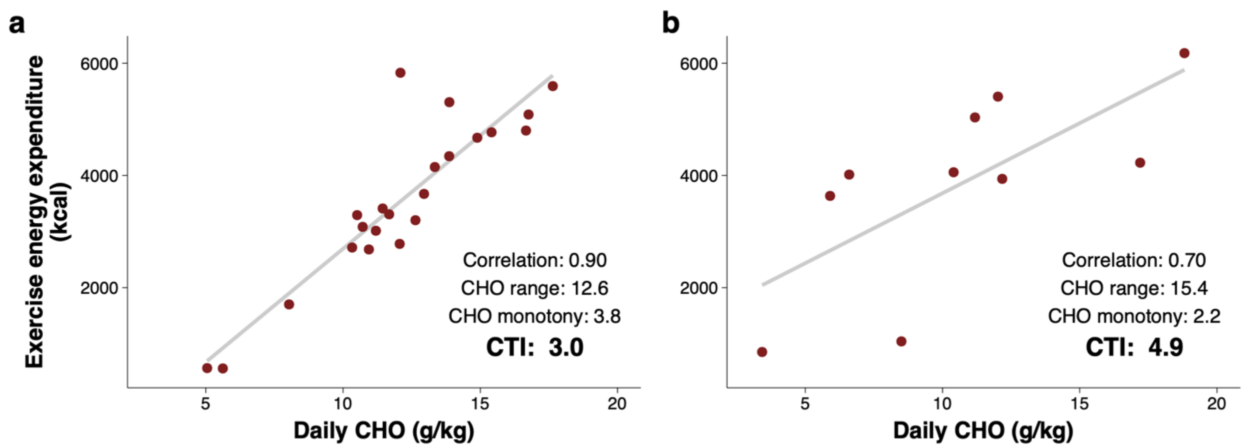


Figure J.4. Practical application of the Carbohydrate Training Index (CTI) using data collected from professional cyclists during a Grand tour. Carbohydrate (CHO) range is calculated as the difference between the highest and lowest days, monotony is calculated as the mean divided by the standard deviation, and CTI is calculated as the correlation * CHO range divided by CHO monotony

J.7. Considerations

To be useful, any metric needs to be both reliable and valid. Reliability is assured in this context, as an athlete would have the same CTI during multiple periods if they had similar eating and exercise patterns. Establishing validity requires being able to compare the CTI to some "ground truth”. However, to our knowledge, there are not any measures of fueling for the work required, or any type of carbohydrate periodization, to compare with. More than ten iterations of the index were considered, with the equation established based on a combination of domain expertise and experience of the research team, using simulated and real-world data. Despite the lack of gold-standard method for quantifying an athlete's carbohydrate-training practices, the approach is widely used by athletes and practitioners. Therefore, it is of interest to quantify it.

It would be tempting to want to link the CTI metric to a functional or practical outcome such as performance or training adaptation, but that is not the point of this paper or the metric itself. Furthermore, it is beyond the scope of this paper to suggest athletes aim for a certain value, or that increasing the value will lead to specific outcomes. Indeed, that is the role of the individual practitioner, coach, and athlete to decide. Rather, we believe it is important to have a tool to measure and characterize the practices of athletes in the field. Future research is needed to determine any relationships between the CTI and specific performance or adaptation outcomes.

A potential limitation of the CTI relates to the 24-h periods used for analysis. The CTI is therefore unable to discern within-day carbohydrate manipulation, such as twice-daily training without carbohydrate between sessions [12] or an overnight sleep-low approach [165, 178]. However, aligning with the "fuel for the work required" paradigm [2], appropriate daily intake should be ensured regardless of the within-day timing. The CTI does not attempt or aim to categorize various periodization strategies. Future work could consider a within-day index to work alongside or as part of the CTI. It is also conceivable that carbohydrate intake over rolling 3- to 4-day periods could be beneficial. However, recent data from our lab [572] showing athletes adjust their carbohydrate intake based on the current day's training but not the prior or subsequent days, implies periods longer than 24 h would reduce the resolution of dietary intake without adding much practical significance.

An additional limitation is that the correlation values could be influenced by outliers, in which case an alternative to the Pearson calculation such as the Spearman correlation might seem more appropriate. Although the correlation coefficient for each athlete can be adjusted based on the distribution and existence of outliers, we recommend against this because outlier values matching a very large training load are both appropriate and important, in the context of fueling for the work required. Furthermore, extremely high correlations were found ($r = 0.96$) when calculating the CTI using the Pearson and Spearman correlation values, on 12 weeks of training data from 40 endurance athletes [652]. Finally, beyond its utility in the research setting the CTI was created as a practical tool for athletes and coaches. Therefore, the required calculations should be easily performed on widely available spreadsheet software, without needing to calculate and choose from multiple correlation coefficients.

J.8. Conclusion and Future Directions

It is recommended that athletes modulate their daily carbohydrate intake according to the demands of their training, a concept referred to as "fueling for the work required". Although this concept is easy for an athlete to understand, objective measures of the dietary variation occurring with respect to an athlete's training load are not readily available. We introduce a Carbohydrate Training Index (CTI), a single metric to capture the combination of how tightly an athlete's carbohydrate intake is adjusted based on training load, the magnitude of adjustments, and how frequently these adjustments occur. The CTI can serve as a tool for researchers, coaches, and athletes to better understand how an athlete is varying their carbohydrate intake based on training, perform measurable dietary interventions, and improve accuracy when characterizing athlete practices.

Future research can determine normative values across a wider population of endurance athletes, if CTI values are robust to different training load metrics, if normative values are comparable across various endurance sports and competitive levels, and how the CTI can be applied outside of endurance sports. It would also be valuable for future studies to report the CTI to quantify differences in intake patterns between groups (e.g., studies comparing chronically high- or low-carbohydrate with periodized carbohydrate intake, or studies reporting self-selected athlete intake). This could allow the CTI to be used as a moderator in meta-analyses, which have thus far found no effect on performance from periodized carbohydrate intakes but have lacked a way to quantify the periodization [653].